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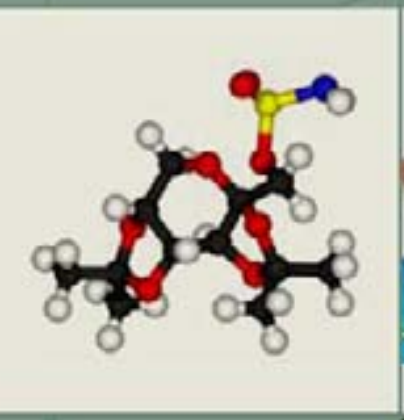

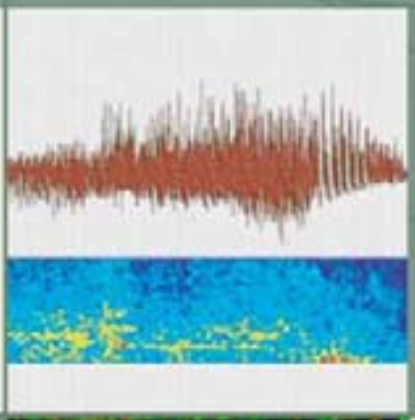

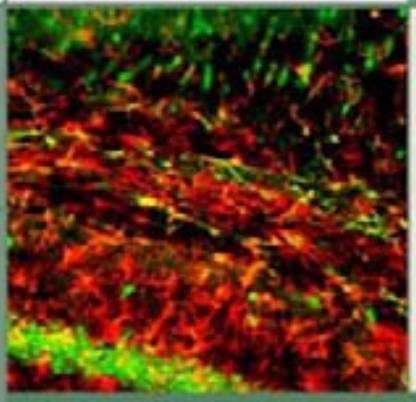

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Dedication

Jasper's Basic Mechanisms of the Epilepsies, Fourth Edition, is dedicated to two groups: those who have committed their lives to the cause of epilepsy and those who live with epilepsy. Dr. H.H. Jasper, the preeminent founder of the neuroscience of epilepsy, is chief among the first, and this book is most appropriately named in his honor. We also dedicate this book to Dr. Frank Risch, committed in a different but no less important way in the service of those with epilepsy. Through his efforts as chief of rehabilitation at the Los Angeles VA Hospital and as an originator of the Epi-Hab training facilities, he improved the lives of countless adults with epilepsy. More than 50 to 60 million families worldwide struggle with epilepsy every day. In their honor, we recognize 21-year-old Zachary Brigido and his family in Lincoln, Rhode Island. Zachary has a severe and uncontrolled seizure disorder; he needs help with all aspects of daily living. Zachary's mother, LeeAnn has crusaded to raise awareness of epilepsy research and to find a cure for 19 years. Zachary's parents stepped forward to help sponsor the Jasper's Basic Mechanisms of the Epilepsies Workshops in Yosemite through their support of CURE—Citizens United for Research in Epilepsy. Proud to sponsor our research conference, LeeAnn wrote: "Together, maybe we can make it possible for me to someday say: My son *had* epilepsy." Zachary and LeeAnn remind us of the families who live with epilepsy and seizures. They give purpose to our work. Thus, in the spirit of Dr. H.H. Jasper, this Fourth Edition of *Jasper's Basic Mechanisms of the Epilepsies* is dedicated to those who must battle epilepsy in their daily lives and also to the community of researchers and caregivers who commit their own lives to make a positive difference. We hope this book inspires others to join our search for cures. Perhaps among them will be those who find a way to end the epilepsies.

—The Editors

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Preface

When H. Houston Merritt was appointed by the Surgeon General of the Public Health Service in May 1966 to chair the Public Health Service Advisory Committee on the Epilepsies, he formed two subcommittees—one on Research and Research Training, led by Arthur A. Ward, Jr., and one on Service and Service Training, led by David D. Daly. A basic research task force was established under Herbert H. Jasper's leadership. "It was apparent to this task force that basic research in the epilepsies needed stimulation."[1] Thus, they developed plans for a workshop held in Colorado Springs in February 1968 and an open symposium in November 1968. A "modern definitive statement on current knowledge regarding fundamental aspects of the epilepsies" was the goal. Such a statement would "serve as a benchmark against which future progress can be measured."

Since the original workshop and symposium of 1968, basic and clinical researchers in epilepsy have gathered together every 14 years to hold workshops (at the Kroc Foundation Headquarters in Santa Ynez in 1982, at the Marina Yacht Club in San Diego in 1996, and in Yosemite National Park in 2009) with these goals in mind: to assess where epilepsy research has been, what it has accomplished, and where it should go. In a Foreword written for the Second Edition of *Basic Mechanisms of the Epilepsies*, published in 1983, Jasper reminded us that the original and ultimate goal of the Public Health Service subcommittee on Research and Research Training was to search for a "better understanding of the epilepsies and seek more rational methods of their prevention and treatment."[2] The third edition of the book was named in honor of Jasper.[3]

In line with the enormous expansion in the understanding of basic epilepsy mechanisms over the past four decades, this Fourth Edition of *Jasper's Basic Mechanisms of the Epilepsies* is the most ambitious yet. In 90 chapters, the book considers the role of interactions between neurons, synapses, and glia in the initiation, spread, and arrest of seizures. It examines mechanisms that underlie excitability, synchronization, seizure susceptibility, and ultimately epileptogenesis. It provides a framework for expanding the epilepsy genome and understanding the complex heredity responsible for common epilepsies as it explores disease mechanisms of ion channelopathies and developmental epilepsy genes. It considers the mechanisms of conditions that are comorbid with epilepsy. And, for the first time, fulfilling the original Merritt and Jasper goals of "seeking rational methods of prevention and treatment," this Fourth Edition describes the current efforts to translate the discoveries in epilepsy disease mechanisms into new therapeutic strategies. In the Foreword for the First Edition, Merritt wrote, "...it is commonly assumed that epilepsy is inherited and numerous studies attest to the importance of hereditary factors." The current edition further justifies Merritt's view of the inherited epilepsies as beacons illuminating basic disease mechanisms, which in turn define molecular targets for designing novel treatments. In keeping with the original justification of Merritt and Jasper for basic epilepsy research, there are now numerous examples of discoveries in the laboratory that intersect with and impact clinical practice. Among these are the application of epilepsy gene discoveries for clinical genetic testing/screening, as well as the use of rapamycin for the treatment of tuberous sclerosis, gentamicin to overcome nonsense mutations in Lafora progressive myoclonus epilepsy, and enzyme replacement therapy for Gaucher's disease.

In its previous editions, this book has been considered the bible of basic epilepsy research, essential to students and research scientists who conduct laboratory-based experimental epilepsy research using cellular, brain slice, and animal models, and also of interest to neurologists and clinical epileptologists. We hope this new edition will honor its venerable legacy by being as useful as these previous volumes.

—The Editors

REFERENCES

1. Jasper HH, Ward AA, Pope A, eds. *Basic Mechanisms of the Epilepsies*. Boston: Little, Brown; 1969.
2. Delgado-Escueta AV, Ward AA, Woodbury DM, Porter RJ. *Basic Mechanisms of the Epilepsies: Cellular and Molecular Approaches*. Advances in Neurology Series, Vol. 44. New York: Raven Press; 1986.
3. Delgado-Escueta AV, Wilson WA, Olsen RW, Porter RJ. *Jasper's Basic Mechanisms of the Epilepsies*. 3rd ed. Advances in Neurology Series, Vol. 79. Philadelphia: Lippincott Williams & Wilkins; 1999.

Acknowledgments

The editors are grateful for the contributions of many capable people who generously offered their time and energy to this project in the belief that a periodic assessment of progress in basic epilepsy research is useful to moving the field forward. Their motivation, like that of the editors, is to advance the basic science of epilepsy in the service of those suffering with the condition in its diverse manifestations.

We are especially indebted to the distinguished members of the international editorial advisory board—Giuseppe Biagini, Amy Brooks-Kayal, Wolfgang Löscher, Helen Scharfman, Phil Schwartzkroin, John Swann, and Annamaria Vezzani—who assisted with the selection of topics and the peer review of each chapter. They were our indispensable editorial partners, but we assume responsibility for errors. We also thank Maxime Levesque and Gabriella Panuccio, who helped with the editorial work of Section 2. Brief summaries of each chapter were published in December 2010 as a supplement to *Epilepsia*; we are grateful again to Phil Schwartzkroin who, this time in his role as the journal's coeditor, made this opportunity available.

Essential to the development of this book was the Jasper's Basic Mechanisms of the Epilepsies International Workshops held in March 2009 at the Yosemite Lodge at the Falls in Yosemite National Park. The workshops at Yosemite provided a foundation for each of the sections of the book. The road to the Yosemite meeting included planning sessions at the annual meetings of the American Epilepsy Society in 2006 (San Diego), 2007 (Philadelphia), and 2008 (Seattle). Development of the book began in earnest at a breakfast with the members of the editorial advisory board at the end of the Yosemite meeting, with inspiration from majestic pines and glaciated peaks. It reached a crescendo at a gathering of the editors on a glorious day in August 2009 in Malibu, California—including a walk to clear the mind on cliffs overlooking the Pacific Ocean.

We thank Susan Gayle Pietsch Escueta and the Epilepsy Foundation of Greater Los Angeles, Kathy West and the Epilepsy Foundation of San Diego, and members of the international GENESS consortium who volunteered their time to staff the Yosemite meeting because they believed in the importance of this project. Financial support for the meeting was provided by Citizens United for Research in Epilepsy, the National Institute of Neurological Disorders and Stroke, the National Institute of Child Health and Human Development, the American Epilepsy Society, the Children's Neurobiological Solutions Foundation, the Savoy Foundation, and Johnson and Johnson's Scientific Affairs Division.

Finally, the editors salute our predecessors who conceived of a book on basic mechanisms of the epilepsies and, most importantly, the authors of the 90 chapters compiled in this volume who admirably rose to the challenge when we asked.

—The Editors

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SECTION 1

Introduction

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Chapter 1

The Next Decade of Research in the Basic Mechanisms of the Epilepsies

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SECTION 1: INTRODUCTION

In 1969, H.H. Jasper, A.A. Ward, and A. Pope and the U.S. Public Health Service Advisory Committee on the Epilepsies of the National Institutes of Health published the first volume on *Basic Mechanisms of the Epilepsies*.[1] Since then, basic and clinical researchers in epilepsy have gathered together each decade to assess where epilepsy research has been, what it has accomplished, and where it should go.[2] In 1999, the third edition of the book was named in honor of H.H. Jasper.[3] In February 2010, the International League Against Epilepsy (ILAE) Commission on Classification and Terminology revised the classification of seizures and the many forms of epilepsy. Epilepsies are now classified into three types: genetic, structural/metabolic, and unknown.[4] Independently, the World Health Organization (WHO) is in the process of revising the International Code of Diseases-11 Codes (ICD-11 Codes) and may well follow the same general scheme. Conceived with an emphasis on basic mechanisms, these two new international classifications represent important changes in concept and approach, changes that the third edition of *Jasper's Basic Mechanisms of the Epilepsies* had espoused in its 1999 classification of epilepsies as necessary for answering the essential questions of how seizures start and stop, and for aligning the underlying causes of epilepsy phenotypes with their molecular and cellular disease pathways.[3] The fourth edition of *Jasper's Basic Mechanisms of the Epilepsies* continues in this strategic direction. Four major sections of this edition have been organized to address a multidisciplinary range of issues: the fundamentals of neuronal network excitability that produce seizures and epilepsy; how seizure mechanisms contribute to epileptogenesis;

Table 1–1 **Top Research Priorities: Where Should Epilepsy Research Go in the Next 10 Years?***

1. Understand mechanisms of epileptogenesis; create methods for monitoring the development of seizure susceptibility; identify approaches to prevent epilepsy
2. Understand mechanisms of neuronal synchronization and the triggering of seizures
3. Determine the genetic risk factors for the non-Mendelian epilepsies and the role of genetic background in determining epilepsy phenotype; understand how mutations produce human epilepsy phenotypes; explore the role of epigenetic factors
4. Understand the role of brain development in epilepsy and the influence of epilepsy on the developing brain
5. Characterize the role of glia and immune mechanisms in seizures and epileptogenesis
6. Develop drugs targeted to disease mechanisms
7. Explore the potential of nonconventional treatment approaches, including brain stimulation, cell and gene therapy, and approaches for influencing brain remodeling
8. Understand mechanisms of pharmacoresistance to antiepileptic drugs and develop methods to overcome refractoriness
9. Develop new targeted approaches to deliver therapies

*Top research priorities as proposed and voted on by the authors of the fourth edition of *Jasper's Basic Mechanisms of the Epilepsies* during the Yosemite workshops and subsequently modified by the editors.

genetic mechanisms of heritable susceptibility to epilepsy in the developing brain; and new mechanisms in epilepsy therapeutics.

As in previous editions of *Jasper's Basic Mechanisms of the Epilepsies*[1–3], the first chapter of this edition looks toward the next decade and prioritizes research areas where efforts should be accelerated and enhanced (see Table 1–1). For the first time, basic research in the epilepsies foresees the promise of personalized medicine based on genomic information as current efforts are described to translate the discoveries concerning epilepsy disease mechanisms into molecular and cellular therapeutic strategies that bring repairs and cures to specific epilepsies.

SECTION 2: FUNDAMENTALS OF NEURONAL EXCITABILITY RELEVANT TO SEIZURES AND EPILEPSY

Neurobiological research has progressed swiftly during the last decade, providing detailed information on the molecular, pharmacological, and functional characteristics of voltage- and ligand-gated membrane signaling mechanisms regulating neuronal network excitability. Analysis of the mechanisms of high-frequency oscillations has furthered our understanding of how perturbations in fundamental neuronal activity patterns—often overlapping those involved in learning and memory—become relevant in the generation of partial epileptic discharges as well as in epileptogenesis. Along with the views originally formulated by Herbert Jasper and his former student Peter Gloor, thalamocortical and reticulocortical mechanisms have been firmly established as the substrate for primary generalized seizures. An eminent example is the absence seizure phenotype, which is circuitry dependent, with exclusive thalamocortical expression, and where details of cellular and molecular events are defined. Burst firing in the thalamocortical circuitry of GABAergic reticular thalamus neurons is activated by low -voltage activated T-type calcium channels producing electroencephalographic (EEG) spike-wave discharges and absence seizures. Excessive gamma-aminobutyric acid (GABA) release from reticular thalamus hyperpolarizes thalamocortical neurons through an increased tonic $GABA_A$ receptor current. $GABA_B$ receptors, in turn, modulate this tonic current, explaining the antiabsence action of $GABA_B$ antagonists. The seeds of this concept were planted in the laboratory of David Prince when it was shown that the thalamic T-type voltage-dependent calcium current was a target of the antiabsence drug ethosuximide. This concept continued to flower when P/Q calcium channel mutations in mice were shown to lead to downstream elevations in thalamic T-type voltage-dependent calcium channels that, in turn, produced spike waves, and when it was shown that overexpression of T-type channels produces a spike-wave epilepsy phenotype. The animal model evidence

for thalamic T-type calcium channel involvement in human epilepsy is further supported by the association of mutations in genes for T-type channels, P/Q type channels, and *GABRB3* in individuals with absence epilepsy. In *GABRB3* knockout (KO) mice, a model of human remitting absence epilepsy, downstream changes also lead to elevations in thalamic T-type calcium channel activity. However, and in line with Gloor's corticoreticular concept that attributed to both thalamus and cortex fundamental roles in absence seizure generation, evidence obtained from rat genetic models of spike-wave discharge indicates that this epileptic discharge can also have a cortical origin.

Myoclonic and tonic-clonic convulsive seizures may also be circuitry dependent with reticulocortical expression. Audiogenic kindling in genetically epilepsy-prone rats induces tonic-clonic seizures that initially require only activation of brainstem nuclei. After audiogenic kindling, the "seizure network" permanently expands into the forebrain, involving *N*-methyl-D-aspartate (NMDA) receptor-mediated cyclic adenosine monophosphate (cAMP) changes in amygdala. Imaging and computer-based modeling of neuronal networks underlying epileptic discharges are also providing valuable information defining neuronal network synchronization under physiological and pathological conditions.

In this context, several studies have recently pointed to a peculiar role played by $GABA_A$ receptor-mediated mechanisms in synchronizing neuronal networks during some type of high-frequency oscillations (termed *ripples*) as well as during interictal discharges in partial epileptic disorders. As these basic advances continue, new properties of abnormal signaling in brain networks, such as pathological high-frequency oscillations (20–80 Hz Gamma frequencies and 200–600 Hz 'ripples'), provide useful electrocorticographic diagnostic and prognostic markers and point to disease mechanisms that link to human epilepsies and that then may translate into new therapeutic tools and antiepileptogenesis drugs.

SECTION 3: MECHANISMS OF SEIZURE SUSCEPTIBILITY AND EPILEPTOGENESIS

Major strides have been made in the last decade in describing how brain damage leads to the chronic and enduring condition of spontaneous seizures, a process defined as *epileptogenesis*. Accumulating evidence for major biological events occurring during the period of epileptogenesis in human and experimental temporal lobe epilepsy includes (1) the progressive formation of new recurrent excitatory circuits such as mossy fiber sprouting; (2) the selective and progressive loss of specific, vulnerable GABAergic interneurons; (3) the extensive molecular plasticity at the transcriptional, translational, and trafficking levels of protein expression; (4) the loss, dispersion, and proliferation of dentate granule cells; and (5) the pathogenic appearance of dentate granule cells in ectopic locations or with abnormal hilar basal dendrites.

Mossy fiber sprouting of excitatory axons and establishment of new synaptic connectivity is now accepted as a ubiquitous epileptogenic response to neocortical or hippocampal damage; the typical sprouted axon projecting into the granule cell layer and the inner molecular layer forms >500 synapses, of which >95% are with other granule cells. Granule cells with basal dendrites are highly interconnected with each other and with adult granule cells, and may act as hubs for excitatory activity and enhance hyperexcitability in the dentate network. While axonal sprouting is accepted as a common neuropathological feature in acquired temporal lobe epilepsy produced by trauma, hypoxia/ischemia, stroke, febrile seizures, status epilepticus, and infections, the time course of granule cell hyperexcitability and spontaneous epileptiform discharges following epileptogenic injuries, molecular mechanisms preceding and leading to axonal sprouting, and the construction of epileptogenic circuits within the reorganized networks are the focus of chapters in this section

Section 3 of this book should be of particular interest to clinical epileptologists who study the syndrome of febrile convulsions/hippocampal reorganization/mesial temporal lobe epilepsy. This latter syndrome has a >90% success rate in the elimination of seizures after resection of hippocampal epileptogenic zones. Excitotoxic, necrotic, and programmed (apoptotic) cell death are being analyzed experimentally in brain tissue from identified human focal epilepsy cases. Within this tissue, the roles of neurogenesis and neurotrophin biology continue to provide essential information on the mechanisms underlying reorganization and synaptogenesis. Increases of brain derived neurotrophic factor (BDNF) expression and enhanced activation of tyrosine kinase B (TrkB) in the mossy fiber pathway of hippocampus contribute to hyperexcitability,

while the integration of adult-born dentate granule cells may restore inhibition. These disease pathways may illuminate novel targets for the repair of hyperexcitable neural circuits, a new strategic goal in epilepsy therapy.

Another long-standing focus of interest discussed in this section is the field of GABA receptor plasticity. Differing approaches with new insights into the roles of pre- and postsynaptic GABA receptor-mediated transmission and chloride homeostasis are described. In temporal lobe epilepsy models, expression of the $GABA_A$ receptor δ subunit is substantially decreased in principal cells but increased in interneurons, and a change in the localization of the γ2 subunit from primarily synaptic to perisynaptic sites suggests a subunit switch. In alcohol-kindled seizures, remodeling of $GABA_A$ receptors also occurs. Alcohol-sensitive extrasynaptic α4/δ-containing $GABA_A$ receptor-mediated tonic currents in hippocampal and other cells are downregulated. Benodiazepine-sensitive synaptic α1/γ2-mediated synaptic currents are downregulated, and compensatory α4/γ2 extrasynaptic and synaptic GABA receptors are elevated in parallel with increased sensitivity to alcohol. Finally, the biology of glia-neuron interactions is assuming an increasingly central position in current pathological models of epilepsy and also provides novel therapeutic targets for antiepileptogenesis. Reactive astrocytosis leads to reduced adenosine- and GABA-dependent inhibition. Astrocytes regulate surface expression of neuronal NMDA receptors and release of glutamate and D-serine, and they are involved in the pathophysiology of brain inflammation (e.g., cytokines produce toxic autocrine or paracrine mechanisms and alter glutamate and GABA receptor function).

SECTION 4: EPILEPSY GENES AND DEVELOPMENT

The full potential of personalized medicine to revolutionize patient care in the epilepsies will be attained once the epilepsy genome is completed and treatments specific to individual patients are based on pharmacogenomic information. As widely predicted, the creation of physical and genetic maps of the human genome in 2000 led to an explosive increase in knowledge of the number of single genes linked to epilepsy phenotypes over the last decade. In human epilepsies, more than 20 syndromes previously considered idiopathic (now called *genetic generalized epilepsies* in the 2010 ILAE classification) have been shown to arise from mutations in some 45 identified epilepsy genes (Fig. 1–1 and Table 1–2). The distinction between genes expressed primarily as epilepsies and genetic disease whose phenotype includes epilepsy is becoming more blurred as cases that were previously considered primary and idiopathic epilepsies now show brain magnetic resonance imaging and fluorodeoxyglucose (FDG)–positron emission tomography scan abnormalities. Additional chromosomal loci continue to be mapped from clinical pedigrees, and we foresee more than 100 epilepsy genes identified in the coming decade. The low cost and high throughput of deep gene sequencing to discover allelic variants, and the massively parallel whole exome DNA resequencing of individual epilepsy patients, promise to enormously expand the epilepsy genome. With the identification of more epilepsy genes, basic mechanisms of epilepsies will assume even greater importance as proof of causality. The identification of genes will also inspire studies of molecular and cellular function, and of synaptic and network disease mechanisms, and will provide opportunities for the development of novel approaches for prevention, repairs, and cures. In the laboratory, genotype-phenotype relationships continue to emerge at an even faster pace with the use of a reverse strategy, namely, mutating newly discovered human epilepsy genes in engineered mouse models and screening for epileptic phenotypes. These approaches will steadily unlock a collection of epilepsy genes from a broad array of functional biological pathways that exert major control over brain excitability and synchronization. As examples, mice with mutations in auxiliary voltage-activated calcium channel subunit genes *Cacnb4* (*lethargic*) and *Cacna2d2* (*ducky*) show downstream elevations in thalamic T-type currents, enhancing rhythmicity in thalamocortical networks subserving spike-wave oscillations and producing absence; human mutations in $Na_V1.1$ channels reduce Na^+ currents and decrease electrical excitability in GABAergic interneurons and inhibition, producing downstream epilepsy, ataxia, and cognitive decline of Dravet's severe mycoclonic epilepsy. In animal models of tuberous scleroses and cortical

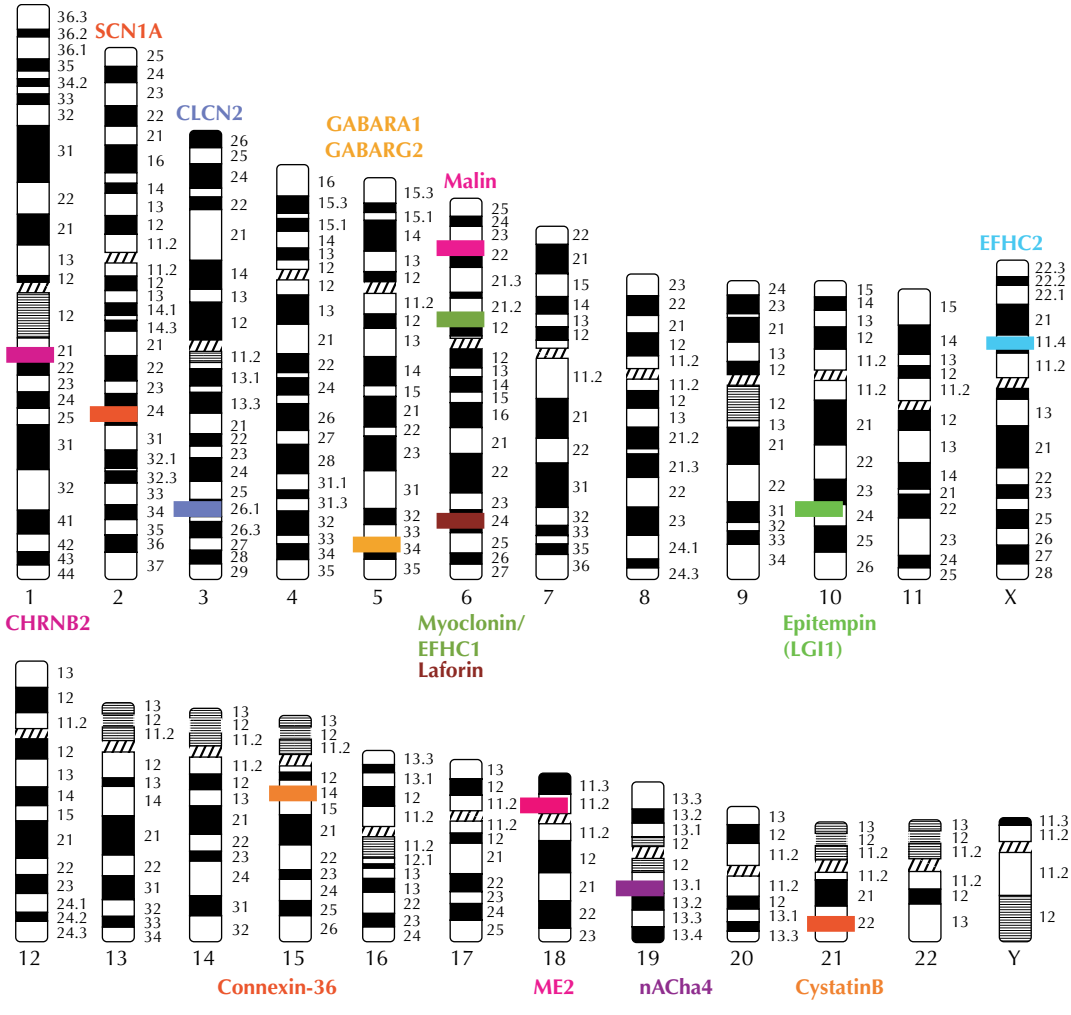

Figure 1–1. Some of the human epilepsy genes and their chromosome loci (based on chapters of this edition of *Jasper's Basic Mechanisms of the Epilepsies*).

dysplasia, hyperactivation of the mammalian target of rapamycin (mTOR) pathway promotes epileptogenesis and neuropathological abnormalities, and the mTOR inhibitor, rapamycin, prevents or reverses epilepsy and associated phenotypes. For many other epilepsy genes, much will be learned in the laboratory about how both the gain and loss of function lead to epilepsy in the developing brain and how they affect neuronal migration, proliferation, differentiation, genesis, and maintenance of synapses and dendrites. Three genes are leading examples of developmental pathways in epilepsy causing lissencephaly, epilepsy, and mental retardation in infants and young children. *LIS1*, *DCX*, and *TUBA1A* encode microtubule-related proteins involved in neuronal migration and synaptogenesis. Exploration of these functional pathways is accelerating the identification of additional candidate genes, as well as novel targets for the development of antiepileptogenesis drugs. As the revolution in genomic technology continues, the analysis of complex heredity becomes more tractable. The next decade will see increased exploration of genetic risk variants that contribute to common sporadic epilepsies and the extent to which they can modify epileptic phenotypes. The analysis of epilepsy comorbidities will become an important aspect of this search, since their association may point to new common gene pathways, converging mechanisms, and

Table 1–2 **Human Epilepsy Genes and Engineered Mouse Models***

Chromosome	Phenotype and Gene	KO Mouse Model	Kin Mouse Model
1p35-31.1	Early childhood absence epilepsy; also in epilepsy with paroxysmal exercise-induced dyskinesia; *SLC2A1*/Glut1, glucose transporter type I	Yes	None
1p36	Generalized epilepsy with febrile seizures plus (GEFS+) with absence seizures; GABRD, GABA receptor variants E177A and R220H and Arg220His	None	None
1q21-23	Familial hemiplegic migraine with epilepsy; *ATP1A2*, sodium potassium adenosine triphosphatase (ATPase) alpha 2 subunit	None	None
1q21	Autosomal dominant nocturnal frontal lobe epilepsy 3; *CHRNB2*, beta 2 subunit of nicotinic acetylcholine receptor	Yes	None
2q22-23	Idiopathic generalized epilepsy, juvenile myoclonic epilepsy; *CACNB4* with *R482X* mutation, calcium channel beta 4 subunit	Yes	None
2q24	Severe myoclonic epilepsy of infancy (SMEI) of Dravet syndrome or simple febrile seizures, intractable epilepsy, complex partial seizures with hippocampal scleroses, pain syndromes; *SCN9A*, sodium channel alpha 9 subunit	Yes	None
2q24	Intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC); generalized epilepsies with febrile seizures plus (GEFS+); SMEI of Dravet, febrile seizures 3 (FEB3); de novo mutation in *SCN1A*, sodium channel alpha 1 subunit	Yes	Yes
2q24	Partial epilepsy; *SCN3A*, sodium channel alpha 3 subunit	None	None
2q23-24.3	Benign familial neonatal infantile seizures; febrile and afebrile seizures; *SCN2A*, sodium channel alpha 2 subunit	None	None
4q13-31	Progressive myoclonus epilepsy, Unverricht-Lundborg type, also in action myoclonus-renal failure syndrome; *SCARB2/LIMP2*, scavenger receptor class B, member 2	Yes	None
5p13	Episodic ataxia with seizures, migraine, alternating hemiplegia; *EAAT1*, excitatory amino acid transporter 1; glial glutamate transporter	Yes	None
5q34	Juvenile myoclonic epilepsy, EJM3; *GABRA1*, GABA receptor alpha 1 subunit	Yes	None
5q34	Generalized epilepsy with febrile seizures plus (GEFS+3) and childhood absence; febrile seizures only, Dravet syndrome; *GABRG2*, GABA A receptor gamma 2 subunit	None	*GABARG2* (R43Q)
6p12-p11	Juvenile myoclonic epilepsy, EJM1; myoclonin1/ EFHC1, EF hand containing 1	Yes	None
6p-21.3	Juvenile myoclonic epilepsy, EJM2; *BRD2*, bromodomain-containing 2, mitogen-activated kinase	Yes	None
6p-22	Lafora progressive myoclonus epilepsy; *EPM2B/ NHLRC1*, malin ubiquitin ligase	Yes	Yes
7q36	Focal epilepsy; developmental delay, cortical migration defects; *CNTNAP2*, contactin-associated protein-like 2	None	None

(continued)

Table 1–2 **(Continued)**

Chromosome	Phenotype and Gene	KO Mouse Model	Kin Mouse Model
8q24	Benign familial neonatal convulsions (EBN); *KCNQ3*, potassium channel	None	Yes (Kcnq3/ *G311V*)
9q33.3-q34.11	Sporadic Ohtahara syndrome of early infantile epileptic encephalopathy with EEG suppression bursts; de novo missense or nonsense mutation in *STXBP1/Munc18–1*, syntaxin-binding protein 1	Embryonic lethal	None
9q33.3-q34.11	Early-onset epileptic encephalopathy (early-onset West syndrome) with severe hypomyelination; *SPTAN1*, alpha-II spectrin de novo deletion, duplication	None	None
10q22	Generalized epilepsy and paroxysmal dyskinesia; nonconvulsive absence seizures; *KCNMA1*, BK potassium channel alpha subunit	Yes	None
10q24	Autosomal dominant partial epilepsy with auditory features; *LGI1*, leucine-rich, glioma inactivated 1	Yes	Yes
11p13	Rolandic epilepsy with centrotemporal spikes; *ELP4*, elongator complex gene	None	None
11p15.5	Neonatal seizures with EEG suppression-burst and hypotonia; *SLC25A22/GC1* encodes mitochondrial glutamate/H^+ symporters	None	None
11q14-q23	Tuberous sclerosis; *TSC1* (hamartin) gene	Yes	Yes-conditional *Tsc1* in glia
12p13	Partial epilepsy, partial epilepsy in episodic ataxia 1; *KCNA1*, voltage-gated potassium channel	Yes	None
12q22-q24.1	Tuberous sclerosis; *TSC2* (tuberin) gene	Yes	None
16p13	Autosomal recessive familial infantile myoclonic epilepsy (FIME); *TBC1D24*, binds ARF6, which is involved in neurite branching and extension	None	None
15q21	Idiopathic generalized epilepsy; *ME2*, malic enzyme involved in GABA synthesis	None	None
15q11–15	Remitting childhood absence epilepsy; *GABRB3*, GABA receptor beta 3 subunit	Yes	None
19p13	Absence epilepsy, familial hemiplegic migraine, dominant episodic ataxia2, acetazolamide-responsive spinocerebellar degeneration type 6; *CACNA1A*, voltage-gated P/Q calcium channel alpha subunit	Yes	Yes: *Cacna1a R91Q*
19q13	Generalized epilepsies with febrile seizures plus; *SCN1*β, sodium channel beta 1 subunit	Yes	None
19q13.2	Autosomal dominant nocturnal frontal lobe epilepsy 1; *NACHR*, nicotinic acetylcholine receptor alpha 4 subunit	None	*Chrna4-S284L*; *S280F*; *865–873insGCT*
20q	Benign familial neonatal convulsions (EBN1); *KCNQ2*, potassium channel subunit	Yes	*Kcnq2G279S*; *Kcnq2A306T*
21q22.3	Unverricht-Lundborg disease; progressive myoclonus epilepsy Baltic myoclonus (EPM1); *CSTB*, cystatin B (stefin B)	Yes	None
Xp22	Aicardi's syndrome with early infantile epileptic encephalopathy and EEG burst suppression; *TREX1*, three prime repair exonuclease 1, 3′-5′ DNA exonuclease	None	None

(continued)

Table 1–2 **Human Epilepsy Genes and Engineered Mouse Models* (Continued)**

Chromosome	Phenotype and Gene	KO Mouse Model	Kin Mouse Model
Xp22	X-linked infantile spasms of West syndrome, spasticity, mental retardation, Partington syndrome (ataxia, mental retardation and dystonia), Ohtahara syndrome, X-linked myoclonic epilepsy; *ARX*, Aristaless-related homeobox	Yes *lethal*	truncation, polyalanine expansion
Xp22	Infantile spasms, hypsarrhythmia, overlapping phenotypes of Angelman syndrome and atypical Rett syndrome; *CDKL5*, cyclin- dependent kinase like 5	None	None
Xq22.1	Dravet syndrome, epilepsy and mental retardation in females (EFMR); *PCDH19*, protocadherin 19	None	None
Xq22	Rolandic epilepsy with oral and speech dyspraxia; bilateral perisylvian polymicrogyria; *SPRX2*, secreted sushi-repeat containing protein	None	None
Xq22.3-q23	X-linked lissencephaly and subcortical band heteotopia, double cortex syndrome; *DCX*, doublecortin	Yes	None

* These epilepsy genes are based on chapters in this edition of *Jasper's Basic Mechanisms of the Epilepsies*, but these are not all the known monogenic causes of epilepsy or all the de novo mutations in epilepsy.

studies on pharmacogenomics and personalized medicine.

SECTION 5: EPILEPSY THERAPEUTICS

The accelerating pace of discovery in basic epilepsy research is beginning to deliver the molecular pathways and cellular mechanisms that can be targeted in the development of novel therapies. Therefore, for the first time, the editors devote a major section of *Jasper's Basic Mechanisms of the Epilepsies* to the treatment of epilepsy, with an emphasis on the scientific underpinnings. Since publication of the work of Merritt and Putnam in the 1940s, the discovery of antiepileptic drugs has been based on screening candidate drugs in predictive animal models without regard to mechanisms of drug actions. Drugs often reach the market with little understanding of their pharmacodynamic effects. Once they are well established in clinical practice, information eventually emerges on the molecular targets through which they act to protect against seizures. In contrast, over the past decade, several entirely novel antiepileptic drug targets, notably $\alpha_2\delta$, SV2A, and KCNQ potassium channels, have been identified in studies with the newer drugs levetiracetam, gabapentin, pregabalin, and ezogabine (retigabine). The efficacy of these drugs enables expanded discovery programs directed at other members within these molecular families as well as target-based screening of chemical libraries for active compounds, a new paradigm in antiepileptic drug discovery.

Even with the availability of a steady stream of new antiepileptic drugs, many of which act on unique molecular targets, many patients still do not achieve adequate seizure control. This has led investigators to look beyond drugs to nontraditional treatment approaches. Dramatic progress has been made in at least one older nonconventional treatment approach, deep brain stimulation, which has shown promise in clinical trials. Other older nontraditional approaches that continue to be investigated include hormonal and dietary strategies. At the same time, emerging cell and gene-based treatment strategies are being applied in epilepsy. Promising results have been obtained in animal models, and by the time the next edition of this book is published, we will have a better sense of the practical feasibility of these approaches. As researchers open new frontiers in biological therapies, there has been continuing interest in understanding the neurobiological bases of pharmacoresistance

to conventional small-molecule antiepileptic drugs, which is leading to new ideas on how to overcome drug refractoriness, some of which are already being tested in the clinic.

CLOSING SUMMARY

Finally, in line with the view that understanding epileptogenesis is a critical research priority (Table 1–1), investigators are seeking antiepileptogenic treatment strategies with the ambitious objective of preventing the onset of epilepsy or reversing it once it has become established. New animal models based on mutations of human genetic generalized epilepsies designed to investigate antiepileptogenic strategies are under development in order to study disease modification and cures. Successful approaches will likely have little similarity to drugs that are currently used to protect against seizures. Even with the recognition that symptomatic treatments for neurological disorders have been easier to come by than those that correct an underlying brain defect, there is nevertheless optimism that these new approaches will eventually lead to the prevention, repair, cure, and eradication of some forms of epilepsy.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Jasper HH, Ward AA, Pope A. *Basic Mechanisms of the Epilepsies*. Boston: Little, Brown; 1969.
2. Delgado-Escueta AV, Ward AA, Woodbury DM, Porter RJ. *Basic Mechanisms of the Epilepsies: Cellular and Molecular Approaches*. Advances in Neurology Series, Vol. 44. New York: Raven Press; 1986.
3. Delgado-Escueta AV, Wilson WA, Olsen RW, Porter RJ. *Jasper's Basic Mechanisms of the Epilepsies*. 3rd ed. Advances in Neurology Series, Vol. 79. Philadelphia: Lippincott Williams and Wilkins; 1999.
4. Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Moshé SL, Nordli D, Plouin P, Scheffer IE. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia*. 2010;51(4):676–685.

Chapter 2

Herbert H. Jasper and the Basic Mechanisms of the Epilepsies

Massimo Avoli

THE EARLY YEARS

Born in La Grande, Oregon, on July 27, 1906, Herbert H. Jasper was strongly influenced by his father, whom he later described as a "thoroughly dedicated minister who excelled in mathematics and philosophy as well as practical engineering."[1] His great-grandfather arrived in Oregon by covered wagon. As a young girl, his mother emigrated with her family from Switzerland. Her ancestors were French Huguenots who had fled France to escape persecution in the 1700s. We may wish to assume that these pioneer genes provided Jasper with the exploring mind that allowed for his outstanding research achievements.

Both in his undergraduate and initial graduate studies he majored in psychology (Fig. 2–1). His first published paper, entitled "Optimism and Pessimism in College Environments,"[2] was based on his undergraduate thesis at Reed College, and soon afterward, other papers followed on similar subjects. The turning point from a sociopsychological approach to understanding the intricacies of the human mind toward more fundamental physiological questions, presumably, coincided with Jasper's move from Oregon to the University of Iowa, where he obtained his first doctorate at the age of 25, in 1931. The first publication that testifies to this new orientation appeared in *Science* in 1931 and was titled "The Iowa Eye-Movement Camera."[3] Gloor commented on Jasper's change of research orientation as follows: "To a cynic, this new approach may appear like turning from the sublime to the trivial. I suspect that for Herbert Jasper, nurtured in philosophy and psychology, it must have been a difficult step to take, but it testifies to his courageous acceptance of the belief that we shall never be able to understand the human mind unless we are willing to deal first with very fundamental, measurable processes, starting with paradigms which may appear simplistic, but provide objectively verifiable and measurable data."[4]

During the winter of 1929–1930, at a meeting of the American Physiological Society in Chicago, Jasper had a fortunate meeting with Alexandre and Andrée Monnier, who had been studying in St. Louis with Gasser. He accepted

Figure 2–1. Herbert H. Jasper in 1926, while at Willamette University in Salem, Oregon. This university was where his father had also graduated in the school of theology and social studies.

their invitation to join them in Paris, for which he obtained a fellowship from the Rockefeller Foundation. During 1931 and 1932, he worked in Paris with the Monniers, as well as with Louise Lapicque, studying the phenomenon of chronaxie in peripheral nerves, using their cathode ray oscilloscope that had been acquired in St. Louis. At that time, chronaxie was hoped to disclose basic mechanisms of neuronal function that could be applicable to central mechanisms as well.

Jasper then returned to the United States, where he worked at Brown University in Providence, Rhode Island, developing an electroencephalographic (EEG) laboratory at the Emma Pendleton Bradley Home, and in 1935 he published, in *Science*, the first paper in the United States on human EEG[5] (Fig. 2–2A). There, working with Carmichael, he described "spontaneous" fluctuations in magnitude of the alpha waves, the reduction of these waves by visual stimulation, and their consistent frequency in the same individual upon repeated examinations. He also reported that "In one or two pathological cases which we have studied, a frequency of alpha waves as low as 2–3 per second has been observed." As proposed by Feindel,[6] these were presumably delta waves associated with brain damage. Jasper and Carmichael[5] cautiously predicted: "It may well be that the electroencephalograms of the sort described in this note may prove significant in psychology and clinical neurology. It is even possible that this technique may provide information in regard to brain action which will be comparable in significance to the information in regard to heart function which is provided by the electrocardiograph." A few months later, the Harvard group (including Fredric Gibbs, Hallowell Davis, Alexander Forbes,

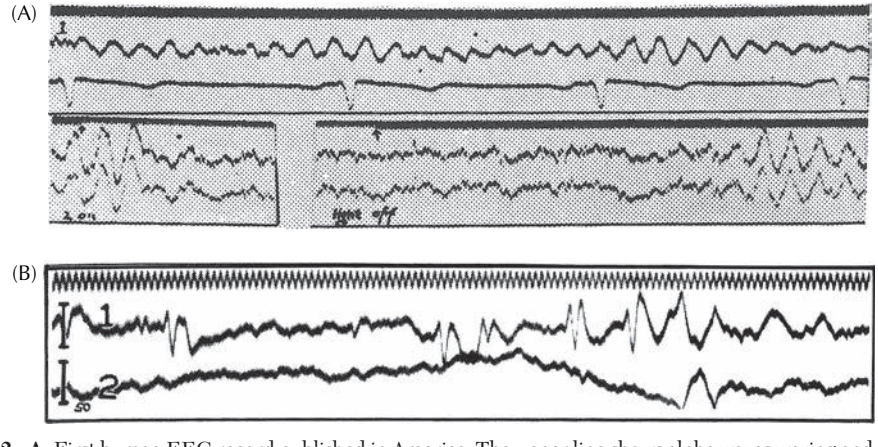

Figure 2–2. A. First human EEG record published in America. The upper line shows alpha waves waxing and waning; the bottom two lines show blocking of the alpha waves with visual stimulation.[5] **B.** Interictal activity recorded from a patient with posttraumatic Jacksonian seizures; note that spikes or sharp waves are recorded from the precentral region (1) but not from the homologous area (2).[8]

and William Lennox) reported that the EEG activity of epileptic patients was abnormal. Shortly afterward, Jasper and his team confirmed this finding[7,8] (Fig. 2–2B) and proposed that the EEG examination, in addition to being useful for the diagnosis of epilepsy, could be employed for the localization of the epileptic area in the brain. Indeed, this pioneering work led to the later development of two schools of EEG with differing emphasis and perspectives. Gibbs' school used a holistic approach, as it considered any highly integrated activity as the expression of brain function as a whole. The opposing view, by reflecting a kind of Cartesian or topistic concept of cerebral organization, was that used by Jasper: it associated even highly complex brain functions with localized neuronal substrates that had to be defined anatomically.

Jasper returned to Paris in 1935 to defend his primary thesis, titled "Recherches sur l'excitabilité et les caractères de la reponse dans le systeme neuromusculaire des crustacés. Influence des centres ganglionaires." These studies had been completed over the summers of 1932 and 1933 at the Woods Hole Marine Biological Laboratories, where he had been able to interact with many leading neurobiologists, such as Harry Grundfest, Alan Hodgkin, and Francis Schmitt. He added, however, a supplementary thesis titled "Electroencéphalographie chez l'homme" related to the EEG work he had carried out with his team at Brown University. In his autobiography Jasper recalled that "the examining committee seemed satisfied with the principle thesis on the crustacean neuromuscular system," but "All were enthusiastic about my second thesis on *electroencéphalographie chez l'homme*" and "I had to treat everyone to a champagne reception afterward as is the custom."[9]

Shortly after his thesis presentation, Jasper traveled to Germany and met Hans Berger, whose early reports on the discovery of EEG had been received with skepticism, and then visited Edgar Adrian and Brian Matthews in Cambridge, who had expanded Berger's original findings. As remarked by Andermann,[10] Jasper was not insensitive to the political turmoil of those years. He wrote of Berger's persecution by the Nazis, a preamble to Berger's suicide in 1941, and of Marthe Vogt, the daughter of Cecile and Oscar Vogt, whom he advised to leave Germany and for whom he helped to obtain a fellowship in England; Marthe Vogt, a neuropharmacologist, became a fellow of the Royal Society and a professor at Cambridge University.

WORK AT THE MONTREAL NEUROLOGICAL INSTITUTE

While at Brown University, Jasper and his colleagues had heard of Wilder Penfield's observations on electrical stimulation to map the cortex in conscious patients during surgery for the treatment of focal epilepsy at the Montreal Neurological Institute (MNI, also called "the Neuro"). They invited Penfield to give a seminar in the Psychology Department at Brown and also to visit the EEG laboratory located in the basement of the Bradley Hospital. Penfield himself described his first meeting with Jasper as follows: "He [Jasper] could, he said, localize the focus of an epileptic seizure by the disturbance of brain rhythms outside the skull. I doubted that but hoped it might be true."[11] Although skeptical at first, Penfield agreed to operate on two patients who had been studied by Jasper in Providence, and found lesions underlying the area where the EEG abnormalities had been localized. As described in detail in the letter written by Jasper to Penfield on February 5, 1938 (Fig. 2–3), this initial success started a collaboration that Penfield defined as "our almost unthinkable commuters research project." Each week, Jasper loaded a portable EEG machine into his car and on arrival at the MNI studied Penfield's patients, returning later in the week to Brown to continue his work. This was not an easy task, as there are more than 600 km between Providence and Montreal, and those were late 1930s roads (several years before the creation of the interstate highway system by President Dwight Eisenhower). Jasper's pioneer spirit was once more put to the test.

Penfield was finally convinced by Jasper that it was possible to identify the site of origin of a patient's seizure, even between attacks, by recording the EEG through the unopened skull (i.e., by studying the location of the interictal epileptic discharge), and also that it was possible to refine this localization by recording the brain activity directly from the exposed surface during neurosurgery. Penfield invited

EMMA PENDLETON BRADLEY HOME
EAST PROVIDENCE
RHODE ISLAND

February 5, 1938

Dr. W. G. Penfield
Montreal Neurological Institute
3801 University Street
Montreal, Canada

My dear Dr. Penfield:

The more I think about your proposal that we should join forces in our respective fields of research, the more I believe that such a collaboration should result in some very important progress in the interpretation of electroencephalograms, and I should hope that also it would make some significant contributions to our general understanding of epilepsy.

Incidentally, this should give us an opportunity for an excellent comparison of the relative value of the Roentgen encephalogram and the electroencephalogram in the localization of brain pathology. I am also very much interested in following up the operated cases to obtain a good record of the change in the electrical activity of the brain. This should yield important information in regard to some general questions of brain physiology as well as test the efficacy of the operation in removing a discharging focus and possibly result in the discovery of new focii in some cases.

I feel that this is such an excellent opportunity that you have suggested, that I would like very much to spend the next three months in Montreal, but I do not see how that can be arranged in the immediate future. I have arranged my teaching so that it will occupy only Monday, Tuesday, and Wednesday of each week, and our own research could also be carried out during this time. This would leave Thursday, Friday, and Saturday free for work in Montreal, and occasionally I could arrange also to spend an entire week with you if necessary. Do you think that our collaboration might be satisfactory if I could plan on being in Montreal Thursday, Friday, and Saturday of each week? This would necessitate considerable expense for travel, but in view of the importance of the work I believe that it would be worth the expense.

I have written Dr. Lambert to get his impressions of this arrangement, and hope to hear from him in the near future. I understand that Dr. Gregg has been on a trip west,

so that you have probably not as yet heard from him. In checking over our budget for this year, I find that I could pay the necessary traveling expenses without exceeding the total amount allowed in our grant for the year if the expenditure was authorized by the Rockefeller Foundation.

In regard to equipment, I have a very good portable set-up which could be very easily transported to Montreal in my car. It is now being used for some of our own work in the hospitals, but I will be glad to remove it temporarily in case you wish to have some cases run before your equipment is completed.

Very sincerely yours,

Herbert H. Jasper

Herbert H. Jasper
D. ès Sc.

HHJ:JMT

Figure 2–3. Letter sent by Jasper to Penfield in 1938 in which he describes possible options for establishing a closer collaboration at the Neuro.

Jasper to join him at the MNI, where he was to work for the next three decades. With funds provided by the Rockefeller Foundation, McConnell, Sir Herbert Holt, and Duccan, an annex off the MNI basement dedicated to clinical EEG was built. It was dedicated to "research on the physiology of the brain with special reference to epilepsy and dementia." The EEG Department, which is still located in the same area of the building 70 years later, was opened by a symposium in February 1939. The meeting continued in the Laurentians, the first of the annual ski meets of the Eastern EEG Association. Of this period in Montreal, Jasper wrote, "My time with Wilder Penfield and his family, in which I became an adopted member, working with his splendid enthusiastic staff and hundreds of colleagues and students from all over the world who worked with us, was certainly the most pleasant and productive 27 years of my life."[12] As Penfield was fond of saying, epilepsy was their great teacher.

Shortly after the inauguration of the EEG laboratory at the MNI, World War II broke out, and both research and clinical care had to be reoriented toward war-related medical issues. Jasper became involved in research on air transport for head injuries, antibiotics for the treatment of brain wounds, studies on air-pilot blackout, and seasickness prevention. Problems related to numerous cases of nerve injury were also studied with electrophysiological techniques, and Jasper became one of the initiators of another branch of neurophysiology: electromyography.[13] Around 1940—but actually, as indicated by his curriculum vitae (see the Appendix), in 1939— feeling the need for more medical training, Jasper enrolled as a medical student at McGill University and completed the 3-year wartime curriculum while continuing with his clinical and laboratory work.

At the beginning of 1944, he was required to fulfill his military service (Fig. 2–4A). After a few weeks of formal military training at Camp Borden (Ontario), he was assigned to the MNI, where he continued to work on the various projects related to the war effort. It is fascinating to read in a handwritten note sent from Jasper to Penfield on March 9, 1944: "The stretcher drill and PT on the assault course is taken seriously—not to mention the route marches with

(A)

(B)

The Canadian Medical Procurement and Assignment Board.

Ottawa.

Sept.17, 1945

Col. Wilder Penfield,
Montreal Neurological Institute,
3801 University St.,
Montreal 2, P.Q.

Re: Captain Herbert Jasper, RCAMC

Dear Colonel Penfield:

Your letter of 11 September 45 to Major General Fenwick has been passed to us for attention as this Board is immediately concerned in priority of release.

As Captain Jasper has had a fairly short term of service it will be of very great assistance if this officer is placed on the McGill Univeristy priority list.

The University priority list is intended to include those who are urgently required to make possible the rehabilitation training of demobilized medical officers. It is a very high priority. If the name is not placed on the list, the request will be considered in the same way as all other requests for premature release -- on the merits of the individual case.

We will delay action until you have discussed this matter with Dean Meakins who supervises the University priority list and await a further communication from Dean Meakins or yourself.

H.A. Proctor, Major,
Exec.Sec. CMPAB

Figure 2–4. A. H.H. Jasper as an officer in the Canadian Army. **B.** Letter sent by Major Proctor of the Canadian Army in response to Penfield's inquiry about Jasper's release from duty at the end of the war.

full pack—5 miles the first week—10 miles the second, 15 miles the third, up to 18–20 miles the final week. . . . I shall return to Montreal on Easter. . . . After that I am anxious to settle down to some serious work with you." Although Jasper was a captain (and Penfield a colonel, as is evident by reading the correspondence reported in Fig. 2–4B) in the Canadian Army, he did not sign the declaration of allegiance to the king; he maintained his American citizenship to allow his children to choose their citizenship until 1950, when he decided to stay in Canada and became a Canadian citizen.

Once the war was over, Jasper refocused his interest on epilepsy and on what epilepsy could reveal about brain function. The laboratories of EEG and of experimental neurophysiology (the latter are, in fact, still located on the seventh floor of the MNI six decades later) enjoyed a period of extraordinary growth, attracting bright young men and women from all parts of the globe (Fig. 2–5). As Jasper wrote: "We had more than 100 research fellows during the 10 years following the war."[9] Their publications covered a broad range of research topics, including the reticular formation as well as the thalamocortical and limbic systems using EEG and unit cell recordings aimed at unraveling the fundamental mechanisms of epilepsy as well as of behavior. The functional organization of the thalamocortical system as an integrating unit subserving physiological functions represented, in those years, a hot topic of research, one of the crucial points being the identification of the functional connectivity of the thalamic nuclei and their role in the phenomenon of incrementing (also termed *recruiting* and *augmenting*) responses that were recorded from the neocortex during low-frequency stimulation of the thalamus.[14] This search led Jasper to discover that generalized spike-and-wave discharges at 3 Hz—which are typically seen in human petit

Figure 2–5. Herbert Jasper and research fellows in the laboratory of neurophysiology at the MNI (1952). Top row, left to right: J. Courtois (Canada), Gloor (Switzerland), Hunter (Australia), Tukel (Turkey), Ingvar (Sweden), Mrs. Tukel (Turkey), Hanbery (USA); middle row: Stoll (USA), Jasper (Canada), Ajmone-Marsan (Italy); front row: A. Courtois (France), Oeconomos (Greece), Feindel (Canada). The empty space between Mrs. Tukel and Hanbery was left for Li (China)—who was not present when the photo was taken—to be filled by a photomontage.

mal epilepsy—can be elicited in the cat by stimulating the midline thalamic nuclei.[15] Indeed, Jasper's views on the role of thalamocortical mechanisms in generalized epilepsy have been seminal to the development of important concepts and experimental work in this area.[16–18] I should add that, as often happens in scientific research, these data were at times controversial; however, Jasper was always keen to settle on unclear scientific issues. One of these cases occurred when he agreed with Horace Magoun (another pioneer of research on the thalamocortical system) to test an ongoing dispute between the MNI and UCLA laboratories by having Robert Naquet—at that time a fellow in Jasper's laboratory—sent to California to repeat some salient experiments; these data were later published in the *EEG Journal*.[19]

In this period, Jasper was also interested in the function of the limbic system, and he asked one of his fellows, Peter Gloor, to explore, by electrophysiological means, the connections of the amygdala. These experiments revealed that this limbic area projects intensely to a large brain region comprising the basal core of the forebrain and midbrain extending to the hypothalamus and the brainstem tegmentum. These data—which were included in Gloor's Ph.D. thesis—were so novel that they became a chapter in the first edition of the *Handbook of Physiology* published by the American Physiological Society.[20] Jasper's interest in charting the brain, both anatomically and functionally, is also mirrored by the publication of an atlas of the cat diencephalon[21] that, 30 years later, was still in use in many laboratories around the world.

Jasper's school of EEG was flourishing during his years of association with Penfield at the MNI, investigating patients with intractable epilepsy being considered for surgery. The scientific output was prodigious and culminated in Penfield's and Jasper's masterpiece, *Epilepsy and the Functional Anatomy of the Human Brain*,[22] which remains an invaluable reference in our search for understanding epileptic disorders. At around this time, Jasper published another comprehensive book on the brain mechanisms of consciousness that also became a classic in the EEG literature.[23]

In the early 1950s, Jasper's pioneer genes pushed him toward new frontiers. He was no longer satisfied with the information obtained with EEG recordings, and he wanted to know more about the activity of single neuronal cells and of their contribution to the EEG rhythms. He tackled this problem with several of the young scientists who were coming to the MNI (such as Chu-Lu Li, Hughes McLennan and, a few years later, Costa Stefanis), and clarified the complex relationships between gross surface EEG potentials and cortical neuron activity that were recorded with extracellular or intracellular microelectrodes. However, although he wanted to gain a better understanding of the mechanisms underlying neuronal excitability, he did not lose his interest in how the mind works. Therefore, he began to study in the late 1950s the involvement of cortical neurons in conditioning in monkeys. To this end, Jasper sought help from his former student (and later Nobel laureate) David Hubel, who was working in Washington, D.C., and developed tungsten microelectrodes to record from single neocortical cells in waking experimental animals and humans. As Gloor remarked, "this was the first time anyone has attempted to employ single-unit recording techniques in the investigation of behavior."[4] This methodology was later applied, in collaboration with Gill Bertrand, to analyze the activity of single human thalamic neurons in patients undergoing stereotaxic treatment for the relief of parkinsonian tremor.[24]

In 1996 Jasper wrote, "It was Penfield's dream to create a multidisciplinary neuroscience institute in which the basic scientists worked closely with clinicians and the laboratories of radiology, neuropathology, neurochemistry, neuroanatomy, neuropsychology, and, of course, with electroencephalography and neurophysiology, in a fusion of clinical and basic research. This was a forerunner of what soon became what we now know as neuroscience. I was delighted to take part in the realization of Penfield's dream, which soon became my own as well; it became for me an international as well as an interdisciplinary dream."[9]

Such multidisciplinary effort received impetus from the recruitment of Allan Elliott, a brain chemist who expanded the biochemistry laboratories into the first research unit for neurochemistry. In 1953 Elliott invited Ernst and Elizabeth Florey to the MNI to study an inhibitory factor that they had found in brain extract. In 1955, the Floreys and McLennan characterized the inhibitory effects of "Factor I" on peripheral and central synaptic transmission.[25] One year

later, Florey's Factor I was identified as gamma-aminobutyric acid (GABA). This was quickly followed by the work of Iwama and Jasper, who confirmed its inhibitory action on the cerebral cortex.[26] As Jasper noted, "the reports from Drs Florey and McLennan on the isolation of the substances from brain tissue which have strong inhibitory or excitatory effects on the activity of the central nervous system were outstanding…the isolation of a naturally occurring inhibitory substance in the brain, if confirmed by further study, may be a discovery of major consequence, not only for our understanding of normal brain function, but also for the rational treatment of brain disorders."[12]

THE FOUNDING OF IBRO AND THE BME PROJECT

Jasper was keen on organizing and participating in international societies and conferences. Thus, at a meeting of the International Federation of EEG and Clinical Neurophysiology in Moscow in 1958 (Fig. 2–6), he and others voted unanimously for a resolution proposing the creation of an international organization that could cut across world boundaries and improve communication and collaboration among brain researchers. Jasper participated in the founding of the International Brain Research Organization (IBRO) and in 1960 became its first executive secretary, moving with his family to Paris for a sabbatical year. IBRO, which was linked to the United Nations Educational, Scientific, and Cultural Organization (UNESCO), was incorporated on March 29, 1961, by the Senate of Canada. At the peak of the Cold War, IBRO provided a powerful scientific platform for closer collaboration between Western and Iron Curtain neuroscientists. Jasper wrote in 1996 that "after the close of the Moscow Colloquium in 1958 I had had a private conference with the president of the Soviet Academy of Science which resulted in his agreement to collaborate

PARTICIPANTS OF THE MOSCOW INTERNATIONAL COLLOQUIUM ON ELECTROENCEPHALOGRAPHY OF HIGHER NERVOUS ACTIVITY

Photographed in the court yard of the House of Scientists
Moscow, October 10, 1958

Front Row: N. N. Das (India), H. W. Magoun (USA), A. Fessard (France), F. Bremer (Belgium), I. S. Beritashvili (USSR), H. Gastaut (France), H. H. Jasper (Canada), M. Brazier (USA), V. S. Rusinov (USSR), G. Moruzzi (Italy), H.-T. Chang (China), K. Lissak (Hungary).

Second Row: D. S. Voronzov (USSR), W. Grey Walter (England), J. Matsumoto (Japan), P. K. Anokhin (USSR), J. Bureš (Czechoslovakia), S. A. Sarkisov (USSR), V. V. Parin (USSR), E. A. Asratian (USSR), G. D. Smirnov (USSR), N. I. Grashenkov (USSR), A. V. Kogan (USSR), M. N. Livanov (USSR), A. Kreindler (Rumania), J. Konorsky (Poland), F. Morrell (USA), G. V. Gershuni (USSR), L. G. Trofimov (USSR).

Third Row: W. Storm van Leeuwen (Holland), V. A. Kozhevnikov (USSR), P. Dell (France), A. L. Jus (Poland), G. T. Sakhiulina (USSR), N. V. Golikov (USSR), P. G. Kostiuk (USSR), R. Hernández-Peón (Mexico), E. Atsev (Bulgaria), P. S. Kupalov (USSR), K. Hrbek (Czechoslovakia), N. N. Dsidsishvili (USSR), S. P. Narikashvili (USSR), R. Galambos (USA), E. N. Sokolov (USSR).

Figure 2–6. Participants to the International Federation of EEG and Clinical Neurophysiology conference held in Moscow in 1958. This was the event where the foundation for the creation of IBRO was laid.

with our international efforts in this direction."[9] Fifty years later, IBRO continues to promote neuroscience, with special emphasis on assisting young investigators in the developing world.

In the 1960s, Jasper was also called on to chair the Basic Resarch Task Force of the Public Health Service Advisory Committee on the Epilepsies, which had been created by the Surgeon General of the U.S. Public Health Service. The Task Force concluded that basic epilepsy research needed stimulation, and thus it was decided to hold a workshop/symposium and to publish a comprehensive monograph. Jasper was given the assignment of developing the first workshop, which was held in Colorado Springs, Colorado, and of editing the book. The first volume of *Basic Mechanisms of the Epilepsies* was published in 1969 with H.H. Jasper, A.A. Ward, and A. Pope as editors.[27] As discussed in Chapter 1 of this book, basic and clinical researchers in epilepsy gather together approximately each decade to review their accomplishments and to identify future directions.

MOVING TO THE OTHER SIDE OF THE MOUNTAIN (MONT ROYAL, OF COURSE!)

After returning to Montreal from his IBRO assignment with UNESCO in Paris, Jasper decided to focus on experimental neurophysiology, concentrating on the combination of neurochemical and microelectrode techniques that he had developed so effectively with Elliott and his team. These studies revealed that certain functional states of the brain (e.g., arousal mediated through the activation of the brainstem reticular formation) are associated with changes in the release of amino acid transmitters and with an increased release of acetylcholine.[28,29]

In 1965 Jasper moved to the Université de Montréal, where an enthusiastic new group in the neurosciences, supported by the Medical Research Council of Canada, had been formed. There, continuing his research on the relation between neurochemical changes and brain activity, he reported that there was an increased release of acetylcholine during rapid eye movement (REM) sleep compared to that occurring during slow wave stages.[30] Moreover, he discovered that acetylcholine, when applied to the cortex after preventing its hydrolysis with eserine, caused sustained epileptiform discharges that could be triggered by weak sensory stimulation.[31] Later, with Koyama and van Gelder, he extended the analysis of the neurochemical mechanisms causing seizure activity and epileptogenesis to amino acid neurotransmitters.[32] Jasper himself reviewed these data in a paper that was written while approaching his 90th birthday.[33]

Jasper's final years of active research focused on catecholaminergic mechanisms in the cerebral cortex. At that time, there was little evidence for a central role of catecholamines. With Reader, de Champlain, and Descarries, he found a reciprocal relation between acetylcholine, on one side, and noradrenaline and dopamine release, on the other, during activation of the cerebral cortex by sensory inputs. As commented by Gloor, "at this stage of his career Jasper's search for the mind had become a search for molecules. He has traveled a long road since his early college days when he had attempted to understand why some people approach[ed] life in an optimistic frame of mind, while others were subject to a gloomy pessimism."[4] It should be emphasized that while being a key figure in the development of this new department at Université de Montréal, Jasper continued to work as consultant in neurophysiology at the MNI.

HERBERT JASPER'S LATE YEARS

During his 70s and 80s, Jasper persisted tirelessly in his research at the Université de Montréal and continued to visit the MNI. His attendance at conferences and lectures was always characterized by sharp comments on the topics under discussion. Because of his fundamental training in laboratory neurobiology, he offered valuable critiques of new work that were sometimes based on research topics which he and his collaborators had previously examined. He gave superb summaries of symposia from his well-informed perspective, and he continued to write review papers that, while being historical in their nature, also provided original and inspiring concepts on future directions.[34]

Herbert Jasper collected prizes, awards, distinctions, honorary degrees, and worldwide recognition. Among these were the Ralph Gerard Prize of the Society for Neuroscience (Fig. 2–7), the McLaughlin Medal of the Royal

Figure 2–7. Peter Gloor, Herbert Jasper, Brenda Milner, and Ted Jones at the event awarding the Ralph Gerard Prize of the Society for Neuroscience in Washington, D.C., in 1993.

Society of Canada, the FNG Starr Award of the Canadian Medical Association, and the Albert Einstein World Science Award of the World Cultural Council. He was appointed an Officer of the Order of Canada, elected to the Canadian Medical Hall of Fame, and appointed as Le Grand Officier de l'Ordre National du Québec. His work in epilepsy was recognized by the William G. Lennox Award of the American Epilepsy Society, the Carl Spencer Lashley Award of the American Philosophical Society, and the research award sponsored by the Milkin Family Medical Foundation and the American Epilepsy Society.

Gloor has remarked that from the very beginning, Jasper wanted "to use the EEG as a means to investigate fundamental aspects of brain function, rather than to merely employ it as a diagnostic test."[4] Gloor stated: "He always hoped that research directed at fundamental and even molecular mechanisms in the brain may help us to better understand the human mind." However, contrary to other giants of neurobiology research such as Eccles or Penfield, he never discussed brain, mind, and behavior from a philosophical perspective.

Herbert H. Jasper continued to be in good health up to a few months before his 93rd birthday, when on March 11, 1999, he succumbed to a sudden cardiac attack. I am sure that his prodigious contributions will continue to be appreciated by the neuroscience community of the third millennium, thus making this pioneer's life span more than two centuries.

ACKNOWLEDGMENTS

I am indebted to Ms. Marylou Jasper for reading this paper and for providing invaluable information on Dr. H. Jasper as scientist and husband. I also thank Ms. Toula Papadopoulos for skillful editorial assistance. Finally, I want to dedicate this chapter to the memory of the late Drs. Peter Gloor and Robert Naquet, two of Jasper's former fellows, who nurtured my interests in epilepsy research and neuroscience.

DISCLOSURE STATEMENT

The author has no conflicts of interest to disclose.

REFERENCES

1. Jasper HH. Philosophy or physics—mind or molecules. In: Worden FG, Swazey JP, Adelman G, eds. *The Neurosciences: Paths of Discovery*. Cambridge, MA: MIT Press; 1975:403–422.

2. Jasper HH. Optimism and pessimism in college environments. *Am J Sociol*. 1928–1929;34:856–873.
3. Jasper HH, Walker RY. The Iowa eye-movement camera. *Science*. 1931;74:291–294.
4. Gloor P, Jasper HH. Neuroscientist of our century. In: Avoli M, Reader TA, Dykes RW, Gloor P, eds. *Neurotransmitters and Cortical Function: From Molecules to Mind*. New York: Plenum; 1988:1–13.
5. Jasper HH, Carmichael L. Electrical potentials from the intact human brain. *Science*. 1935;81:51–53.
6. Feindel W. Herbert Henri Jasper (1906–1999): an appreciation. *Can J Neurol Sci*. 1999;26:224–229.
7. Jasper HH. Cortical excitatory state and variability in human brain rhythms. *Science*. 1936;83:259–260.
8. Jasper HH, Hawke WA. Electroencephalography. IV. Localization of seizure waves in epilepsy. Arch Neurol Psychiatry. 1938;39:885–901.
9. Jasper HH. Some highlights of 70 years in neuroscience research. In: Squire LR, ed. *The History of Neuroscience in Autobiography*. Vol. 1. Washington, DC: Society for Neuroscience; 1996:318–346.
10. Andermann F. Herbert Henri Jasper: an appreciation and tribute to a founder of modern neuroscience 1906–1999. *Epilepsia*. 2000;41:113–120.
11. Penfield W. Herbert Jasper. Recent Contributions to Neurophysiology: International Symposium in Neurosciences in Honor of Herbert H. Jasper. In: Cordeau JP, Gloor P, eds. *EEG Clin Neurophysiol*. 1972;31:9–12.
12. Jasper HH. History of the early development of electroencephalography and clinical neurophysiology at the Montreal Neurological Institute: the first 25 years, 1939–1964. *Can J Neurol Sci*. 1991;18:533–548.
13. Jasper HH. The rate of re-innervation of muscle following nerve injury in man as determined by the electromyogram. *Trans R Can Sect*. 1946;40:81–92.
14. Jasper HH, Ajmone-Marsan C. Thalamocortical integrating mechanisms. *Res Publ Assoc Res Nerv Ment Dis*. 1952;30:493–512.
15. Jasper HH, Drooglever-Fortuyn J. Experimental studies on the functional anatomy of petit mal epilepsy. *Proc Assoc Res Nerv Ment Dis*. 1946;26:272–298.
16. Avoli M, Gloor P, Kostopoulos G, Naquet R. *Generalized Epilepsy: Neurobiological Approaches*. Boston, Basel and Berlin: Birkhäuser; 1990.
17. Jasper HH. Historical introduction. In: Avoli M, Gloor P, Kostopoulos G, Naquet R, eds. *Generalized Epilepsy: Neurobiological Approaches*. Boston, Basel, and Berlin: Birkhäuser; 1990:
18. Crunelli V, Leresche N. Childhood absence epilepsy: genes, channels, neurons and networks. *Nat Rev Neurosci*. 2002;3:371–382.
19. Jasper H, Naquet R, King EV. Thalamocortical recruiting responses in sensory receiving areas in the cat. *Electroencephalogr Clin Neurophysiol*. 1955;7:99–114.
20. Gloor P. Amygdala. In: Field J, Magoun HW, Hall EV, eds. *Handbook of Physiology: II. Neurophysiology*. Washington, DC: American Physiological Society; 1960:1395–1420.
21. Jasper HH, Ajmone-Marsan C. *A Stereotaxic Atlas of the Diencephalon of the Cat*. National Research Council of Canada; 1954.
22. Penfield W, Jasper H. *Epilepsy and the Functional Anatomy of the Human Brain*. Boston: Little, Brown; 1954.
23. Adrian ED, Bremer F, Jasper HH, Delasfresnaye JF. *Brain Mechanisms and Consciousness*. Oxford: Blackwell Scientific Publications; 1954.
24. Jasper HH, Bertrand G. Recording from microelectrodes in stereotaxic surgery for Parkinson's disease. *J Neurosurg*. 1966;24:219–221.
25. Florey E, McLennan H. Effects of an inhibitory factor (factor I) from brain on central synaptic transmission. *J Physiol*. 1955;130:446–455.
26. Iwama K, Jasper HH. The action of gamma aminobutyric acid upon cortical electrical activity in the cat. *J Physiol*. 1957;138:365–380.
27. Jasper HH, Ward AA, Pope A. *Basic Mechanisms of the Epilepsies* Boston: Little, Brown; 1969.
28. Jasper HH, Khan RT, Elliott KA. Amino acids released from the cerebral cortex in relation to its state of activation *Science*. 1965;147:1448–1449.
29. Celesia GG, Jasper HH. Acetylcholine released from cerebral cortex in relation to state of activation. *Neurology*. 1966;16:1053–1063.
30. Jasper HH, Tessier J. Acetylcholine liberation from cerebral cortex during paradoxical (REM) sleep. *Science*. 1971;172:601–602.
31. Ferguson JH, Jasper HH. Laminar DC studies of acetylcholine-activated epileptiform discharge in cerebral cortex. *Electroencephalogr Clin Neurophysiol*. 1971;30:377–390.
32. van Gelder NM, Koyama I, Jasper HH. Taurine treatment of spontaneous chronic epilepsy in a cat. *Epilepsia*. 1977;18:45–54.
33. Jasper HH. Historical introduction: early efforts to find neurochemical mechanisms in epilepsy. In: Avanzini G, Engel J, Fariello R, Heinemann U, eds. *Neurotransmitters in Epilepsy*. New York: Elsevier Science Publishers; 1992:1–8.
34. Jasper HH, Reader TA, Avoli M, Dykes RW, Gloor P. Molecular control and communication in cerebral cortex: an overview. In: Avoli M, Reader TA, Dykes RW, Gloor P, eds. *Neurotransmitters and Cortical Function: From Molecules to Mind*. New York: Plenum; 1988:593–605.

APPENDIX: ORIGINAL CURRICULUM VITAE OF DR. H. JASPER, DATED APPROXIMATELY 1954

JASPER, Herbert H.
M.A., Ph.D. (Iowa), D.es Sci, (Paris), M.D. (McGill)
Hon. Doctorate (Bordeaux), C.M.
McGill University
Montreal Neurological Institute
Born: July 27th, 1906
Educated: '23–'26 Willamette University, Portland Or.
'27 Reed College, Portland, Or. B.A.
'27–'29 University of Oregon, M.A.

'29–'31 University of Iowa, Ph.D.
'31–'33 University of Paris, D.es Sci, Psychology. Also Fellow, National Research Council (U.S.)
'33 Laureat, French Academy of Science
'39–'43 McGill University, M.D., C.M.
'47 Doctorate, honoris causea, University of Bordeaux.

Positions: '29 University of Iowa, Instructor in Psychology
'33–'38 Asst. Prof. Psychology and Director, Psychological clinic and Neurophysiology labs. Brown University, Providence, R.I.
'39 Seminar in Neurophysiology, McGill University
'49 Professor Experimental Neurology, McGill Neurophysiologist and Electroencephalopgrapher, Montreal Neurological Institute.
'49 First President, Am. Soc. of Electroencephalographers.
'49–'53 First President, Internat. Fed. of Societies for EEG and clinical Neurophysiology
Editor-in-chief, Journal of EEG and clinical neurophysiology.

FIELDS OF WORK:
Description of transmission of nerve impulses across artificial synapses (crustacea).
Development of techniques of localization of epileptic discharge.
Demonstrated: "psychomotor epilepsy" as a form of focal epilepsy.
Demonstration of a separate diencephalic projection system in animals, regulating electrical activity in the cortex.
Cortico-subcortical connections and action of the reticular network on the brain stem.

Chapter 3

Why—and How—Do We Approach Basic Epilepsy Research?

Philip A. Schwartzkroin

This volume and its predecessors[1–3] focus on basic mechanisms of the epilepsies. Most of us, when we open such a volume, expect to see a discussion of studies on experimental animal models and/or reduced brain preparations (i.e., not studies in humans), with an emphasis on cellular, molecular, and genetic variables that influence neuronal excitability, synaptic interactions, and circuitry dynamics. These studies are typically carried out in a laboratory, often by individuals who employ basic research skills that are quite different from those used in taking care of patients with a neurological disorder. We basic scientists are trained in exacting research techniques, use highly specialized methods, and employ critical faculties that help us avoid the potential pitfalls of experimental approaches.

These features of basic research often separate the associated laboratory activities from research carried out on patients with medical disorders—so-called clinical research. As we all know, the separation between basic and clinical research is not always easy to make. Physician scientists may move easily from the laboratory to the clinic and blur the distinctions. Highly technical approaches developed in the laboratory may be applied to patient populations, and technical advances developed for clinical assessments may eventually be applied in the laboratory. And cellular, molecular, and genetic variables that once were investigated primarily (or exclusively) in animals and in vitro preparations are now routinely applied to clinical populations. Indeed, many of the basic imaging and genetic insights into neurological disorders were identified initially in clinical studies.

Given how blurred this basic-clinical distinction is, it has become increasingly important for basic scientists to break down the basic-clinical separation, and particularly to give up the idea that clinical research is somehow inferior—lacking, perhaps, the rigor or the insights associated with laboratory work. There is, however, an important aspect of research that often (not always) separates basic from clinical studies—the availability of normal control groups. One of the major advantages of laboratory work—aside from enabling the researcher to apply invasive approaches that would not be ethically appropriate in human subjects—is the possibility of separating variables of interest and therefore creating control groups that differ only in the variable of interest. This laboratory advantage provides the basic scientist with an especially powerful (but narrow) means of drawing strong conclusions from his or her work. It is important to recognize, however, that depending on the goal of the study, isolation of single variables may not provide answers that are of clinical value, since real-life pathologies rarely appear to be dependent on single variables.

WHAT DO WE MEAN BY "BASIC" RESEARCH?

Basic research can take a number of different pathways in the attempt to provide insights and advances. The basic research enterprise can be divided into at least three somewhat different activities, each of which involves different approaches and different goals.

Exploration and Discovery

Historically and conceptually, scientific research begins with exploration, an attempt to see what's out there. Exploration has not always been respected as a scientific research activity, especially since it may be poorly focused or biased, so that the explorer finds what he or she is looking for. Yet, modern research is almost inconceivable without the exploration of a Galileo or a Darwin. Indeed, where would today's disciplines of molecular genetics be without the exploratory research that resulted in the description of the human genome?

In years past, a grant review that included a phrase such as "this is an exploratory study" or "this is a purely descriptive study" was an inevitable death knell. More recently, we have begun to appreciate the importance of exploratory studies and to understand that exploration can be pursued on many different levels (e.g., within a large animal population or within the DNA components of the genome). We now seem to allow exploratory research if it is sufficiently molecular (i.e., if the study uses sophisticated modern molecular/genetic tools), but we still reject such studies if they use more ordinary approaches (e.g., macroscopic or even microscopic observation). Is there a good rationale for accepting descriptive studies at one level but not at another? Why reject detailed descriptive studies (or label them as second-rate science) if such studies provide a basis for asking important questions?

Hypothesis Testing

Scientific training usually emphasizes the need for well-designed experiments, which typically involve the development of clear hypotheses that can be tested using established (or newly developed) scientific methods. Key features of this type of research include (1) asking questions (posing hypotheses) that can be definitively addressed in the laboratory; (2) applying appropriate control/comparison groups; (3) replicating the results of the study; (4) analyzing the results with appropriate statistical tests;

and (5) being careful not to overinterpret the data. We are all taught that, in the laboratory, hypotheses can never be proved, only disproved. We are taught that experimental data can be used to support a hypothesis. We are cautioned against making the jump from one well-controlled experimental context to a general statement of truth. Thus, this type of research is carried out in a very narrow, specialized context—and yet it is now the gold standard for what we think of as good science. Why should that be the case?

Hypothesis testing is a means by which one can use the results of exploration and discovery (i.e., observations and descriptions) to make predictions about our world. A hypothesis often reads "If…, then…." That is, given a certain initial condition and/or manipulation, one can reasonably expect a given outcome. Prediction is, indeed, the basis for much of scientific research, and has been the driving force underlying the development of the scientific method and techniques. Providing a reliable basis for prediction is the job of the researcher.

Invention and Technological Advancement

Inventors have been among our most illustrious scientists, but modern invention—with its association with financial gain—has often been viewed as a nonscientific function (or at least a less than respectable scientific activity). Inventors have been looked at as tinkerers or engineers—but not as true researchers. Yet, technological invention drives modern science, and the findings from basic laboratory (and clinical) research drive technical innovation. Why, then, would one want to separate the technological enterprise from research (either exploratory or hypothesis testing)? It is often the technological advance that is the ultimate goal of even the most basic forms of research.

WHAT CAN WE ACCOMPLISH BY ENGAGING IN BASIC RESEARCH?

Even if we know *what* we're doing, it is often unclear *why* we should be doing it. Why would a bright young student want to spend his or her lifetime doing research in the laboratory? What rewards await the researcher? What value does this activity have for our society? The answers to these questions are often assumed by teachers and students, by researchers and granting agencies, by grant applicants and reviewers. But the answers are not simple. Indeed, at least at the general level, they may vary dramatically from individual to individual, from discipline to discipline, and from generation to generation.

Knowledge for the Sake of Knowledge

At every point in history, in every civilization, there have always been individuals who were inherently curious about their environment, who inevitably asked "why" and "how" questions. In modern society, for those who just "want to know," there is no better way to pursue that goal than in the laboratory. In this sense, scientific research is the modern religion. The young research scientist is provided with powerful tools, introduced to complex and intriguing questions, and encouraged to attack difficult problems as a challenge (for the sake of obtaining an answer). While some young scientists enter the field with specific practical goals in mind, most are simply intrigued by the opportunity to study something "cool," to become good at what they do—and eventually to be recognized for their expertise and contributions. Obtaining knowledge for its own sake is an old and sacred pursuit, one that is respected (implicitly) in the philosophy of many granting agencies that have very practical agendas. Accordingly, since it is impossible to know how a given discovery or insight might be used (in a practical way) in the future, we should encourage and support even research that doesn't seem to offer any immediate application or have any obvious relevance to real issues and problems. Because of the uncertainty about what insights might become important in the future, because what seems irrelevant or unusable at one time might provide the basis for important future developments, basic research without a specific practical rationale should be encouraged and supported. This position is certainly not universally held, and it represents a somewhat fragile rationale for basic research in an environment in which resources are scarce.

Control

Inasmuch as research studies allow us to make more and more accurate predictions, they provide us with better and better control over our environment. With an understanding of how things work (i.e., "If…, then…") comes the opportunity to develop more effective interventions and means for exploiting these mechanisms. Exploratory research provides a starting point for such developments; for example, the description of the human genome has opened up important possibilities for treating—and even curing—many diseases. However, real control (e.g., effective treatments) depends on an understanding of basic mechanisms (e.g., those processes that connect genetic structure with behavioral phenotype)—which, in turn, comes as the result of hypothesis-testing research.

One can, to be sure, rely on serendipitous data to obtain control of one's world. For example, many effective medical treatments have been discovered without an understanding of the underlying mechanisms of the disease. Given this history, there is an ongoing debate about the relative efficacy of research strategies that depend on insights into basic mechanisms. This debate is an important one within the context of epilepsy research. Regardless of the strategy, however, the key rationale for basic research is that the chosen approach will improve our ability to predict outcomes—and to intervene to alter those outcomes.

Quality of Life

This capability of effective intervention leads, in theory, to a better quality of life—more effective energy generation, better methods for dealing with climate change, or development of improved drugs/treatments that make us healthier and help us live longer. While it may not always have been the case that research could affect the quality of life of the average person, public support for research is now based on this assumption. We, as scientists, should be aware of this assumption, which brings with it a social responsibility that perhaps did not exist in the past. Research scientists can no longer think of themselves as isolated agents. As scientists, our goals (as well as our approaches) are shaped by the interrelatedness and interdependence of research laboratories and by our ties and obligations to the supporting society. We do not really have a *choice* about whether our research should be geared to making improvements for our society. This goal is now an obligatory aspect of the research enterprise.

WHY DO BASIC *EPILEPSY* RESEARCH?

How does all of this general philosophical musing relate to our specific discipline, epilepsy research? In particular, what justifications can we give—to ourselves, each other, and the society that supports us—for pursuing this line of work? The usual rationale involves some reference to the identification of better treatments and cures. If that's the case, then we should be prepared to point to specific accomplishments in our history that show how basic research has led to a better quality of life for people with epilepsy. Indeed, in today's world, our answers to these questions need to be not only theoretically satisfying (and correct) but also practical. If one accepts our obligation, as scientists, to contribute to the society that supports us, then the "Because…" response must reflect a feasible activity and achievable goals.

Because It's Interesting

There's no getting around the fact that many young researchers are attracted to epilepsy research simply because the problems are intellectually fascinating, the phenomena are dramatic, and the potential experimental approaches allow/encourage the use of a broad array of technical weaponry. There are few neurological phenomena as dramatic as a seizure—whether you monitor it behaviorally, electrophysiologically, or molecularly. Further, there is a broad range of still unexplored but very important questions that beckon enticingly to the ambitious young scientist anxious to make his or her mark in a complex field. In recruiting young investigators, and in discussing the advances in our field with the public, we need to take advantage of the inherent

drama of epilepsy, of its complex nature, even of the beauty of so many different systems interacting to produce a clinically important disorder. In short, the traditional stigma associated with epilepsy can (and should) be turned on its head to reveal a scientifically compelling mystery.

Because It Sheds Light on General Brain Function

For better or worse, one could make the argument that basic epilepsy research has shed more light on normal brain function than on the underlying bases of seizures (and their treatments) (e.g., see [4, 5]). Because seizure-related phenomena appear to use normal brain mechanisms, but involve a dramatic exaggeration of these processes, it is sometimes easier to see what's going on in the epileptic brain. Nowhere is this relationship more obvious than in the general area of brain plasticity. Synaptic modification (e.g., as in kindling[6]), anatomical reorganization (e.g., sprouting[7]), and changes in gene expression associated with seizure activity[8] have shed light on normal plasticities associated with development, learning and memory, and aging. This interplay between normal and pathological was evident early in studies using strychnine neuronography to map out functional connections in the brain (e.g., see ref. 9) and the Jacksonian march of epileptic activity to determine the topography of brain motor areas (as explored by Jasper, among others; see ref. 10). Epilepsy patients subjected to split brain procedures have given investigators the opportunity to further explore brain localization of function (e.g., see ref. 11), and imaging techniques developed to localize seizure onset zones have been refined to explore higher brain processes (e.g., see ref. 12). Cellular mechanisms importantly associated with normal brain function—such as soma/dendritic calcium flux,[13] regulation of extracellular potassium by astrocytes,[14] and recurrent excitation[15]—were studied early (and in some cases first identified) in seizure models in which these processes were exaggerated. Research approaches can be honed and investigative tools optimized in studies of seizure phenomenology to gain the sensitivity needed for studying more subtle changes in normal brain function.

In many epilepsies, the brain functions normally most of the time. Since a variety of research protocols—including invasive procedures—can be justified to study the epilepsy in those brains (because they are epileptic), it may be possible to gain insight into normal brain function by examining the baseline state. A clear understanding of the normal neurological baseline is absolutely critical if we are to test theories of epileptogenesis (i.e., how the brain changes from its normal state to the epileptic state); hypotheses focusing on aberrations of normal brain development,[16] reversion of the mature brain to an immature state,[17] loss of homeostatic controls,[18] and uncontrolled plastic processes[19] all implicitly demand that we define normal brain function that is pathological in the epileptic state.

Not only do epilepsy studies shed light on normal brain function, they also often have considerable relevance for understanding other neurological disorders. For example, epilepsy research now includes studies of mechanisms that are key to neurodegenerative disorders (e.g., mechanisms of cell damage/death,[20] traumatic brain injury such as postinjury reorganization,[21] and gene-related change [loss or increase] in function[22]). The overlap is apparent not only at the laboratory level but also clinically, where it is now clear that epilepsy can/should be viewed as a syndrome that often includes important comorbidities beyond the seizure itself (e.g., see refs. 23, 24).

Because It Offers Real Opportunities for Research Career Development

In modern biomedical research, an investigator's choice of a research focus is molded not only by his or her interests, but also by opportunities for making a significant impact and for obtaining long-term funding for his or her research activities. One can, of course, argue about whether basic epilepsy research has been adequately funded (relative to other neurological disorders? relative to other conditions that affect such a large percentage of the population? relative to the impact of the epileptic condition on medical costs/spending?). In the United States, the National Institutes of Health and many private funding

agencies provide significant research support, not only for the well-established investigator with a track record of productivity, but also for young investigators (presumably with fresh ideas). And relevant support is available in somewhat unexpected places if one looks for it (e.g., the Department of Defense). Despite these funding opportunities, it often appears that the field has lost, or failed to attract and maintain, many high-quality researchers; these individuals have chosen to focus their work in other research areas, presumably because they see more opportunity to participate at the cutting edge of biomedical research or because they view disease-related research as second-class (compared to pure basic research). One of the major challenges to the epilepsy field is to lure outstanding young investigators into this area of research—and retain them for the long term. Success in this effort will be determined by how well we can promote epilepsy as an exciting field of opportunity.

What features of a basic research program are likely to attract top-quality investigators? A list of such features is likely to include the following: (1) interesting problems that have research-based solutions; (2) opportunities to learn and apply modern technologies; (3) activities that involve links/collaborations with outstanding researchers in related fields; (4) high-profile publication opportunities that will gain the respect of colleagues and thus enhance one's career; and (5) the potential for making contributions to a significant health/societal issue. In the epilepsy research field, we have generally relied upon the intrinsic interest of the problem and have done relatively little to present relevant research opportunities in a way that explicitly targets these goals. Indeed, until recently, we have been rather imprecise about the research challenges that energize our research efforts, and have not been effective in presenting them in a way that is understandable and intriguing to an investigator who is not already committed to this area of research. For example: How many would-be researchers have any idea of the prevalence, varieties, and neurological consequences of clinical seizure disorders? Do we simply want to suppress seizure activity—and if that is our goal, haven't we already solved the problem with available antiepileptic drugs? What are the long-term opportunities and challenges in the field, and how are they related to general neuroscience (or other neurological disorders) research?

Because It May Lead to Development of Better Treatments and Cures

However an epilepsy researcher might start out (whatever the feature that first attracts him or her to this field), sooner or later the researcher is likely to realize that his or her laboratory activities could have significant consequences for people with seizure disorders. The realization that one's work in the laboratory could (should?) provide the bases for new treatments (and even cures) is a potent motivator. Given that motivation, a particularly exciting feature of the current state of epilepsy research is the proximity of the laboratory to the clinic. While that potential has always existed in theory (the epilepsy field has always implicitly involved translational research), it has never been so real as it is today. Modern neuroscience offers the tools and the concepts that can link, in a direct and impactful manner, laboratory insights with clinical practice/treatment. Enhancing that relationship by encouraging interactions between basic and clinical researchers provides a strong answer to the question "Why do basic epilepsy research?"

HOW DO WE CHOOSE AND PRIORITIZE RESEARCH GOALS?

It's fine to make a theoretical commitment to epilepsy research—but what does that commitment really mean? What are the strategies for pursuing meaningful research goals in our field? There are many ways of approaching the challenge, many directions and means for generating meaningful data. Rather than (or in addition to) encouraging a search for the "silver bullet" treatment/cure, it is important to recognize the diversity inherent in the problem and the need for many different hypotheses and approaches. Researchers may find themselves considering the following issues as they determine how they will choose and pursue their experimental goals.

Identification of Important Problems

In a field as diverse and complex as epilepsy research, a search for "the" critical research questions can be a daunting task. Further, the focus of research tends to shift from decade to decade (indeed, from year to year)—a normal result of changes that occur as our research tools and conceptual understanding change and become more powerful. In the recent past, as a result of evolving techniques in molecular genetics and of our growing appreciation of the social impact of epileptic disorders, there has been an emphasis on such issues as genetic causes of the epilepsies, the involvement of molecular pathways recruited during seizure activity, and the cognitive alterations associated with seizures (or brain conditions that give rise to seizures). In recent publications devoted to an exploration of current and future research priorities, there has been a shift in our research focus to exploring the underlying bases of epileptogenesis (mechanisms and treatments), to examining brain development and catastrophic epilepsies (aberrant processes during brain development that lead to difficult-to-control epilepsies and to associated long-term cognitive deterioration), to studying comorbidities associated with seizure conditions, and to developing new therapeutic strategies (new targets for antiepileptic drugs and nondrug treatment/cure strategies).[25,26] The shift in focus is driven, at least in part, by new insights at the clinical level, as well as by an appreciation for what can be productively investigated in the laboratory. And the new strategies and targets are made possible by insights and achievements with respect to previous research targets.[27] Given the rich complexity of the field, there is no reason to think that there will not be new sets of research priorities when the next edition of *Jasper's Basic Mechanisms of the Epilepsies* is published.

Understanding Basic Mechanisms versus Empirical Testing

The issues that call for our research attention can be attacked in what appear to be two fundamentally different ways. On the one hand, investigators can seek the underlying bases of a seizure-related phenomenon. The rationale for this approach seems obvious: If we understand the underlying mechanism, we can design an appropriate intervention/treatment. On the other hand, investigators may choose to test various treatment strategies without knowledge of the underlying mechanisms. This approach has been historically quite productive; for example, the identification of most current antiepileptic drugs (AEDs) was carried out without an understanding of their potential mechanisms of action. There is a strong tendency in the current research environment to give priority to the first approach—even though there remains considerable disagreement about the feasibility of, for example, rationale drug therapy based on pharmacological mechanisms.[28,29] Part of the problem, of course, is that although we may understand the potential molecular targets of a given drug, we don't understand (in most cases) the underlying abnormality that gives rise to the epileptic condition. An additional difficulty is that most treatments (pharmacological and nonpharmacological) have multiple effects, and it is difficult to determine which action is the antiepileptic one. Does that matter? Should the fact that we don't understand, for example, the anticonvulsant basis of deep brain stimulation or vagus nerve stimulation lead to a discriminatory bias against (or delay in) using such treatments? Some may argue that most of our current antiepileptic treatments (whether pharmacological, surgical, or otherwise) have come out of empirical *clinical* experiences, not from laboratory research and insights into basic mechanisms. The value of elucidating underlying mechanisms is rarely contested. But there is a basic research tendency to dismiss empirical clinical studies as bad science. This point of view may have a discouraging effect on the development of novel treatments.

Detailed Analyses versus the Big Picture

A related dichotomy that tends to divide research strategies in different laboratories is whether to seek detailed mechanistic analysis of a narrow variable or invest one's resources in gathering data that will yield a descriptive picture of a broader nature. Obviously, these

two approaches are not mutually exclusive, and most research involves aspects of both. A given laboratory (or researcher) will tend to emphasize one over the other but will operate somewhere in the middle of this continuum. Interestingly, in most Ph.D. programs, we work hard to train young investigators to focus narrowly on a well-defined problem, to generate a fine-grained analysis of a narrow research target. This mechanistic approach has obvious strengths—offering would-be researchers an opportunity to make a significant mark in the field and thus establish a reputation. This approach suffers, however, from the danger of producing rather myopic researchers who don't really understand the broader context in which they work. It is also clear that what one laboratory sees as mechanistic may be simply descriptive to another. Finally, given that mechanistic analysis is dependent on accurate description as a first step, and given that most clinically relevant problems in the epilepsy field are complex and involve multiple variables, careful descriptive studies are critical. Critique of a research program as unacceptably descriptive or irrelevantly narrow ignores the important question of if/how such studies might contribute to an important research goal.

WHAT APPROACHES—CONCEPTUAL OR TECHNICAL—ARE LIKELY TO YIELD SIGNIFICANT RESULTS?

All research—in particular, all basic epilepsy research—is not created equal. One's goals will determine, in large measure, how one approaches the problem(s) of interest and the techniques/methods that will most effectively move the study forward. While this point seems obvious, it is not so easy to realize in practice. Indeed, it is often the case that an investigator's methodological expertise will largely determine the types of experiments that are carried out in his or her laboratory—and thus will limit the investigator's ability to address the real goals of the research. While a single investigator cannot be expected to have sufficiently broad expertise to allow his or her laboratory to choose the most appropriate methodological approaches to a problem of interest, we can expect—and should urge—that the methods and experimental design employed to address a research problem are consistent with the investigator's stated goals. In the epilepsy field, one might also argue that those goals should provide insights into the mechanisms, behaviors, and treatments of epilepsy *as a real clinical target*.

Conceptual Goals

As is the case in any field, the conceptual goal(s) of the basic epilepsy researcher should ideally be well focused, clearly articulated, and significant. As a journal editor and grant reviewer, I routinely ask why the author/applicant has chosen to carry out the experiments described in his or her manuscript or grant application. If the researchers can't answer that question, then I rapidly lose interest. Because epilepsy is a complex set of disorders, because it is common, and because it has many connections to normal brain function, it is sometimes difficult for an investigator to articulate a clear rationale for his or her work—not only for the reader (or reviewer) but also for him- or herself. Why is it important to be focused and clear in one's goals? There are a number of ways to answer that question. Clear research goals lead to (1) well-designed experimental protocols; (2) less confusion in choosing what variables to study and what experimental models to use; and (3) a baseline from which one can discuss the significance of the research. Confusion about what one can conclude from an experimental study is a common result of insufficient clarity with respect to one's experimental goals. Conceptual fuzziness in basic epilepsy research often involves such confusions as (1) identifying a cellular/molecular feature in the epileptic brain and concluding that—simply because it's there—it contributes to the epileptic (or epileptogenic) process; (2) assuming that some abnormality described in an animal model of epilepsy (or in an in vitro/reduced preparation) is a feature of a human clinical condition (or is responsible for the development or expression of the epilepsy); or (3) drawing conclusions about epilepsy (or the epileptic brain) based on findings associated with seizures in a normal brain. Establishing clear experimental goals and drawing appropriate, well-supported conclusions from experimental data, are not always

easy—especially since there may be significant considerations that limit what one can actually study. While such methodological limitations are a fact of experimental life, there is no such restriction on elaboration of one's conceptual goals.

Technical Approaches

As mentioned above, technology (and methodology) often limits what we *can* study, and the technical capabilities of a given laboratory often determine what that laboratory actually does study. Collaborative interactions enlarge a given investigator's experimental scope. But the investigator is constantly challenged to be sure that the methods and techniques offer an appropriate approach to the problem of interest. Laboratory techniques seem to go in and out of fashion, and there has been a tendency for research directions to change as a function of the availability of new technologies. The investigator must therefore make decisions about if/how to change the direction of his or her research, based not only on whether a given technique has the appropriate power to address the experimental question but also on whether it is currently fashionable. Grant and manuscript reviewers are unquestionably biased in favor of studies that employ state-of-the-art technologies and may question the value of research based on old- fashioned approaches. For example, advances in molecular/genetic techniques have given us a new capability for assessing phenomenology and mechanisms at these levels—but molecular/genetic studies do not always provide better answers to all questions. Identifying a gene associated with a certain type of epilepsy does not tell us how that epilepsy occurs or how it should be treated; gene discovery is, in fact, only the start of an investigation to answer those questions. Similarly, gene array studies that show changes in gene expression in the epileptic brain provide only a starting point. These studies do tell us that the epileptic brain is different from the normal brain—but we know that. The real issue is to determine the differences that are critical to (causal of) the epileptic (or epileptogenic) condition. That is not to say that we should not take advantage of new technologies. There is no question that modern technologies have provided the researcher with previously unheard-of opportunities for addressing questions that might provide significant insights into important issues. These technologies should certainly be incorporated into our arsenal of research tools. But in doing so, investigators need to remain aware that these techniques do not make it any easier to ask, or answer, the right questions.

Model Development

Among the many challenges and opportunities faced by the basic epilepsy researcher is the often bewildering proliferation of models. The investigator almost invariably will need to determine which model(s) best addresses his or her experimental goals, which includes asking such questions as: What is this a model of? What questions can be effectively addressed? What laboratory techniques are compatible with studying this model? What variables and endpoints can be measured? Depending on their research goals, some experimenters may not need to work with an animal/tissue model at all (i.e., the investigation can focus on epilepsy in the human patient[30] or can employ computer modeling approaches[31]). One must ask also: What should be modeled? To find out what? How? Why? These questions have been addressed in some detail in the recent volume *Models of Seizures and Epilepsy*,[32,33] so the following discussion will summarize only key points that are directly relevant to the general question "Why do basic epilepsy research?"

BECAUSE IT'S INTERESTING AND FUNDABLE (PROVIDES OPPORTUNITIES FOR CAREER DEVELOPMENT)

As indicated above, there are many features of the epileptic brain that are attractive because of their general relevance to normal brain function and/or to other neurological disorders, and/or because of the ease with which they can be approached with modern techniques. The choice of appropriate models for this type of research can be overwhelming. Investigators routinely use in vitro preparations of single neurons (or neuron-like cells) and glia to study the function of channels, receptors, intracellular messenger systems, protein trafficking,

and so on, all with the rationale that alterations in the function of these variables can give rise—in some way, at some point in a complex process—to the abnormal electrical activity that defines seizures. Some critics have disputed whether such reduced preparations are accurately labeled *epileptic* (or whether individual neurons can produce epileptiform activities or seizures). It is nevertheless certainly worthwhile to study the underlying bases of aberrant cellular development and activity on their own merits. And arguably, this type of information will provide insights into epileptic phenomenology that could not be obtained from more complex systems. Simplified preparations also often allow for the application of powerful genetic, molecular, and electrophysiological methods that are difficult to employ in complex models. A striking example of successful pursuit of an epilepsy-relevant problem, using molecular genetic insights applied at the cellular level, is the recent identification of the mTOR pathway in tuberous sclerosis.[34] Analysis of many epilepsy-relevant mechanisms is facilitated by reduced preparation models, but relevant insight may require a more complex system of synaptically interacting neurons (or neurons and glia), as provided in acute slice and organotypic culture preparations (e.g., modulation of tonic inhibition[35]). Because of the methodological sophistication often associated with studies on reduced preparations, these models are often seen as particularly attractive when applying for research support. Many investigators have developed successful and productive epilepsy research careers based on a focus on simplified preparations, and there is a temptation to assume that these models are somehow superior to more complex models (because they are amenable to sophisticated manipulations, one can control and study a small number of variables, etc.). We need constant reminders about the limitations involved in interpreting data from such experiments with respect to our goal of explaining epilepsy phenomena or elucidating epilepsy mechanisms. For example, to study the interactions between neurons and endogenous inflammatory molecules requires a system that includes immune system elements.[36] Similarly, recent interest in the role of the blood-brain barrier[37] or of hormonal modulation of epileptogenicity[38] requires models in which those elements are present and normally functional.

Seizures are inherently interesting as a dramatic neurological output, and many laboratory manipulations can induce seizure-like phenomena. It is not always clear, however, that all of these experimentally induced seizure-like activities have anything to do with epilepsy. In past years, much of the basic research focus in epilepsy laboratories has been on models in which seizure-like electrical activities (or molecular changes) were induced in an otherwise normal brain. While we now know that seizure discharge in the normal brain may be different from output from an epileptic brain, the ease of inducing such activities and the strong attraction to the seizure output per se have encouraged studies in normal tissue. There is no doubt that many of these investigations have provided important insights into potential epileptic (and epileptogenic) mechanisms. But investigators who choose to focus on these types of models (i.e., in which seizure phenomenology is induced in normal brain tissue) should be aware, at the outset, that these studies may be of primarily intrinsic interest, since the results may not be relevant to clinical epilepsy phenomena.

BECAUSE IT SHEDS LIGHT ON HOW THE BRAIN WORKS

Many investigators have, over the years, argued that epilepsy provides a unique opportunity to understand normal brain function. For example, the key role of calcium—so much the key to research on synaptic plasticity and excitotoxicity—was an early focus of epilepsy researchers studying neurons that produce epileptiform burst discharge.[39,40] Comorbidities associated with epilepsy have begun to receive more attention, and investigators can now look to a large list of epilepsy models to study, for example, cognitive deficits, psychiatric abnormalities, and aberrations in brain development (e.g., see ref. 41). Investigations in animal models of seizure-related anxiety, depression, and other mood/psychiatric disorders[38,42,43] parallel the clinical focus on these comorbidities.[23,44] Historically, lesions that have been associated with temporal lobe epilepsy have also led to a greater understanding of memory mechanisms,[45] of the cognitive decline associated with neurodegenerative disorders such as Alzheimer's disease,[46] and of neurogenesis.[47] It is important to realize, in approaching studies

of brain function through the use of epilepsy models, that it is critical to place such studies into a relevant theoretical framework in order to make any sense of experimental results. The observation of seizures in an animal model of Alzheimer's disease doesn't necessarily tell you anything about epilepsy *or* about Alzheimer's disease. But if approached within a framework that links seizure mechanisms and Alzheimer's disease–related neurodegeneration and/or circuitry reorganization, this observation may provide the basis for interesting and insightful investigation.

BECAUSE IT CAN LEAD TO BETTER TREATMENTS AND CURES FOR A COMMON (AND DISABLING) NEUROLOGICAL DISORDER

Historically, it has been very difficult to model human epilepsy in animals, and investigators have developed a variety of strategies for making findings from animal models relevant to the human disorder. Most commonly, the approach has been to choose some feature of epilepsy (or, more accurately, one of the many different types of epilepsy) to study in detail. For example, many basic research investigations have employed models of temporal lobe epilepsy, examining such features as mossy fiber sprouting,[7] changes in inhibition in various hippocampal brain regions,[48,49] recurrent excitation,[50] and so on. This approach provides a host of research possibilities, offering many different methodological approaches. One can look at genetic, molecular, electrophysiological, pathological, and behavioral variables. One can study seizure events, interictal activities, postictal behaviors, or even phenomena that are not apparently linked to a seizure but that occur in the brain of an epileptic individual. The investigator can create the relevant phenomenon experimentally (e.g., by injecting a drug) or look at phenomena that occur spontaneously in a model system (e.g., a genetically epileptic mouse).

There is a long ongoing argument about how completely a model of epilepsy should recapture the key features of the human clinical disorder in order to be considered a relevant model. This argument has had no winner. Ideally, a relevant animal model should exhibit spontaneous seizure discharges that resemble—behaviorally, clinically, and electrically—the seizure type(s) of interest. Such a rigorous criterion may be unrealistic, however, when one considers how different the human brain is from a rodent brain (in which most such models are developed), the differences in developmental maturation and aging, and the difficulties in assessing seizure expression in nonhuman subjects. Particularly if the epilepsy type is complex, holding out for such a relevant model may impede research rather than encourage it (see ref. 51 for an interesting discussion of modeling infantile spasms). Alternatively, many investigators have turned to rodent models in which the key defining feature is a gene mutation that parallels a clinically occurring genetic mutation in humans. In these models, one can certainly make the case for the genetic cause of the epilepsy—but not necessarily for the relevance of subsequent gene expression. A number of drug treatments (and other experimental interventions) also give rise, over the long term, to spontaneous seizures, often associated with the pathological features that resemble those seen in human epilepsy (e.g., hippocampal sclerosis, as seen in temporal lobe epilepsy[52,53]).

The decision to use one of these models should certainly be based on the question the investigator wants to answer. For example, a genetic model may provide important insight into the molecular pathways that determine brain epileptogenicity (e.g., in models of cortical dysplasia). But is such a model relevant if one is to use it to study/understand the cognitive dysfunction seen in the epileptic brain? Is the time frame of seizure onset important (e.g., how well does the rodent recapitulate the human maturational sequence with respect to the mechanism under study)? If one uses such models to examine, for example, seizure-related changes in gene expression, can one successfully dissociate expression changes associated with seizure activity from expression patterns that characterize epileptogenicity (i.e., the seizure-sensitive state) or epileptogenesis (i.e., the development of the seizure-sensitive state) or an underlying brain lesion? One might choose a genetic model based on its clinically relevant neuropathological characteristics, as opposed to a model that mimics seizures discharge/electrical excitability. The long-term functional consequences of the associated structural abnormality, with respect to epileptogenicity or cognitive comorbidity, may

be explored in such a model even if the animal is not spontaneously epileptic. Under what conditions would such a model be relevant? Whatever approach one takes, it is important that the choices be made on the basis of a coherent theoretical framework.

Modern epilepsy research has yielded a new set of variables that appear to be characteristic of the epileptic brain, ranging from high levels of neurogenesis to robust expression of immune system variables to subtle changes in baseline tonic inhibition.[36,54,55] That these changes are seen in epileptic tissue seems clear. The salience of these phenomena for epileptogenesis and/or seizure activity remains to be established. Further, as is often the case in research, the more we learn, the less clear are our assumptions about epilepsy mechanisms. For example, it is becoming increasingly clear that many (most?) epileptic conditions are not easily attributable to a single genetic/molecular/electrophysiological abnormality, but are reflections of multiple changes—each one often subtle—that together give rise to aberrant brain activities. Thus, the models we choose must eventually provide us with the possibility of exploring the interaction among contributing variables. Two-hit hypotheses have become fashionable when thinking about developmental insults that give rise to seizure activities,[56,57] and they provide examples of how models may be manipulated to explore multifactorial contributions to the epileptic state.

Much of the research focusing on basic mechanisms of the epilepsies has revolved around identifying new treatment options—whether involving new drugs or focusing on novel therapeutic strategies. Both strategies can be pursued in a plethora of models. A major concern in these studies is whether treatment efficacy must be assessed against spontaneously occurring seizure activity in an epileptic animal, or whether some other measure of excitability (or synchrony) would provide an equally useful assessment. The verdict is still out, and investigators are looking for appropriate epilepsy-related phenomena—biomarkers—against which they can measure the effects of their treatments (e.g., a stable frequency of spontaneous seizures[58]; fast ripples[59]). In drug studies, it is also important to understand the relative concentration effects (absorption, blood-brain barrier penetrations, uptake into parenchyma, etc.) in humans (and particularly epileptic humans) compared to the chosen animal model and at different developmental ages. Since many animal models involve a normal brain in which seizures are generated, the question of if/how such neural tissue compares to epileptic tissue with respect to pharmacokinetic and pharmacodynamic issues must be addressed.

CONCLUDING THOUGHTS

There are multiple factors that go into the decision to carry out basic research, as well as additional influences that will determine whether an investigator decides to devote a lifetime to the study of epilepsy-related issues. The motivation for these decisions, and the subsequent choices that must be made about what problems to tackle and what techniques to employ, often play out in the absence of conscious attention. Just as it is important to give careful consideration to the choice of laboratory techniques or animal models, it is also helpful to be aware of the factors influencing one's research directions. As indicated above, there are no correct choices to be made. What is important is that the choices be consistent, coherent, and defensible.

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REFERENCES

1. Jasper HH, Ward AA Jr, Pope A, eds. *Basic Mechanisms of the Epilepsies*. Boston: Little, Brown; 1969.
2. Delgado-Escueta AV, Ward AA Jr, Woodbury DM, Porter RJ, eds. *Basic Mechanisms of the Epilepsies: Molecular and Cellular Approaches*. New York: Raven Press; 1986.
3. Delgado-Escueta AV, Wilson WA, Olsen RW, Porter RJ, eds. *Jasper's Basis Mechanisms of the Epilepsies.* 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 1999.
4. Lockard JS, Ward AA Jr, eds. *Epilepsy: A Window to Brain Mechanisms*. New York: Raven Press; 1980.
5. Engel J Jr, Schwartzkroin PA, Moshé SL, Lowenstein DH, eds. *Brain Plasticity and Epilepsy*. San Diego, CA: Academic Press; 2001.
6. Goddard GV. Separate analysis of lasting alteration in excitatory synapses, inhibitory synapses and cellular

excitability in association with kindling. *Electroencheph Clin Neurophysiol*. 1982;36:288–294.
7. Tauck DL, Nadler JV. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. *J Neurosci*. 1985;5:1016–1022.
8. Gall C, Lauterorn J, Isackson P, White J. Seizures, neuropeptide regulation, and mRNA expression in the hippocampus. *Prog Brain Res*. 1990;83:371–390.
9. Dusser de Barenne JG. The mode and site of action of strychnine in the nervous system. *Physiol Rev*. 1933;13:325–335.
10. Penfield W, Jasper H. Functional localization in the cerebral cortex. In: Penfield W, Jasper H, eds. *Epilepsy and the Functional Anatomy of the Human Brain*. London: Churchill; 1954:88–102.
11. Gazzaniga MS, Sperry RW. Language after section of the cerebral commissures. *Brain.* 1967;90:131–138.
12. Englot DJ, Blumenfeld H. Consciousness and epilepsy: why are complex partial seizures complex? *Prog Brain Res*. 2010;177:147–170.
13. Traub RD. Neocortical pyramidal cells: a model with dendritic calcium conductance reproduces repetitive firing and epileptic behavior. *Brain Res*. 1979;173:243–257.
14. Lux HD, Heinemann U, Dietzel I. Ionic changes and alterations in the size of the extracellular space during epileptic activity. *Adv Neurol*. 1986;44:619–639.
15. Wong RK, Traub RD, Miles R. Cellular basis of neuronal synchrony in epilepsy. *Adv Neurol*. 1986;44:583–592.
16. Rakhade SN, Jensen FE. Epileptogenesis in the immature brain: emerging mechanisms. *Nat Rev Neurosci*. 2009;5:380–391.
17. Ben-Ari Y, Holmes GL. Effects of seizures on developmental processes in the immature brain. *Lancet Neurol*. 2006;5:1055–1063.
18. Turrigiano GG. Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci*. 1999;22:221–227.
19. Schwartzkroin PA. Origins of the epileptic state. *Epilepsia.* 1997;38:853–858.
20. Henshall DC, Simon RP. Epilepsy and apoptosis pathways. *J Cereb Blood Flow Metab*. 2005;25:1557–1572.
21. Pitkänen A, Immonen RJ, Gröhn OH, Kharatishvili I. From traumatic brain injury to posttraumatic epilepsy. What animal models tell us about the process and treatment options. *Epilepsia.* 2009;50(suppl 2):1–9.
22. Aronica E, Gorter JA. Gene expression profile in temporal lobe epilepsy. *Neuroscientist.* 2007;13:100–108.
23. LaFrance WC Jr, Kanner AM, Hermann B. Psychiatric comorbidities in epilepsy. *Int Rev Neurobiol*. 2008;83:347–383.
24. Jacobs MP, Leblanc GG, Brooks-Kayal A, Jensen FE, Lowenstein DH, Noebels JL, Spencer DD, Swann JW. Curing epilepsy: progress and future directions. *Epilepsy Behav*. 2009;14:438–445.
25. Baulac M, Pitkänen A. Research priorities in epilepsy for the next decade: a representative view of the European scientific community. *Epilepsia.* 2009;50:571–578.
26. Kelly MS, Jacobs MP, Lowenstein DH. The NINDS epilepsy research benchmarks. *Epilepsia.* 2009;50:579–582.
27. Jacobs MP, Fischbach GD, Davis MR, Dichter MA, Dingledine R, Lowenstein DH, Morrell MJ, Noebels JL, Rogawski MA, Spencer SS, Theodore WH. Future directions for epilepsy research. *Neurology.* 2001;57:1536–1542.
28. Perucca E. Current trends in antiepileptic drug therapy. *Epilepsia.* 2003;44(suppl 4):41–47.
29. French JA, Faught E. Rational polytherapy. *Epilepsia.* 2009;50(suppl 8):63–68.
30. Köhling R, Avoli M. Methodological approaches to exploring epileptic disorders in the human brain in vitro. *J Neurosci Methods.* 2006;155:1–19.
31. Santhakumar V, Aradi I, Soltesz I. Role of mossy fiber sprouting and mossy cell loss in hyperexcitability: a network model of the dentate gyrus incorporating cell types and axonal topography. *J Neurophysiol*. 2005;93:437–453.
32. Engel J Jr, Schwartzkroin PA. What should be modeled? In: Pitkänen A, Schwartzkroin PA, Moshé SL, eds. *Models of Seizures and Epilepsy*. San Diego, CA: Elsevier/Academic Press; 2006:1–14.
33. Schwartzkroin PA, Engel J Jr. What good are animal models? In: Pitkänen A, Schwartzkroin PA, Moshé SL, eds. *Models of Seizures and Epilepsy*. San Diego, CA: Elsevier/Academic Press; 2006:659–668.
34. Wong M. Mammalian target of rapamycin (mTOR) inhibition as a potential antiepileptogenic therapy: from tuberous sclerosis to common acquired epilepsies. *Epilepsia.* 2010;51:27–36.
35. Zhang N, Wei W, Mody I, Houser CR. Altered localization of $GABA_A$ receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *J Neurosci.* 2007;27:7520–7531.
36. Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. *Epilepsia.* 2005;46:1724–1743.
37. Marchi N, Angelov L, Masaryk T, Fazio V, Granata T, Hernandez N, Hallene K, Diglaw T, Franic L, Najm I, Janigro D. Seizure-promoting effect of blood-brain barrier disruption. *Epilepsia.* 2007;48:732–742.
38. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in $GABA_A$ receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci*. 2005;8:797–804.
39. Schwartzkroin PA, Slawsky M. Probable calcium spikes in hippocampal neurons. *Brain Res*. 1977;135:157–161.
40. Traub RD, Wong RK. Synchronized burst discharge in disinhibited hippocampal slice. II. Model of cellular mechanism. *J Neurophysiol*. 1983;49:459–471.
41. Holmes GL. Effects of seizures on brain development: lessons from the laboratory. *Pediatr Neurol*. 2005;33:1–11.
42. Post RM. Neurobiology of seizures and behavioral abnormalities. *Epilepsia.* 2004;45(suppl 2):5–14.
43. Mazarati AM, Pineda E, Shin D, Tio D, Taylor AN, Sankar R. Comorbidity between epilepsy and depression; role of hippocampal interleukin-1β. *Neurobiol Dis*. 2010;37:461–467.
44. Kanner AM. Mood disorder and epilepsy: a neurobiologic perspective of their relationship. *Dialogues Clin Neurosci*. 2008;10:39–45.
45. Milner B. Psychological aspects of focal epilepsy and its neurosurgical management. *Adv Neurol*. 1975;8:299–321.
46. Palop JJ, Mucke L. Epilepsy and cognitive impairments in Alzheimer disease. *Arch Neurol.* 2009;66:435–440.

47. Kernie SG, Parent JM. Forebrain neurogenesis after focal ischemic and traumatic brain injury. *Neurobiol Dis*. 2010;37:267–274.
48. Coulter DA. Mossy fiber zinc and temporal lobe epilepsy: pathological association with altered "epileptic" gamma-aminobutyric acid A receptors in dentate granule cells. *Epilepsia.* 2000;41(suppl 6): 96–99.
49. Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, DeFelipe J, Ben-Ari Y, Escapez M, Bernard C. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. *Nat Neurosci*. 2001;4:52–62.
50. Christian EP, Dudek FE. Characteristics of local excitatory circuits studied with glutamate microapplication in the CA3 area of rat hippocampal slices. *J Neurophsyiol.* 1988;59:90–109.
51. Stafstrom CE, Moshe SL, Swann JW, Nehlig A, Jacobs MP, Schwartzkroin PA. Models of pediatric epilepsies: strategies and opportunities. *Epilepsia.* 2006;47:1407–1414.
52. Majores M, Schoch S, Lie A, Becker AJ. Molecular neuropathology of temporal lobe epilepsy: complementary approaches in animal models and human disease tissue. *Epilepsia.* 2007;48(suppl 2):4–12.
53. Curia G, Longo D, Biagini G, Jones RS, Avoli M. The pilocarpine model of temporal lobe epilepsy. *J Neurosci Methods.* 2008;172:143–157.
54. Semyanov A, Walker MC, Kullmann DM, Silver RA. Tonically active $GABA_A$ receptors: modulating gain and maintaining the tone. *Trends Neurosci*. 2004;27:262–269.
55. Parent JM, Murphy GG. Mechanisms and functional significance of aberrant seizure-induced hippocampal neurogenesis. *Epilepsia.* 2008;49(suppl 5): 19–25.
56. Hoffmann AF, Zhao Q, Holmes GL. Cognitive impairment following status epilepticus and recurrent seizures during early development: support for the "two-hit hypothesis." *Epilepsy Behav.* 2004;5:873–877.
57. Serbanescu I, Cortez, MA, McKerlie CK, Snead OC 3rd. Refractory atypical absence seizures in rat: a two hit model. *Epilepsy Res*. 2004;62:53–63.
58. Williams PA, White AM, Clark S, Ferraro DF, Swiercz W, Staley KJ, Dudek FE. Development of spontaneous recurrent seizures after kainate-induced status epilepticus. *J Neurosci*. 2009;29:2103–2112.
59. Engel J Jr, Bragin A, Staba R, Mody I. High-frequency oscillations: what is normal and what is not? *Epilepsia.* 2009;50:598–604.

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SECTION 2

Fundamentals of Neuronal Excitability Relevant to Seizures and Epilepsy

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Chapter 4

Voltage-Gated Na^+ Channels

Structure, Function, and Pathophysiology

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Voltage-gated Na^+ channels initiate action potentials in neurons and other excitable cells, and they are responsible for propagation of action potentials along nerves, muscle fibers, and the neuronal somatodendritic compartment.[1] They are complexes of a large pore-forming α subunit and smaller auxiliary β subunits.[2,3] Multiple genes encode Na^+ channel subunits, and the distinct Na^+ channel subtypes have subtle differences in functional properties, differential expression in excitable cells, and differential distribution in subcellular compartments.[2,4] These differences in function and localization contribute to the specialized functional roles of Na^+ channels in neuronal physiology and pharmacology.

Na^+ CHANNEL SUBUNIT STRUCTURE

Na^+ channel proteins in mammalian brain are complexes of a 260 kD α subunit in association with one or two auxiliary β subunits (β1–β4) 33 to 36 kD in size.[2,3]. The primary sequence predicts that the Na^+ channel α-subunit folds into four internally repeated domains (I–IV), each of which contains six α-helical transmembrane segments (S1–S6) (Fig. 4–1). In each domain, the S1 through S4 segments serve as the voltage-sensing module, and the S5 and S6 segments and the reentrant P loop that folds into the transmembrane region of the channel between them serve as the pore-forming module. A large extracellular loop connects the S5 or S6 transmembrane segments to the P loop in each domain, whereas the other extracellular loops are small. Large intracellular loops link the four homologous domains, and the large N-terminal and C-terminal domains also contribute substantially to the mass of the internal face of Na^+ channels. This view of Na^+ channel architecture, derived originally from hydrophobicity analysis and structural modeling,[5] has been largely confirmed by

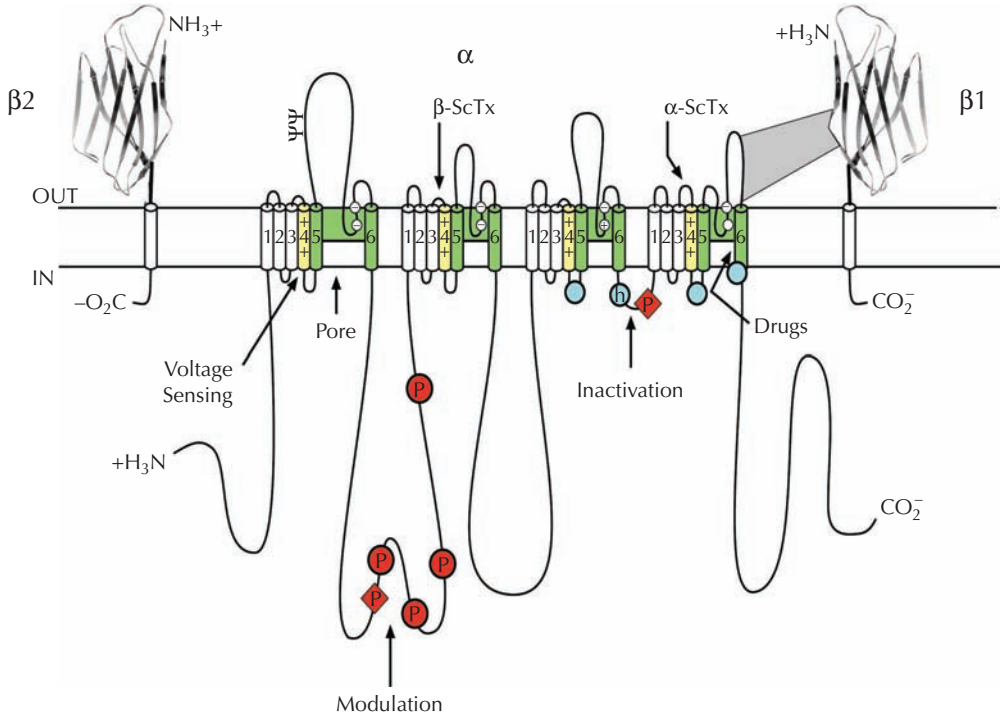

Figure 4–1. Transmembrane organization of Na^+ channel subunits. The primary structures of the subunits of the voltage-gated ion channels are illustrated as transmembrane folding diagrams. Cylinders represent probable alpha-helical segments. Bold lines represent the polypeptide chains of each subunit, with the length approximately proportional to the number of amino acid residues in the brain Na^+ channel subtypes. The extracellular domains of the β1- and β2-subunits are shown as immunoglobulin-like folds. Ψ, sites of probable N-linked glycosylation; P, sites of demonstrated protein phosphorylation by protein kinase A (PKA, circles) and protein kinase C (PKC, diamonds); shaded, pore-lining S5-P-S6 segments; white circles, the outer (EEDD) and inner (DEKA) rings of amino residues that form the ion selectivity filter and the tetrodotoxin binding site; ++, S4 voltage sensors; h in the shaded circle, inactivation particle in the inactivation gate loop; open shaded circles, sites implicated in forming the inactivation gate receptor. Sites of binding of α- and β-scorpion toxins and a site of interaction between α- and β1-subunits are also shown.

biochemical, electrophysiological, and structural experiments.[3]

Na^+ CHANNEL GENES

Na^+ channels are the founding members of the ion channel superfamily that includes voltage-gated Ca^{2+} channels, TRP channels, voltage-gated, inward rectifying, and two-pore-domain K^+ channels, and cyclic nucleotide-regulated CNG and HCN channels.[6] In evolution, the four-domain Na^+ channel was last among the voltage-gated ion channels to appear, and it is only found in multicellular organisms. It is thought that Na^+ channels evolved by two rounds of gene duplication from ancestral single-domain bacterial Na^+ channels like NaChBac found in *Bacillus halodurans,* which may also be the ancestral single-domain channel from which Ca^{2+} channels arose.[6,7] Voltage-gated Na^+ channel genes are present in a variety of metazoan species including fly, leech, squid, and jellyfish. The biophysical properties, pharmacology, gene organization, and even intron-splice sites of these invertebrate Na^+ channels are largely similar to those of the mammalian Na^+ channels, adding further support for the idea that the primordial Na^+ channel was established before the evolution of vertebrates.[8]

The human, mouse, and rat Na^+ channel genes and proteins are the best characterized to date. Ten related Na^+ channel genes are found in mammals (Fig. 4–2), which map to four paralogous chromosome segments.[8] More than 20 exons comprise each of the nine Na^+ channel α-subunit genes. Genes encoding Na^+

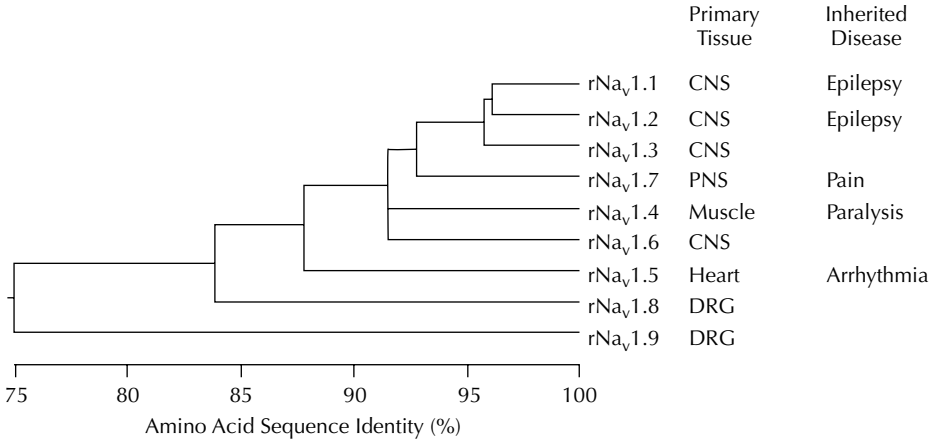

Figure 4–2. Amino acid sequence similarity of voltage-gated Na^+ channel α-subunits. Phylogenetic relationships of rat Na^+ channel sequences Nav1.1–Nav1.9. To perform the analysis, the amino acid sequences for all of the isoforms were aligned using Clustal W. Divergent portions of the terminal regions and the cytoplasmic loops between domains I–II and II–III were excluded. Primary tissue of expression and diseases caused by mutations are indicated.

channels $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, and $Na_v1.7$ are localized on chromosome 2 in the human and mouse, and these channels share similarities in sequence, biophysical characteristics, block by nanomolar concentrations of the potent pore-blocking toxin tetrodotoxin (TTX), and broad expression in neurons. A second cluster of genes encoding $Na_v1.5$, $Na_v1.8$, and $Na_v1.9$ channels is localized to human chromosome 3p21–24 (and to the orthologous region of chromosome 3 in the mouse). Although they are more than 75% identical in sequence to the group of channels whose genes are located on chromosome 2, these Na^+ channels all contain amino acid substitutions that confer varying degrees of resistance to TTX. In $Na_v1.5$, the principal cardiac isoform, a single amino acid change from phenylalanine to cysteine in the pore region of domain I is responsible for a 200-fold reduction in TTX sensitivity compared to those channels whose genes are located on chromosome 2.[9] At the identical position in $Na_v1.8$ and $Na_v1.9$, the amino acid residue is serine, and this change results in even greater resistance to TTX.[10] These two channels are preferentially expressed in peripheral sensory neurons.[11] In comparison to the Na^+ channels on chromosomes 2 and 3, $Na_v1.4$, which is expressed in skeletal muscle, and $Na_v1.6$, which is highly abundant in the central nervous system (CNS), have greater than 85% sequence identity and similar functional properties, including TTX sensitivity in the nanomolar concentration range.[4] In spite of these similarities, phylogenetic analysis by parsimony comparisons suggests a more distant evolutionary relationship, consistent with their distinct chromosomal localizations (Fig. 4–2). $Na_v1.4$ and $Na_v1.6$ are located on human chromosome 11 (mouse chromosome 17) and human chromosome 15 (mouse chromosome 12), respectively.[4]

A 10th Na^+ channel, Na_x, whose gene is located near the Na^+ channels of chromosome 2, is evolutionarily more distant. Key differences in functionally important regions of voltage sensor and inactivation gate and lack of functional expression of voltage-gated Na^+ currents in heterologous cells suggest that Na_x may not function as a voltage-dependent Na^+ channel. Consistent with this conclusion, targeted deletion of the Na_x gene in mice causes functional deficits in sensing plasma salt levels.[12]

The Na_vβ-subunits are encoded by four distinct genes in mammals.[13–15] The gene encoding β1 maps to 19q13, whereas β2 and β4 are located on 11q22–23 and β3 is located nearby at 11q24. The β1 and β3 subunits associate noncovalently with the α subunits, whereas β2 and β4 are covalently linked by a disulfide bond. All four β subunits have a single transmembrane segment, a large N-terminal extracellular domain, and a small intracellular C-terminal domain (Fig. 4–1). The Na_vβ subunits are dual-function proteins. Coexpression

of $Na_V\beta$ subunits alters the kinetics and voltage dependence of activation and inactivation of Na^+ channel complexes.[15] Moreover, their extracellular domain forms an immunoglobulin-like fold, similar to many cell adhesion molecules, and the β subunits are involved in interactions of Na^+ channels with proteins in the extracellular matrix, with other cell adhesion molecules, and with intracellular regulatory proteins like protein kinases and phosphoprotein phosphatases.[13,15,16] The importance of the functions of the $Na_V\beta$ subunits is underscored by the dramatic effects of deletion of the genes encoding $Na_V\beta1$ and $Na_V\beta2$, which include failure of myelination and axonal conduction, epileptic seizures, and premature death.[17,18]

MOLECULAR BASIS OF Na^+ CHANNEL FUNCTION

Classical work by Hodgkin and Huxley defined the three key features of Na^+ channels: (1) voltage-dependent activation, (2) rapid inactivation, and (3) selective ion conductance.[19] Building on this foundation, extensive structure-function studies employing molecular, biochemical, structural, and electrophysiological techniques have provided a clear understanding of the molecular basis of Na^+ channel function.[2] The narrow outer pore is formed by the reentrant P loops between transmembrane segments S5 and S6 of each domain (Fig. 4–1). Four amino acid residues in analogous positions in the P loops in each domain (aspartate in domain I, glutamate in domain II, lysine in domain III, and alanine in domain IV, DEKA) are crucial for Na^+ selectivity. Mutation of these four residues to glutamates confers Ca^{2+} selectivity, indicating that the side chains of these amino acid residues are likely to interact with Na^+ ions as they are conducted through the ion selectivity filter of the pore.

Similar to other voltage-gated ion channels, the voltage dependence of activation of Na^+ channels derives from outward movement of gating charges driven by changes in the transmembrane electrical potential. The S4 segments of each homologous domain serve as the primary voltage sensors for activation.[2] They contain repeated motifs of a positively charged amino acid residue followed by two hydrophobic residues in S4 segments creating a transmembrane spiral of positive charges. Upon depolarization, outward movement and rotation of S4 is thought to initiate a conformational change that opens the Na^+ channel pore.[5,20,21] This *sliding helix* or *helical screw* model is supported by strong evidence.[22] For example, neutralization of the positively charged residues in S4 reduces the voltage dependence of gating. The outward and rotational gating movement of S4 segment has been detected directly by reaction of substituted cysteine residues in S4 segments with extracellular sulfhydryl reagents following channel activation. A structural model of the voltage-sensing and pore-forming modules of Na^+ channels in the resting and activated states is presented in Fig. 4–3.

Fast inactivation of the Na^+ channel is a critical process that occurs within milliseconds of channel opening. The generally accepted model of this process involves a conserved inactivation gate formed by the intracellular loop connecting domains III and IV, which serves as a hinged lid that binds to the intracellular end of the pore and blocks it[2] (Fig. 4–1). Intracellular perfusion of proteases[23] or intracellular application of antibodies directed to this loop[24] prevents fast inactivation. The latch of this fast inactivation gate is formed by three key hydrophobic residues (isoleucine, phenylalanine, methionine, IFM), and an adjacent threonine (T).[25,26] Mutations of these amino acid residues destabilize the inactivated state, and peptides harboring the IFM motif can restore inactivation to Na^+ channels having mutated inactivation gates.[27,28] The closed inactivation gate is thought to make multiple interactions with hydrophobic amino acid residues near the intracellular mouth of the pore that may constitute the inactivation gate receptor. Scanning mutagenesis experiments implicate hydrophobic residues in intracellular S4-S5 loops of domains III and IV, as well as the intracellular end of the S6 transmembrane segment in domain IV in forming the inactivation gate receptor.[29–31]

The other intracellular domains of Na^+ channels are also functionally important. The C-terminal cytoplasmic domain is important for setting the properties of fast inactivation and contains several binding sites for interacting proteins,[32,33] and the cytoplasmic loop between DII and DIII binds to ankyrin G, which is important for clustering Na^+ channels in axon initial segments through interactions with the cytoskeleton.[34]

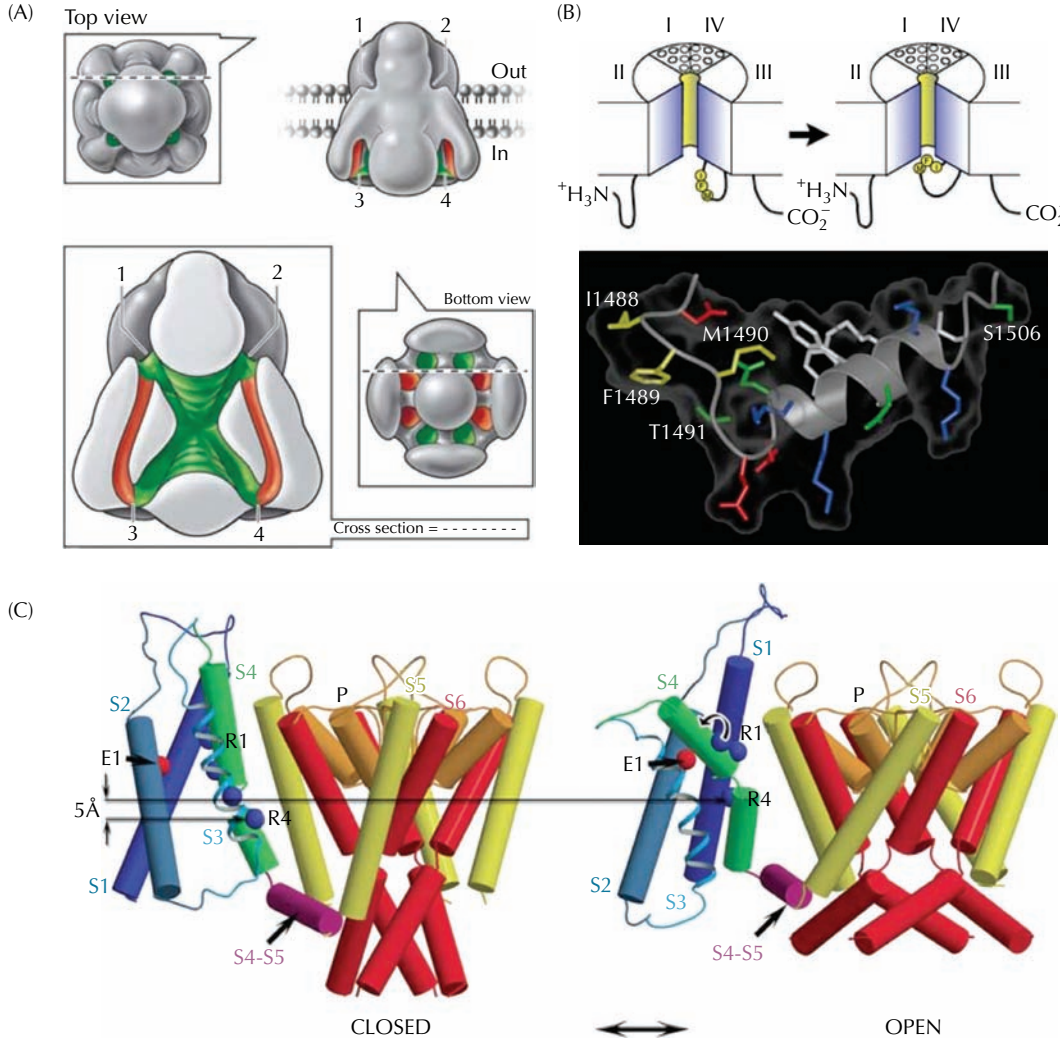

Figure 4–3. Three-dimensional structure of Na^+ channels and a related potassium channel. **A.** The three-dimensional structure of the Na_V channel α-subunit at 20 Å resolution, compiled from electron micrograph reconstructions. Adapted from ref. 43. **B.** Three-dimensional structure of the central segment of the inactivation gate as determined by multidimensional NMR. Isoleucine 1488, phenylalanine 1489, and methionine 1490 (IFM) are illustrated in yellow . Threonine 1491, which is important for inactivation, and serine 1506, which is a site of phosphorylation and modulation by protein kinase C, are also indicated in the lower panel. **C.** $K_V1.2$ models of the closed and open states shown in cylinder representation. Only a single voltage-sensing module is shown attached to the tetramer of the pore-forming module for clarity. Transmembrane segments S1 through S6 and the P loop are colored by a rainbow scheme from blue to red. The S4-S5 linker is colored purple. Approximate positions of the alpha carbon atoms of the first and fourth gating-charge-carrying arginines in the S4 (labeled R1 and R4 and colored blue) and E226 in the S2 (labeled E1 and colored red) are shown in sphere representation. All intracellular and extracellular loops, except for the S4-S5 linker, are represented by curved lines for simplicity. Vertical translation of the R4 between the closed and open states along the membrane normal vector and relative to the plane of the membrane is indicated by arrows. The S3 is represented by a ribbon to show clearly the positions of the gating-charge-carrying arginines in the S4 segment.

Na^+ channel functions are dramatically modulated by phosphorylation.[35,36] The large intracellular linker between domains I and II contains at least five well-characterized phosphorylation sites for protein kinases A and C, which induce a reduction in Na^+ currents by enhancement of the slow inactivation process.[37,38] Moreover, a recent mass spectrometry study has suggested that the number of active phosphorylation sites in brain in vivo may be much larger than was

previously thought and may be isoform-specific.[39] Serine residues in the intracellular linker between domains II and III are phosphorylated by casein kinase-2, which can modulate the interaction between ankyrin G and Na^+ channel α subunits and therefore the clustering of the channel at the axon initial segment.[40] Na^+ channels are also regulated by protein tyrosine kinases, which reduce the current through multisite phosphorylation and modification of fast inactivation.[41,42]

THREE-DIMENSIONAL STRUCTURE OF Na^+ CHANNELS

The three-dimensional structure of the Na^+ channel is not yet completely known, but some limited structural information has emerged. Structural determination at 19 Å resolution by cryoelectron microscopy and image reconstruction techniques show that Na^+ channels have a bell shape (side view), a fourfold symmetry of transmembrane masses, and large intracellular and extracellular masses through which several inlets and outlets allow aqueous access[43] (Fig. 4–3A). Unexpectedly, this image of Na^+ channels reveals a central pore that does not directly connect the intracellular and extracellular spaces, instead splitting into four branches near the membrane surface. Moreover, it suggests the presence of four peripherally located transmembrane pores, which may function as gating pores for voltage-sensor movement, as described below.

The only high-resolution structure of Na^+ channels is the solution structure of the inactivation gate in the intracellular loop connecting domains III and IV, as determined by nuclear magnetic resonance (NMR) analysis of this intracellular loop expressed in bacteria[44] (Fig. 4–3B). This structural analysis reveals a rigid alpha helical structure, preceded by two turns that position the key hydrophobic sequence motif IFMT such that it can interact with and block the inner mouth of the pore.

The global fold of the Na^+ channel pore region is predicted to be similar to that of voltage-gated potassium channels[21,45,46] (Fig. 4–3C). The structure of the open state of this channel has been determined by x-ray crystallography, and the structure of the closed state has been determined by structural modeling using the Rosetta method. This structural view shows the S5 and S6 regions of the Na^+ channel arrayed in fourfold symmetry around the aqueous ion-conduction pathway, the pore being lined mostly by S6 and the reentrant loop that form the ion-selectivity filter. A single voltage-sensor module is illustrated for simplicity. In the resting (closed) state of the channel, the S4 segment is in an inward position. Activation of the voltage sensor causes outward and rotational movement of the S4 segment and complementary movement of the S1, S2, and S3 segments around it. These two coupled molecular movements translocate the gating charges in the S4 segment from exposure to the intracellular milieu to exposure to the extracellular milieu, as required for voltage-dependent gating.

Comparison of the primary structures of the auxiliary β subunits to those of other proteins reveals a close structural relationship to the family of proteins that contain immunoglobulin-like folds, which include many cell adhesion molecules.[47, 48] The extracellular domains of these type I single-membrane-spanning proteins are predicted to fold in a manner similar to that of myelin protein P0, whose immunoglobin-like fold is known to be formed by a sandwich of two beta sheets held together by hydrophobic interactions. Myelin P0 is a cell adhesion molecule involved in tight wrapping of myelin sheets, and many related cell adhesion molecules with extracellular Ig folds and a single membrane-spanning segment are involved in cell-cell interactions among neurons and glia. As expected from their structure, $Na_V\beta$ subunits interact with extracellular matrix molecules, other cell adhesion molecules, and intracellular cytoskeletal proteins and signaling proteins.[13] These interactions are thought to be important for localization and stabilization of Na^+ channels in specific subcellular compartments such as axon initial segments and nodes of Ranvier, where they are present in high density.

EXPRESSION, LOCALIZATION, AND FUNCTION OF Na^+ CHANNEL SUBTYPES IN THE NERVOUS SYSTEM

Na^+ channel subtypes are differentially expressed in tissues and differentially localized in subcellular domains of individual cells. $Na_V1.3$ is

highly expressed in rodent fetal nervous tissues, whereas $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ are abundant in juvenile and adult CNS.[49–51] Subcellular distribution and properties of Na^+ channels are essential for neuronal functions.[34,51,52] Excitatory and inhibitory synaptic inputs are integrated at the axon initial segment (AIS), where Na^+ channels are clustered at particularly high density and generate the depolarizing phase of action potentials. Excitability propagates along the axon through the activity of Na^+ channels, which in myelinated fibers are clustered at nodes of Ranvier, and reaches synaptic terminals where Na^+ channels regulate excitability and thus the activation of Ca^{2+} channels and the initiation of neurotransmitter release. Na^+ channels are also present in the somatodendritic compartment, where they boost synaptic inputs and generate action potentials that backpropagate from the AIS into the dendrites. These action potentials can be considered retrograde signals of the integrated neuronal output that are sent to the dendritic tree in order to modulate synaptic plasticity.

The AIS is a proximal part of the axon about 40 µm in length in which the high density of Na^+ channels (about 20–50 times greater than in the soma or proximal dendrites[34,53]) produces a high Na^+ current density and a particularly low action potential threshold. These properties make the AIS the primary site of initiation of action potentials in neurons. Most of the studies on AIS have focused on excitatory cells, in which the action potential initiation site is located in the distal region of the AIS. Interestingly, the AIS contains specialized subdomains; the distal part is enriched in $Na_V1.6$, whereas the proximal part is enriched in Nav1.2.[34] This subdivision is functionally important. In fact, patch-clamp recordings from axon cut ends have revealed a higher density of Na^+ current and a progressive reduction in the half-activation voltage in the AIS with increasing distance from the soma. A recent study has shown that $Na_V1.6$ channels in the distal AIS promote action potential initiation, whereas $Na_v1.2$ channels in the proximal AIS promote its backpropagation to the soma.[54]

Myelinated axons are characterized by regularly spaced gaps of about 1 µm in the myelin sheath, the nodes of Ranvier, in which Na^+ channels are clustered at high density and allow saltatory conduction. During development, $Na_v1.6$ replaces $Na_v1.2$ in maturing nodes of Ranvier in myelinated axons.[55,56] Similar to neurons with myelinated axons, the AIS is also the site of action potential initiation in unmyelinated glutamatergic neurons, which contain $Na_v1.2$ and, to a lesser extent, Nav1.6 channels.[57] However, in these neurons, the AIS lacks the structural and morphological characteristics of myelinated axons, and action potentials are usually initiated in the proximal part.

Recent studies have shown that $Na_v1.1$ is particularly important for the excitability of GABAergic neurons[58,59] (see Chapter 58), and these channels are clustered at the AIS of some types of interneurons, including parvalbumin-positive, fast-spiking interneurons. However, several studies have shown that $Na_V1.1$ (or $Na_V1.3$ in immature neurons) is also present in the somatodendritic compartment of several types of neurons, including glutamatergic pyramidal cells in cortical systems, where it may control neuronal excitability through integration of synaptic impulses and regulation of the threshold for action potential propagation to the dendritic and axonal compartments.[51,57,58,60,61] At axon terminals, the Na^+ channel subtype that is most often found concentrated near presynaptic terminals in cortical areas is $Na_V1.2$. Some discrepancies in the results of immunocytochemical studies of Na^+ channel subcellular localization in the AIS may be explained by the high density of proteins that makes it difficult for antibodies to reach epitopes, which can be better exposed with antigen retrieval techniques.[62]

Na^+ channels in central neurons do not only generate the classical fast-inactivating Na^+ current. In fact, fast inactivation of Na^+ current in neurons is generally incomplete, resulting in a small, persistent Na^+ current (I_{NaP}) with kinetics of inactivation on the order of tens of seconds.[63] I_{NaP} can be generated by all of the neuronal Na^+ channel isoforms and can be modulated by several neurotransmitter systems.[61,64–66] Although I_{NaP} is usually only about 1% of the transient Na^+ current, it is important for several neuronal functions because it is the main depolarizing current in the subthreshold voltage range. It contributes to amplifying postsynaptic potentials, shaping repetitive firing, generating rhythmicity, and maintaining prolonged depolarized plateau potentials.[63,67,68] Thus, even small modifications of I_{NaP} amplitude can substantially modify neuronal properties.

Another subthreshold current generated by Na^+ channels is the resurgent current,[64]

an unusual transient Na^+ current elicited by repolarizations that follow strong depolarizations and that can contribute to spontaneous and to high-frequency firing. It has been proposed that this current is produced by a putative intracellular blocking factor (possibly the intracellular domain of the β4 accessory subunit) that binds to open Na^+ channels and prevents fast inactivation; the blocking factor would unbind during repolarizations generating a transient current that decays because channels deactivate.[69] The resurgent current is substantially reduced in neurons isolated from $Na_V1.6$ or $Na_V1.1$ knockout mice,[61,64] indicating that both of these Na^+ channel types can contribute to its generation. Slowing of Na^+ channel inactivation with application of toxins can induce resurgent currents in neurons from $Na_V1.6$ knockout mice, providing further evidence that other Na^+ channel subtypes can generate resurgent currents.[69] In fact, a recent study has shown that pathogenic mutations of $Na_V1.4$, $Na_V1.5$, and $Na_V1.7$ that cause slowing and destabilization of inactivation can induce increased resurgent Na^+ current at least in some cell types.[70] Thus, increased resurgent current may be a common feature of several pathogenic mutations of Na^+ channels.

Na^+ channels can also have important roles in glia. In microglia they can regulate phagocytic activity,[71] whereas in oligodendrocytes and astrocytes their functional roles are not clear yet, but may involve the regulation of cytoplasmic Na^+ homeostasis.[72] Glial cells usually are not excitable, but action potential-like events have been observed in glial precursor cells and in cultured astrocytes. In addition, a subset of glial cells can generate action potentials when depolarized, receive excitatory and inhibitory synaptic inputs from neurons, and are especially sensitive to excitotoxicity.[73]

$Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ are also expressed at low levels in the peripheral nervous system. However, the three isoforms that have been cloned from sympathetic and dorsal root ganglion neurons, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$, are much more abundant.[74] The $Na_v1.7$ channel is localized in axons, where it functions in initiating and conducting the action potential. More restricted expression patterns are observed for $Na_v1.8$ and $Na_v1.9$; these channels are highly expressed in small sensory neurons of dorsal root ganglia and trigeminal ganglia, where they have a key role in nociception.[75–77] $Na_v1.4$ and $Na_v1.5$ are the primary muscle Na^+ channels that control the excitability of the skeletal and cardiac myocytes, respectively.[11] $Na_v1.5$ is transiently expressed in developing skeletal muscle but is replaced by $Na_v1.4$ in the adult.

Na^+ CHANNEL PHARMACOLOGY

Na^+ channels are the molecular targets for drugs used in the treatment of epilepsy, cardiac arrhythmias, bipolar disorder, and migraine and in the prevention of acute pain; Na^+ channel-blocking drugs are also in development for the treatment of chronic pain.[78,79] Local anesthetics bind to a specific receptor within the pore of Na^+ channels, formed by the S6 segments in domains I, II, and IV[80,81] (Fig. 4–4). Their binding blocks ion movement through the pore and stabilizes the inactivated state of Na^+ channels.[82] Antiarrhythmic drugs and antiepileptic drugs share similar overlapping receptor sites.[78,81] Complete blockage of Na^+ channels would be lethal. However, these drugs selectively block Na^+ channels in depolarized and/or rapidly firing cells, such as axons carrying high-intensity pain information and rapidly firing nerve and muscle cells that drive epileptic seizures or cardiac arrhythmias.[82,83] This selective block arises because the drugs can reach their binding site in the pore of the Na^+ channel more rapidly when the pore is repetitively opened, and they bind with high affinity to inactivated Na^+ channels that are generated in rapidly firing or depolarized cells. This use-dependent action of the Na^+ channel blocking drugs is essential for their therapeutic efficacy. Notably, I_{NaP} is also reduced by antiepileptics and other Na^+ channel blockers, often at concentrations that are lower than those required for reducing the transient component.[79] Na^+ channel blockers used in epilepsy therapy include phenytoin, carbamazepine, lamotrigine, valproate, topiramate, and lacosamide; phenytoin, carbamazepine, lamotrigine, and lacosamide are thought to act selectively on Na^+ channels, whereas the other drugs have multiple targets.[79] Na^+ channels are also the molecular targets for several classes of neurotoxins that bind at six or more distinct receptor sites on the channel, blocking it or modifying its gating properties.[84,85]

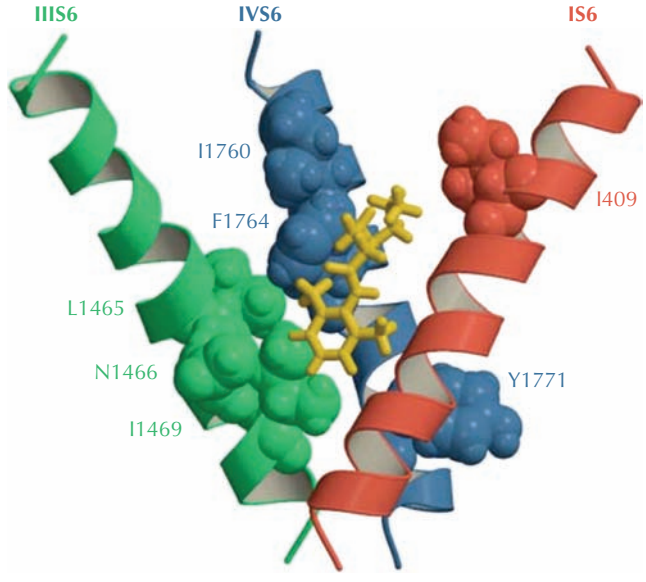

Figure 4–4. Model of etidocaine binding to the local anesthetic receptor site formed by transmembrane segments IS6, IIIS6, and IVS6 of the $Na_v1.2$ channel. A three-dimensional model of the proposed orientation of amino acid residues within the Na^+ channel pore with respect to the local anesthetic etidocaine is presented. Only transmembrane segments IS6 (red), IIIS6 (green), and IVS6 (blue) are shown. Residues important for etidocaine binding are shown in a space-filling representation.

Na^+ CHANNEL AND EPILEPSY

Growing experimental evidence points to a role for abnormal expression and/or function of Na^+ channels in the pathophysiology of epilepsy. Numerous studies have shown altered levels of mRNA and protein for α-subunits $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$ and for β subunits in animal models of epilepsy[86–89] and in human epileptic brain tissue.[90, 91] An upregulation of Na^+ channels has also been reported in astrocytes from human epileptic tissue, suggesting that pathological glial excitability may contribute to epileptic disorders.[92]

Induction of epileptiform activity can also be correlated with changes in Na^+ channel function. For instance, temporal lobe neurons exhibit larger than normal I_{NaP} in both the pilocarpine[93] and kindling[89] animal models of epilepsy and in the human temporal lobe surgically resected from patients.[94] An increase of I_{NaP} may predispose these neurons to hyperexcitability, as demonstrated in transgenic mice expressing an incompletely inactivating $Na_v1.2$ mutant, which show chronic seizures and hippocampal sclerosis.[95] These data show that Na^+ channels can be implicated in acquired epilepsy, either in the process of epileptogenesis and/or in the maintenance of the epileptic state. Their implication in epileptogenesis has been indisputably demonstrated by the identification of several hundred mutations in Na^+ channel genes leading to genetic epileptic syndromes.

GENETIC Na^+ CHANNELOPATHIES

Mutations that alter Na^+ channel function lead to human diseases of hyperexcitabilty (Table 4–1). Multiple mutations of $Na_v1.4$ that yield hyperactive skeletal Na^+ channels have been shown to cause hyperkalemic periodic paralysis and paramyotonia congenita.[96] In contrast, mutations in S4 gating charges that cause reduced function of these channels and induce a Na^+ or proton leak through their voltage-sensing domains (termed the *gating pore current*) cause hypokalemic periodic paralysis.[97,98] Mutations of $Na_v1.5$ that impair inactivation or alter activation lead to inherited long QT syndrome type 3 and Brugada syndrome, in which

Table 4–1 **Major Inherited Diseases of Na^+ Channels**

Disease	Gene	Channel
Generalized epilepsy with febrile seizures plus	*SCN1A*	$Na_V1.1$
	SCN1B	$Na_V\beta1$
Dravet syndrome (severe myoclonic epilepsy in infancy)	*SCN1A*	$Na_V1.1$
	SCN1B	$Na_V\beta1$
Familial hemiplegic migraine type III	*SCN1A*	$Na_V1.1$
Benign familial neonatal-infantile seizures	*SCN2A*	$Na_V1.2$
Hypokalemic periodic paralysis type II	*SCN4A*	$Na_V1.4$
K^+-sensitive normokalemic periodic paralysis	*SCN4A*	$Na_V1.4$
Hyperkalemic periodic paralysis	*SCN4A*	$Na_V1.4$
Paramyotonia congenita	*SCN4A*	$Na_V1.4$
Long QT syndrome type III	*SCN5A*	$Na_V1.5$
Brugada syndrome	*SCN5A*	$Na_V1.5$
Erythermalgia	*SCN9A*	$Na_V1.7$
Paroxysmal extreme pain disorder	*SCN9A*	$Na_V1.7$
Congenital indifference to pain	*SCN9A*	$Na_V1.7$

the risk of fatal cardiac arrhythmias is greatly increased.[99] Gain-of-function mutations in the gene that encodes $Na_V1.7$ channels cause erythermalgia and paroxysmal extreme disorder, and loss-of-function mutations cause congenital indifference to pain.[76]

Like these channelopathies in cardiac and skeletal muscle and in peripheral nerve, mutations in Na^+ channels expressed in the brain cause multiple forms of epilepsy, as well as familial hemiplegic migraine (FHM), a rare severe autosomal dominant inherited subtype of migraine with aura characterized by hemiparesis during the attacks.[79,100,101] Even though the functional effect of some of these mutations is still debated and often depends on the experimental system used for the study,[101] it has been indisputably demonstrated that they have a causative pathogenic role. The vast majority of epileptogenic mutations are in $Na_v1.1$, which is thus far the most clinically relevant epilepsy gene (for databases of $Na_v1.1$ mutations see http://www.molgen.ua.ac.be/SCN1AMutations/ and http://www.scn1a.info/). In fact, generalized (genetic) epilepsy with febrile seizures plus (GEFS+) type II and severe myoclonic epilepsy of infancy (SMEI or Dravet syndrome) result from mutations of $Na_v1.1$ channels, as well as from one mutation of the $\beta1$-subunit[100,102] (and see Chapter 58). Severe myoclonic epilepsy of infancy is caused by inactivation of one allele of the gene encoding $Na_V1.1$ channels, and the hyperexcitability and comorbidities of that disease result from disinhibition caused by selective loss of Na^+ current and action potential firing in GABAergic inhibitory neurons[100] (and see Chapter 58). Generalized epilepsy with febrile seizures plus []may be caused by milder loss-of-function mutations and, at least in some families, by folding defects that can be rescued with pharmacological chaperones and interacting proteins[100,103,104] (and see Chapter 58). Notably, FHM mutations could cause a gain of function.[105]. Benign familial neonatal-infantile seizures (BFNIS) are caused by mutations in $Na_V1.2$ channels.[106,107] The relationship between the functional alterations in $Na_V1.2$ channels, the hyperexcitability, and epilepsy is not completely clear, but several mutations induce a gain of function.[107] These inherited epilepsy syndromes are discussed in detail in Chapter 58.

FUTURE DEVELOPMENTS AND CHALLENGES

We expect that in the future, Na^+ channels will be found to be involved in a larger spectrum of epileptic phenotypes and in other diseases of the CNS. Besides epilepsy, mutations of $Na_v1.1$ can cause FHM, as noted above. Moreover, $Na_V1.1$ missense mutations have recently been identified in two unrelated families presenting with elicited repetitive daily blindness (ERDB), a visual phenotype that cosegregates with FHM but is independent of FHM attacks,[108] suggesting that $Na_V1.1$ mutations may alter retinal functions. Mutations

of $Na_v1.1$ channels have also been found in families presenting with both seizures and psychiatric phenotypes, and possibly predisposing genetic variants of Na^+ channels have been found in autistic patients.[109] Interestingly, SMEI patients carrying $Na_v1.1$ mutations can show cognitive impairment even if they present with infrequent seizures.[110] Therefore, modification of Na^+ channel functions might cause psychiatric disorders or confer susceptibility, consistent with the increased use of Na^+ channel-blocking drugs in the treatment of psychiatric diseases.[111] Interestingly, a loss-of-function mutation of $Na_v1.6$ has been identified in a single patient with cerebellar atrophy, behavioral deficits, and ataxia, further extending the spectrum of pathogenic Na^+ channel genes and diseases.[109] Moreover, Na^+ channels may be involved in the generation of altered neuronal excitability in Alzheimer's disease, in which Na^+ channel β subunits can be cleaved by the same enzymes that process amyloid precursor protein, generating active peptides that can modulate Na^+ channel expression and functions, leading to pathological excitability.[112] Furthermore, Na^+ channels have a pathogenic role in the inflammation and neurodegeneration that occur in multiple sclerosis and other demyelinating diseases, as well as in hypoxia-induced neuronal damage.[79] Future challenges in the field will be to identify the properties that differentiate Na^+ channels from other voltage-gated channels at the molecular level, to reveal the interacting partners that localize different isoforms in defined subcellular domains and modulate their functions, and to shed light on the functions that different Na^+ channel isoforms have in the physiology of different neurons and different subcellular regions. Na^+ channel blockers, which have been used for decades as antiepileptic drugs, poorly discriminate between Na^+ channel isoforms and have similar mechanisms of action. The development of new compounds that selectively target different isoforms and/or differently modify Na^+ channel functions will probably allow the treatment of some diseases that are thus far drug-resistant, including epilepsy.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Hille B. *Ionic Channels of Excitable Membranes*. 3rd ed. Sunderland, MA: Sinauer Associates; 2001.
2. Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron*. 2000;26:13–25.
3. Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacol Rev*. 2005;57:397–409.
4. Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Berwald Netter Y, Noda M, Tamkun MM, Waxman SG, Wood JN, Catterall WA. Nomenclature of voltage-gated sodium channels. *Neuron*. 2000;28:365–368.
5. Guy HR, Seetharamulu P. Molecular model of the action potential sodium channel. *Proc Natl Acad Sci USA*. 1986;508:508–512.
6. Yu FH, Catterall WA. The VGL-chanome: a protein superfamily specialized for electrical signaling and ionic homeostasis. *Sci STKE*. 2004;2004:re15.
7. Ren D, Navarro B, Xu H, Yue L, Shi Q, Clapham DE. A prokaryotic voltage-gated sodium channel. *Science*. 2001;294:2372–2375.
8. Plummer NW, Meisler MH. Evolution and diversity of mammalian sodium channel genes. *Genomics*. 1999;57:323–331.
9. Satin J, Kyle JW, Chen M, Bell P, Cribbs LL, Fozzard HA, Rogart RB. A mutant of TTX-resistant cardiac sodium channels with TTX-sensitive properties. *Science*. 1992;256:1202–1205.
10. Sivilotti L, Okuse K, Akopian AN, Moss S, Wood JN. A single serine residue confers tetrodotoxin insensitivity on the rat sensory-neuron-specific sodium channel SNS. *FEBS Lett*. 1997;409:49–52.
11. Goldin AL. Diversity of mammalian voltage-gated sodium channels. *Ann NY Acad Sci*. 1999;868: 38–50.
12. Watanabe E, Fujikawa A, Matsunaga H, Yasoshima Y, Sako N, Yamamoto T, Saegusa C, Noda M. Nav2/NaG channel is involved in control of salt-intake behavior in the CNS. *J Neurosci*. 2000;20:7743–7751.
13. Isom LL. Sodium channel β-subunits: anything but auxiliary. *Neuroscientist*. 2001;7:42–54.
14. Yu FH, Westenbroek RE, Silos-Santiago I, McCormick KA, Lawson D, Ge P, Ferriera H, Lilly J, DiStefano PS, Catterall WA, Scheuer T, Curtis R. Sodium channel beta-4, a new disulfide-linked auxiliary subunit with similarity to beta-2. *J Neurosci*. 2003;23:7577–7585.
15. Isom LL, De Jongh KS, Catterall WA. Auxiliary subunits of voltage-gated ion channels. *Neuron*. 1994;12:1183–1194.
16. Ratcliffe CF, Qu Y, McCormick KA, Tibbs VC, Dixon JE, Scheuer T, Catterall WA. A sodium channel signaling complex: modulation by associated receptor protein tyrosine phosphatase b. *Nat Neurosci*. 2000;3:437–444.
17. Chen C, Bharucha V, Chen Y, Westenbroek RE, Brown A, Malhotra JD, Jones D, Avery C, Gillespie PJ 3rd, Kazen-Gillespie KA, Kazarinova-Noyes K, Shrager P, Saunders TL, Macdonald RL, Ransom BR, Scheuer T, Catterall WA, Isom LL. Reduced sodium channel density, altered voltage dependence of inactivation,

and increased susceptibility to seizures in mice lacking sodium channel beta 2-subunits. *Proc Natl Acad Sci USA*. 2002;99:17072–17077.
18. Chen C, Westenbroek RE, Xu X, Edwards CA, Sorenson DR, Chen Y, McEwen DP, O'Malley HA, Bharucha V, Meadows LS, Knudsen GA, Vilaythong A, Noebels JL, Saunders TL, Scheuer T, Shrager P, Catterall WA, Isom LL. Mice lacking sodium channel beta 1 subunits display defects in neuronal excitability, sodium channel expression, and nodal architecture. *J Neurosci*. 2004;24:4030–4042.
19. Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol*. 1952;117:500–544.
20. Catterall WA. Molecular properties of voltage-sensitive sodium channels. *Annu Rev Biochem*. 1986;55:953–985.
21. Yarov-Yarovoy V, Baker D, Catterall WA. Voltage sensor conformations in the open and closed states in ROSETTA structural models of K^+ channels. *Proc Natl Acad Sci USA*. 2006;103(19):7292–7297.
22. Catterall WA. Ion channel voltage sensors: structure, function, and pathophysiology. *Neuron*. 2010;67:915–928.
23. Armstrong CM, Bezanilla F, Rojas E. Destruction of sodium conductance inactivation in squid axons perfused with pronase. *J Gen Physiol*. 1973;62: 375–391.
24. Vassilev PM, Scheuer T, Catterall WA. Identification of an intracellular peptide segment involved in sodium channel inactivation. *Science*. 1988;241:1658–1661.
25. West JW, Patton DE, Scheuer T, Wang Y, Goldin AL, Catterall WA. A cluster of hydrophobic amino acid residues required for fast Na^+ channel inactivation. *Proc Natl Acad Sci USA*. 1992;89:10910–10914.
26. Hayward LJ, Brown RH Jr., Cannon SC. Inactivation defects caused by myotonia-associated mutations in the sodium channel III-IV linker. *J Gen Physiol*. 1996;107:559–576.
27. Eaholtz G, Scheuer T, Catterall WA. Restoration of inactivation and block of open sodium channels by an inactivation gate peptide. *Neuron*. 1994;12: 1041–1048.
28. Kellenberger S, West JW, Scheuer T, Catterall WA. Molecular analysis of the putative inactivation particle in the inactivation gate of brain type IIA Na^+ channels. *J Gen Physiol*. 1997;109:589–605.
29. McPhee JC, Ragsdale DS, Scheuer T, Catterall WA. A critical role for transmembrane segment IVS6 of the sodium channel α subunit in fast inactivation. *J Biol Chem*. 1995;270:12025–12034.
30. Smith MR, Goldin AL. Interaction between the sodium channel inactivation linker and domain III S4-S5. *Biophys J*. 1997;73:1885–1895.
31. McPhee JC, Ragsdale D, Scheuer T, Catterall WA. A critical role for the S4-S5 intracellular loop in domain IV of the sodium channel a subunit in fast inactivation. *J Biol Chem*. 1998;273:1121–1129.
32. Mantegazza M, Yu FH, Catterall WA, Scheuer T. Role of the C-terminal domain in inactivation of brain and cardiac sodium channels. *Proc Natl Acad Sci USA*. 2001; 98:15348–15353.
33. Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. *Pharmacol Rev*. 2005;57:411–425.
34. Rasband MN. The axon initial segment and the maintenance of neuronal polarity. Nat Rev Neurosci. 2010;11:552–562.
35. Cantrell AR, Catterall WA. Neuromodulation of Na^+ channels: an unexpected form of cellular plasticity. *Nat Rev Neurosci*. 2001;2:397–407.
36. Scheuer T. Regulation of sodium channel activity by phosphorylation. *Semin Cell Dev Biol*. 2010;22(2): 160–165.
37. Carr D, Day M, Cantrell AR, Scheuer T, Catterall WA, Surmeier DJ. G-protein coupled receptors modulate sodium channel gating by promoting entry into a slow inactivated state a novel form of activity-dependent plasticity. Neuron. 2003;39:793–806.
38. Chen Y, Yu FH, Surmeier DJ, Scheuer T, Catterall WA. Neuromodulation of Na^+ channel slow inactivation via cAMP-dependent protein kinase and protein kinase C. *Neuron*. 2006;49:409–420.
39. Berendt FJ, Park KS, Trimmer JS. Multisite phosphorylation of voltage-gated sodium channel alpha subunits from rat brain. *J Proteome Res*. 2010;9: 1976–1984.
40. Brechet A, Fache MP, Brachet A, Ferracci G, Baude A, Irondelle M, Pereira S, Leterrier C, Dargent B. Protein kinase CK2 contributes to the organization of sodium channels in axonal membranes by regulating their interactions with ankyrin G. *J Cell Biol.* 2008;183:1101–1114.
41. Ahn M, Beacham D, Westenbroek RE, Scheuer T, Catterall WA. Regulation of Na(v)1.2 channels by brain-derived neurotrophic factor, TrkB, and associated Fyn kinase. *J Neurosci*. 2007;27: 11533–11542.
42. Beacham D, Ahn M, Catterall WA, Scheuer T. Sites and molecular mechanisms of modulation of Na(v)1.2 channels by Fyn tyrosine kinase. *J Neurosci*. 2007;27:11543–11551.
43. Sato C, Ueno Y, Asai K, Takahashi K, Sato M, Engel A, Fujiyoshi Y. The voltage-sensitive sodium channel is a bell-shaped molecule with several cavities. *Nature*. 2001;409:1047–1051.
44. Rohl CA, Boeckman FA, Baker C, Scheuer T, Catterall WA, Klevit RE. Solution structure of the sodium channel inactivation gate. *Biochemistry*. 1999;38: 855–861.
45. Long SB, Campbell EB, Mackinnon R. Voltage sensor of Kv1.2: structural basis of electromechanical coupling. *Science*. 2005;309:903–908.
46. Long SB, Campbell EB, Mackinnon R. Crystal structure of a mammalian voltage-dependent Shaker family K^+ channel. *Science*. 2005;309:897–903.
47. Isom LL, Ragsdale DS, De Jongh KS, Westenbroek RE, Reber BFX, Scheuer T, Catterall WA. Structure and function of the beta-2 subunit of brain sodium channels, a transmembrane glycoprotein with a CAM-motif. *Cell*. 1995;83:433–442.
48. Isom LL, Catterall WA. Na^+ channel subunits and Ig domains. *Nature*. 1996;383:307–308.
49. Beckh S, Noda M, Lübbert H, Numa S. Differential regulation of three sodium channel messenger RNAs in the rat central nervous system during development. *EMBO J*. 1989;8:3611–3616.
50. Gordon D, Merrick D, Auld V, Dunn R, Goldin AL, Davidson N, Catterall WA. Tissue-specific expression of the RI and RII sodium channel subtypes. *Proc Natl Acad Sci USA*. 1987;84:8682–8686.

51. Vacher H, Mohapatra DP, Trimmer JS. Localization and targeting of voltage-dependent ion channels in mammalian central neurons. *Physiol Rev*. 2008;88:1407–1447.
52. Lai HC, Jan LY. The distribution and targeting of neuronal voltage-gated ion channels. *Nat Rev Neurosci*. 2006;7:548–562.
53. Catterall WA. Localization of sodium channels in cultured neural cells. *J Neurosci*. 1981;1:777–783.
54. Hu W, Tian C, Li T, Yang M, Hou H, Shu Y. Distinct contributions of Na(v)1.6 and Na(v)1.2 in action potential initiation and backpropagation. *Nat Neurosci*. 2009;12:996–1002.
55. Kaplan MR, Cho MH, Ullian EM, Isom LL, Levinson SR, Barres BA. Differential control of clustering of the sodium channels Na(v)1.2 and Na(v)1.6 at developing CNS nodes of Ranvier. *Neuron*. 2001;30: 105–119.
56. Boiko T, Rasband MN, Levinson SR, Caldwell JH, Mandel G, Trimmer JS, Matthews G. Compact myelin dictates the differential targeting of two sodium channel isoforms in the same axon. *Neuron*. 2001;30:91–104.
57. Westenbroek RE, Merrick DK, Catterall WA. Differential subcellular localization of the RI and RII Na^+ channel subtypes in central neurons. *Neuron*. 1989;3:695–704.
58. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci*. 2006;9:1142–1149.
59. Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, Takeuchi T, Itohara S, Yanagawa Y, Obata K, Furuichi T, Hensch TK, Yamakawa K. Na(v)1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an *Scn1a* gene mutation. *J Neurosci*. 2007;27:5903–5914.
60. Westenbroek RE, Noebels JL, Catterall WA. Elevated expression of type II Na^+ channels in hypomyelinated axons of shiverer mouse brain. *J Neurosci*. 1992;12:2259–2267.
61. Kalume F, Yu FH, Westenbroek RE, Scheuer T, Catterall WA. Reduced sodium current in Purkinje neurons from Nav1.1 mutant mice: implications for ataxia in severe myoclonic epilepsy in infancy. *J Neurosci*. 2007;27:11065–11074.
62. Lorincz A, Nusser Z. Specificity of immunoreactions: the importance of testing specificity in each method. *J Neurosci*. 2008;28:9083–9086.
63. Crill WE. Persistent sodium current in mammalian central neurons. *Annu Rev Physiol*. 1996;58:349–362.
64. Raman IM, Bean BP. Resurgent sodium current and action potential formation in dissociated cerebellar Purkinje neurons. *J Neurosci*. 1997;17:4517–4526.
65. Mantegazza M, Yu FH, Powell AJ, Clare JJ, Catterall WA, Scheuer T. Molecular determinants for modulation of persistent sodium current by G-protein betagamma subunits. *J Neurosci*. 2005;25:3341–3349.
66. Fleidervish IA, Friedman A, Gutnick MJ. Slow inactivation of Na^+ current and slow cumulative spike adaptation in mouse and guinea-pig neocortical neurones in slices. *J Physiol*. 1996;493(pt 1):83–97.
67. Mantegazza M, Franceschetti S, Avanzini G. Anemone toxin (ATX II)-induced increase in persistent sodium current: effects on the firing properties of rat neocortical pyramidal neurones. *J Physiol*. 1998;507(pt 1):105–116.
68. Stafstrom CE. Persistent sodium current and its role in epilepsy. *Epilepsy Curr*. 2007;7:15–22.
69. Grieco TM, Malhotra JD, Chen C, Isom LL, Raman IM. Open-channel block by the cytoplasmic tail of sodium channel beta4 as a mechanism for resurgent sodium current. *Neuron*. 2005;45:233–244.
70. Jarecki BW, Piekarz AD, Jackson JO 2nd, Cummins TR. Human voltage-gated sodium channel mutations that cause inherited neuronal and muscle channelopathies increase resurgent sodium currents. *J Clin Invest*. 2010;120:369–378.
71. Black JA, Liu S, Waxman SG. Sodium channel activity modulates multiple functions in microglia. *Glia*. 2009;57:1072–1081.
72. Sontheimer H, Black JA, Waxman SG. Voltage-gated Na^+ channels in glia: properties and possible functions. *Trends Neurosci*. 1996;19:325–331.
73. Karadottir R, Hamilton NB, Bakiri Y, Attwell D. Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. *Nat Neurosci*. 2008;11:450–456.
74. Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. *Nature*. 1996;379:257–262.
75. Akopian AN, Souslova V, England S, Okuse K, Ogata N, Ure J, Smith A, Kerr BJ, McMahon SB, Boyce S, Hill R, Stanfa LC, Dickenson AH, Wood JN. The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. *Nat Neurosci*. 1999;2:541–548.
76. Dib-Hajj SD, Cummins TR, Black JA, Waxman SG. From genes to pain: Na v 1.7 and human pain disorders. *Trends Neurosci*. 2007;30:555–563.
77. Black JA, Dib-Hajj S, McNabola K, Jeste S, Rizzo MA, Kocsis JD, Waxman SG. Spinal sensory neurons express multiple sodium channel α-subunit mRNAs. *Mol Brain Res*. 1996;43:117–131.
78. Catterall WA. Common modes of drug action on Na^+ channels: local anesthetics, antiarrhythmics and anticonvulsants. *Trends Pharmacol Sci*. 1987;8:57–65.
79. Mantegazza M, Curia G, Biagini G, Ragsdale DS, Avoli M. Voltage-gated sodium channels as therapeutic targets in epilepsy and other neurological disorders. *Lancet Neurol*. 2010;9:413–424.
80. Ragsdale DS, McPhee JC, Scheuer T, Catterall WA. Molecular determinants of state-dependent block of sodium channels by local anesthetics. *Science*. 1994;265:1724–1728.
81. Ragsdale DR, McPhee JC, Scheuer T, Catterall WA. Common molecular determinants of local anesthetic, antiarrhythmic, and anticonvulsant block of voltage-gated Na^+ channels. *Proc Natl Acad Sci USA*. 1996;93:9270–9275.
82. Hille B. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J Gen Physiol*. 1977;69:497–515.
83. Willow M, Gonoi T, Catterall WA. Voltage clamp analysis of the inhibitory actions of diphenylhydantoin and carbamazepine on voltage-sensitive sodium channels in neuroblastoma cells. *Mol Pharmacol*. 1985;27:549–558.
84. Catterall WA. Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Annu Rev Pharmacol Toxicol*. 1980;20:15–43.
85. Catterall WA, Cestele S, Yarov-Yarovoy V, Yu FH, Konoki K, Scheuer T. Voltage-gated ion channels and gating modifier toxins. *Toxicon*. 2007;49:124–141.

86. Bartolomei F, Gastaldi M, Massacrier A, Planells R, Nicolas S, Cau P. Changes in the mRNAs encoding subtypes I, II and III sodium channel alpha subunits following kainate-induced seizures in rat brain. *J Neurocytol*. 1997;26:667–678.
87. Aronica E, Yankaya B, Troost D, van Vliet EA, Lopes da Silva FH, Gorter JA. Induction of neonatal sodium channel II and III alpha-isoform mRNAs in neurons and microglia after status epilepticus in the rat hippocampus. *Eur J Neurosci*. 2001;13:1261–1266.
88. Klein JP, Khera DS, Nersesyan H, Kimchi EY, Waxman SG, Blumenfeld H. Dysregulation of sodium channel expression in cortical neurons in a rodent model of absence epilepsy. *Brain Res*. 2004;1000:102–109.
89. Blumenfeld H, Lampert A, Klein JP, Mission J, Chen MC, Rivera M, Dib-Hajj S, Brennan AR, Hains BC, Waxman SG. Role of hippocampal sodium channel Nav1.6 in kindling epileptogenesis. *Epilepsia*. 2009;50:44–55.
90. Lombardo AJ, Kuzniecky R, Powers RE, Brown GB. Altered brain sodium channel transcript levels in human epilepsy. *Mol Brain Res*. 1996;35:84–90.
91. Whitaker WR, Faull RL, Dragunow M, Mee EW, Emson PC, Clare JJ. Changes in the mRNAs encoding voltage-gated sodium channel types II and III in human epileptic hippocampus. *Neuroscience*. 2001;106:275–285.
92. Steinhauser C, Seifert G. Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. *Eur J Pharmacol*. 2002;447:227–237.
93. Agrawal N, Alonso A, Ragsdale DS. Increased persistent sodium currents in rat entorhinal cortex layer V neurons in a post–status epilepticus model of temporal lobe epilepsy. *Epilepsia*. 2003;44:1601–1604.
94. Vreugdenhil M, Hoogland G, van Veelen CW, Wadman WJ. Persistent sodium current in subicular neurons isolated from patients with temporal lobe epilepsy. *Eur J Neurosci*. 2004;19:2769–2778.
95. Kearney JA, Plummer NW, Smith MR, Kapur J, Cummins TR, Waxman SG, Goldin AL, Meisler MH. A gain-of-function mutation in the sodium channel gene *Scn2a* results in seizures and behavioral abnormalities. *Neuroscience*. 2001;102:307–317.
96. Venance SL, Cannon SC, Fialho D, Fontaine B, Hanna MG, Ptacek LJ, Tristani-Firouzi M, Tawil R, Griggs RC. The primary periodic paralyses: diagnosis, pathogenesis and treatment. *Brain*. 2006;129:8–17.
97. Sokolov S, Scheuer T, Catterall WA. Gating pore current in an inherited ion channelopathy. *Nature*. 2007;446:76–78.
98. Struyk AF, Cannon SC. A Na^+ channel mutation linked to hypokalemic periodic paralysis exposes a proton-selective gating pore. *J Gen Physiol*. 2007;130:11–20.
99. Clancy CE, Kass RS. Defective cardiac ion channels: from mutations to clinical syndromes. *J Clin Invest*. 2002;110:1075–1077.
100. Catterall WA, Kalume F, Oakley JC. NaV1.1 channels and epilepsy. *J Physiol*. 2010;588:1849–1859.
101. Mantegazza M, Rusconi R, Scalmani P, Avanzini G, Franceschetti S. Epileptogenic ion channel mutations: from bedside to bench and, hopefully, back again. *Epilepsy Res*. 2010;92:1–29.
102. Meisler MH, O'Brien JE, Sharkey LM. Sodium channel gene family: epilepsy mutations, gene interactions and modifier effects. *J Physiol*. 2010;588:1841–1848.
103. Rusconi R, Combi R, Cestele S, Grioni D, Franceschetti S, Dalpra L, Mantegazza M. A rescuable folding defective Nav1.1 (SCN1A) sodium channel mutant causes GEFS+: common mechanism in Nav1.1 related epilepsies? *Hum Mutat*. 2009;30:E747–E760.
104. Rusconi R, Scalmani P, Cassulini RR, Giunti G, Gambardella A, Franceschetti S, Annesi G, Wanke E, Mantegazza M. Modulatory proteins can rescue a trafficking defective epileptogenic Nav1.1 Na^+ channel mutant. *J Neurosci*. 2007;27:11037–11046.
105. Cestele S, Scalmani P, Rusconi R, Terragni B, Franceschetti S, Mantegazza M. Self-limited hyperexcitability: functional effect of a familial hemiplegic migraine mutation of the Nav1.1 (SCN1A) Na^+ channel. *J Neurosci*. 2008;28:7273–7283.
106. Heron SE, Crossland KM, Andermann E, Phillips HA, Hall AJ, Bleasel A, Shevell M, Mercho S, Seni MH, Guiot MC, Mulley JC, Berkovic SF, Scheffer IE. Sodium-channel defects in benign familial neonatal-infantile seizures. *Lancet*. 2002;360:851–852.
107. Scalmani P, Rusconi R, Armatura E, Zara F, Avanzini G, Franceschetti S, Mantegazza M. Effects in neocortical neurons of mutations of the Na(v)1.2 Na^+ channel causing benign familial neonatal-infantile seizures. *J Neurosci*. 2006;26:10100–10109.
108. Vahedi K, Depienne C, Le Fort D, Riant F, Chaine P, Trouillard O, Gaudric A, Morris MA, Leguern E, Tournier-Lasserve E, Bousser MG. Elicited repetitive daily blindness: a new phenotype associated with hemiplegic migraine and SCN1A mutations. *Neurology*. 2009;72:1178–1183.
109. Marini C, Mantegazza M. Sodium channelopathies and epilepsy: recent advances and novel perspectives. *Expert Rev Clin Pharmacol*. 2010;3:371–384.
110. Riva D, Vago C, Pantaleoni C, Bulgheroni S, Mantegazza M, Franceschetti S. Progressive neurocognitive decline in two children with Dravet syndrome, de novo SCN1A truncations and different epileptic phenotypes. *Am J Med Genet A*. 2009;149A:2339–2345.
111. Landmark C. Antiepileptic drugs in non-epilepsy disorders: relations between mechanisms of action and clinical efficacy. *CNS Drugs*. 2008;22:27–47.
112. Kovacs DM, Gersbacher MT, Kim DY. Alzheimer's secretases regulate voltage-gated sodium channels. *Neurosci Lett*. 2010;486:68–72.

Chapter 5

Potassium Channels (Including KCNQ) and Epilepsy

Edward C. Cooper

The brain runs on electricity. Because K^+ channels contribute to nearly all aspects of cellular electrical signaling, they were already prime suspects when the search for molecular causes of epilepsy began a quarter of a century ago. Slow initially, efforts to identify K^+ channel genes grew into a flood of clones. At the latest count, the human genome includes about 100 K^+ channel subunits;[1,2] most are expressed in brain. Some of the cloned brain K^+ channels have been the subjects of intense multidisciplinary studies combining structural biology, genetics, electrophysiology, biochemistry, and imaging techniques. Those efforts have yielded many long-sought answers. For example, mechanisms of ion permeation, selectivity, voltage gating, and modulation by posttranslational modifications, drugs, and neurotransmitter receptors have been elucidated with spectacular detail.[3,4] We have learned how specific signals and interactions cause individual channel types to be distributed in unique patterns within neural circuits and how that localization underlies distinctive contributions to excitability.[5,6] Currently, the pace of progress in knowledge and technical power shows no signs of diminishing; instead, it seems to be increasing exponentially.

However, the scope and expectations of epilepsy research have changed during this same time. Demonstrable improvement in seizure control has been replaced as a measure of success by the goals of "no seizures, no side effects," prevention of epilepsy per se, and amelioration of cognitive, motor, and emotional disabilities that limit opportunities for many patients with recurrent seizures.[7] Fundamentally new approaches have emerged, for example through exploitation of stem cells, miniaturized prosthetic electronic stimulators, or diet, which aim to complement or supplant improved molecular pharmacology as a strategy

in epilepsy therapy. Our understanding of epilepsy has also deepened dramatically through clinical and genetic scholarship. The diversity of "the epilepsies" is now much more broadly appreciated. So, assessment of research in channels (including K^+ channels) is now viewed in a context that is at once more rigorous, competitive, and demanding than a decade ago.

This chapter aims to provide a perspective on the achievements and future potential of studies of potassium channels in epilepsy research. The thesis is that the understanding obtained from recent studies justifies continued investment in K^+ channels by academic and industry-based researchers. We first reintroduce the K^+ channels, highlighting progress that appears particularly relevant to epilepsy. Then, as an example, we provide a detailed account of one K^+ channel subfamily, the KCNQ/Kv7 family. Members of this family are mutated in benign familial neonatal seizures, an autosomal dominant syndrome. Moreover, these channels have been found to be practical targets for antiepileptic drug development, perhaps providing a model for studies of other K^+ channel subgroups. We conclude with a discussion of directions for future work.

K^+ CHANNELS: A DIVERSE SUPERFAMILY DERIVED FROM AN EXTRAORDINARILY USEFUL TEMPLATE

Considered together, the recent cloning of the entire genomes from many species of eukaryotes, and of many additional K^+ channels from prokaryotes, now provides a previously unseen view of how the great diversity apparent in the human K^+ channel set emerged. Remarkably, all human K^+ channel pore-forming subunits are derived by gene duplication from a single, likely prokaryotic, ancestor gene. What is gene duplication?[8,9] It is now understood that, in addition to the more familiar deletion, insertion, missense, and nonsense mutations that can occur DNA, duplications of entire genes can occur through errors during replication. The frequency of such gene duplications is low but occurs continuously, at a rate on the order of once per 100 million years for every gene.[9] Given the billion-year history of animals and this exponential process, why aren't the genomes and gene families of living animals larger than they are? Because, in most instances, gene duplications are deleterious or of no advantage. In such unfavorable duplications, random mutations gradually inactivate one of the duplicate genes. Many so-called pseudogenes are the relics of such unselected gene duplicates. Eventually, the pseudogenic region accumulates enough mutations to become undetectable—lost in the genomic background. However, in cases where two duplicated genes can evolve distinct functions—for example, through mutations that cause different patterns of expression or divergence in functions—both may survive. The K^+ channels are an example (kinases and G-protein coupled receptors are others) in which an ancient gene provided an exceptionally useful template for variation. In fact, for several generations, K^+ channel offspring themselves produced successfully divergent duplicates, resulting in exponential growth in the size of the family. Through this process, the current superfamily of human K^+ channel pore subunit genes was derived. Along the way, other mutations took place as well, resulting in channels that lost their permeability to K^+ and/or gained permeability to other ions. Thus, the voltage-gated calcium and sodium, transient receptor potential, and hyperpolarization-activated cyclic adenosine monophosphate (cAMP)–gated nonselective (HCN) channels, and ionotropic glutamate receptors all arose from ancient K^+ channels through gene duplication and divergence.

I think this genomics-based, top-down view is exceptionally important as we attempt to discern how alterations in excitability result in epilepsy. This view highlights how individual channel subtypes expressed in human brain are survivors of a lengthy, rigorous, evolutionary winnowing. A channel gene's survival per se requires both that it has unique function(s) and that it functions in the context of other surviving channels. Although we have made progress in recent years in understanding individual channel functions through in vitro and in vivo mutagenesis studies, including the study of *channelopathies* in both human and model animals,[10,11] we have barely begun to explore how groups of channels, ancient companions in the genomes of animals, have evolved in parallel to jointly carry out novel functions through the combination of their activities.

K^+ Channel Variant 1: Two-Transmembrane (2TM)

The most basic (and ancestral) functional and genetic component of a K^+ channel is the pore itself. The ion-conducting pores of all K^+ channels are built similarly, namely, by four polypeptide domains arranged symmetrically around a central water-filled transmembrane channel. Each of the four pore-forming domains consists of intracellular N and C termini, two transmembrane segments, and an extracellular loop that dips partially back into the pore to line its narrowest part, forming the K^+-selective filter (Fig. 5–1A; see also Fig. 5–2D). All K^+ channels are variants or elaborations of this common template.

Humans possess 15 genes for K^+ channel subunits that adhere to this simplest two- transmembrane (2TM) topology. In spite of this relative simplicity, these channels are important and quite diverse in their functions, patterns of expression, and modes of regulation. Some of these channels exhibit *inward rectification*, a property that means that they are closed by membrane depolarization. Strongly inward-rectifying channels close completely when the membrane is depolarized near the action potential threshold but open at the resting or hyperpolarized potentials. This allows them to function like a magnetic catch on a cabinet door, that is, they hold the membrane hyperpolarized when it is at or near rest, but when Na^+ and Ca^{2+} channels cause a sufficiently strong depolarization, they become inactive.

One group of channels, with weak inward rectification, are regulated by metabotropic G-protein coupled receptors, such as the $GABA_B$, and (subsets of the) dopamine and acetylcholine receptors. In the absence of ligand, these receptors bind G-protein trimers intracellularly. When ligand activates the receptor, the linked βγ G-protein subunits are released, translocate to the nearby GIRK (g-protein coupled, inwardly rectifying K^+) channel, and bind, causing the channel to open. K^+ channels in glia, including inward rectifiers, allow transient elevations in extracellular K^+ resulting from high levels of neuronal firing to be rapidly buffered. Human and rodent data implicate mutations in KCNJ10, a 2TM channel subunit expressed most prominently in glia, in epilepsy and seizure susceptibility, highlighting that channels may cause seizures through multiple mechanisms, even non-neuronal ones.[12]

Structural Variant 2: Two-Pore (4TM)

Humans possess 15 genes for K^+ channels of the two-pore, or 4TM type. These channels are derived from an ancestor in which a 2TM channel gene underwent a tandem duplication (Fig. 5–1B). Channels are formed by two polypeptides, each containing a pair of pore domains. These channels function prominently in setting the resting membrane potential and often underlie the majority of the resting membrane K^+ conductance. Mutations in these genes have not yet been found in human epilepsy syndromes, and limited pharmacological tools are available. Nonetheless, the effects of inhaled general anesthetics depend in part on these channels, and some members of this group are regulated by metabotropic neurotransmitters, highlighting their roles in plasticity and their potential as drug targets.

Structural Variant 3: Voltage-Gated (6TM)

The largest subgroup of the K^+ channels are formed by subunits in which the gating of the 2TM pore is controlled by a linked voltage sensor domain (Fig. 5–1C; see also Fig. 5–2). Genetically, the voltage sensor is inserted upstream of the pore-encoding region, so the resulting subunit polypeptide includes the 4TM segments of the voltage sensor on the N-terminal side. Critically, a short, very highly conserved length of peptide links the voltage sensor to the pore on the intracellular side of the membrane, allowing movement of the voltage sensor to pull open the pore upon depolarization and push it closed at rest (Fig. 5–2).

Voltage-sensor domains arose very early in evolution and are present in some prokaryotic Na^+ and K^+ channels. It is not known whether the voltage sensor evolved by incremental mutation of a preexisting channel or as a separate protein that then was fused upstream of a channel gene in a random recombination event. Support for the second alternative came from the discovery of a 4TM voltage sensor protein that functions as a proton channel, without an

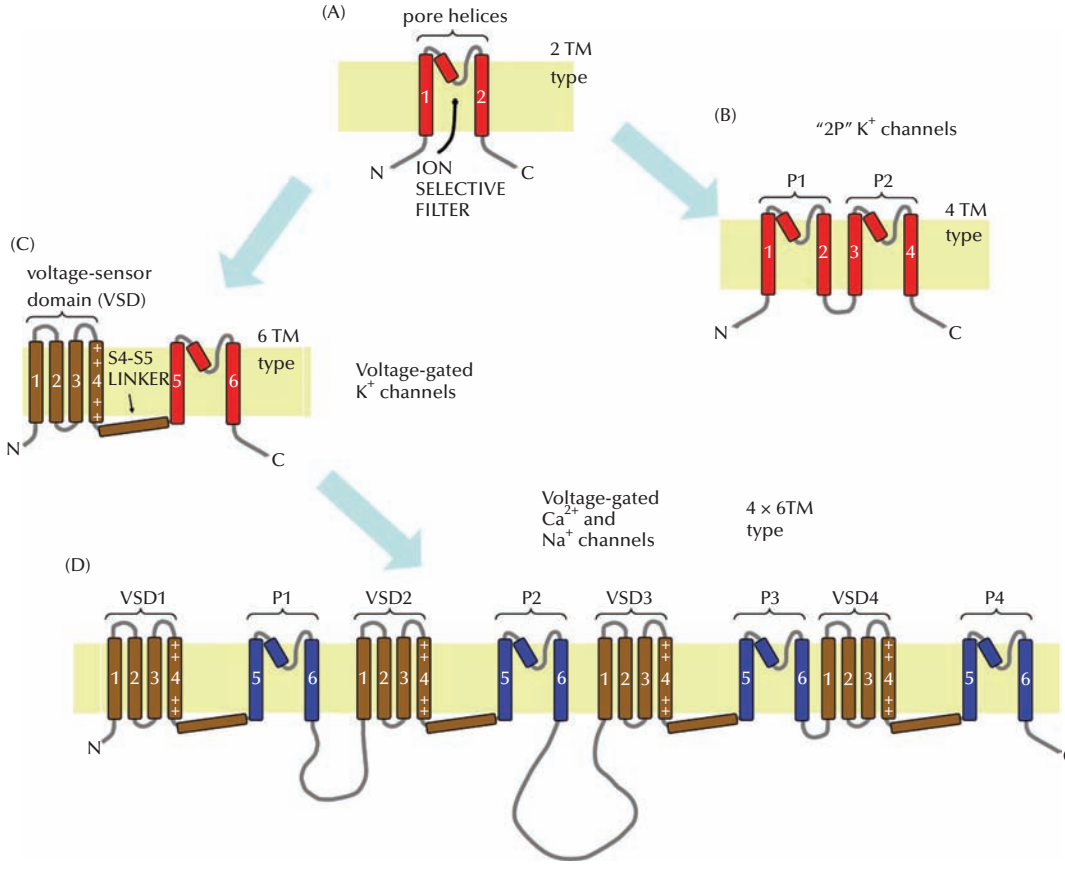

Figure 5–1. Structural features and evolutionary relationships among major groups of K^+, Na^+, and Ca^{2+} channels. **A.** Structure of the simplest ancestral K^+ channel, consisting of 2TM (transmembrane segments), a pore loop, and intracellular N and C terminals. This structure is found in a large group of human 2TM channels, including inward rectifier and G-protein coupled families. **B.** Structure of the 4TM group, produced by tandem duplication and fusion of two 2TM channels. This structure is preserved in mammalian leak channels. **C.** Structure of the 6TM K^+ channels, produced by fusion of a 4TM voltage-sensor domain and a 2TM channel. The fourth transmembrane segment (S4) bears positively charged arginine residues and moves in response to changes in the membrane potential. Movement of S4 is communicated to the pore, resulting in voltage-sensitive opening and closing of the transmembrane ion path. **D.** The structure of human voltage-gated Na^+ and Ca^{2+} channels, produced by two duplications of a 6TM channel. In addition, the ion selectivity of the pore has been altered through mutations.

(*continued from page 59*)

in green (labeled "r"). K^+ molecules, and the direction of net ion flow is indicated in red. **C.** Ribbon diagram showing the crystal structure of a part of one subunit, as seen from within the pore. For clarity, S1 through S3 are not shown. Ions passing through the channel are shown in red. The S4-S5 linker is covalently associated with S5, and side chains (indicated by colored arrowheads) of the linker and S6 interact noncovalently. This allows outward movement of S4 in response to membrane depolarization to pull S6 outward and upward, opening the channel gate (drawing generated using Swiss PDB Viewer, using coordinates of Kv1.2[62]). The position corresponding to the tryptophan residue, critical for activation of KCNQ channels by retigabine, is shown (green side chain on S5). **D.** Side view of the assembled pore domain. In the open state (left), S6 helices (blue) appear bent and the S4-S5 linker tips toward the extracellular side of the membrane. In the closed state (right), S6 helices are straight and the linker tips downward toward the cytoplasm. A stick model of the retigabine molecule is shown (within the black box), approximately to scale. As in C, the positions of the tryptophan residues required for retigabine's effects on KCNQ channels are indicated (green). An added gray (dashed line) box delimits a region on the outside surface of the pore domain adjoining the retigabine-critical residue. Modified from ref. 63.

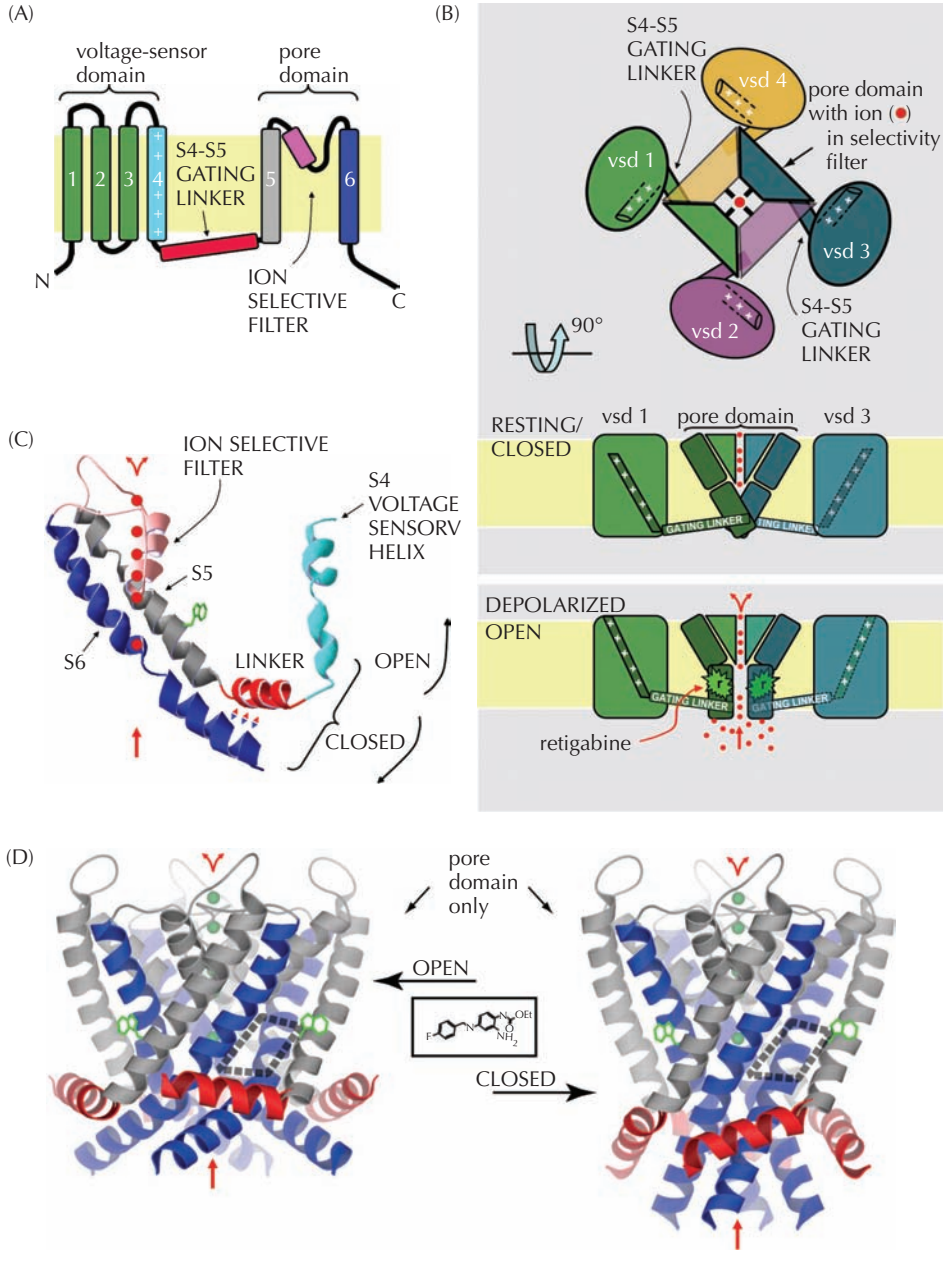

Figure 5–2. Mapping the site of retigabine binding on the structure of a voltage-gated K^+ channel. A. Cartoon showing the membrane folding pattern (topology) and functional domains of a voltage-gated K^+ channel subunit. The first four transmembrane segments constitute the voltage- sensor domain, with the S4 segment carrying a series of positively charged arginine residues (+ signs). The S5 and S6 segments, along with the loop that connects them, form a part of the pore. The two domains are connected by an intracellular helical linker, the S4-S5 linker. Amino and carboxy terminal regions are intracellular. For S4-S6, the color scheme matches that of panel C. **B.** Cartoons showing four voltage-gated K^+ channel subunits assembled into a functional tetrameric channel as viewed from outside the cell (upper) and cutaway sections through the center of the channel showing movements associated with open and closed states (lower). The subunits are color coded, so that the distance between each subunit's pore-forming and voltage-sensor helices, and the critical role of the S4-S5 gating linker, are clear. The positions of the positive-charged S4 helix within each voltage-sensor domain (vsd) are indicated. In the lower cartoon, channel opening results when the S4 helices move outward, pulling the linker up and causing the inner portions of the pore to pull apart. The proposed location of retigabine interaction with KCNQ2–5 subunits is indicated

(*continues on page 58*)

associated K^+ channel-like pore.[13] Regardless of its mechanism of origin, the 6TM voltage-gated template has exhibited extraordinary evolutionary success.[1,2] Humans possess 34 6TM voltage-gated K^+ channel subunit genes. In addition, genes for the pores of Ca^{2+} gated K^+ channels (8 in humans), HCN channels (4), cyclic-nucleotide gated channels (6), transient receptor potential channels (TRPs, 29), voltage-gated Na^+ channels (10), and voltage-gated Ca^{2+} channels (10) are all derived from this template.

The diversity in the voltage-gated K^+ channels includes differences in several separate categories of features. Among these are the midpoint voltage and steepness of voltage-dependent opening and closing; the rates at which transitions from resting to open and open to inactivated states occur; the ability to interact with particular scaffolding proteins that control subcellular localization; the ability to be modulated by receptors, phosphorylation, and intracellular messengers; and the ability to bind accessory subunits.

Structural Variant 4: Non-Pore-Forming Accessory Subunits

In addition to the pore-forming subunits, some K^+ channels include accessory subunits. The accessory subunits are a genetically and structurally diverse collection, all unrelated to the pore subunits and incapable of conducting K^+ ions independently. What these peptides have in common is that they have evolved the ability to bind specifically to particular subtypes of K^+ channel pores. Association is thought to be cotranslational, and the accessory subunits have functions that include modulation of voltage gating and subcellular targeting.

KCNQ CHANNELS: FROM CHANNELOPATHY TO NOVEL THERAPY

The fundamental goal of epilepsy research involving K^+ channels is the development of novel therapies, preventions, and cures. The most complete example of this process so far is research concerning the KCNQ/Kv7 family of voltage-gated K^+ channels and their genes. In 1964, Andreas Rett, memorialized as the discoverer of Rett's syndrome, published the first description of an autosomal dominant epilepsy syndrome later called *benign familial neonatal convulsions* (BFNCs).[14] In this syndrome, onset of partial or generalized clonic convulsions occurs at around 3 days of age; these attacks remit within about 3 months. Although affected infants have been thought to grow and develop normally, a significant proportion (16%) experience additional isolated or recurrent seizures as children or adults.[15] Benign familial neonatal convulsions was the first idiopathic epilepsy for which a genetic locus was identified.[16] Two loci were ultimately associated with the syndrome, with most pedigrees linked to chromosome 20q13.3 and a few linked to 8q24.[17,18]

While these clinicogenetic studies were progressing, completely independent work by cellular electrophysiologists revealed the existence of two unusual voltage-gated K^+ currents. Brown and Adams found in sympathetic neurons a noninactivating voltage-dependent K^+ current with much slower opening and closing kinetics than the classical action potential *delayed rectifier* current.[19] They called this the *M-current*, because it could be transiently suppressed by application of muscarinic cholinergic agonists. Such muscarinic suppression caused membrane depolarization and increased responsiveness during subsequent excitatory synaptic stimulation. M-current was later found in many central neurons.[20] Soon after the first studies of M-current appeared, voltage-clamp recording of peripheral nerves of frogs revealed the presence of a similar K^+ current in myelinated axons.[21] Subsequent work showed that this axonal K^+ current, termed I_{Ks} (slow K^+ current) was concentrated at the nodes of Ranvier.[22] In mammalian myelinated nerves, I_{Ks} is particularly important, because mammalian myelinated axons have a very low density of fast K^+ channels at their nodes.[23] Although the similarities between I_M and I_{Ks} were noted early on,[24] subsequent cloning efforts failed to reveal the genes underlying either current.

In 1998, two novel closely related related K^+ channel subunit genes, *KCNQ2* and *KCNQ3*, were identified at the two BFNC loci. *KCNQ2* and *KCNQ3* were shown to be expressed exclusively by neurons and to be mutated in patients with BFNC.[25–27] Soon after the genes were cloned, study of the kinetics, pharmacology, and expression patterns of heteromeric

KCNQ2/KCNQ3 channels showed that they represented the basis of the M-current in sympathetic[28] and hippocampal neurons. An additional insight was gained when a family was described in which patients with BFNC due to a mutation in *KCNQ2* also suffered from myokymia, a symptom indicative of hyperexcitability in myelinated motor axons.[29] Immunostaining of brain sections and peripheral nerves showed that KCNQ2 was universally present at nodes of Ranvier, and KCNQ3 was also present at a fraction of nodes.[30–32] Indeed, a detailed physiological study using KCNQ-selective blockers and openers showed that, in large myelinated nerves from rat sciatic nerve that stain for KCNQ2 but not KCNQ3, homomeric KCNQ2 channels mediate the slow nodal K^+ current.[31]

Heteromeric KCNQ2/KCNQ3 channels were also detected widely in the central nervous system (CNS) at the axonal initial segments, which are believed to be the site of action potential initiation in most neurons.[30] Although the KCNQ channels found at the axonal initial segments remain to be recorded directly, a growing body of indirect physiological evidence indicates that these channels contribute to control of action potential initiation and firing frequency in the CNS.[33–35] It is also possible that the BFNC phenotype results partly from a loss of channel activity in somatodendritic domains where M-channels have also been recorded in some cell types.[35,36]

Two additional closely related KCNQ channel subunits have also been identified (KCNQ4 and KCNQ5).[37] *KCNQ4* expression is apparently somewhat restricted, including auditory hair cells and neurons in the central auditory pathways (mutations in KCNQ4 give rise to a dominant progressive hearing loss syndrome). KCNQ5 is widely expressed, but its neuronal function is just beginning to be explored.[38] In vitro, KCNQ2–5 subunits can form functional homotetrameric channels. In addition, a variety of heteromeric combinations—including KCNQ2/3, KCNQ3/4, or KCNQ3/5—can assemble functionally. The subunit combinations found in the CNS are only beginning to be explored, although evidence for both KCNQ2 monomers and KCNQ2/3 heteromers has been found.[32,39–41]

The KCNQ family includes one more distantly related gene, *KCNQ1*. Owing to its greater divergence, KCNQ1 cannot form heteromers with KCNQ2–5.[42] Unlike KCNQ2–5, however, KCNQ1 does heteromerize with accessory subunits of the KCNE family. KCNQ1 was first found in the heart, in gastrointestinal tract epithelial cells, and the cochlea. KCNQ1/KCNE1 heteromeric channels underlie an important current in cardiomyocytes that activates very slowly to end the Ca^{2+}-mediated plateau phase of the action potential, promotes ventricular relaxation, and prevents aberrant re-excitation.[43] Mutations in *KCNQ1* are associated with abnormal ventricular repolarization, causing the long-QT syndrome of arrhythmia and sudden death.[44] Recessive mutations in either *KCNQ1* or *KCNE1* cause a related syndrome combining this arrhythmia with congenital deafness.[45] Recently, Goldman et al. used electroencephalography (EEG) to study mutant mice bearing *KCNQ1* mutations known to cause human long-QT syndrome.[46] Remarkably, in addition to cardiac abnormalities, these mice exhibited frequent seizures and, occasionally, death in association with a seizure. Further, biochemical and immunohistochemical experiments provided evidence of previously overlooked *KCNQ1* expression in brain. The implications of these findings are broad, and more studies of *KCNQ1* function in the CNS are merited.

In contrast to the *KCNQ1* example, where murine studies revealed a broader phenotype than was previously appreciated in humans, introduction of *KCNQ2* and *KCNQ3* mutations associated with BFNC has resulted so far in mice without seizures or any described behavioral changes beyond somewhat increased sensitivity to seizure provocation by electrical or chemical treatments.[47–49] Because the human phenotype is so developmentally restricted, it is apparently difficult to recapitulate it in a species as distant as the mouse. At the cellular level, however, functional consequences of *KCNQ2* and *KCNQ3* mutations responsible for BFNC have been studied by expression of mutant channels in cell lines and neurons. Intriguingly, some mutations caused very slight reductions (20%–30%) in channel activity when expressed in *Xenopus* oocytes, which has been interpreted as suggesting that very modest reductions in the M-current might be sufficient to augment or cause seizures.[37] Recent studies show, however, that some mutations dramatically disrupt the trafficking of channels to axons, and this may be the main factor contributing to the disease phenotype.[50] Although the developmental factors

leading to the seizure remission at around 3 months are not understood, delayed postnatal maturation of GABAergic inhibition is a potential contributing factor, rendering neonatal brain especially dependent on K^+ channel activity to prevent excessive excitation.[51]

In parallel with the early studies of BFNC and the M-channel, retigabine emerged as a candidate antiepileptic drug (AED) after successful results in the National Institute of Neurological Diseases and Stroke (NINDS) antiepileptic drug screening program.[52,53] Retigabine was derived from flupirtine, a compound approved for use as an analgesic in Europe but not used in the United States. Retigabine was found to possess the ability to open K^+ channels in neurons.[54,55] Once *KCNQ2* and *KCNQ3* were cloned, independent efforts by several groups rapidly showed that they were molecular target of retigabine.[56–58] Retigabine shifts the voltage dependence of the channel to hyperpolarized potentials, speeds the rate of activation in response to membrane depolarization, and slows the rate of closing (deactivation). Testing retigabine more broadly with combinations of expressed subunits revealed that the degree of potentiation varied, depending on channel composition, in the order (most potent) KCNQ3 > KCNQ2/KCNQ3 > KCNQ2 > KCNQ4, KCNQ5 (least). The KCNQ1 subunit is retigabine insensitive.[59] This observation is clinically significant, since this eliminates one potential source of cardiac toxicity. Swapping domains between the retigabine-sensitive KCNQ2 or KCNQ3 subunits and retigabine-insensitive KCNQ1 made it possible to identify the pore domain as the site of retigabine action.[60,61] Mutagenesis of individual pore residues showed that the action of retigabine requires the presence of a key tryptophan (located near the cytoplasmic end of the fifth transmembrane segment, or S5) that is conserved in KCNQ2–5 but absent in KCNQ1.

To better appreciate the significance of this tryptophan, I aligned the KCNQ2 and Kv1.2 amino acid sequences, and then mapped the location of the crucial residue on the published three-dimensional coordinates of the Kv1.2 crystal structure[62,63] using the Swiss PDBviewer software.[64] This revealed that the residue is located near the components of the channel protein involved in the coupling of movement of the voltage sensor to opening of the gate (Fig. 5–2B–D). The critical tryptophan forms one side of a pocket that also include surfaces of S6, the S4–5 linker, and the S5 and S6 of the adjoining subunit (indicated in dashed grey boxed region in Fig. 5–2D; the molecular structure of retigabine is also indicated at equal scale). Clearly, more than the indicated tryptophan is important, since, for example, both KCNQ3 and KCNQ5 possess the residue but differ more than 10-fold in their sensitivity to retigabine. High-throughput screening has revealed additional chemical classes of KCNQ channel openers that are also potential antiepileptic drugs, including various benzanilides and acrylamides.[65] Their activity in preclinical models of epilepsy provides further proof of the concept of KCNQ2–5 as AED targets. An additional compound (Maxipost, BMS-204352) has been shown to open both KCNQ channels and high-conductance Ca^{2+}-activated K^+ channels.[66,67] This drug blocks ischemic brain damage in rodent models, but a large stage III trial for stroke failed due to lack of efficacy, though subsequent analysis suggests that the trial dosing may have been insufficient.

Retigabine has recently been approved for use in parts of Europe, and by the Food and Drug Administration (FDA) in the USA. Because clinical drug development is slow and expensive, retigabine was studied in American and European clinical drug trials that were designed and set in motion before molecular studies had revealed much about its mechanism of action.[68] A number of recent preclinical studies have attempted to build on molecular findings in order to both enhance the utility of retigabine and aid the rational design of next-generation KCNQ channel openers. One rat study showed that retigabine was particularly effective in suppressing seizures in immature animals, suggesting that it may be useful in pediatric cases.[69] Another suggested that this class of drugs may be potently effective in symptomatic neonatal seizures and status epilepticus.[70]

KCNQ CHANNELS AND BEYOND: PROSPECTS FOR FUTURE POTASSIUM CHANNEL STUDIES IN EPILEPSY RESEARCH

Progress in exploiting the KCNQ2–5 channels as anticonvulsant drugs was hastened, very fortuitously, by the coincidence of independent

efforts to identify the genes, identify the channels, and exploit already discovered channel ligands. However, general and encouraging implications about the prospects for developing K^+ channels further as targets can be gleaned from the KCNQ outcomes so far. Exploitation of the growing number of available K^+ channel crystal structures should facilitate follow-up mutagenesis studies aimed at further defining the mechanisms through which retigabine and other compounds open neuronal KCNQ channels. Crystallization of a KCNQ channel (perhaps in the presence of drug) would also be of enormous utility. Detailed understanding of this drug-channel interaction may serve as a path for rational design of more selective KCNQ activators, and ultimately of drugs able to activate other K^+ channel types. Future basic studies will seek to understand how channels interact to generate neuronal excitability at the level of the subcellular domain and circuit.

DISCLOSURE STATEMENT

The author has no conflicts of interest to disclose.

REFERENCES

1. Yu FH, Catterall WA. The VGL-chanome: a protein superfamily specialized for electrical signaling and ionic homeostasis. *Sci STKE*. 2004;2004:re15.
2. Jegla TJ, Zmasek CM, Batalov S, Nayak SK. Evolution of the human ion channel set. *Comb Chem High Throughput Screen*. 2009;12:2–23.
3. Bezanilla F. Ion channels: from conductance to structure. *Neuron*. 2008;60:456–468.
4. Delmas P, Brown DA. Pathways modulating neural KCNQ/M (Kv7) potassium channels. *Nat Rev Neurosci*. 2005;6:850–862.
5. Lai HC, Jan LY. The distribution and targeting of neuronal voltage-gated ion channels. *Nat Rev Neurosci*. 2006;7:548–562.
6. Vacher H, Mohapatra DP, Trimmer JS. Localization and targeting of voltage-dependent ion channels in mammalian central neurons. *Physiol Rev*. 2008;88:1407–1447.
7. Kelley MS, Jacobs MP, Lowenstein DH. The NINDS epilepsy research benchmarks. *Epilepsia*. 2009;50:579–582.
8. Zhang J. Evolution by gene duplication: an update. *Trends Evolution Ecology*. 2003;18:292–298.
9. Lynch M, Conery JS. The evolutionary fate and consequences of duplicate genes. *Science*. 2000;290:1151–1155.
10. Ptacek L. The familial periodic paralyses and nondystrophic myotonias. *Am J Med*. 1998;105: 58–70.
11. Noebels JL. The biology of epilepsy genes. Annu Rev Neurosci 2003;26:599–625.
12. Inyushin M, Kucheryavykh LY, Kucheryavykh YV, Nichols CG, Buono RJ, Ferraro TN, Skatchkov SN, Eaton MJ. Potassium channel activity and glutamate uptake are impaired in astrocytes of seizure-susceptible DBA/2 mice. *Epilepsia*. 2010;51:1707–1713.
13. Sasaki M, Takagi M, Okamura Y. A voltage sensor-domain protein is a voltage-gated proton channel. *Science*. 2006;312:589–592.
14. Zimprich F, Ronen GM, Stogmann W, Baumgartner C, Stogmann E, Rett B, Pappas C, Leppert M, Singh N, Anderson VE. Andreas Rett and benign familial neonatal convulsions revisited. *Neurology*. 2006;67:864–866.
15. Ronen GM, Rosales TO, Connolly M, Anderson VE, Leppert M. Seizure characteristics in chromosome 20 benign familial neonatal convulsions. *Neurology*. 1993;43:1355–1360.
16. Leppert M, Anderson VE, Quattlebaum T, Stauffer D, O'Connell P, Nakamura Y, Lalouel JM, White R. Benign familial neonatal convulsions linked to genetic markers on chromosome 20. *Nature*. 1989;337: 647–648.
17. Ryan SG, Wiznitzer M, Hollman C, Torres MC, Szekeresova M, Schneider S. Benign familial neonatal convulsions: evidence for clinical and genetic heterogeneity. *Ann Neurol*. 1991;29:469–473.
18. Steinlein O, Schuster V, Fischer C, Haussler M. Benign familial neonatal convulsions: confirmation of genetic heterogeneity and further evidence for a second locus on chromosome 8q. *Hum Genet*. 1995;95:411–415.
19. Brown DA, Adams PR. Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neuron. *Nature*. 1980;283:673–676.
20. Brown D. M-currents: an update. *Trends Neurosci*. 1988;11:294–299.
21. Dubois JM. Evidence for the existence of three types of potassium channels in the frog Ranvier node membrane. *J Physiol*. 1981;318:297–316.
22. Roeper J, Schwarz JR. Heterogeneous distribution of fast and slow potassium channels in myelinated rat nerve fibres. *J Physiol*. 1989;416:93–110.
23. Chiu SY, Ritchie JM, Rogart RB, Stagg D. A quantitative description of membrane currents in rabbit myelinated nerve. *J Physiol*. 1979;292:149–166.
24. Dubois JM. Potassium currents in the frog node of Ranvier. *Prog Biophys Mol Biol*. 1983;42:1–20.
25. Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, Steinlein OK. A potassium channel mutation in neonatal human epilepsy. *Science*. 1998;279:403–406.
26. Charlier C, Singh NA, Ryan SG, Lewis TB, Reus BE, Leach RJ, Leppert M. A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family [see comments]. *Nat Genet*. 1998;18:53–55.
27. Singh NA, Charlier C, Stauffer D, Dupont BR, Leach RJ, Melis R, Ronen GM, Bjerre I, Quattlebaum T, Murphy JV, Mcharg ML, Gagnon D, Rosales TO, Peiffer A, Anderson VE, Leppert M. A novel potassium channel gene, *KCNQ2*, is mutated in an inherited epilepsy of newborns. *Nat Genet*. 1998;18: 25–29.
28. Wang H-S, Pan Z, Shi W, Brown BS, Wymore RS, Cohen IS, Dixon JE, Mckinnon D. KCNQ2 and KCNQ3 potassium channel subunits: molecular

correlates of the M-channel. *Science*. 1998;282: 1890–1893.
29. Dedek K, Kunath B, Kananura C, Reuner U, Jentsch TJ, Steinlein OK. Myokymia and neonatal epilepsy caused by a mutation in the voltage sensor of the KCNQ2 K^+ channel. *Proc Natl Acad Sci USA*. 2001;98:12272–12277.
30. Pan Z, Kao T, Horvath Z, Lemos J, Sul JY, Cranstoun SD, Bennett V, Scherer SS, Cooper EC. A common ankyrin-G-based mechanism retains KCNQ and NaV channels at electrically active domains of the axon. *J Neurosci*. 2006;26:2599–2613.
31. Schwarz JR, Glassmeier G, Cooper EC, Kao TC, Nodera H, Tabuena D, Kaji R, Bostock H. KCNQ channels mediate IKs, a slow K^+ current regulating excitability in the rat node of Ranvier. *J Physiol*. 2006;573:17–34.
32. Devaux JJ, Kleopa KA, Cooper EC, Scherer SS. KCNQ2 is a nodal K^+ channel. *J Neurosci*. 2004;24:1236–1244.
33. Shah MM, Migliore M, Valencia I, Cooper EC, Brown DA. Functional significance of axonal Kv7 channels in hippocampal pyramidal neurons. *Proc Natl Acad Sci USA*. 2008;105:7869–7874.
34. Hu H, Vervaeke K, Storm JF. M-channels (Kv7/KCNQ channels) that regulate synaptic integration, excitability, and spike pattern of CA1 pyramidal cells are located in the perisomatic region. *J Neurosci*. 2007;27:1853–1867.
35. Yue C, Yaari Y. Axo-somatic and apical dendritic Kv7/M channels differentially regulate the intrinsic excitability of adult rat CA1 pyramidal cells. *J Neurophysiol*. 2006;95:3480–3495.
36. Chen X, Johnston D. Properties of single voltage-dependent K^+ channels in dendrites of CA1 pyramidal neurones of rat hippocampus. *J Physiol*. 2004;559:187–203.
37. Jentsch TJ. Neuronal KCNQ potassium channels: physiology and role in disease. *Nat Rev Neurosci*. 2000;1:21–30.
38. Tzingounis AV, Heidenreich M, Kharkovets T, Spitzmaul G, Jensen HS, Nicoll RA, Jentsch TJ. The KCNQ5 potassium channel mediates a component of the afterhyperpolarization current in mouse hippocampus. *Proc Natl Acad Sci USA*. 2010;107:10232–10237.
39. Cooper EC, Aldape KD, Abosch A, Barbaro NM, Berger MS, Peacock WS, Jan YN, Jan LY. Colocalization and coassembly of two human brain M-type potassium channel subunits that are mutated in epilepsy. *Proc Natl Acad Sci USA*. 2000;97:4914–4919.
40. Shah MM, Mistry M, Marsh SJ, Brown DA, Delmas P. Molecular correlates of the M-current in cultured rat hippocampal neurons. *J Physiol*. 2002;544:29–37.
41. Shen W, Hamilton SE, Nathanson NM, Surmeier DJ. Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. *J Neurosci*. 2005;25:7449–7458.
42. Howard RJ, Clark KA, Holton JM, Minor DL Jr. Structural insight into KCNQ (Kv7) channel assembly and channelopathy. *Neuron*. 2007;53:663–675.
43. Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MT. Coassembly of K(V) LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. *Nature*. 1996;384:80–83.
44. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, Vanraay TJ, Shen J, Timothy KW, Vincent GM, De Jager T, Schwartz PJ, Toubin JA, Moss AJ, Atkinson DL, Landes GM, Connors TD, Keating MT. Positional cloning of a novel potassium channel gene: *KVLQT1* mutations cause cardiac arrhythmias. *Nat Genet*. 1996;12:17–23.
45. Neyroud N, Tesson F, Denjoy I, Leibovici M, Donger C, Barhanin J, Faure S, Gary F, Coumel P, Petit C, Schwartz K, Guicheney P. A novel mutation in the potassium channel gene *KVLQT1* causes the cardioauditory syndrome [see comments]. *Nat Genet*. 1997;15:186–189.
46. Goldman AM, Glasscock E, Yoo J, Chen TT, Klassen TL, Noebels JL. Arrhythmia in heart and brain: *KCNQ1* mutations link epilepsy and sudden unexplained death. *Sci Transl Med*. 2009;1:2ra6.
47. Yang Y, Beyer BJ, Otto JF, O'Brien TP, Letts VA, White HS, Frankel WN. Spontaneous deletion of epilepsy gene orthologs in a mutant mouse with a low electroconvulsive threshold. *Hum Mol Genet*. 2003;12:975–984.
48. Singh NA, Otto JF, Dahle EJ, Pappas C, Leslie JD, Vilaythong A, Noebels JL, White HS, Wilcox KS, Leppert MF. Mouse models of human *KCNQ2* and *KCNQ3* mutations for benign familial neonatal convulsions show seizures and neuronal plasticity without synaptic reorganization. *J Physiol*. 2008;598:3405–3423.
49. Otto JF, Singh NA, Dahle EJ, Leppert MF, Pappas CM, Pruess TH, Wilcox KS, White HS. Electroconvulsive seizure thresholds and kindling acquisition rates are altered in mouse models of human *KCNQ2* and *KCNQ3* mutations for benign familial neonatal convulsions. *Epilepsia*. 2009;50:1752–1759.
50. Chung HJ, Jan YN, Jan LY. Polarized axonal surface expression of neuronal KCNQ channels is mediated by multiple signals in the KCNQ2 and KCNQ3 C-terminal domains. *Proc Natl Acad Sci USA*. 2006;103:8870–8875.
51. Ben-Ari Y. Excitatory actions of GABA during development: the nature of the nurture. *Nat Rev Neurosci*. 2002;3:728–739.
52. Porter RJ, Gratz E, Narang PK, Mirsky AE, Schwerdt P, Smallber S, Theodore W, Weingartner H, White BG. Effect of flupirtine on uncontrolled partial or absence seizures. *Epilepsia*. 1983;24:253–254.
53. Porter RJ. Mechanisms of action of new antiepileptic drugs. *Epilepsia*. 1989;30(suppl 1):S29–S34; discussion S64–S28.
54. Rundfeldt C. The new anticonvulsant retigabine (D-23129) acts as an opener of K^+ channels in neuronal cells. *Eur J Pharmacol*. 1997;336:243–249.
55. Rundfeldt C. Characterization of the K^+ channel opening effect of the anticonvulsant retigabine in PC12 cells. *Epilepsy Res*. 1999;35:99–107.
56. Rundfeldt C. The new anticonvulsant retigabine (D-23129) activates M-currents in Chinese hamster ovary-cells transfected with human KCNQ2/3 subunits. *Neurosci Lett*. 2000;282:73–76.
57. Main MJ, Cryan JE, Dupere JR, Cox B, Clare JJ, Burbidge SA. Modulation of KCNQ2/3 potassium channels by the novel anticonvulsant retigabine. *Mol Pharmacol*. 2000;58:253–262.
58. Alekov A, Rahman MM, Mitrovic N, Lehmann-Horn F, Lerche H. A sodium channel mutation causing epilepsy in man exhibits subtle defects in fast inactivation

and activation in vitro. *J Physiol*. 2000;529(pt 3): 533–539.
59. Tatulian L, Delmas P, Abogadie FC, Brown DA. Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. *J Neurosci*. 2001;21:5535–5545.
60. Wuttke TV, Seebohm G, Bail S, Maljevic S, Lerche H. The new anticonvulsant retigabine favors voltage-dependent opening of the Kv7.2 (KCNQ2) channel by binding to its activation gate. *Mol Pharmacol*. 2005;67:1009–1017.
61. Schenzer A, Friedrich T, Pusch M, Saftig P, Jentsch TJ, Grotzinger J, Schwake M. Molecular determinants of KCNQ (Kv7) K^+ channel sensitivity to the anticonvulsant retigabine. *J Neurosci*. 2005;25:5051–5060.
62. Long SB, Campbell EB, Mackinnon R. Crystal structure of a mammalian voltage-dependent Shaker family K^+ channel. *Science*. 2005;309:897–903.
63. Long SB, Campbell EB, Mackinnon R. Voltage sensor of Kv1.2: structural basis of electromechanical coupling. *Science*. 2005;309:903–908.
64. Guex N, Peitsch MC, Schwede T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: a historical perspective. *Electrophoresis*. 2009;30(suppl 1):S162–S173.
65. Wu YJ, He H, Sun LQ, L'Heureux A, Chen J, Dextraze P, Starrett JE Jr, Boissard CG, Gribkoff VK, Natale J, Dworetzky SI. Synthesis and structure-activity relationship of acrylamides as KCNQ2 potassium channel openers. *J Med Chem*. 2004;47:2887–2896.
66. Gribkoff VK, Starrett JE Jr, Dworetzky SI, Hewawasam P, Boissard CG, Cook DA, Frantz SW, Heman K, Hibbard JR, Huston K, Johnson G, Krishnan BS, Kinney GG, Lombardo LA, Meanwell NA, Molinoff PB, Myers RA, Moon SL, Ortiz A, Pajor L, Pieschl RL, Post-Munson DJ, Signor LJ, Srinivas N, Taber MT, Thalody G, Trojnacki JT, Wiener H, Yeleswaram K, Yeola SW. Targeting acute ischemic stroke with a calcium-sensitive opener of maxi-K potassium channels. *Nat Med*. 2001;7: 471–477.
67. Schroder RL, Jespersen T, Christophersen P, Strobaek D, Jensen BS, Olesen SP. KCNQ4 channel activation by BMS-204352 and retigabine. *Neuropharmacology*. 2001;40:888–898.
68. Porter RJ, Partiot A, Sachdeo R, Nohria V, Alves WM. Randomized, multicenter, dose-ranging trial of retigabine for partial-onset seizures. *Neurology*. 2007;68:1197–1204.
69. Mazarati A, Wu J, Shin D, Kwon YS, Sankar R. Antiepileptogenic and antiictogenic effects of retigabine under conditions of rapid kindling: an ontogenic study. *Epilepsia*. 2008;49:1777–1786.
70. Raol YH, Lapides DA, Keating JG, Brooks-Kayal AR, Cooper EC. A KCNQ channel opener for experimental neonatal seizures and status epilepticus. *Ann Neurol*. 2009;65:326–336.

Chapter 6

Voltage-Gated Calcium Channels in Epilepsy

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Voltage-gated calcium channels are integral membrane proteins that form calcium-selective pores in the plasma membrane (Fig. 6–1). Calcium ions flowing into the cell are driven by an electrochemical gradient generated by a high concentration of calcium outside the cell to a low calcium concentration inside. In neurons the rapid influx of calcium depolarizes the cell membrane potential due to its divalent positive charge and mediates biophysical processes such as action potential firing and membrane potential oscillations. A second effect of calcium ion influx is to regulate the intracellular signaling pathways and biochemical machinery required for physiological functions such as neurotransmitter release. Cells contain numerous calcium-sensitive proteins, such as enzymes and DNA transcription factors

that can be up- or downregulated by the binding of calcium ions. Due to the highly complex and widespread effects of calcium channels, even small alterations in their expression or biophysical properties can induce pathophysiological changes in the brain with the potential to induce epileptic seizures.

CALCIUM CHANNEL NOMENCLATURE

Calcium channels are generally classed as either high voltage-activated (HVA) or low voltage-activated (LVA), depending on whether they open at more positive (e.g., –40 mV) or more negative (e.g., –60 mV) membrane potentials, respectively[1,2] (Fig. 6–1). High voltage-activated channels can be further classified according to their pharmacological sensitivities and genetic α_1 subunit protein (Ca_V) composition into L-type ($Ca_V1.1$–$Ca_V1.4$), P/Q-type ($Ca_V2.1$), N-type ($Ca_V2.2$), and R-type ($Ca_V2.3$). Low voltage-activated channels, also known as *T-type*, for their comparatively "tiny" or "transient" currents are further classified to according to their α_1 subunit composition ($Ca_V3.1$–$Ca_V3.3$).[1] Additional structural and functional variants of each Ca_V subtype can be generated by alternative splicing to produce a large number of different *splice variants* and therefore increase the repertoire and complexity of calcium channel properties. It should be noted that $Ca_V1.3$ L-type and $Ca_V2.3$ R-type channels can exhibit characteristics of *mid-voltage-activated* channels, opening at membrane potentials that are more negative than HVA channels and more positive than LVA channels. For simplicity in this chapter, $Ca_V3.1$–$Ca_V3.3$ will be referred to as *T-type channels* and all other calcium channels will be referred to as *HVA channels*.

While each calcium channel α_1 subunit contains the molecular machinery necessary to conduct calcium ions (calcium-selective pore, voltage-sensing and -gating mechanisms), a number of ancillary proteins (β, $\alpha_2\delta$, and γ subunits) are associated with the HVA channel types and modify channel biophysical properties and expression[2] (Fig. 6–1). Four β subunit genes ($\beta1$–$\beta4$), four $\alpha_2\delta$ subunit genes ($\alpha_2\delta_1$–$\alpha_2\delta_4$), and eight γ subunit genes ($\gamma1$–$\gamma8$) have been identified in vertebrates. There is no firm biochemical evidence as yet that T-type calcium channels require ancillary subunits for native functioning. Nine of the ten calcium α_1 subunits (all but $Ca_V1.1$) are widely expressed in the central and peripheral nervous systems, and several have been implicated in contributing to epilepsy pathophysiology.

CALCIUM CHANNEL BIOPHYSICAL PROPERTIES

From their closed/resting state, calcium channels open once the membrane potential depolarizes to a threshold point, at which the internal voltage sensor moves and the channel conformation changes to an open-pore calcium-conducting state. Calcium channels conduct ions only in the open state, and with ongoing depolarization an internal inactivation mechanism induces additional conformational changes to prevent further conduction. Once in the inactivated state, the channels can only be reopened by repolarization to hyperpolarized membrane potentials, allowing the voltage sensor to return to its original closed conformation and the inactivation machinery to return to its deinactivated position. Only from this state can further membrane depolarization reopen the channels to their ion-conducting state. The membrane potentials and the rates at which these steps occur vary among the calcium channel subtypes and splice variants, producing channel variants with widely differing conducting properties.[2–4]

Calcium channels are generally slower at opening (activation) and closing (deactivation) than typical voltage-activated sodium channels. Among the calcium channel subtypes, HVA channels generally display slower activation and faster deactivation than LVA channels. Further, HVA channels generally inactivate much more slowly than LVA channels. Together these properties result in HVA channels generating longer-lasting calcium influxes upon sustained depolarizations, with T-type channels conducting more rapid and shorter calcium influxes under both brief and sustained depolarizations (Fig. 6–1). Of particular note, T-type channels also exhibit a distinct overlap of the membrane potentials at which they both activate and inactivate, uniquely enabling them to regulate subthreshold excitability including mediating intrinsic oscillatory behaviors and firing rates.

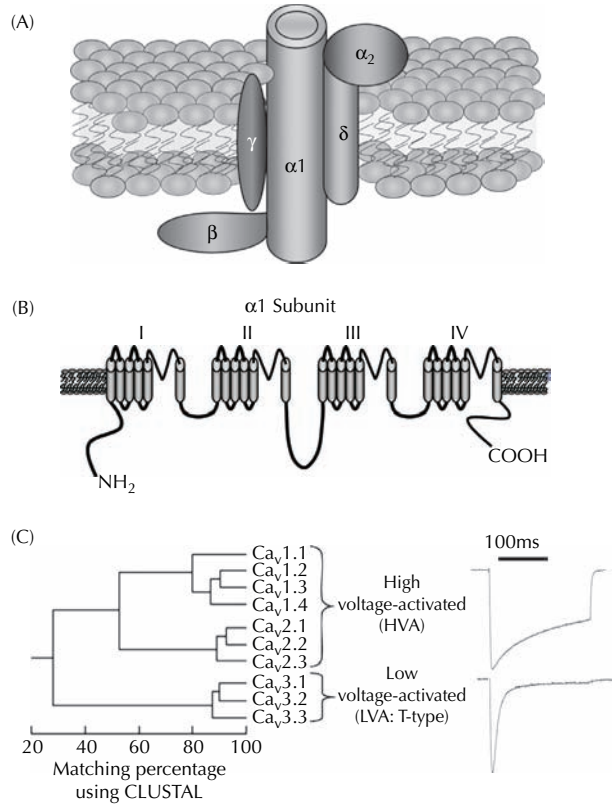

Figure 6–1. Voltage-gated calcium channels. **A.** Schematic illustrating the topography of the HVA calcium channel complex showing the main pore-forming α_1 subunit and ancillary subunits. The α_1 and δ subunits are integral membrane proteins, the β subunit is intracellular and binds directly to the α_1, and the α_2 subunit is thought to be largely extracellular. **B.** Schematic diagram showing the structure of the calcium channel α_1 subunit with its four-domain structure. **C.** The left panel shows the phylogenic relationship between the 10 known calcium channel α_1 subunits. Ca_V1 subunits form the L-type subfamily; Ca_V2 channels form the P/Q-type, N-type, and R-type; and the Ca_V3 subunits form the LVA T-type calcium channels. The right panel shows representative traces of calcium currents recorded from reticular thalamic neurons in response to depolarization of the membrane potential. The upper trace shows a slow inactivating HVA current, and the lower trace shows a fast inactivating LVA current.

CALCIUM CHANNELS AND EXCITABILITY

T-Type Channels and Excitability

T-TYPE CALCIUM CHANNELS AND BURST FIRING

T-type channels typically open at membrane potentials of about –70 to –50 mV, more negative than those required to open both typical HVA calcium channels and sodium channels (~ –40 to –30 mV).[4] The comparatively smaller depolarization required to open T-type channels from resting bestows a particular importance with regard to cellular excitability. Small depolarizations induced by, for example, *N*-methyl-D-aspartate (NMDA) receptor activation can cause T-type calcium channels to open, leading to further membrane depolarization, which in turns leads to the opening of additional T-type calcium channels.[5–7] If the expression of these channels is of sufficient density, this cascade depolarization induces a *calcium spike*, also known as a *low- threshold spike* (LTS), similar to an action potential, but slower in activation and inactivation rate and peaking at more hyperpolarized membrane potentials (~ –45 to –35 mV).[8,9]

This calcium spike can depolarize the membrane to a level whereby sodium channels and potassium channels then open and initiate high-frequency action potential (AP) firing on the crest of the LTS. The AP firing can continue until the T-type calcium channels inactivate and the membrane is repolarized by

small-conductance calcium-activated potassium (sK) channels. This type of event is known as a *burst*, and burst firing is thought to underlie the spike-and-wave discharges (SWDs) that are both the hallmark of absence epilepsy seizures on electroencephalography (EEG) recordings and that can also be observed in some other generalized and partial epilepsies (Fig. 6–2).[10,11] The "spikes" in these events are thought to correspond to summated neurotransmission, whereas the "wave" complexes are predicted to correspond to a period of neural quiescence. Together, they represent the oscillatory nature of absence seizures as they progress and resonate in the brain.

Different T-type channel subtypes contribute to particular parts of the burst due to their differing activation/inactivation kinetics (*fastest* $Ca_V3.1 > Ca_V3.2 >> Ca_V3.3$ *slowest*), deactivation kinetics (*fastest* $Ca_V3.3 > Ca_V3.1 > Ca_V3.2$ *slowest*), and rate of recovery from inactivation (*fastest* $Ca_V3.1 > Ca_V3.3 > Ca_V3.2$ *slowest*).[8,12] $Ca_V3.1$ channels are predicted to generate very-fast-activating, short-lasting bursts, $Ca_V3.2$, to generate fast-activating, longer-lasting bursts and $Ca_V3.3$ slow-activating and very-long-lasting bursts. Neuronal bursting properties likely depend on the relative proportion of the three T-subtypes that are expressed within a given neuron.

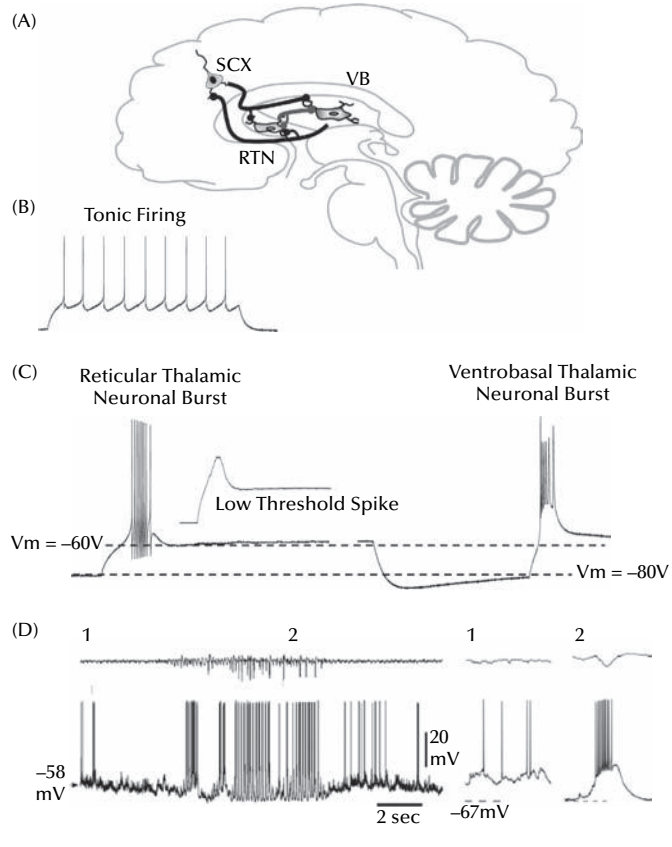

Figure 6–2. The thalamocortical network and burst firing. **A.** Diagram of the thalamocortical network showing connections between the somatosensory cortex (SCX), the sensory relay neurons of the ventrobasal posterior thalamic groups (VB), and the reticular thalamic nucleus (RTN). **B.** Under normal physiological conditions, as sensory signals from the periphery are relayed to the cortex, the VB and RTN neurons fire tonically in response to depolarization. In this state, there is minimal T-type calcium channel activity. During epileptiform activity, burst firing becomes predominant (**C**) and the TC network becomes locked in a self-propagating oscillatory loop (Vm = membrane potential). **C.** Inset: In the absence of sodium channel activity (600 nM tetrodotoxin applied), the low-threshold spike that underlies burst firing is evident. **D.** Burst firing in the RTN of the GAERS epileptic rat model of absence epilepsy correlates with burst firing in the RTN. Upper panels in show EEG recordings, and lower panels show corresponding intracellular recordings in an RTN neuron with expanded time scales on right side. Note that tonic neuronal firing does not correspond to spikes in the EEG trace (1), however burst firing correlates closely with spikes observed on the EEG trace during spike-wave discharges (2).

T-TYPE CALCIUM CHANNELS AND SLOW OSCILLATIONS

In addition to the oscillations generated by burst firing, T-type calcium channels are involved in generating a number of other types of oscillations, especially in the thalamocortical network, which are of particular importance in some epileptic disorders. The membrane potentials at which T-type channel variants open, close, inactivate and deinactivate are known to overlap and vary among subtypes. At potentials of overlap in conducting and nonconducting states, some percentage of channels is always open, although the entire population is constantly shifting between open, closed, and inactivated states. This produces a constant inward calcium current known as a *window current*.[4,8,13,14] Whether a given neuron is at a membrane potential where the window current is on or off can have a great effect on excitability, and the switching between these states, controlled by different leak, hyperpolarization-activated depolarizing and cation-activated depolarizing conductances, is thought to underlie a number of neural rhythms and oscillations.[15–19] While burst firing is a critical propagator of seizure activity, intrinsic oscillations within cells and networks potentially underlie the actual initiation of seizures.[20] This can be observed by artificial enhancement of T-type channel expression in inferior olivary neurons using computer modeling combined with patch clamp (known as *dynamic clamp*) and is sufficient to induce spontaneous oscillations.[21] This likely occurs because subtle changes in T-type channel current density can lead to large changes in electrophysiological oscillatory behavior.[21,22] For example, overexpression of the $Ca_V3.3$ channel alone in neuroblastoma cells induces spontaneous oscillatory activity and low-threshold firing.[23]

HVA Calcium Channels and Excitability

The HVA channels are involved in many different aspects of neuronal excitability, and a comprehensive discussion is beyond the scope of this chapter. However, some of their roles relative to epilepsy are of particular note. Postsynaptically expressed HVA channels generate large, long-lasting depolarization, and modification of their biophysical properties or expression can have substantial effects on the intrinsic firing properties of neurons. While HVA channels all play a role in low-threshold burst firing in that they conduct large amounts of calcium during bursts, especially during action potentials, they do not appear to be crucial for bursting activity to occur.[6,24] R-type channels, however, are becoming increasingly linked to a role in burst firing, as they have a lower threshold for activation than typical HVA channels, are capable of transient surges of current, and are linked to afterdepolarization, which is required for repetitive bursting.[25,26] Furthermore, R-type channels may be involved in generating adequate activation of sK channels to ensure sufficient repolarization following a burst, which is requirement for T-type deinactivation over a series of multiple bursts. In addition, R-type channels have been proposed to contribute to sustained depolarizations, known as *plateau potentials*, which have been implicated in pro-epileptic neuronal activity.[27]

A number of the HVA calcium channel subtypes are also expressed presynaptically and are critically involved in neurotransmitter release.[28–30] With an absolute dependence of neural functions on synaptic neurotransmission, it follows that even small alterations in the biophysical properties of presynaptic calcium channels could have a significant impact on the firing properties of nerve cells and neural networks, with the potential to lead to epileptic seizure activity.

CALCIUM CHANNELS IN ABSENCE EPILEPSY

T-Type Calcium Channels in the Thalamocortical Network and Absence Seizures

The thalamocortical (TC) network components involved in absence seizures appear to be comprised of three primary nuclei: sensory relay neurons including those located in the ventrobasal posterior thalamic groups (VB), the corticothalamic pyramidal neurons in layers V–VI of the sensory cerebral cortex

(SCX), and the reticular thalamic nucleus (RTN; Fig. 6–2). In this network, glutamatergic axonal efferents from the SCX synapse on VB neurons, which send reciprocal glutamatergic projections back to the SCX. The RTN forms a shell around the dorsal-anterior face of the thalamus, and as axons from SCX and VB neurons by de-inactivating the T-type calcium channels expressed in VB neurons the RTN, they synapse upon RTN neurons, inducing depolarization. Reticular thalamic nucleus neurons are GABAergic and send projections both to VB neurons and to other RTN neurons, inducing hyperpolarization in both neuronal types. Ventrobasal thalamic neurons are thought to respond more faithfully to hyperpolarizing inputs via the RTN than to those directly from depolarizing inputs from the SCX.[31]

During wakefulness or seizure-free periods, VB neurons act as a simple relay by forwarding sensory signals from the periphery to the cortex. In this mode, thalamic neurons are relatively depolarized and T-type channels in both VB and RTN neurons are, in general, inactivated. In this state, thalamic neurons follow a generally "tonic" or repetitive firing pattern of variable frequency with regular action potentials and little bursting (Fig. 6–2).

However, during absence seizures (which follow a pattern similar to that of nonrapid eye movement [REM] sleep) the SCX, VB, and RTN neurons become locked in a self-propagating oscillatory loop. During this state, RTN neurons are more hyperpolarized, allowing burst firing to occur via the deinactivation of $Ca_V3.2$ and $Ca_V3.3$ T-type channels expressed in these cells in response to depolarization from VB and SCX collaterals[32–35] (Fig. 6–2). Close correlation is observed between the timing of burst firing in neurons of the RTN and the spikes observed in (SWDs) on EEG recordings during absence seizures in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model of absence epilepsy[36,37] (Fig. 6–2). Burst firing in the RTN induces hyperpolarization of VB neurons via $GABA_A$ and/or $GABA_B$ receptor activation, deinactivating $Ca_V3.1$ T-type channels, and as hyperpolarization-activated channels (HCN; I_h) and corticothalamic inputs depolarize the neuron back toward the resting potential, a *rebound burst* is induced by opening of the deinactivated $Ca_V3.1$ channels[33,38,39] (Fig. 6–2). This process, in turn, induces depolarization in the SCX, which sends excitatory signals back to the thalamus, and the absence seizure propagates.[18,39] Overall, although RTN neurons are GABAergic, they actually drive excitatory behavior in their burst-firing state by de-inactivating the T-type calcium channels expressed in VB neurons.

The role of T-type calcium channels in the SCX is less clear. All three T-subtypes exist in the cortex, with $Ca_V3.1$ and $Ca_V3.3$ being expressed throughout, but with greatest expression in layer V; also, this layer is the only layer to express $Ca_V3.2$.[33] While oscillatory activity and low-threshold spiking have been identified in the cortex, burst firing is generally uncommon in cortical neurons.[40–44] Correspondingly, T-type currents are generally either absent or of small magnitude in cortical neurons, while HVA calcium channels are abundant.[45–50] However, evidence from genetic rat models of absence epilepsy suggests that seizures actually initiate in the SCX, which then recruits the thalamus and spreads to other cortices.[43,51–53] Whether intrinsic normal oscillatory activity in this region recruits a hyperexcitable thalamus to induce seizures, or whether pathophysiological hyperexcitability in the cortex is responsible for inducing seizures directly, is still unknown.[20,54,55]

Calcium Channels in Human Epilepsy

T-TYPE CALCIUM CHANNEL MUTATIONS IN HUMAN EPILEPSY

A number of apparent mutations have been identified in the human *CACNA1G* ($Ca_V3.1$) and *CACNA1H* ($Ca_V3.2$) genes within subpopulations of idiopathic generalized and childhood absence epilepsy patients.[56–59] Some of the alterations in $Ca_V3.2$ have been shown to induce altered biophysical properties or increase channel expression when examined in exogenous expression systems. However, some have no apparent effect, potentially reflecting the polygenic nature of idiopathic generalized epilepsies and/or that a subset of the changes represent single nucleotide polymorphisms.[60–68] Taken together these findings provide a strong indication that T-type calcium channels play a role in human idiopathic generalized epilepsies, supporting a large volume of data provided by experiments on animal models.

P/Q-TYPE CALCIUM CHANNEL MUTATIONS IN HUMAN EPILEPSY

The $Ca_V2.1$ subunit encodes both P-type and Q-type channels through an alternate splicing mechanism.[69] These channels are highly expressed presynaptically, where they are critically involved in neurotransmission and synaptic efficacy and therefore have a great influence on neuronal excitability.[70, 71] This aspect is reflected by a number of mutations in the $Ca_V2.1$ gene identified in patients suffering from severe neurological disorders, including ataxias and congenital migraine.[66] While instances in which HVA channel mutations have been identified in human absence epilepsy patients are rare, such cases do exist for $Ca_V2.1$. Within three generations of a single family, five members suffered from a combination of absence seizures/episodic ataxia and were found to possess a missense mutation (*E147K*) in the $Ca_V2.1$ subunit gene.[72] In another study, an 11-year-old boy was identified with primary generalized epilepsy, episodic and progressive ataxia, and mild learning difficulties. Analysis of his genome revealed a truncation mutation (*R1820*-stop) in the $Ca_V2.1$ channel.[73] Both of these distinct mutations result in a loss in P/Q-type channel function. Another missense mutation (*I712V*) has been reported in the $Ca_V2.1$ subunit gene of an 11-year-old girl suffering from episodes of seizures, ataxia, and other neural disorders, although no functional effects have yet been observed on the channel properties.[74] Furthermore, a small proportion of patients with familial hemiplegic migraine type-1 and with underlying mutations in the $Ca_V2.1$ channel also display both generalized and complex partial seizures. [66,67,75–79]

T-Type Calcium Channels in Absence Epilepsy Animal Models

GENETIC ABSENCE EPILEPSY RATS FROM STRASBOURG (GAERS)

Genetic rodent models of epilepsy have been useful in understanding the mechanisms that underlie absence seizures. Some of these models are generated by inbreeding rats that have developed epilepsy naturally to produce fully epileptic strains. The GAERS inbred Wistar rat model displays spontaneous absence seizures with characteristics similar to those of the human condition, with the exception that SWDs occur at a higher frequency (3–4 Hz in humans vs. 7–9 Hz in GAERS).[80,81] Calcium channels have been implicated in SWDs in GAERS since an early experiment wherein the injection of cadmium into the RTN at a concentration that blocks all calcium channel subtypes (1 mM) was found to abolish SWDs.[82] An increase in T-type current density has been found in the RTN neurons of GAERS with a corresponding increase in expression of $Ca_V3.2$ but not $Ca_V3.3$ mRNA.[83,84] Furthermore, GAERS has been shown to possess a missense mutation (*R1584P*) in the *Cacna1h* gene encoding $Ca_V3.2$ that correlates closely with seizure expression when GAERS rats are outcrossed with nonepileptic control rats[85] (Fig. 6–3). The *R1584P* mutation induces a gain of function in a particular $Ca_V3.2$ splice variant (+exon 25), increasing the rate at which channels recover from inactivation and allowing enhanced charge conduction during high-frequency depolarizations such as those that occur during burst firing. Since a greater number of $Ca_V3.2$ channels will recover from inactivation during multiple bursting in GAERS, the LTS magnitude is predicted to decrease less over a series of bursts. As LTS magnitude has been shown to correlate directly with the number of APs per burst,[86] the resultant effect in GAERS RTN neurons is that over a series of multiple bursts, the number of APs per burst decreases to a lesser degree throughout a burst train. In addition to the *R1584P* mutation's effect on $Ca_V3.2$ channel biophysical properties, the thalamic expression of the affected splice variant (+exon 25) also increases with development, potentially exacerbating hyperexcitability and underlying the temporal nature of seizure expression in GAERS animals.

WISTAR ALBINO GLAXO RATS FROM RIJSWIJK (WAG/RIJ)

Wistar Albino Glaxo Rats from Rijswijk (WAG/Rij) comprise another well-studied genetic absence epilepsy model that displays spontaneous seizures.[87] Like GAERS, these rats also display upregulation of T-type calcium channel expression, although in WAG/Rij this involves the $Ca_V3.1$ subtype in thalamic centrolateral and lateral geniculate (visual cortex projecting) neurons and $Ca_V3.3$ in centrolateral and RTN neurons (Fig. 6–3).[88] Despite increased T-type currents in all three neuron types, no differences

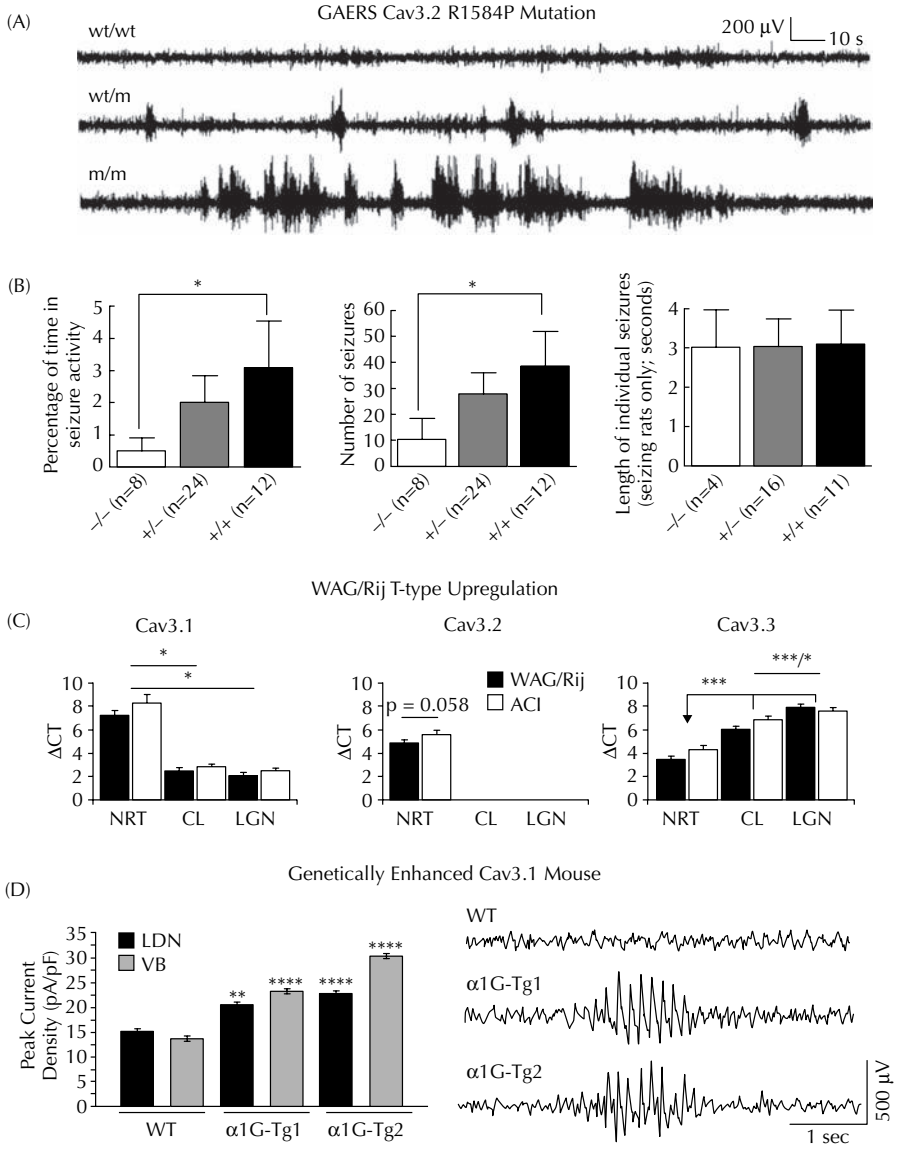

Figure 6–3. T-type calcium channels and absence epilepsy. **A, B.** In the GAERS rodent model of absence epilepsy, an arginine to proline missense mutation at position 1584 (*R1584P*) correlates with the expression of seizure activity. Mating GAERS with a nonepileptic strain (NEC) through two generations produces offspring that have no copies (*wt/wt*), one copy (*wt/m*), or two copies (*m/m*) of the *R1584P* mutation in an otherwise similar genetic background. Animals with two copies of the mutation spend increased time in seizure activity and experience more seizures than animals with no copies of the *R1584P* mutation. The missense GAERS mutation does not affect either the duration or the morphology of individual seizures. **C.** The WAG/Rij model of absence epilepsy displays increased expression of the $Ca_V3.1$ T-type calcium channel in thalamic centrolateral (CL) and lateral geniculate (LGN) neurons and of the $Ca_V3.3$ T-type in CL and RTN neurons. **D.** In mice with a genetically enhanced expression of $Ca_V3.1$ channels (*Tg1* and *Tg2*), larger T-type currents are observed in lateral dorsal (LDN) and ventrobasal (VB) thalamic neurons, and the mice display spontaneous bilateral spike-wave discharges in EEG recordings.

have been observed in the number of APs generated per burst. However, modeling studies predict that smaller depolarizations would be required to induce burst firing in lateral geniculate neurons of WAG/Rij animals. In addition, as with the $Ca_V3.2$ T-type in GAERS, alterations in the expression of specific splice variants of $Ca_V3.1$ have been noted in the WAG/Rij model, which is of particular interest since this occurs in the same , domain III-IV linker region where

seizure-related splice variation was observed with $Ca_V3.2$ in GAERS (exon 25–26).[89] In support of the involvement of $Ca_V3.1$ channels in seizure generation in this model, specific block of $Ca_V3.1$ using indomethacin-related compounds has been shown to attenuate seizures in WAG/Rij.[90] Interestingly, the systemic administration of the L-type calcium channel blocker, nimodipine, apparently exacerbates seizures in this model.[91]

MANIPULATION OF $CA_V3.1$ CHANNELS AND ABSENCE SEIZURES

In support of a role for $Ca_V3.1$ T-type channels in absence seizures, genetic enhancement of $Ca_V3.1$ expression in mice results in spontaneous bilateral SWDs[92] (Fig. 6–3). Accordingly, genetic knockout of the $Ca_V3.1$ channel in mice generates a phenotype whereby thalamic relay neurons cannot burst fire, and *in vivo* the mice show resistance to classic pharmacologically induced absence seizures using $GABA_B$ agonists, baclofen, and butyrolacetone (a prodrug of γ-hydroxybutyric acid).[38]

Overall, it appears that an increase in the activity of any of the three T-type channel subtypes in the TC system may have the effect of enhancing or inducing absence seizures as a direct result of increased burst firing in any of the TC regions, whether it occurs from increased expression or increased function of a T-type calcium channel subtype. Certainly, enhancement of either $Ca_V3.1$ or $Ca_V3.2$ channels seems to have strong pro-epileptic effects in the TC system.

HVA Channels in Absence Epilepsy Animal Models

HVA CHANNELS IN WISTAR ALBINO GLAXO RATS FROM RIJSWIJK

In the WAG/Rij model of absence epilepsy, in addition to an increase in T-type currents, an increase in the expression of P/Q-type channel protein occurs in the RTN.[93] This appears to occur presynaptically, although experiments have yet to be conducted to determine the functional significance of this expression change on synaptic neurotransmission and absence seizure activity.

HVA CHANNELS IN ABSENCE EPILEPTIC MICE MODELS

Mice generated or identified with mutations that suppress P/Q-type channel function exhibit many features of absence epilepsy. $Ca_V2.1$ gene knockout mice suffer from severe ataxia and seizures and die in early life following massive neuronal damage, in particular in the cerebellum, where P/Q-type channels play a vital role in movement control.[66,67,94–97] *Tottering* mice ($Ca_V2.1$ *P601L*) display absence and motor seizures, whereas *Leaner* (C-terminal truncation) and *Rolling Nagoya* mice (*R1262G*) display absence seizures only.[98–100] Each of these mutations results in decreased P/Q-type current density and a number of other biophysical alterations, generally considered as loss of function.[66,101] The reduced activity can be observed physiologically as attenuated excitatory synaptic neurotransmission in cortical and thalamic neurons.[102,103] Similarly, *Rocker* mice (*T1310K*) display absence-like seizures; however, the effects on channel biophysics are not currently known.[104] It should be noted that all of these mouse models display ataxia, likely due to the critical role of cerebellar P/Q-type channels in movement control, although they may have pro-epileptic downstream effects concerning the production of secondary seizures. In this regard, it should be noted that with the *Tottering* mouse, an increase is observed in the T-type currents ($Ca_V3.1$) in thalamic relay neurons.[105]

Of further note, seizures in $Ca_V2.1$ knockout mice can be abolished by introducing a second mutation to also knock out $Ca_V3.1$ T-type channel function.[106] Although similar investigations have not been reported for the other absence model mice carrying $Ca_V2.1$ mutations, this finding implies that decreased activity of P/Q-type channels may lead to a compensatory increase in T-type currents, which may be responsible for the absence seizures observed. However, since in the WAG/Rij model an *increase* in P/Q-type channel expression is observed in conjunction with increased T-type activity, a compensatory decrease in P/Q-type/increase in T-type model cannot be accepted as absolute. In addition, combined knockout studies must be treated with caution since the absence seizures observed in $Ca_V2.1$ knockout mice can be abolished by a second mutation to knock out shaker-like potassium channels,

which normally increases excitability.[107] Thus, it might be argued that any number of mutations that interfere with excitability in the TC network may block the epileptic phenotype caused by a first mutation.

The mid-voltage activated R-type channel has been shown to play a role in modulating TC rhythmicity, altering the frequencies displayed during pharmacologically induced SWD.[108] Mice lacking $Ca_V2.3$ channels do not display spontaneous absence seizures; however, they do exhibit increased susceptibility to the absence seizures and motor arrest induced by systemic administration of γ-hydroxybutyrolactone.[108] Conversely, a more recent study suggests that $Ca_V2.3$ deficient mice display reduced sensitivity to γ-butyrolactone-induced absence seizures as a result of attenuated sK channel-mediated after-hyperpolarization, and therefore T-type calcium channel de-inactivation, following a burst.[109] This is of particular interest since these mice also demonstrate resistance to generalized convulsive and limbic seizures (discussed in "$Ca_V2.3$ R-type channels in the kainic acid-Induced limbic epilepsy model" and "Generalized seizures in $Ca_V2.3$ transgenic mice").[110,111]

Calcium Channel Ancillary Subunits in Absence Epilepsy Models

The β, $\alpha_2\delta$, and γ ancillary calcium channel subunits that modulate the biophysical properties and expression of the HVA α_1 subunits have also been implicated in absence epilepsy.[112] Mice containing a mutation that genetically deletes the β4 subunit, known as *lethargic*, express SWDs and ataxia, along with defects in presynaptic function.[113] In addition, two strains of mice with mutations in the $\alpha_2\delta_2$ subunit, known as *ducky* and *ducky*^{2J}, both also display SWDs and ataxia.[114,115] Furthermore, a mutation that renders the $\alpha_2\delta_2$ subunit nonfunctional (the *entla* mouse) has also been linked to SWDs.[116] All three $\alpha_2\delta_2$ mutated mice models possess reduced P/Q-type currents and display absence and/or ataxia phenotypes similar to those of the $Ca_V2.1$ gene knockout/mutated mice described above, suggesting that at least part of the mechanism by which they induce seizures may be due to attenuated $\alpha_2\delta_2$ -mediated modulation of the $Ca_V2.1$ P/Q-type calcium channel. A similar mechanism may underlie the β4 knockout *lethargic* mice since β subunits are essential for P/Q-type channel function.

Mutations in the γ_2 subunit protein, also known as *stargazin*, have been found in *stargazer* and *waggler* mouse epilepsy models and result in increased inactivation of P/Q-type channels.[117,118] These mice both display SWDs as well as head-tossing behavior, which are exacerbated in *waggler* mice due to an additional knockout of the γ_4 subunit.[119] As a note of caution, in addition to the modulatory role of this subunit on HVA calcium channels, stargazin is known to be involved in the synaptic trafficking and biophysical modulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Therefore, some or all of the phenotypes associated with mutations in these mice could involve AMPA-mediated signaling. This may also be reflected in the GAERS model, where the stargazin subunit is upregulated in both the SCX and thalamus, key areas involved in absence seizures, although no alterations in P/Q-type channel activity have yet been reported in this model.[120]

CALCIUM CHANNELS IN TEMPORAL LOBE/COMPLEX PARTIAL EPILEPSY

T-Type Calcium Channels in the Pilocarpine Model of Temporal Lobe Epilepsy/Complex Partial Epilepsy

In the pilocarpine model of temporal lobe epilepsy (TLE), status epilepticus is induced by systemic administration of the muscarinic receptor agonist, pilocarpine.[121,122] During an initial acute phase lasting for up to approximately 24 hours, rodents suffer from seizures resembling those of TLE. Following this event, a seizure-free period lasting for a few days to weeks occurs until a chronic phase resembling that of complex-partial seizures develops (Fig. 6–4). Immediately after the acute phase, significant pathophysiological damage can be observed in hippocampal, thalamic, cortical, and striatal structures. During this period, $Ca_V3.2$ expression is upregulated and a corresponding upregulation of T-type currents

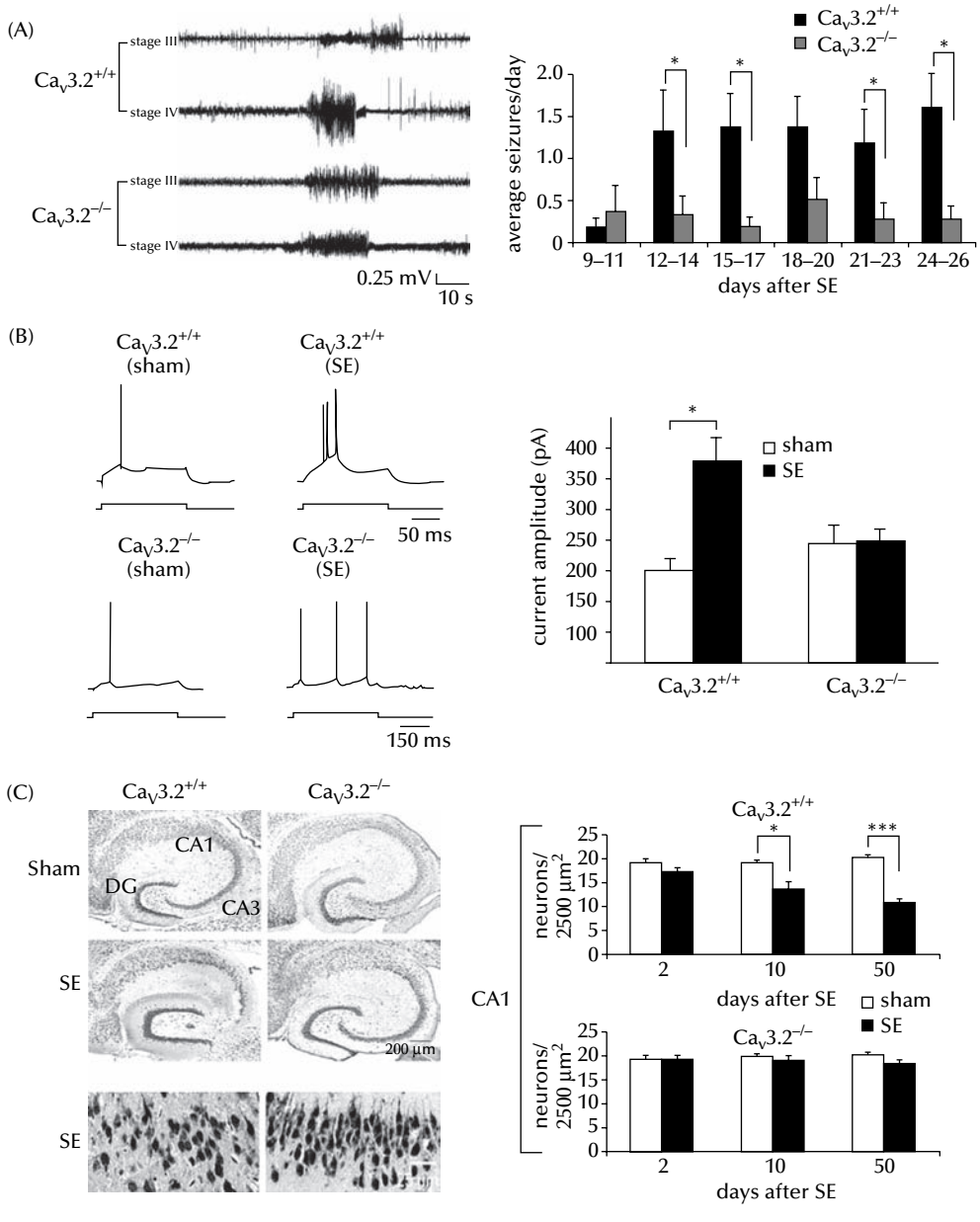

Figure 6–4. T-type calcium channels in temporal lobe/complex-partial epilepsy. **A.** Systemic injection of pilocarpine in mice induces the development of complex-partial seizures, as observed with EEG recording. Seizure morphology and duration are similar in wild-type ($Ca_V3.2^{+/+}$) and $Ca_V3.2$ gene knockout mice ($Ca_V3.2^{-/-}$); however, $Ca_V3.2^{-/-}$ mice display fewer seizures per day than $Ca_V3.2^{+/+}$ mice following status epilepticus (SE). **B.** CA1 hippocampal neurons do not display burst-firing activity in response to depolarization in sham-treated $Ca_V3.2^{+/+}$ mice (top left panel); however, in $Ca_V3.2^{+/+}$ mice displaying pilocarpine-induced seizures 7–15 days after SE (top right panel), clear burst firing is observed. Conversely, in $Ca_V3.2^{-/-}$ mice, burst firing is not observed in either sham-treated (bottom left panel) or pilocarpine-treated (bottom right panel) animals. **B,** far right panel: Increased T-type tail currents are detected in CA1 neurons of $Ca_V3.2^{+/+}$ mice treated with pilocarpine but not in CA1 neurons of sham-treated controls or $Ca_V3.2^{-/-}$ mice. **C.** Neuronal cell loss occurs via a $Ca_V3.2$-dependent mechanism. Representative hippocampal sections from sham-control and SE-experienced $Cav3.2^{+/+}$and $Cav3.2^{-/-}$ mice stained with an antibody directed against the neuron-specific epitope NeuN 50 days after treatment. Note the pronounced neuronal cell loss in CA1 and CA3 areas after SE in Cav3.2 mice but not in $Cav3.2^{-/-}$ mice. The higher-magnification micrographs of representative CA1 subfields of SE-experienced animals highlight the substantial neuronal degeneration in $Cav3.2^{+/+}$ but not $Cav3.2^{-/-}$ mice (bottom). Neuronal cell loss is quantified in the right panel.

is thought to occur in the apical dendrites of hippocampal CA1 neurons.[123–126] Some small changes have also been observed in the biophysical properties of the T-type currents in these neurons.[126] Burst firing is increased in CA1 neurons after the induction of status epilepticus, as would be expected with increased T-type conductance, and can be reversed by specific blockade of $Ca_V3.2$ channels (Fig. 6–4). Furthermore, in $Ca_V3.2$ knockout mice, the number of seizures is attenuated, burst firing is abolished, and neuronal damage in the CA1 region (cell loss and mossy fiber sprouting) is reduced.[125] Therefore, T-type channels, specifically $Ca_V3.2$, appear to be upregulated by temporal lobe seizures and/or have a strong influence on the development of complex-partial seizures in the pilocarpine model. In addition, there is a direct correlation between seizure-induced neuronal damage and upregulated expression of $Ca_V3.2$ channels; however, whether increased $Ca_V3.2$ expression induces neuronal damage or if damage itself increases the expression of $Ca_V3.2$ is unknown.

T-Type Channels in the Electrical Kindling Model of Limbic Epilepsy

Seizure *kindling* is another established model for studying epilepsy in both rodents and higher animals.[127] In this model, low-intensity focal electrical stimulation of a particular area of the brain is used to induce seizures, which increase in intensity and duration as the induction is repeated, due to the fact that seizures lower the threshold for further seizures. For example, electrical stimulation of limbic structures can induce TLE. In rats, similar to what is seen in the pilocarpine model, kindling increases T-type currents in CA1 hippocampal neurons following stimulation applied to CA3 hippocampal efferents (Schaffer collaterals) that innervate the CA1.[128] The increase in T-type currents remains 6 weeks following the cessation of kindling stimulation. Simultaneous increases in neuronal hyperexcitability and damage are also observed in this model, implying that increased T-type currents may drive neurons into a pathophysiological, hyperexcitable state wherein overexcitability induces neuronal damage. Conversely, and again similar to the pilocarpine model, the reverse may be true: seizure-induced damage may lead to an upregulation of T-type currents.

HVA Channels in Electrical Kindling Models of Limbic Epilepsy

In the same electrical kindling model using rats, HVA currents increase by ~50% in comparison to controls in hippocampal CA1 neurons.[128] Correspondingly, $Ca_V1.3$, $Ca_V2.1$, and $Ca_V2.3$ channel mRNA is increased in the CA1 and dentate gyrus hippocampal regions in the initial stages of epileptogenesis as seizures are developing.[129] In contrast, at these stages, the expression of $Ca_V2.2$ N-type channel mRNA is decreased. However, once kindling is fully developed, a significant *increase* is observed in expression of the $Ca_V2.2$ subtype alone. Therefore, alterations in both HVA and T-type calcium channel expression occur at different levels and rates in the kindling model in a subtype-specific manner, making elucidation of the specific role of each subtype somewhat complicated. Nonetheless, there is a clear correlation of calcium channel expression with the development and maintenance of seizures in this limbic epilepsy model.

$Ca_V2.3$ R-Type Channels in the Kainic Acid-Induced Limbic Epilepsy Model

$Ca_V2.3$ knockout mice exhibit altered susceptibility to absence seizures and decreased susceptibility to generalized seizures, as discussed in detail in "HVA channels in absence epileptic mice models" and in "Generalized Seizures in $Ca_V2.3$ transgenic mice" and are thought to contribute to epileptogenic plateau potentials in CA1 hippocampal neurons.[27,108–111] Further studies have revealed that $Ca_V2.3$-deficient mice are also resistant to limbic seizures and secondary generalized seizures induced by systemic administration of the glutamate receptor agonist, kainic acid.[110] It should be noted that this only applies to the more severe stages of seizure in this model and that less severe seizures actually display signs of increased sensitivity, as has been suggested for absence seizures in these mice.[108] In addition, these mice show reduced neuronal cell loss and neurodegeneration within the CA3

region in response to seizures, and their survival rate is significantly improved.

CALCIUM CHANNELS IN GENERALIZED CONVULSIVE SEIZURES

HVA Calcium Channels in Genetic Epilepsy-Prone Rats

Genetic Epilepsy-Prone rats (GEPRs) are inbred Sprague-Dawley strains that develop either a moderate (GEPR-3) or severe (GEPR-9) predisposition to, and expression of, spontaneous as well as audiogenic and kindling-induced complex-partial seizures leading to secondary tonic-clonic seizures. Seizures are thought to originate from the forebrain and/or brainstem circuitry as well as the inferior colliculus.[130,131] High voltage-activated calcium currents have been shown to be increased in inferior colliculus neurons of the less severe seizure-expressing GEPR-3 strain.[132] Corresponding increases in $Ca_V1.3$ L-type and $Ca_V2.3$ R-type protein levels are observed in neurons from this region in GEPR-3 rats that have not yet suffered seizures.[133] Following a single audiogenic seizure, GEPR-3 rats display a further increase in the expression of both $Ca_V1.3$ and $Ca_V2.3$ calcium channels and also an increase in the expression of the $Ca_V2.1$ P/Q-type. Interestingly, these are the same calcium channel types upregulated in limbic electrical kindling models, further supporting their role in epileptic susceptibility.

Generalized Seizures in $Ca_V2.3$ Transgenic Mice

The $Ca_V2.3$ knockout mouse model displays altered susceptibility to pharmacologically induced absence and limbic seizures.[108,109,110] While these mice display no spontaneous seizures, it has been demonstrated that $Ca_V2.3$ knockout mice show resistance to generalized convulsive seizures and reduced lethality induced by systemic administration of the GABA receptor antagonist, pentylenetetrazol.[111] However, these mice do not show any altered susceptibility to seizures induced by administration of the potassium channel blocker, 4-aminopyrridine.

Calcium Channel Ancillary Subunits in Genetic Convulsive Animal Models

In addition to the alterations in HVA channel α_1 subunit expression in GEPRs, expression abnormalities of the calcium channel ancillary subunits have been observed in this model. Expression of the β_3 subunit is increased in seizure-naive GEPR-3 rats and increases further following induction of a single audiogenic seizure.[133] In contrast, expression of the $\alpha_2\delta$ subunit is decreased in seizure-naive GEPR-3 rats and decreases further following induction of a single audiogenic seizure. The overall effect on calcium currents remains difficult to establish due to the altered expression of the HVA channel subunits that also occurs in GEPRs.

CONCLUSIONS

A number of currently used antiepileptic drugs (AEDs) have been shown to block calcium channels.[134] These include front-line absence treatments such as ethosuximide[49,135–150] and valproic acid,[49,138,151–152] zonisamide[153–157] and leviteracetam[160,172,173] in the treatment of partial-onset and generalized seizures, lamotrigine[49,158–163] and gabapentin/pregabalin[174–182] for partial seizures and primary/secondary generalized convulsive seizures and phenytoin,[49,164,] and carbamazepine[49, 165,166] and topiramate[160, 167–170] to control complex-partial and tonic-clonic seizures. In many cases, the exact relevance of the *in vitro* pharmacological findings is difficult to interpret since the cells in which the AEDs have been tested *in vitro* are often not from the region where the drug has its intended effect *in vivo*. Further difficulties arise from the drug concentrations used since the accurate measurement of clinical AED concentrations in specific human brain areas is often not possible; as a result, the drug concentrations for *in vitro* testing are estimated from human plasma concentrations combined with animal cerebral spinal fluid concentration to plasma concentration ratios. The result is often that higher or lower concentrations may be tested in comparison to those existing in the brains of epileptic patients. Within these limitations, in most *in vitro* studies, 100% block of calcium channel activity is rarely observed with clinical concentrations of AEDs. Despite this, convincing evidence for

the involvement of subtype-selective calcium channels in AED pharmacology is mounting for some of the currently used AEDs. As a result, calcium channels are more commonly being viewed as attractive targets for novel epileptic therapies. While small molecules with the ability to specifically block individual calcium channel subtypes are not presently available, considerable effort is ongoing to develop new and selective calcium channel–blocking compounds aimed at the treatment of epilepsy.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose

REFERENCES

1. Ertel EA, Campbell KP, Harpold MM, Hofmann F, Mori Y, Perez-Reyes E, Schwartz A, Snutch TP, Tanabe T, Birnbaumer L, Tsien RW Catterall, WA. Nomenclature of voltage-gated calcium channels. *Neuron*. 2000;25:533–535.
2. Snutch TP, Peloquin J, Mathews E, McRory JE. Molecular properties of voltage-gated calcium channels. In: Zamponi GW, ed. *Voltage-Gated Calcium Channels.* Springer, U.S.A., Landes Biosciences; 2005:61–94.
3. Catterall WA, de Jongh K, Rotman E, Hell J, Westenbroek R, Dubel SJ, Snutch TP. Molecular properties of calcium channels in skeletal muscle and neurons. *Ann NY Acad Sci*. 1993;681:342–355.
4. Perez-Reyes E. Molecular physiology of low-voltage-activated T-type calcium channels. *Physiol Rev*. 2003;83:117–161.
5. Turner JP, Leresche N, Guyon A, Soltesz I, Crunelli V. Sensory input and burst firing output of rat and cat thalamocortical cells: the role of NMDA and non-NMDA receptors. *J Physiol*. 1994;480(pt 2):281–295.
6. Xu J, Clancy CE. Ionic mechanisms of endogenous bursting in CA3 hippocampal pyramidal neurons: a model study. *PLoS One*. 2008;3:e2056.
7. Jahnsen H, Llinas R. Voltage-dependent burst-to-tonic switching of thalamic cell activity: an in vitro study. *Arch Ital Biol*. 1984;122:73–82.
8. Cain SM, Snutch TP. Contributions of T-type calcium channel isoforms to neuronal firing. *Channels*. 2010;4:44–51.
9. Llinas R, Jahnsen H. Electrophysiology of mammalian thalamic neurones in vitro. *Nature*. 1982;297: 406–408.
10. Blumenfeld H. Cellular and network mechanisms of spike-wave seizures. *Epilepsia*. 2005;46(suppl 9): 21–33.
11. Destexhe A, Sejnowski TJ. The initiation of bursts in thalamic neurons and the cortical control of thalamic sensitivity. *Philos Trans R Soc Lond B Biol Sci*. 2002;357:1649–1657.
12. Chemin J, Monteil A, Perez-Reyes E, Bourinet E, Nargeot J, Lory P. Specific contribution of human T-type calcium channel isotypes (alpha(1G), alpha(1H) and alpha(1I)) to neuronal excitability. *J Physiol*. 2002;540:3–14.
13. Coulter DA, Huguenard, JR Prince DA. Calcium currents in rat thalamocortical relay neurones: kinetic properties of the transient, low-threshold current. *J Physiol*. 1989;414:587–604.
14. Carbone E, Lux HD. A low voltage-activated, fully inactivating Ca channel in vertebrate sensory neurones. *Nature*. 1984;310:501–502.
15. Crunelli V, Cope DW, Hughes SW. Thalamic T-type Ca^{2+} channels and NREM sleep. *Cell Calcium.* 2006;40:175–190.
16. Contreras D. The role of T-channels in the generation of thalamocortical rhythms. *CNS Neurol Disord Drug Targets*. 2006;5:571–585.
17. Huguenard JR, Prince DA. Intrathalamic rhythmicity studied in vitro: nominal T-current modulation causes robust antioscillatory effects. *J Neurosci*. 1994;14:5485–5502.
18. Huguenard JR. Anatomical and physiological considerations in thalamic rhythm generation. *J Sleep Res*. 1998;7(suppl 1):24–29.
19. Williams SR, Toth TI, Turner JP, Hughes SW, Crunelli V. The 'window' component of the low threshold Ca^{2+} current produces input signal amplification and bistability in cat and rat thalamocortical neurones. *J Physiol*. 1997;505(pt 3):689–705.
20. Pinault D, Slezia A, Acsady L. Corticothalamic 5–9 Hz oscillations are more pro-epileptogenic than sleep spindles in rats. *J Physiol*. 2006;574:209–227.
21. Chorev E, Manor Y, Yarom Y. Density is destiny. On the relation between quantity of T-type Ca^{2+} channels and neuronal electrical behavior. *CNS Neurol Disord Drug Targets*. 2006;5:655–662.
22. McCormick DA, Huguenard JR. A model of the electrophysiological properties of thalamocortical relay neurons. *J Neurophysiol*. 1992;68:1384–1400.
23. Chevalier M, Lory P, Mironneau C, Macrez N, Quignard JF. T-type CaV3.3 calcium channels produce spontaneous low-threshold action potentials and intracellular calcium oscillations. *Eur J Neurosci*. 2006;23:2321–2329.
24. McCobb DP, Beam KG. Action potential waveform voltage-clamp commands reveal striking differences in calcium entry via low and high voltage-activated calcium channels. *Neuron*. 1991;7:119–127.
25. Metz AE, Jarsky T, Martina M, Spruston N. R-type calcium channels contribute to afterdepolarization and bursting in hippocampal CA1 pyramidal neurons. *J Neurosci*. 2005;25:5763–5773.
26. Randall AD, Tsien RW. Contrasting biophysical and pharmacological properties of T-type and R-type calcium channels. *Neuropharmacology*. 1997;36:879–893.
27. Tai C, Kuzmiski JB, MacVicar BA. Muscarinic enhancement of R-type calcium currents in hippocampal CA1 pyramidal neurons. *J Neurosci*. 2006;26: 6249–6258.
28. Catterall WA. Structure and function of neuronal Ca^{2+} channels and their role in neurotransmitter release. *Cell Calcium*. 1998;24:307–323.
29. Neher E, Sakaba T. Multiple roles of calcium ions in the regulation of neurotransmitter release. *Neuron*. 2008;59:861–872.
30. Wadel K, Neher E, Sakaba T. The coupling between synaptic vesicles and Ca^{2+} channels determines fast neurotransmitter release. *Neuron*. 2007;53:563–575.

31. Landisman CE, Connors BW. VPM and PoM nuclei of the rat somatosensory thalamus: intrinsic neuronal properties and corticothalamic feedback. *Cereb Cortex*. 2007;17:2853–2865.
32. Huguenard JR, Prince DA. A novel T-type current underlies prolonged Ca(2+)-dependent burst firing in GABAergic neurons of rat thalamic reticular nucleus. *J Neurosci*. 1992;12:3804–3817.
33. Talley EM, Cribbs LL, Lee JH, Daud A, Perez-Reyes E, Bayliss DA. Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels. *J Neurosci*. 1999;19:1895–1911.
34. Meis S, Biella G, Pape HC. Interaction between low voltage-activated currents in reticular thalamic neurons in a rat model of absence epilepsy. *Eur J Neurosci*. 1996;8:2090–2097.
35. Joksovic PM, Bayliss DA, Todorovic SM. Different kinetic properties of two T-type Ca^{2+} currents of rat reticular thalamic neurones and their modulation by enflurane. *J Physiol*. 2005;566:125–142.
36. Pinault D, Leresche N, Charpier S, Deniau JM, Marescaux C, Vergnes M, Crunelli V. Intracellular recordings in thalamic neurones during spontaneous spike and wave discharges in rats with absence epilepsy. *J Physiol*. 1998;509(pt 2):449–456.
37. Slaght SJ, Leresche N, Deniau JM, Crunelli V, Charpier S. Activity of thalamic reticular neurons during spontaneous genetically determined spike and wave discharges. *J Neurosci*. 2002;22:2323–2334.
38. Kim D, Song I, Keum S, Lee T, Jeong MJ, Kim SS, McEnery MW, Shin HS. Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking alpha(1G) T-type Ca^{2+} channels. *Neuron*. 2001;31:35–45.
39. Manning JP, Richards DA, Bowery NG. Pharmacology of absence epilepsy. *Trends Pharmacol Sci*. 2003;24:542–549.
40. Amitai Y. Membrane potential oscillations underlying firing patterns in neocortical neurons. *Neuroscience*. 1994;63:151–161.
41. Beierlein M, Gibson, JR Connors BW. A network of electrically coupled interneurons drives synchronized inhibition in neocortex. *Nat Neurosci*. 2000;3:904–910.
42. Beierlein M, Gibson JR, Connors BW. Two dynamically distinct inhibitory networks in layer 4 of the neocortex. *J Neurophysiol*. 2003;90:2987–3000.
43. Polack PO, Mahon S, Chavez M, Charpier S. Inactivation of the somatosensory cortex prevents paroxysmal oscillations in cortical and related thalamic neurons in a genetic model of absence epilepsy. *Cereb Cortex*. 2009;19:2078–2091.
44. Charpier S, Leresche N, Deniau JM, Mahon S, Hughes SW, Crunelli V. On the putative contribution of GABA(B) receptors to the electrical events occurring during spontaneous spike and wave discharges. *Neuropharmacology*. 1999;38:1699–1706.
45. Brown AM, Schwindt PC, Crill WE. Voltage dependence and activation kinetics of pharmacologically defined components of the high-threshold calcium current in rat neocortical neurons. *J Neurophysiol*. 1993;70:1530–1543.
46. Lorenzon NM, Foehring RC. Characterization of pharmacologically identified voltage-gated calcium channel currents in acutely isolated rat neocortical neurons. I. Adult neurons. *J Neurophysiol.* 1995;73:1430–1442.
47. Lorenzon NM, Foehring RC. Characterization of pharmacologically identified voltage-gated calcium channel currents in acutely isolated rat neocortical neurons. II. Postnatal development. *J Neurophysiol*. 1995;73:1443–1451.
48. Almog M, Korngreen A. Characterization of voltage-gated Ca(2+) conductances in layer 5 neocortical pyramidal neurons from rats. *PLoS ONE*. 2009;4:e4841.
49. Sayer RJ, Brown AM, Schwindt PC, Crill WE. Calcium currents in acutely isolated human neocortical neurons. *J Neurophysiol*. 1993;69:1596–1606.
50. Sayer RJ, Schwindt PC, Crill WE. High- and low-threshold calcium currents in neurons acutely isolated from rat sensorimotor cortex. *Neurosci Lett*. 1990;120:175–178.
51. Polack PO, Guillemain I, Hu E, Deransart C, Depaulis A, Charpier S. Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *J Neurosci*. 2007;27:6590–6599.
52. Nersesyan H, Hyder F, Rothman DL, Blumenfeld H. Dynamic fMRI and EEG recordings during spike-wave seizures and generalized tonic-clonic seizures in WAG/Rij rats. *J Cereb Blood Flow Metab*. 2004;24:589–599.
53. Meeren HK, Pijn JP, Van Luijtelaar EL, Coenen AM, Lopes da Silva FH. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci*. 2002;22:1480–1495.
54. Pinault D. Cellular interactions in the rat somatosensory thalamocortical system during normal and epileptic 5–9 Hz oscillations. *J Physiol*. 2003;552:881–905.
55. Blumenfeld H, McCormick DA. Corticothalamic inputs control the pattern of activity generated in thalamocortical networks. *J Neurosci*. 2000;20:5153–5162.
56. Singh B, Monteil A, Bidaud I, Sugimoto Y, Suzuki T, Hamano S, Oguni H, Osawa M, Alonso ME, Delgado-Escueta AV, Inoue Y, Yasui-Furukori N, Kaneko S, Lory P, Yamakawa K. Mutational analysis of *CACNA1G* in idiopathic generalized epilepsy. *Hum Mutat.* 2007;28:524–525.
57. Chen Y, Lu J, Pan H, Zhang Y, Wu H, Xu K, Liu X, Jiang Y, Bao X, Yao Z, Ding K, Lo WH, Qiang B, Chan P, Shen Y, Wu X. Association between genetic variation of *CACNA1H* and childhood absence epilepsy. *Ann Neurol*. 2003;54:239–243.
58. Heron SE, Khosravani H, Varela D, Bladen C, Williams TC, Newman MR, Scheffer IE, Berkovic SF, Mulley JC, Zamponi GW. Extended spectrum of idiopathic generalized epilepsies associated with *CACNA1H* functional variants. *Ann Neurol*. 2007;62:560–568.
59. Liang J, Zhang Y, Chen Y, Wang J, Pan H, Wu H, Xu K, Liu X, Jiang Y, Shen Y, Wu X. Common polymorphisms in the *CACNA1H* gene associated with childhood absence epilepsy in Chinese Han population. *Ann Hum Genet*. 2007;71:325–335.
60. Vitko I, Chen Y, Arias JM, Shen Y, Wu XR, Perez-Reyes E. Functional characterization and neuronal modeling of the effects of childhood absence epilepsy variants of *CACNA1H*, a T-type calcium channel. *J Neurosci*. 2005;25:4844–4855.
61. Vitko I, Bidaud I, Arias JM, Mezghrani A, Lory P, Perez-Reyes E. The I-II loop controls plasma membrane expression and gating of $Ca_v3.2$ T-type Ca^{2+} channels: a paradigm for childhood absence epilepsy mutations. *J Neurosci*. 2007;27:322–330.

62. Khosravani H, Altier C, Simms B, Hamming KS, Snutch TP, Mezeyova J, McRory JE, Zamponi GW. Gating effects of mutations in the Cav3.2 T-type calcium channel associated with childhood absence epilepsy. *J Biol Chem*. 2004;279:9681–9684.
63. Khosravani H, Bladen C, Parker DB, Snutch TP, McRory JE, Zamponi GW. Effects of Cav3.2 channel mutations linked to idiopathic generalized epilepsy. *Ann Neurol*. 2005;57:745–749.
64. Peloquin JB, Khosravani H, Barr W, Bladen C, Evans R, Mezeyova J, Parker D, Snutch TP, McRory JE, Zamponi GW. Functional analysis of Ca3.2 T-type calcium channel mutations linked to childhood absence epilepsy. *Epilepsia*. 2006;47: 655–658.
65. Wang JL, Han CY, Jing YH, Chen YC, Feng N, Lu JJ, Zhang YH, Pan H, Wu HS, Xu KM, Jiang YW, Liang JM, Wang L, Wang XL, Shen Y, Wu XR. The effect of *CACNA1H* gene G773D mutation on calcium channel function. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 2006;23:369–373.
66. Adams PJ, Snutch TP. Calcium channelopathies: voltage-gated calcium channels. *Subcell Biochem*. 2007;45:215–251.
67. Cain SM, Snutch TP. Voltage-gated calcium channels and disease. *Biofactors*. 2011;37:197–205.
68. Klassen T, Davis C, Goldman A, Burgess D, Chen T, Wheeler D, McPherson J, Bourquin T, Lewis L, Villasana D, Morgan M, Muzny D, Gibbs R, Noebels J. Exome sequencing of ion channel genes reveals complex profiles confounding personal risk assessment in epilepsy. *Cell*. 2011;145:1036–1048.
69. Bourinet E, Soong TW, Sutton K, Slaymaker S, Mathews E, Monteil A, Zamponi GW, Nargeot J, Snutch TP. Splicing of alpha 1A subunit gene generates phenotypic variants of P- and Q-type calcium channels. *Nat Neurosci*. 1999;2:407–415.
70. Trimmer JS, Rhodes KJ. Localization of voltage-gated ion channels in mammalian brain. *Annu Rev Physiol*. 2004;66:477–519.
71. Evans RM, Zamponi GW. Presynaptic Ca^{2+} channels—integration centers for neuronal signaling pathways. *Trends Neurosci*. 2006;29:617–624.
72. Imbrici P, Jaffe SL, Eunson LH, Davies NP, Herd C, Robertson R, Kullmann DM, Hanna MG. Dysfunction of the brain calcium channel CaV2.1 in absence epilepsy and episodic ataxia. *Brain*. 2004;127:2682–2692.
73. Jouvenceau A, Eunson LH, Spauschus A, Ramesh V, Zuberi SM, Kullmann DM, Hanna MG. Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. *Lancet*. 2001;358:801–807.
74. Guerin AA, Feigenbaum A, Donner EJ, Yoon G. Stepwise developmental regression associated with novel *CACNA1A* mutation. *Pediatr Neurol*. 2008;39:363–364.
75. Ducros A, Denier C, Joutel A, Cecillon M, Lescoat C, Vahedi K, Darcel F, Vicaut E, Bousser MG, Tournier-Lasserve E. The clinical spectrum of familial hemiplegic migraine associated with mutations in a neuronal calcium channel. *N Engl J Med*. 2001;345:17–24.
76. Zangaladze A, Asadi-Pooya AA, Ashkenazi A, Sperling MR. Sporadic hemiplegic migraine and epilepsy associated with *CACNA1A* gene mutation. *Epilepsy Behav*. 2010;17:293–295.
77. Kors EE, Melberg A, Vanmolkot KR, Kumlien E, Haan J, Raininko R, Flink R, Ginjaar HB, Frants RR, Ferrari MD, van den Maagdenberg AM. Childhood epilepsy, familial hemiplegic migraine, cerebellar ataxia, and a new *CACNA1A* mutation. *Neurology*. 2004;63:1136–1137.
78. Debiais S, Hommet C, Bonnaud I, Barthez MA, Rimbaux S, Riant F, Autret,A. The *FHM1* mutation *S218L*: a severe clinical phenotype? A case report and review of the literature. *Cephalalgia*. 2009;29:1337–1339.
79. Chan YC, Burgunder JM, Wilder-Smith E, Chew SE, Lam-Mok-Sing KM, Sharma V, Ong BK. Electroencephalographic changes and seizures in familial hemiplegic migraine patients with the *CACNA1A* gene *S218L* mutation. *J Clin Neurosci*. 2008;15:891–894.
80. Danober L, Deransart C, Depaulis A, Vergnes M, Marescaux C. Pathophysiological mechanisms of genetic absence epilepsy in the rat. *Prog Neurobiol*. 1998;55:27–57.
81. Marescaux C, Vergnes M, Depaulis A. Genetic absence epilepsy in rats from Strasbourg—a review. *J Neural Transm Suppl*. 1992;35:37–69.
82. Avanzini G, Vergnes M, Spreafico R, Marescaux C. Calcium-dependent regulation of genetically determined spike and waves by the reticular thalamic nucleus of rats. *Epilepsia*. 1993;34:1–7.
83. Talley EM, Solorzano G, Depaulis A, Perez-Reyes E, Bayliss DA. Low-voltage-activated calcium channel subunit expression in a genetic model of absence epilepsy in the rat. *Brain Res Mol Brain Res*. 2000;75:159–165.
84. Tsakiridou E, Bertollini L, de Curtis M, Avanzini G, Pape HC. Selective increase in T-type calcium conductance of reticular thalamic neurons in a rat model of absence epilepsy. *J Neurosci*. 1995;15:3110–3117.
85. Powell KL, Cain SM, Ng C, Sirdesai S, David LS, Kyi M, Garcia E, Tyson JR, Reid CA, Bahlo M, Foote SJ, Snutch TP, O'Brien TJ. A Cav3.2 T-type calcium channel point mutation has splice-variant-specific effects on function and segregates with seizure expression in a polygenic rat model of absence epilepsy. *J Neurosci*. 2009;29:371–380.
86. Zhan XJ, Cox CL, Sherman SM. Dendritic depolarization efficiently attenuates low-threshold calcium spikes in thalamic relay cells. *J Neurosci*. 2000;20:3909–3914.
87. Coenen AM, Van Luijtelaar EL. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. *Behav Genet*. 2003;33:635–655.
88. Broicher T, Kanyshkova T, Meuth P, Pape HC, Budde T. Correlation of T-channel coding gene expression, IT, and the low threshold Ca^{2+} spike in the thalamus of a rat model of absence epilepsy. *Mol Cell Neurosci*. 2008;39:384–399.
89. Broicher T, Kanyshkova T, Landgraf P, Rankovic V, Meuth P, Meuth SG, Pape HC, Budde T. Specific expression of low-voltage-activated calcium channel isoforms and splice variants in thalamic local circuit interneurons. *Mol Cell Neurosci*. 2007;36:132–145.
90. Rimoli MG, Russo E, Cataldi M, Citraro R, Ambrosino P, Melisi D, Curcio A, De Lucia S, Patrignani P, De Sarro G, Abignente E. T-type channel blocking properties and antiabsence activity of two imidazo[1,2-b] pyridazine derivatives structurally related to indomethacin. *Neuropharmacology*. 2009;56:637–646.
91. van Luijtelaar G, Wiaderna D, Elants C, Scheenen W. Opposite effects of T- and L-type Ca^{2+} channels blockers in generalized absence epilepsy. *Eur J Pharmacol*. 2000;406:381–389.

92. Ernst WL, Zhang Y, Yoo JW, Ernst SJ, Noebels JL. Genetic enhancement of thalamocortical network activity by elevating alpha 1g-mediated low-voltage-activated calcium current induces pure absence epilepsy. *J Neurosci*. 2009;29:1615–1625.
93. van de Bovenkamp-Janssen MC, Scheenen WJ, Kuijpers-Kwant FJ, Kozicz T, Veening JG, van Luijtelaar EL, McEnery MW, Roubos EW. Differential expression of high voltage-activated Ca^{2+} channel types in the rostral reticular thalamic nucleus of the absence epileptic WAG/Rij rat. *J Neurobiol*. 2004;58:467–478.
94. Pietrobon D. Function and dysfunction of synaptic calcium channels: insights from mouse models. *Curr Opin Neurobiol*. 2005;15:257–265.
95. Spacey SD, Hildebrand ME, Materek LA, Bird TD, Snutch TP. Functional implications of a novel EA2 mutation in the P/Q-type calcium channel. *Ann Neurol*. 2004;56:213–220.
96. Adams PJ, Garcia E, David LS, Mulatz KJ, Spacey SD, Snutch TP. Ca(V)2.1 P/Q-type calcium channel alternative splicing affects the functional impact of familial hemiplegic migraine mutations: implications for calcium channelopathies. *Channels* (*Austin*). 2009;3:110–121.
97. Jun K, Piedras-Renteria ES, Smith SM, Wheeler DB, Lee SB, Lee TG, Chin H, Adams ME, Scheller RH, Tsien RW, Shin HS. Ablation of P/Q-type Ca(2+) channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the alpha(1A)-subunit. *Proc Natl Acad Sci USA*. 1999;96:15245–15250.
98. Noebels JL. A single gene error of noradrenergic axon growth synchronizes central neurones. *Nature*. 1984;310:409–411.
99. Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD Jr, Hawkes R, Frankel WN, Copeland NG, Jenkins NA. Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell*. 1996;87:607–617.
100. Green MC, Sidman RL. Tottering—a neuromusclar mutation in the mouse. And its linkage with oligosyndacylism. *J Hered*. 1962;53:233–237.
101. Wakamori M, Yamazaki K, Matsunodaira H, Teramoto T, Tanaka I, Niidome T, Sawada K, Nishizawa Y, Sekiguchi N, Mori E, Mori Y, Imoto K. Single tottering mutations responsible for the neuropathic phenotype of the P-type calcium channel. *J Biol Chem*. 1998;273:34857–34867.
102. Ayata C, Shimizu-Sasamata M, Lo EH, Noebels JL, Moskowitz MA. Impaired neurotransmitter release and elevated threshold for cortical spreading depression in mice with mutations in the alpha1A subunit of P/Q type calcium channels. *Neuroscience*. 2000;95:639–645.
103. Caddick SJ, Wang C, Fletcher CF, Jenkins NA, Copeland NG, Hosford DA. Excitatory but not inhibitory synaptic transmission is reduced in *lethargic* (*Cacnb4*(*lh*)) and *tottering* (*Cacna1atg*) mouse thalami. *J Neurophysiol*. 1999;81:2066–2074.
104. Zwingman TA, Neumann PE, Noebels JL, Herrup K. *Rocker* is a new variant of the voltage-dependent calcium channel gene *Cacna1a*. *J Neurosci*. 2001;21:1169–1178.
105. Zhang Y, Mori M, Burgess DL, Noebels JL. Mutations in high-voltage-activated calcium channel genes stimulate low-voltage-activated currents in mouse thalamic relay neurons. *J Neurosci*. 2002;22:6362–6371.
106. Song I, Kim D, Choi S, Sun M, Kim Y, Shin HS. Role of the alpha1G T-type calcium channel in spontaneous absence seizures in mutant mice. *J Neurosci*. 2004;24:5249–5257.
107. Glasscock E, Qian J, Yoo JW, Noebels JL. Masking epilepsy by combining two epilepsy genes. *Nat Neurosci*. 2007;10:1554–1558.
108. Weiergraber M, Henry M, Ho MS, Struck H, Hescheler J, Schneider T. Altered thalamocortical rhythmicity in Ca(v)2.3-deficient mice. *Mol Cell Neurosci*. 2008;39:605–618.
109. Zaman T, Lee K, Park C, Paydar A, Choi JH, Cheong E, Lee CJ, Shin HS. CaV2.3 channels are critical for oscillatory burst discharges in the reticular thalamic nucleus and absence epilepsy. *Neuron*. 2011;70:95–108.
110. Weiergraber M, Henry M, Radhakrishnan K, Hescheler J, Schneider T. Hippocampal seizure resistance and reduced neuronal excitotoxicity in mice lacking the Cav2.3 E/R-type voltage-gated calcium channel. *J Neurophysiol*. 2007;97:3660–3669.
111. Weiergraber M, Henry M, Krieger A, Kamp M, Radhakrishnan K, Hescheler J, Schneider T. Altered seizure susceptibility in mice lacking the Ca(v)2.3 E-type Ca^{2+} channel. *Epilepsia*. 2006;47:839–850.
112. Zamponi GW, Lory P, Perez-Reyes E. Role of voltage-gated calcium channels in epilepsy. *Pflugers Arch*. 2009;460:395–403.
113. Burgess DL, Jones JM, Meisler MH, Noebels JL. Mutation of the Ca^{2+} channel beta subunit gene *Cchb4* is associated with ataxia and seizures in the *lethargic* (*lh*) mouse. *Cell*. 1997;88:385–392.
114. Barclay J, Balaguero N, Mione M, Ackerman SL, Letts VA, Brodbeck J, Canti C, Meir A, Page KM, Kusumi K, Perez-Reyes E, Lander ES, Frankel WN, Gardiner RM, Dolphin AC, Rees M. *Ducky* mouse phenotype of epilepsy and ataxia is associated with mutations in the *Cacna2d2* gene and decreased calcium channel current in cerebellar Purkinje cells. *J Neurosci*. 2001;21:6095–6104.
115. Brodbeck J, Davies A, Courtney JM, Meir A, Balaguero N, Canti C, Moss FJ, Page KM, Pratt WS, Hunt SP, Barclay J, Rees M, Dolphin AC. The *ducky* mutation in *Cacna2d2* results in altered Purkinje cell morphology and is associated with the expression of a truncated alpha 2 delta-2 protein with abnormal function. *J Biol Chem*. 2002;277:7684–7693.
116. Brill J, Klocke R, Paul D, Boison D, Gouder N, Klugbauer N, Hofmann F, Becker CM, Becker K. *entla*, a novel epileptic and ataxic *Cacna2d2* mutant of the mouse. *J Biol Chem*. 2004;279:7322–7330.
117. Letts VA, Felix R, Biddlecome GH, Arikkath J, Mahaffey CL, Valenzuela A, Bartlett FS 2nd, Mori Y, Campbell KP, Frankel WN. The mouse *stargazer* gene encodes a neuronal Ca^{2+}-channel gamma subunit. *Nat Genet*. 1998;19:340–347.
118. Letts VA, Kang MG, Mahaffey CL, Beyer B, Tenbrink H, Campbell KP, Frankel WN. Phenotypic heterogeneity in the *stargazin* allelic series. *Mamm Genome*. 2003;14:506–513.
119. Letts VA, Mahaffey CL, Beyer B, Frankel WN. A targeted mutation in *Cacng4* exacerbates spike-wave seizures in *stargazer* (*Cacng2*) mice. *Proc Natl Acad Sci USA*. 2005;102:2123–2128.
120. Powell KL, Kyi M, Reid CA, Paradiso L, D'Abaco GM, Kaye AH, Foote SJ, O'Brien TJ. Genetic absence

epilepsy rats from Strasbourg have increased corticothalamic expression of *stargazin*. *Neurobiol Dis*. 2008;31:261–265.
121. Cavalheiro EA. The pilocarpine model of epilepsy. *Ital J Neurol Sci.* 1995;16:33–37.
122. Cavalheiro EA, Santos NF, Priel MR. The pilocarpine model of epilepsy in mice. *Epilepsia*. 1996;37:1015–1019.
123. Su H, Sochivko D, Becker A, Chen J, Jiang Y, Yaari Y, Beck H. Upregulation of a T-type Ca^{2+} channel causes a long-lasting modification of neuronal firing mode after status epilepticus. *J Neurosci.* 2002;22:3645–3655.
124. Yaari Y, Yue C, Su H. Recruitment of apical dendritic T-type Ca^{2+} channels by backpropagating spikes underlies de novo intrinsic bursting in hippocampal epileptogenesis. *J Physiol*. 2007;580:435–450.
125. Becker AJ, Pitsch J, Sochivko D, Opitz T, Staniek M, Chen CC, Campbell KP, Schoch S, Yaari Y, Beck H. Transcriptional upregulation of Cav3.2 mediates epileptogenesis in the pilocarpine model of epilepsy. *J Neurosci*. 2008;28:13341–13353.
126. Graef JD, Nordskog BK, Wiggins WF, Godwin DW. An acquired channelopathy involving thalamic T-type Ca^{2+} channels after status epilepticus. *J Neurosci*. 2009;29:4430–4441.
127. Bertram E. The relevance of kindling for human epilepsy. *Epilepsia*. 2007;48(suppl 2):65–74.
128. Faas GC, Vreugdenhil M, Wadman WJ. Calcium currents in pyramidal CA1 neurons in vitro after kindling epileptogenesis in the hippocampus of the rat. *Neuroscience*. 1996;75:57–67.
129. Hendriksen H, Kamphuis W, Lopes da Silva FH. Changes in voltage-dependent calcium channel alpha1-subunit mRNA levels in the kindling model of epileptogenesis. *Brain Res Mol Brain Res*. 1997;50:257–266.
130. Faingold CL. The genetically epilepsy-prone rat. *Gen Pharmacol*. 1988;19:331–338.
131. Jobe PC, Mishra PK, Adams-Curtis LE, Deoskar VU, Ko KH, Browning RA, Dailey JW. The genetically epilepsy-prone rat (GEPR). *Ital J Neurol Sci*. 1995;16:91–99.
132. N'Gouemo P, Faingold CL, Morad M. Calcium channel dysfunction in inferior colliculus neurons of the genetically epilepsy-prone rat. *Neuropharmacology*. 2009;56:665–675.
133. N'Gouemo P, Yasuda R, Faingold CL. Seizure susceptibility is associated with altered protein expression of voltage-gated calcium channel subunits in inferior colliculus neurons of the genetically epilepsy-prone rat. *Brain Res*. 2010;1308:153–157.
134. Weiergraber M, Stephani U, Kohling R. Voltage-gated calcium channels in the etiopathogenesis and treatment of absence epilepsy. *Brain Res Rev*. 2010;62:245–271.
135. Browne TR, Dreifuss FE, Dyken PR, Goode DJ, Penry JK, Porter RJ, White BG, White PT. Ethosuximide in the treatment of absence (peptit mal) seizures. *Neurology*. 1975;25:515–524.
136. Coulter DA, Huguenard JR, Prince DA. Specific petit mal anticonvulsants reduce calcium currents in thalamic neurons. *Neurosci Lett*. 1989;98:74–78.
137. Coulter DA, Huguenard, JR, Prince, DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann Neurol.* 1989;25:582–593.
138. Broicher T, Seidenbecher T, Meuth P, Munsch T, Meuth SG, Kanyshkova T, Pape HC, Budde T. T-current related effects of antiepileptic drugs and a Ca^{2+} channel antagonist on thalamic relay and local circuit interneurons in a rat model of absence epilepsy. *Neuropharmacology.* 2007;53:431–446.
139. Leresche N, Parri HR, Erdemli G, Guyon A, Turner JP, Williams SR, Asprodin, E, Crunelli V. On the action of the anti-absence drug ethosuximide in the rat and cat thalamus. *J Neurosci.* 1998;18:4842–4853.
140. Crunelli V, Leresche, N. Block of thalamic T-Type Ca(2+) channels by ethosuximide is not the whole story. *Epilepsy Curr.* 2002;2:53–56.
141. Bourinet E, Alloui A, Monteil A, Barrere C, Couette B, Poirot O, Pages A, McRory J, Snutch TP, Eschalier A, Nargeot J. Silencing of the Cav3.2 T-type calcium channel gene in sensory neurons demonstrates its major role in nociception. *EMBO.* 2005;24:315–324.
142. Kostyuk PG, Molokanova EA, Pronchuk NF, Savchenko AN, Verkhratsky AN. Different action of ethosuximide on low- and high-threshold calcium currents in rat sensory neurons. *Neuroscience*. 1992;51:755–758.
143. Todorovic SM, Lingle CJ. Pharmacological properties of T-type Ca2+ current in adult rat sensory neurons: Effects of anticonvulsant and anesthetic agents. *J Neurophysiol.* 1998;79:240–252.
144. Mudado MA, Rodrigues AL, Prado VF, Beirao PS, Cruz JS. CaV 3.1 and CaV 3.3 account for T-type Ca2+ current in GH3 cells. *Braz J Med Biol Res.* 2004;37:929–935.
145. Herrington J, Lingle CJ. Kinetic and pharmacological properties of low voltage-activated Ca2+ current in rat clonal (GH3) pituitary cells. *J Neurophysiol.* 1992;68:213–232.
146. Gomora JC, Daud AN, Weiergraber M, Perez-Reyes E. Block of cloned human T-type calcium channels by succinimide antiepileptic drugs. *Mol Pharmacol.* 2001;60:1121–1132.
147. Richards DA, Manning JP, Barnes D, Rombola L, Bowery NG, Caccia S, Leresche N, Crunelli V. Targeting thalamic nuclei is not sufficient for the full anti-absence action of ethosuximide in a rat model of absence epilepsy. *Epilepsy Res.* 2003;54: 97–107.
148. Manning JP, Richards DA, Leresche N, Crunelli V, Bowery NG. Cortical-area specific block of genetically determined absence seizures by ethosuximide. *Neuroscience.* 2004;123:5–9.
149. Polack PO, Charpier S. Ethosuximide converts ictogenic neurons initiating absence seizures into normal neurons in a genetic model. *Epilepsia.* 2009;50:1816–1820.
150. Gulhan Aker R, Tezcan, K, Carcak, N, Sakalli, E, Akin, D, Onat, FY. Localized cortical injections of ethosuximide suppress spike-and-wave activity and reduce the resistance to kindling in genetic absence epilepsy rats (GAERS). *Epilepsy Res.* 2009;89:7–16.
151. Kelly KM, Gross RA, Macdonal, RL. Valproic acid selectively reduces the low-threshold (T) calcium current in rat nodose neurons. *Neurosci Lett.* 1990;116:233–238.
152. Rogawski MA, Porter RJ. Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. *Pharmacol Rev.* 1990;42:223–286.

153. Kwan P, Sills GJ, Brodie MJ. The mechanisms of action of commonly used antiepileptic drugs. *Pharmacol Ther.* 2001;90:21–34.
154. Biton V. Clinical pharmacology and mechanism of action of zonisamide. *Clin Neuropharmacol.* 2007;30:230–240.
155. Suzuki S, Kawakami K, Nishimura S, Watanabe Y, Yagi K, Seino M, Miyamoto K. Zonisamide blocks T-type calcium channel in cultured neurons of rat cerebral cortex. *Epilepsy Res.* 1992;12:21–27.
156. Kito M, Maehara M, Watanabe K. Mechanisms of T-type calcium channel blockade by zonisamide. *Seizure.* 1996;5:115–119.
157. Matar N, Jin W, Wrubel H, Hescheler J, Schneider T, Weiergraber M. Zonisamide block of cloned human T-type voltage-gated calcium channels. *Epilepsy Res.* 2009;83:224–234.
158. Rambeck B, Wolf, P. Lamotrigine clinical pharmacokinetics. *Clin Pharmacokinet.* 1993;25:433–443.
159. Walker MC, Tong X, Perry H, Alavijeh MS, Patsalos PN. Comparison of serum, cerebrospinal fluid and brain extracellular fluid pharmacokinetics of lamotrigine. *Br J Pharmacol.* 2000;130:242–248.
160. Martella G, Costa C, Pisani A, Cupini LM, Bernardi G, Calabresi P. Antiepileptic drugs on calcium currents recorded from cortical and PAG neurons: Therapeutic implications for migraine. *Cephalalgia.* 2008;28:1315–1326.
161. Stefani A, Spadoni F, Siniscalchi A, Bernardi G. Lamotrigine inhibits Ca2+ currents in cortical neurons: Functional implications. *Eur J Pharmacol.* 1996;307:113–116.
162. Wang SJ, Huan, CC, Hsu KS, Tsai JJ, Gean PW. Inhibition of N-type calcium currents by lamotrigine in rat amygdalar neurones. *Neuroreport.* 1996;7:3037–3040.
163. Hainsworth AH, McNaughton NC, Pereverzev A, Schneider T, Randal, AD. Actions of sipatrigine, 202W92 and lamotrigine on R-type and T-type Ca2+ channel currents. *Eur J Pharmacol.* 2003;467:77–80.
164. Twombly DA, Yoshii M, Narahashi T. Mechanisms of calcium channel block by phenytoin. *J Pharmacol Exp Ther.* 1988;246:189–195.
165. Liu L, Zheng T, Morris MJ, Wallengren C, Clarke AL, Reid CA, Petrou S, O'Brien TJ. The mechanism of carbamazepine aggravation of absence seizures. *J Pharmacol Exp Ther.* 2006;319:790–798.
166. Ambrosio AF, Silva AP, Malva JO, Soares-da-Silva P, Carvalho AP, Carvalho CM. Carbamazepine inhibits L-type Ca2+ channels in cultured rat hippocampal neurons stimulated with glutamate receptor agonists. *Neuropharmacology.* 1999;38:1349–1359.
167. Zhang X, Velumian AA, Jones OT, Carlen PL. Modulation of high-voltage-activated calcium channels in dentate granule cells by topiramate. *Epilepsia.* 2000;41:S52–S60.
168. Christensen J, Andreasen F, Poulsen JH, Dam M. Randomized, concentration-controlled trial of topiramate in refractory focal epilepsy. *Neurology.* 2003;61:1210–1218.
169. Christensen J, Hojskov CS, Dam M, Poulsen JH. Plasma concentration of topiramate correlates with cerebrospinal fluid concentration. *Ther Drug Monit.* 2001;23:529–535.
170. Russo E, Constanti A, Ferreri G, Citraro R, De Sarro G. Nifedipine affects the anticonvulsant activity of topiramate in various animal models of epilepsy. *Neuropharmacology.* 2004;46:865–878.
171. Kuzmiski JB, Barr W, Zamponi GW, MacVicar BA. Topiramate inhibits the initiation of plateau potentials in CA1 neurons by depressing R-type calcium channels. *Epilepsia.* 2005;46:481–489.
172. Kasteleijn-Nolst Trenite DG, Marescau, C, Stodieck S, Edelbroek PM, Oosting J. Photosensitive epilepsy: a model to study the effects of antiepileptic drugs. Evaluation of the piracetam analogue, levetiracetam. *Epilepsy Res.* 1996;25:225–230.
173. Lee CY, Chen CC, Liou HH. Levetiracetam inhibits glutamate transmission through presynaptic P/Q-type calcium channels on the granule cells of the dentate gyrus. *Br J Pharmacol.* 2009;158:1753–1762.
174. Sills GJ. The mechanisms of action of gabapentin and pregabalin. *Curr Opin Pharmacol.* 2006;6: 108–113.
175. Marais E, Klugbauer N, Hofmann F. Calcium channel alpha(2)delta subunits-structure and gabapentin binding. *Mol Pharmacol.* 2001;59:1243–1248.
176. Qin N, Yagel S, Momplaisir ML, Codd EE, D'Andrea MR. Molecular cloning and characterization of the human voltage-gated calcium channel alpha(2)delta-4 subunit. *Mol Pharmacol.* 2002;62:485–496.
177. Rock DM, Kelly KM, Macdonald RL. Gabapentin actions on ligand- and voltage-gated responses in cultured rodent neurons. *Epilepsy Res.* 1993;16:89–98.
178. Brown JT, Randall A. Gabapentin fails to alter P/Q-type Ca2+ channel-mediated synaptic transmission in the hippocampus in vitro. *Synapse.* 2005;55:262–269.
179. Stefani A, Spadoni F, Giacomin, P, Lavaroni F, Bernardi G. The effects of gabapentin on different ligand- and voltage-gated currents in isolated cortical neurons. *Epilepsy Res.* 2001;43:239–248.
180. Fink K, Dooley DJ, Meder WP, Suman-Chauhan N, Duffy S, Clusmann H, Gothert M. Inhibition of neuronal Ca(2+) influx by gabapentin and pregabalin in the human neocortex. *Neuropharmacology.* 2002;42:229–236.
181. Fink K, Meder W, Dooley DJ, Gothert M. Inhibition of neuronal Ca(2+) influx by gabapentin and subsequent reduction of neurotransmitter release from rat neocortical slices. *Br J Pharmacol.* 2000;130:900–906.
182. Sutton KG, Martin DJ, Pinnock RD, Lee K, Scott RH. Gabapentin inhibits high-threshold calcium channel currents in cultured rat dorsal root ganglion neurones. *Br J Pharmacol.* 2002;135:257–265.

Chapter 7

Hyperpolarization-Activated Cyclic Nucleotide-Gated (HCN) Ion Channelopathy in Epilepsy

Nicholas P. Poolos

Hyperpolarization-activated, cyclic nucleotide-gated (HCN) ion channels represent a unique class of voltage-gated ion channels. Initially characterized in the heart as "pacemaker" channels,[1] they are now understood to be an essential modulator of neuronal excitability in cortex, hippocampus, and thalamus. Their diverse contributions to neuronal excitability stem from a constellation of unusual features: they are both voltage- and ligand-gated; they open with hyperpolarization of membrane potential rather than with depolarization; and in the principal neurons of cortex and hippocampus, they are localized almost exclusively to the apical dendrites, where they exert a strong influence on the flow of excitatory synaptic inputs to the cell soma. Because of the influence of HCN channels on neuronal physiology, they also play an important role in epilepsy. Genetic deletion of the HCN2 channel subtype causes absence epilepsy in mice,[2] while deletion of the HCN1 subtype exerts a proconvulsive effect and accelerates epileptogenesis.[3] Loss of HCN1 channel expression and function also occurs during epileptogenesis in animal models of acquired epilepsy, contributing to neuronal hyperexcitability and promoting further seizures.[4] Conversely, upregulation of HCN channel function by antiepileptic drugs may constitute part of their anticonvulsant mechanism of action.[5] Thus, substantial new evidence that has emerged just in the past 10 years links HCN channel dysfunction with epilepsy.

In this chapter, I describe how the unique biophysical properties of HCN channels lead to an influential role in seizure generation; whether recent evidence truly supports the existence of HCN channelopathy in human epilepsy; and how the mechanisms underlying acquired HCN dysfunction could be targeted by antiepileptic therapies.

HCN CHANNEL BIOPHYSICAL PROPERTIES

HCN channels are voltage-gated ion channels that structurally resemble K^+ channels, with a six-transmembrane domain topology, including a pore region that conducts ion flow. However, HCN channels possess biophysical properties that make them virtually unique in comparison to other voltage-gated channels.[6] First, despite structural similarity to K^+ channels, HCN channels are relatively less selective for K^+ ions, allowing inward passage of Na^+ ions. Because at typical neuronal resting potential the driving force for Na^+ is so much greater than for K^+, HCN channels primarily conduct Na^+ current under physiological conditions, thus depolarizing neuronal membrane potential. Second, the voltage-dependent activation of HCN channels is also anomalous compared to most other channels: HCN channels are fractionally open at resting potential, and their activation increases with hyperpolarization from rest rather than with depolarization, as is common with other channels. Thus, neuronal depolarization tends to turn off HCN channels, while hyperpolarization tends to activate them. Third, HCN channels do not display inactivation, and thus are constitutively active around the resting potential. The current mediated by HCN channels, I_h, is estimated to comprise about half of the resting conductance of many neuron types. This allows HCN channels to exert a strong influence on the passive properties of the neuron, such as resting potential and input resistance. (The term *passive*, of course, is a misnomer, since these properties are modulated by HCN and other voltage-gated channels that by definition are active conductances.) Fourth, HCN channels open remarkably slowly, with activation time constants that range from tens to hundreds of milliseconds, that is, several orders of magnitude slower than those of most ion channels. Finally, HCN channels are partly gated by intracellular levels of cyclic nucleotides such as cyclic adenosine 3′,5′-monophosphate (cAMP). This allows channel activity to be modulated by both voltage and intracellular second messengers.

The net result of these biophysical features is a current that inherently stabilizes the neuron at its resting potential, minimizing the influence of synaptic inputs. When the neuron becomes depolarized by a synaptic input, the tonic depolarizing Na^+ current mediated by HCN channels is turned off, since the channels deactivate with depolarization. This hyperpolarizes the membrane potential back towards rest. Conversely, a hyperpolarizing input (such as an inhibitory postsynaptic potential, IPSP) will turn on I_h, depolarizing the neuron back toward rest. Thus, I_h displays an inherent negative-feedback property that imparts a stabilizing effect on neuronal excitability. While it might seem that this stabilizing action might equally apply to excitation and inhibition, it turns out that I_h will disproportionately modulate these two types of synaptic inputs, depending on how the conductance is distributed throughout the cell. One of the most intriguing themes to emerge in the last 15 years of research on ion channel function is the nonuniformity of ion channel distribution within the neuron, particularly in pyramidal neurons. HCN channels represent a prime example of this nonuniform or segregated distribution at a subcellular level, causing them to disproportionately diminish the impact of excitatory postsynaptic potentials (EPSPs). This is discussed further below.

HCN channels are encoded by four separate genes, *HCN1–4*.[7] Ion channels encoded by each of the isoforms have differing biophysical properties (such as speed of gating and sensitivity to cAMP), and are differentially distributed throughout the brain. HCN1 and HCN2 are the main brain isoforms, with HCN1 predominant in the neocortex and hippocampus and HCN2 predominant in the thalamus. HCN3 has a diffuse but low-level distribution in the brain, while HCN4 is present mostly in thalamic relay neurons.[6] In this review, we will mainly consider HCN1 as the cortical/hippocampal subtype; it has relatively fast activation times (tens of milliseconds) but virtual insensitivity to cAMP. HCN2 is the main subcortical (e.g., thalamic) isoform, with an intermediate (several hundreds of milliseconds)

activation time constant and a depolarizing shift in its voltage dependence on exposure to cAMP. As described below, these biophysical differences among HCN subtypes account for their functional roles in the brain regions in which they are found.

HCN CHANNEL EFFECTS ON NEURONAL EXCITABILITY

As discussed above, HCN channels tend to stabilize neuronal membrane potential against either excitatory or inhibitory inputs. Interestingly, their slow activation time course, particularly for the HCN2 and HCN4 subtypes, can be exploited to produce membrane potential oscillations. This occurs when an inward current that activates at hyperpolarized potentials, such as the T-type Ca^{2+} current, is paired with I_h. Indeed, the first characterization of HCN channels was in the sinoatrial node of the heart, where HCN2 and HCN4 channels help set the frequency of firing that produces the sinus rhythm. In fact, it is the modulation of I_h by changes in intracellular cAMP concentration that contributes to the autonomic control of heart rate by β-adrenergic and cholinergic receptor activation.[1] A similar oscillatory function occurs in thalamocortical projection neurons that underlie synchronization of cortical rhythms seen in sleep and in primarily generalized seizures such as absence seizures.[8] An interesting feature of this interaction is that HCN channels need to function in a narrow range of activity in order to mediate oscillations. Either downregulation or upregulation of steady-state I_h has the potential to abolish oscillations.[9] Similarly, blockade of the T-type Ca^{2+} channels will abolish the thalamocortical burst firing underlying absence seizures, a well-described mechanism of action of the antiepileptic drug (AED) ethosuximide (ETX).[10] Because of these contributions to oscillatory activity, HCN channels have often been labeled *pacemaker* currents. However, in principal neurons of cortex and hippocampus, their role is quite different.

In neocortical and hippocampal pyramidal neurons, the actions of HCN channels have been intensely investigated over the past decade. As was first described in 1998, HCN channels in pyramidal neurons show a strikingly nonuniform pattern of distribution: rather than being homogeneously distributed across the cell membrane, they are instead arrayed in a gradient pattern along the somatodendritic axis, being present at low levels in the cell body but at increasingly high density (7- to 10-fold compared to the soma) in the apical dendrites.[11] The high dendritic density of HCN channels places them in proximity to excitatory inputs, the vast majority of which arrive in the dendrites. Because HCN channels are open at rest, they diminish the input resistance of the dendrites to incoming synaptic currents, decreasing the voltage change produced by an EPSP; conversely, when HCN channels are inactivated, input resistance is higher, and EPSPs are increased in magnitude. In essence, I_h makes for "leaky" dendrites that do not faithfully transmit excitatory inputs. While I_h also causes resting potential depolarization that opposes its inhibitory effect on action potential firing, it appears than in pyramidal neurons the high dendritic density of I_h and its attenuating action on synaptic inputs (particularly repetitive inputs) predominate. This is illustrated in Fig. 7–1, where it can be seen that the inhibitory actions of I_h on input resistance and EPSP summation outweigh its excitatory actions on resting potential.

In the early years following the first characterization of I_h in central nervous system (CNS) neurons, much was made of its dual influence on neuronal excitability, with suggestions by more than one reviewer of the field that I_h could be described as neither an excitatory nor an inhibitory conductance.[12,13] Now, with many investigations of its action published, it is clear that I_h has a net inhibitory action in the principal neurons of cortex and hippocampus: it reduces temporal summation (TS) and action potential (AP) firing from dendritic EPSPs; it inhibits forward propagation of dendritic APs and increases the threshold for dendritic calcium spikes; and dendritic HCN channels reduce acquisition of learning and memory in several in vivo paradigms. These findings are summarized in Table 7–1.

In nonpyramidal inhibitory interneurons of cortex and hippocampus, it appears that I_h has a predominantly somatic localization; therefore, its influence on excitability is mediated more through its depolarization of the resting potential, opposite to its action in principal neurons.[14] Thus, inhibition of HCN

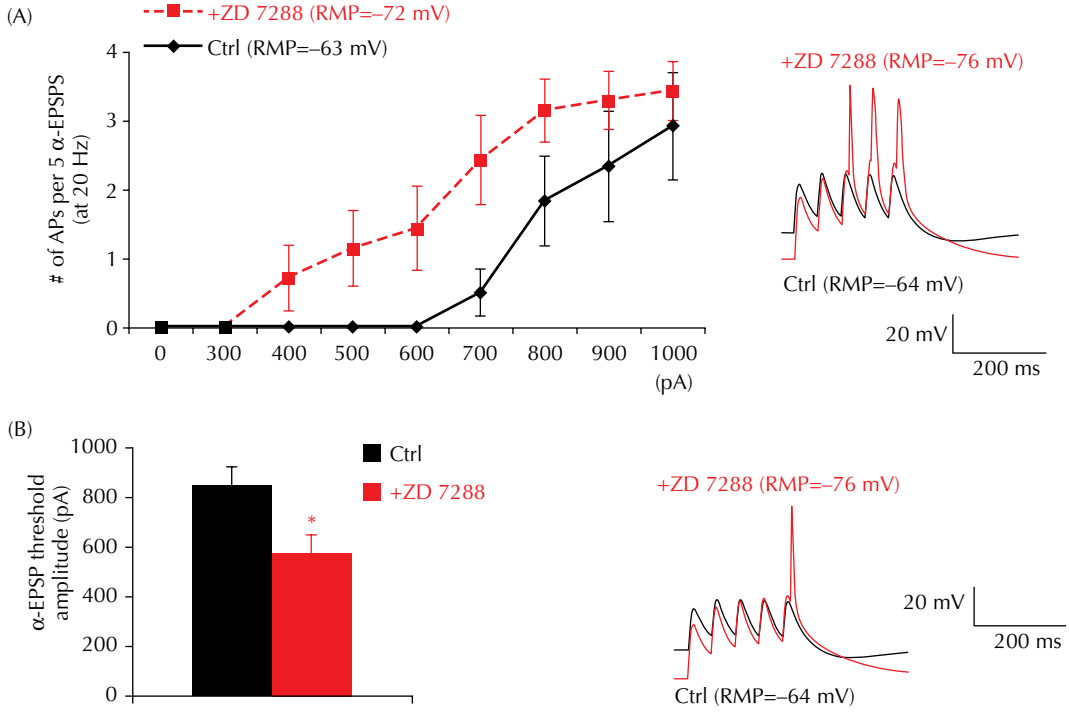

Figure 7–1. Blockade of HCN channels increases excitability in pyramidal neurons. **A**. Action potential (AP) firing elicited by current injection of α waveforms during dendritic current clamp recordings (~200 µm from the soma) under control conditions and after blockade of HCN channels with ZD 7288. Despite the hyperpolarization of the resting membrane potential (RMP) that occurs after HCN channel blockade, increased temporal summation produces increased AP firing through a wide range of α-EPSP amplitudes. **B**. Measurement of the threshold α-EPSP amplitude needed to produce a single AP shows that the threshold is decreased following HCN channel blockade, again showing increased excitability.

Table 7–1 Actions of HCN Channels in Pyramidal Neurons

Neuron	Action of HCN Channels on Excitability
CA1	Reduces and normalizes TS; reduces AP firing from dendritic inputs;[5,11,48,63] inhibits dendritic Ca^{2+} spikes[64]
	Reduces LTP and spatial learning;[65] reduces intrinsic excitability after LTP via CAMKII;[66] loss of I_h increases intrinsic excitability after LTD via mGluR[67]
	Increases rebound AP firing after hyperthermia-induced seizures[31,39]
	Loss of I_h post-SE increases TS and AP firing[4]
NC	Reduces and normalizes TS;[68,69] inhibits dendritic calcium spikes;[70] inhibits dendritic calcium spikes and burst firing in WAG/Rij epileptic rats[26,27]
EC	Inhibits AP firing; loss of HCN post-KA increases excitability;[33] inhibits AP firing after D1 receptor activation[71]
	Knockout of HCN1 increases excitability and sensitivity to convulsants[3]
PFC	Reduces spatial learning in primates and rats; I_h blockers in vivo increase neuronal firing;[72] inhibits excitability and dendritic calcium spikes; α2-NARs inhibit I_h and increase excitability[73,74]

Abbreviations: AP, action potential; CA1, hippocampus cornu ammonis area 1; CAMKII, Ca2+/calmodulin-dependent protein kinase II; D1, dopamine receptor type 1; EC, entorhinal cortex; KA, kainic acid; LTD, long-term depression; LTP, long-term potentiation; mGluR, metabotropic glutamate receptor; NAR, noradrenergic receptor; NC, neocortex; PFC, prefrontal cortex; TS, temporal summation.

channels in interneurons reduces AP output, while activation of HCN channels increases firing. However, this opposite sign of HCN influence in inhibitory neurons is concordant with its excitatory effect in principal neurons: both actions of HCN channels serve to diminish the overall excitability of cortex and hippocampus.

CONTRIBUTION OF HCN CHANNELS TO EPILEPSY

Evidence for Human HCN Channelopathy

Evidence for HCN channelopathy in human epilepsy is thus far limited. Since virtually all clear-cut evidence for any ion channelopathy in epilepsy derives from the inherited or genetic epilepsies, we must first consider these conditions. There are no descriptions yet of genetic epilepsy with Mendelian inheritance of an HCN channel mutation, as have been reported for certain Na^+, K^+, and other voltage-gated ion channels.[15] This, of course, does not mean that such mutations do not exist, and it is possible that large-scale screening projects such as the Epilepsy Phenome-Genome Project will uncover them. Since only a small fraction of all epilepsies display Mendelian patterns of inheritance, if HCN channels are to be implicated in genetic epilepsy, they are more likely to be implicated as a polygenic or susceptibility trait. Several medium-scale screening projects of sporadic epilepsy have reported polymorphisms in HCN genes occurring at higher frequency in case versus control patients. One study of 84 patients with idiopathic generalized epilepsy identified a single *HCN1* polymorphism (*A881T*) that was not identified in 510 controls. There was a far higher degree of sequence variation in the *HCN2* gene; however, there were only two nonsynonymous mutations.[16] One of these, *R527Q*, was analyzed using heterologous expression; I_h generated from the mutant channels was found to have biophysical properties similar to those from wild-type channels. A three-proline deletion in *HCN2* identified in one patient in the first study was also independently observed in another study of patients with idiopathic generalized epilepsy (IGE) or febrile seizures (FS).[17] This variant was found in 3 out of 65 (2.3%) unrelated epilepsy patients, all of whom had FS, but in 0 of 72 patients with IGE and 3 of 772 controls. Analysis of expressed mutant channels suggested an increase in I_h. Again, since this particular mutation has not thus far been found to cosegregate with disease, it cannot at this point be regarded as causative of epilepsy but only as a possible susceptibility trait.

It is worth noting that several studies have identified *HCN4* mutations in association with inherited cardiac arrhythmia.[18,19] Thus, at present, the strongest association of HCN channelopathy and disease is for this subtype, which is minimally expressed in the adult brain.

Investigation of HCN channel expression in human brain tissue from patients with epilepsy has similarly been limited. A comparison of HCN channel mRNA expression from temporal lobe resections overall found no significant change in comparison to autopsy controls; however, a subgroup of patients with the greatest degree of hippocampal sclerosis appeared to have an increase in *HCN1* expression limited to the dentate gyrus (DG).[20] This finding was surprising, since DG neurons normally demonstrate very little I_h, and was interpreted as a potential "compensatory" upregulation of expression in the most severely affected patients. Other investigators found that I_h magnitude measured in neocortical neurons from brain tissue acutely removed during epilepsy surgery inversely depended on the frequency of presurgical seizures, suggesting that more severe epilepsy was associated with a loss of neocortical HCN channel function.[21] In this study, however, no control comparisons were made, a common limitation of studies involving live human tissue.

In summary, the human evidence for genetic HCN channelopathy in epilepsy is thus far anecdotal. However, a significant body of evidence obtained from animal modeling suggests that HCN channelopathy could be causative of genetic epilepsy and develops in the setting of acquired epilepsy as well.

HCN Channels in Animal Models of Genetic Epilepsy

The above human studies provide suggestive, but still anecdotal, evidence for human genetic HCN channelopathy. Studies in animal models

of genetic deletion of HCN channels advance a far more compelling case that this ion channel may be relevant to epilepsy. Constitutive knockout of the *hcn2* gene produced a phenotype consistent with the high density of the HCN2 subunit in the thalamus: a tendency of thalamocortical neurons studied in brain slices to fire bursts of action potentials, and spontaneous absence seizures, marked by generalized 5 Hz spike-wave discharges, detected in the mutant animals.[2] These mice also displayed a cardiac sinus arrhythmia, consistent with loss of HCN2 channel expression from sinoatrial node cells.

Two studies have examined *hcn1* deletion for evidence of epilepsy.[3,22] Neither of these studies detected spontaneous seizures in knockout animals. However, both studies demonstrated that *hcn1* deletion increased the severity of seizures, whether provoked by kindling or chemoconvulsants, with a high rate of death from status epilepticus (SE). In the kainic acid (KA) model of epilepsy, even after the dose of KA was halved to reduce death from SE, the latency period from SE to the occurrence of the first spontaneous seizure was shortened from 386 h to 60 h.[3] This study went one step further to examine pyramidal neuron excitability in *hcn1* knockout mice. Consistent with prior work showing an inhibitory effect of HCN channels on excitability in cortex and hippocampus (Table 7–1), pyramidal neurons lacking the principal HCN subunit mediating I_h demonstrated both increased intrinsic excitability and prolonged excitatory responses to synaptic stimulation. Both studies confirmed the role of HCN channels as exerting an inhibitory and even an anticonvulsant influence on cortical and hippocampal excitability. That *hcn1* deletion produces cortical and hippocampal hyperexcitability while not producing epilepsy is interesting and at this point not subject to easy explanation. One possible explanation is that constitutive deletion of HCN1 channels leads to compensatory upregulation of tonic $GABA_A$ receptor-mediated current that may partially suppress hyperexcitability.[23] Use of conditional knockout animals of HCN channels might help support or disprove this idea. A similar situation is reported for the K^+ channel subunit Kv4.2, a predominantly dendritic subunit that exerts a significant influence on neuronal excitability. In fact, deletion of the gene encoding for Kv4.2 also does not result in epilepsy.[24]

HCN channel dysfunction has also been identified in inbred rodent models of genetic epilepsy. One such model of absence epilepsy, the Wistar Albino Glaxo/Rij (WAG/Rij) rat, shows loss of HCN channel function. WAG/Rij rats display spontaneous spike-wave discharges associated with behavioral absence-like episodes, with seizures appearing to begin from a cortical focus, then generalizing via rapid intracortical spread.[25] The cortical origin of seizures has been found to correlate with loss of I_h in neocortical neurons, accompanied by a loss of HCN1 protein expression;[26] this loss of HCN1-mediated currents was confined to the dendrites of neocortical neurons, progressed during development, and paralleled the onset of behavioral seizures.[27] Neither of these studies addressed the question of whether the changes in HCN channel expression and function were the cause or effect of seizures in this animal model (that most likely has numerous gene mutations contributing to epilepsy); however, a subsequent study suppressed developmental seizures in the WAG/Rij rat with ETX administration for the first 5 months of life, then measured changes in HCN1 expression as well as that of two Na^+ channel proteins known to be dysregulated in this model, Nav1.1 and Nav1.6.[26] Surprisingly, epilepsy-associated changes in all three ion channels were reversed when seizures were chronically suppressed, and although spontaneous seizures recurred when ETX treatment was stopped, the time course of their development was markedly prolonged.[28] These intriguing results suggest that HCN1 channels in the WAG/Rij model of epilepsy, while not causative of epilepsy, may potentially contribute to the course of epileptogenesis by amplifying the effect of spontaneous seizures. This phenomenon in other contexts has been referred to as "seizures beget seizures" and may be relevant in acquired models of epilepsy as well, as discussed below.[29]

HCN Channel Downregulation in Animal Models of Acquired Epilepsy

It is interesting that the first studies to link HCN channels and epilepsy were landmark publications that launched much of the future investigation in the field, yet turned out not to

predict subsequent developments. This work used a newly characterized model of febrile seizures in which immature rats were exposed to high temperature, provoking SE.[30] This stimulus produced an unexpected, long-lasting increase in GABAergic inhibition in CA1 pyramidal neurons accompanied by an increase in I_h measured at the soma. It was suggested that hyperexcitability might result following IPSPs as the increased I_h triggered rebound AP firing.[31] However, follow-up work using the same model but investigating regulation of the *hcn1* gene transcription and protein production found persistent downregulation of expression; *hcn2* was transiently upregulated, then returned to baseline.[32]

Subsequent work in other animal models of acquired epilepsy has consistently found downregulation of HCN channel expression and function, changes that were opposite to the upregulation of I_h initially seen in hyperthermia model. The first study to look at I_h changes in a model of SE induced by KA used whole-cell patch clamp recordings in the dendrites of entorhinal cortical neurons.[33] This was an important advance in methodology since, as described above, the vast majority of HCN channels are localized to the dendrites of pyramidal neurons, raising the possibility that in epilepsy they are differentially regulated compared to somatic channels. (Most subsequent studies have used dendritic patch clamp recording to study changes in I_h in epilepsy.) These authors found that dendritic excitability increased in an HCN channel-dependent fashion within 24 h of KA-induced SE and remained so at 1 week post-SE, demonstrating an early change in HCN channel function that promoted hyperexcitability.

Subsequent work tracked changes in I_h during the development of epilepsy and confirmed the association of decreased I_h during epileptogenesis. When dendritic recordings were made from CA1 pyramidal neurons in animals exposed to pilocarpine-induced SE, I_h was significantly reduced at two different time points, an acute period 1 week post-SE when the animals, as verified by electroencephalographic (EEG) recordings, started to manifest spontaneous seizures, and at 1 month after SE, when the animals were chronically epileptic.[4] In both cases, there were two changes in I_h properties that reduced its overall magnitude: a reduction of dendritic I_h density that was reflected in a loss of HCN protein expression, and a hyperpolarizing shift in I_h activation that reduced the amount of current active at rest. The downregulation of I_h gating progressively worsened as seizure frequency increased from the 1 week to the 1 month time points, while the loss of I_h density was constant. Both of these changes were associated with increased excitability of CA1 pyramidal neurons.

A second study essentially replicated these findings, and further observed that loss of I_h altered the intrinsic resonance of pyramidal neurons for synaptic excitation at theta frequencies,[34] possibly underlying deficits in hippocampal-dependent memory tasks that accompany temporal lobe epilepsy.[35] Similar chronic loss of I_h was observed following KA-induced SE, although the authors also observed a transient (1–2 days post-SE) increase in I_h at the soma.[36] Loss of I_h and HCN channel expression has been observed in other animal models of epilepsy, including perinatal hypoxia[37] and cortical dysplasia.[38] This suggests that the loss of I_h seen in chemoconvulsant models is not model-specific and may be a general feature of animal models of epilepsy.

It is not entirely clear why the discordant result of increased I_h was observed in the initial hyperthermia model. It is probably not explained by recordings done exclusively at the soma in those first studies, as a subsequent report using dendritic recordings in hyperthermia-exposed animals found a similar upregulation of I_h.[39] Possibly the discrepancy results from the mild epilepsy phenotype that results from hyperthermia-provoked SE, yielding only brief electrographic seizures in a minority of animals and at later time points than those studied in the original description.[40] By contrast, the chemoconvulsant models produce a much more robust epilepsy phenotype with a rapid developmental onset.

Mechanisms of HCN Channel Downregulation in Acquired Epilepsy

The findings in the pilocarpine model revealed that HCN channelopathy in acquired epilepsy consists of two separate mechanisms of ion channel dysfunction: a loss of I_h current density manifested by reduced HCN1 protein expression,

and a downregulation of voltage-dependent gating of the remaining channels. It is important to ask whether either or both of these phenomena are the cause or the effect of epilepsy. This question was at least partially answered by controlling seizures with phenobarbital administration for the first week post-SE and then measuring I_h properties.[4] Preventing spontaneous seizures reversed the hyperpolarizing shift in I_h gating, demonstrating that this was a seizure-dependent phenomenon. The loss of I_h density and HCN1 protein expression, however, was independent of ongoing seizures. Recent evidence shows that the loss of HCN channels begins as rapidly as 1 h post-SE, well before the onset of spontaneous seizures, and thus may be a contributor to the development of the epileptic condition.[41] While gating changes in the remaining channels are caused by seizures rather than the reverse, it is conceivable that by promoting neuronal hyperexcitability, this HCN channelopathy mechanism could contribute to the gradual run-up in seizure frequency that occurs during epileptogenesis. Some support for this latter idea comes from the observation that *HCN1* knockout mice have a much more rapid development of epilepsy after chemoconvulsant-induced SE than wild-type mice.[3]

What are the molecular underpinnings of the separate processes producing HCN channelopathy in epilepsy? For the loss of HCN channel expression, it is clear that transcriptional downregulation is at least one mechanism. Several investigators have validated the loss of *HCN1* mRNA expression at time points beginning 3 days after SE and persisting into the period of chronic epilepsy.[32,34,42] In an in vitro model of epilepsy using KA administration in organotypic slice cultures, this loss of *HCN1* transcription was dependent on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) activation,[43] although in vivo involvement of these entities is unknown. Whether transcriptional downregulation is the earliest process leading to loss of HCN channel expression is unclear. HCN channels, like other ion channels, are subject to dynamic regulation of their membrane localization and stability. The best-described mechanism involves an accessory protein, tetratricopeptide-repeat containing Rab8b interacting protein (TRIP8b). This protein, expressed as a multitude of alternatively spliced isoforms, interacts with HCN1 channels and stabilizes their somatodendritic expression.[44–46] It appears that the interaction between Trip8B and HCN1 channels is reduced in epilepsy, but this process does not appear to begin prior to the onset of transcriptional downregulation.[36]

More is known about the processes underlying the downregulation of HCN channel gating in epilepsy. It is well known that although cAMP upregulates the gating of HCN2 and HCN4 channels, the HCN1 isoform is largely insensitive.[47] However, the gating of HCN1 channels is modulated by phosphorylation, notably by p38 mitogen-activated kinase (p38 MAPK), with kinase activation producing a depolarizing (upregulating) shift in gating and kinase inhibition producing the opposite effect.[48] Dephosphorylation by the serine-threonine phosphatase calcineurin (CaN) produces concordant effects on HCN1 gating, with increased phosphorylation upregulating gating and decreased phosphorylation downregulating it.[49] Since HCN channel gating is downregulated in chronic epilepsy, it would be reasonable to ask whether these phosphorylation pathways are dysregulated as well; in fact, p38 MAPK is relatively deactivated in epileptic tissue, while CaN activity in enhanced.[49] These changes in phosphorylation activity were driven by unknown upstream signaling processes and not by changes in the protein expression of the individual entities. This suggests that the epileptic state is associated with dynamic changes in signaling processes that might be amenable to pharmacological targeting, as has been suggested for another phosphorylation pathway, the mammalian target of rapamycin.[50] Phospholipid pathways may also modulate HCN channels, but whether they are altered in epilepsy is unknown.[51,52]

Antiepileptic Drug Actions on HCN Channels

The downregulation of HCN channels in epilepsy suggests that if this process could be pharmacologically reversed, an antiepileptic benefit might be realized. Interestingly, there are several reports of existing AEDs that interact with HCN channels. The first published report showed that acetazolamide (ACZ), a carbonic anhydrase inhibitor with some efficacy in absence epilepsy, upregulated I_h in thalamocortical neurons.[53] This effect was attributed to an

alkalinization of intracellular pH, leading to a 5 mV depolarization of voltage-dependent gating. The AED lamotrigine (LTG) also upregulates I_h through a ~10 mV depolarizing shift of gating in hippocampal pyramidal neurons.[5] In the case of LTG, although its effect was demonstrated in hippocampal neurons, upregulation of I_h in neocortical or thalamic neurons might potentially explain its efficacy against generalized seizures. Application of LTG blocks spontaneous rhythmic firing in combined thalamocortical brain slices.[54] The action of LTG on thalamic neurons is dependent on HCN channels, as was shown in recordings from reticular thalamic neurons, spontaneously bursting-firing cells whose rhythmic output is dependent in part on HCN2 channels. When LTG was superfused on thalamic tissue slices, the frequency of rhythmic firing was markedly reduced; however, this action of LTG was abolished in cells from *HCN2* knockout animals.[55] A similar test of the specificity of LTG action in hippocampus or neocortex using *HCN1* knockout animals has not yet been reported. And although LTG also acts to reduce Na^+ currents in a manner similar to that of the AEDs phenytoin (PHT) and carbamazepine (CBZ),[56] this mechanism of action is unlikely to explain its efficacy in generalized epilepsy, as PHT and CBZ are poorly effective in these syndromes.[57]

Another angle on the action of LTG is seen in its effects in interneurons residing in the stratum oriens that project to pyramidal neuron dendrites.[58,59] In these spontaneously active interneurons, HCN channels are presumably localized perisomatically such that upregulation of I_h depolarizes the resting membrane potential and increases the firing rate; this had the concordant effect of decreasing pyramidal neuron excitability by virtue of an increased frequency of spontaneous inhibitory postsynaptic currents. This result was notable because the actions of AEDs are often considered only from the perspective of inhibition of principal neurons, whereas the same action on interneurons might be expected to be counterproductive on overall brain excitability. This study demonstrated a potentially complementary action of LTG on interneurons compared to their pyramidal counterparts that arose from differing contributions of HCN channels to excitability in the two neuron types.

Aside from LTG and ACZ, the conventional AED gabapentin has also been shown to upregulate I_h.[60] It would seem straightforward to ask whether HCN channel inhibition by a drug such as ZD 7288 might have a proconvulsant effect. Some studies using in vitro models of seizures to address this question have reported a paradoxical anticonvulsant action of HCN channel inhibition.[61] Other studies have reported that ZD 7288 inhibits glutamatergic transmission; therefore, it cannot be considered a selective antagonist for HCN channels when synaptic transmission is studied.[62] Because *hcn1* deletion lowers the threshold for provoked seizures and SE, and *hcn2* deletion results in generalized epilepsy, it seems reasonable to conclude that HCN channels exert an anticonvulsant effect on the brain as a whole. This would suggest that discovery of novel specific HCN channel agonists might be a productive avenue for future AED development.

CONCLUSIONS

The HCN channel has emerged as a compelling new candidate channelopathy in epilepsy. It plays a powerful inhibitory role in cortical and hippocampal excitability, both at single-neuron and network levels, and it influences the development of thalamocortical rhythms as a result of its high expression in thalamic nuclei. In animal models of acquired epilepsy, HCN channel expression is downregulated, contributing to pathological hyperexcitability. Conversely, several AEDs upregulate the function of HCN channels, offering the potential of a novel target for further AED development.

Evidence of human HCN channelopathy is thus far anecdotal. However, given the substantial support from animal models for a pathological role of this channel in acquired epilepsy in particular, understanding the mechanisms by which HCN channels are dysregulated may provide insights applicable to the larger number of epileptic ion channelopathies that are continually being characterized.

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REFERENCES

1. Difrancesco D. Serious workings of the funny current. *Prog Biophys Mol Biol.* 2006;90:13–25.
2. Ludwig A, Budde T, Stieber J, Moosmang S, Wahl C, Holthoff K, Langebartels A, Wotjak C, Munsch T, Zong X, Feil S, Feil R, Lancel M, Chien KR, Konnerth A, Pape HC, Biel M, Hofmann F. Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. *Embo J*. 2003;22:216–224.
3. Huang Z, Walker MC, Shah MM. Loss of dendritic HCN1 subunits enhances cortical excitability and epileptogenesis. *J Neurosci*. 2009;29:10979–10988.
4. Jung S, Jones TD, Lugo J Jr, Sheerin AH, Miller JW, D'Ambrosio R, Anderson AE, Poolos NP. Progressive dendritic HCN channelopathy during epileptogenesis in the rat pilocarpine model of epilepsy. *J Neurosci*. 2007;27:13012–13021.
5. Poolos NP, Migliore M, Johnston D. Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. *Nat Neurosci*. 2002;5:767–774.
6. Biel M, Wahl-Schott C, Michalakis S, Zong X. Hyperpolarization-activated cation channels: from genes to function. *Physiol Rev*. 2009;89:847–885.
7. Santoro B, Chen S, Luthi A, Pavlidis P, Shumyatsky GP, Tibbs GR, Siegelbaum SA. Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. *J Neurosci*. 2000;20:5264–5275.
8. McCormick DA, Bal T. Sleep and arousal: thalamocortical mechanisms. *Annu Rev Neurosci*. 1997;20:185–215.
9. Yue BW, Huguenard JR. The role of H-current in regulating strength and frequency of thalamic network oscillations. *Thalamus Rel Syst*. 2001;1:95–103.
10. Coulter DA, Huguenard JR, Prince DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann Neurol*. 1989;25:582–593.
11. Magee JC. Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *J Neurosci*. 1998;18:7613–7624.
12. Poolos NP. The yin and yang of the h-channel and its role in epilepsy. *Epilepsy Curr.* 2004;4:3–6.
13. Santoro B, Baram TZ. The multiple personalities of h-channels. *Trends Neurosci*. 2003;26:550–554.
14. Lupica CR, Bell JA, Hoffman AF, Watson PL. Contribution of the hyperpolarization-activated current (I_h) to membrane potential and GABA release in hippocampal interneurons. *J Neurophysiol*. 2001;86:261–268.
15. Avanzini G, Franceschetti S, Mantegazza M. Epileptogenic channelopathies: experimental models of human pathologies. *Epilepsia.* 2007;48(suppl 2):51–64.
16. Tang B, Sander T, Craven KB, Hempelmann A, Escayg A. Mutation analysis of the hyperpolarization-activated cyclic nucleotide–gated channels *HCN1* and *HCN2* in idiopathic generalized epilepsy. *Neurobiol Dis*. 2008;29:59–70.
17. Dibbens LM, Reid CA, Hodgson B, Thomas EA, Phillips AM, Gazina E, Cromer BA, Clarke AL, Baram TZ, Scheffer IE, Berkovic SF, Petrou S. Augmented currents of an *HCN2* variant in patients with febrile seizure syndromes. *Ann Neurol*. 2010;67:542–546.
18. Nof E, Luria D, Brass D, Marek D, Lahat H, Reznik-Wolf H, Pras E, Dascal N, Eldar M, Glikson M. Point mutation in the HCN4 cardiac ion channel pore affecting synthesis, trafficking, and functional expression is associated with familial asymptomatic sinus bradycardia. *Circulation.* 2007;116:463–470.
19. Schulze-Bahr E, Neu A, Friederich P, Kaupp UB, Breithardt G, Pongs O, Isbrandt D. Pacemaker channel dysfunction in a patient with sinus node disease. *J Clin Invest*. 2003;111:1537–1545.
20. Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ. Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide–gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. *J Neurosci*. 2003;23:6826–6836.
21. Wierschke S, Lehmann TN, Dehnicke C, Horn P, Nitsch R, Deisz RA. Hyperpolarization-activated cation currents in human epileptogenic neocortex. *Epilepsia*. 2010;51:404–414.
22. Santoro B, Lee JY, Englot DJ, Gildersleeve S, Piskorowski RA, Siegelbaum SA, Winawer MR, Blumenfeld H. Increased seizure severity and seizure-related death in mice lacking HCN1 channels. *Epilepsia.* 2010;51:1624–1627.
23. Chen X, Shu S, Schwartz LC, Sun C, Kapur J, Bayliss DA. Homeostatic regulation of synaptic excitability: tonic GABA(A) receptor currents replace I(h) in cortical pyramidal neurons of HCN1 knock-out mice. *J Neurosci.* 2010;30:2611–2622.
24. Barnwell LF, Lugo JN, Lee WL, Willis SE, Gertz SJ, Hrachovy RA, Anderson AE. Kv4.2 knockout mice demonstrate increased susceptibility to convulsant stimulation. *Epilepsia*. 2009;50:1741–1751..
25. Meeren HK, Pijn JP, Van Luijtelaar EL, Coenen AM, Lopes da Silva FH. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci*. 2002;22:1480–1495.
26. Strauss U, Kole MH, Brauer AU, Pahnke J, Bajorat R, Rolfs A, Nitsch R, Deisz RA. An impaired neocortical Ih is associated with enhanced excitability and absence epilepsy. *Eur J Neurosci*. 2004;19:3048–3058.
27. Kole MH, Brauer AU, Stuart GJ. Inherited cortical HCN1 channel loss amplifies dendritic calcium electrogenesis and burst firing in a rat absence epilepsy model. *J Physiol*. 2007;578:507–525.
28. Blumenfeld H, Klein JP, Schridde U, Vestal M, Rice T, Khera DS, Bashyal C, Giblin K, Paul-Laughinghouse C, Wang F, Phadke A, Mission J, Agarwal RK, Englot DJ, Motelow J, Nersesyan H, Waxman SG, Levin AR. Early treatment suppresses the development of spike-wave epilepsy in a rat model. *Epilepsia.* 2007;49:400–409.
29. Ben-Ari Y, Crepel V, Represa A. Seizures beget seizures in temporal lobe epilepsies: the boomerang effects of newly formed aberrant kainatergic synapses. *Epilepsy Curr*. 2008;8:68–72.
30. Chen K, Baram TZ, Soltesz I. Febrile seizures in the developing brain result in persistent modification of neuronal excitability in limbic circuits. *Nat Med*. 1999;5:888–894.
31. Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I. Persistently modified h-channels after

complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat Med*. 2001;7:331–337.
32. Brewster A, Bender RA, Chen Y, Dube C, Eghbal-Ahmadi M, Baram TZ. Developmental febrile seizures modulate hippocampal gene expression of hyperpolarization-activated channels in an isoform- and cell-specific manner. *J Neurosci.* 2002;22:4591–4599.
33. Shah MM, Anderson AE, Leung V, Lin X, Johnston D. Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. *Neuron*. 2004;44:495–508.
34. Marcelin B, Chauviere L, Becker A, Migliore M, Esclapez M, Bernard C. h channel-dependent deficit of theta oscillation resonance and phase shift in temporal lobe epilepsy. *Neurobiol Dis*. 2009;33:436–447.
35. Chauviere L, Rafrafi N, Thinus-Blanc C, Bartolomei F, Esclapez M, Bernard C. Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy. *J Neurosci*. 2009;29:5402–5410.
36. Shin M, Brager D, Jaramillo TC, Johnston D, Chetkovich DM. Mislocalization of h channel subunits underlies h channelopathy in temporal lobe epilepsy. *Neurobiol Dis*. 2008;32:26–36.
37. Zhang K, Peng BW, Sanchez RM. Decreased I_H in hippocampal area CA1 pyramidal neurons after perinatal seizure-inducing hypoxia. *Epilepsia*. 2006;47:1023–1028.
38. Hablitz JJ, Yang J. Abnormal pyramidal cell morphology and HCN channel expression in cortical dysplasia. *Epilepsia.* 2010;51(suppl 3):52–55.
39. Dyhrfjeld-Johnsen J, Morgan RJ, Foldy C, Soltesz I. Upregulated H-current in hyperexcitable CA1 dendrites after febrile seizures. *Front Cell Neurosci*. 2008;2:1–8.
40. Dube C, Richichi C, Bender RA, Chung G, Litt B, Baram TZ. Temporal lobe epilepsy after experimental prolonged febrile seizures: prospective analysis. *Brain*. 2006;129:911–922.
41. Jung S, Warner LN, Pitsch J, Becker A, Poolos NP. Rapid loss of dendritic HCN channel expresssion in hippocampal pyramidal neurons following status epilepticus. *J Neurosci*. 2011;31:14291–14295.
42. Powell KL, Ng C, O'Brien TJ, Xu SH, Williams DA, Foote SJ, Reid CA. Decreases in HCN mRNA expression in the hippocampus after kindling and status epilepticus in adult rats. *Epilepsia*. 2008;49:1686–1695.
43. Richichi C, Brewster AL, Bender RA, Simeone TA, Zha Q, Yin HZ, Weiss JH, Baram TZ. Mechanisms of seizure-induced "transcriptional channelopathy" of hyperpolarization-activated cyclic nucleotide gated (HCN) channels. *Neurobiol Dis*. 2008;29:297–305.
44. Lewis AS, Schwartz E, Chan CS, Noam Y, Shin M, Wadman WJ, Surmeier DJ, Baram TZ, Macdonald RL, Chetkovich DM. Alternatively spliced isoforms of TRIP8b differentially control h channel trafficking and function. *J Neurosci*. 2009;29:6250–6265.
45. Santoro B, Wainger BJ, Siegelbaum SA. Regulation of HCN channel surface expression by a novel C-terminal protein-protein interaction. *J Neurosci*. 2004;24:10750–10762.
46. Santoro B, Piskorowski RA, Pian P, Hu L, Liu H, Siegelbaum SA. TRIP8b splice variants form a family of auxiliary subunits that regulate gating and trafficking of HCN channels in the brain. *Neuron*. 2009;62:802–813.
47. Chen S, Wang J, Siegelbaum SA. Properties of hyperpolarization-activated pacemaker current defined by coassembly of HCN1 and HCN2 subunits and basal modulation by cyclic nucleotide. *J Gen Physiol*. 2001;117:491–504.
48. Poolos NP, Bullis JB, Roth MK. Modulation of h-channels in hippocampal pyramidal neurons by p38 mitogen-activated protein kinase. *J Neurosci.* 2006;26:7995–8003.
49. Jung S, Bullis JB, Lau IH, Jones TD, Warner LN, Poolos NP. Downregulation of dendritic HCN channel gating in epilepsy is mediated by altered phosphorylation signalling. *J Neurosci*. 2010;30:6678–6688.
50. Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol*. 2008;63:444–453.
51. Fogle KJ, Lyashchenko AK, Turbendian HK, Tibbs GR. HCN pacemaker channel activation is controlled by acidic lipids downstream of diacylglycerol kinase and phospholipase A2. *J Neurosci*. 2007;27:2802–2814.
52. Zolles G, Klocker N, Wenzel D, Weisser-Thomas J, Fleischmann BK, Roeper J, Fakler B. Pacemaking by HCN channels requires interaction with phosphoinositides. *Neuron*. 2006;52:1027–1036.
53. Munsch T, Pape HC. Upregulation of the hyperpolarization-activated cation current in rat thalamic relay neurones by acetazolamide. *J Physiol*. 1999;519(pt 2):505–514.
54. Gibbs JW 3rd, Zhang YF, Ahmed HS, Coulter DA. Anticonvulsant actions of lamotrigine on spontaneous thalamocortical rhythms. *Epilepsia*. 2002;43:342–349.
55. Ying SW, Jia F, Abbas SY, Hofmann F, Ludwig A, Goldstein PA. Dendritic HCN2 channels constrain glutamate-driven excitability in reticular thalamic neurons. *J Neurosci*. 2007;27:8719–8732.
56. Kuo C-C, Lu L. Characterization of lamotrigine inhibition of Na^+ channels in rat hippocampal neurones. *Br J Pharmacol*. 1997;121:1231–1238.
57. Benbadis SR, Tatum WO, Gieron M. Idiopathic generalized epilepsy and choice of antiepileptic drugs. *Neurology*. 2003;61:1793–1795.
58. Peng BW, Justice JA, Zhang K, He XH, Sanchez RM. Increased basal synaptic inhibition of hippocampal area CA1 pyramidal neurons by an antiepileptic drug that enhances I(H). *Neuropsychopharmacology.* 2010;35:464–472.
59. Peng BW, Justice JA, Zhang K, Li JX, He XH, Sanchez RM. Gabapentin promotes inhibition by enhancing hyperpolarization-activated cation currents and spontaneous firing in hippocampal CA1 interneurons. *Neurosci Lett*. 2011;494:19–23.
60. Surges R, Freiman TM, Feuerstein TJ. Gabapentin increases the hyperpolarization-activated cation current I_h in rat CA1 pyramidal cells. *Epilepsia*. 2003;44:150–156.
61. Gill CH, Brown JT, Shivji N, Lappin SC, Farmer C, Randall A, McNaughton NC, Cobb SR, Davies CH. Inhibition of Ih reduces epileptiform activity in rodent hippocampal slices. *Synapse*. 2006;59:308–316.
62. Inaba Y, Biagini G, Avoli M. The H current blocker ZD7288 decreases epileptiform hyperexcitability in the rat neocortex by depressing synaptic transmission. *Neuropharmacology*. 2006;51:681–691.

63. Magee JC. Dendritic I_h normalizes temporal summation in hippocampal CA1 neurons. *Nat Neurosci*. 1999;2:508–514.
64. Tsay D, Dudman JT, Siegelbaum SA. HCN1 channels constrain synaptically evoked Ca^{2+} spikes in distal dendrites of CA1 pyramidal neurons. *Neuron*. 2007;56:1076–1089.
65. Nolan MF, Malleret G, Dudman JT, Buhl DL, Santoro B, Gibbs E, Vronskaya S, Buzsaki G, Siegelbaum SA, Kandel ER, Morozov A. A behavioral role for dendritic integration: HCN1 channels constrain spatial memory and plasticity at inputs to distal dendrites of CA1 pyramidal neurons. *Cell*. 2004;119:719–732.
66. Fan Y, Fricker D, Brager DH, Chen X, Lu HC, Chitwood RA, Johnston D. Activity-dependent decrease of excitability in rat hippocampal neurons through increases in I_h. *Nat Neurosci*. 2005;8:1542–1551.
67. Brager DH, Johnston D. Plasticity of intrinsic excitability during long-term depression is mediated through mGluR-dependent changes in I_h in hippocampal CA1 pyramidal neurons. *J Neurosci*. 2007;27:13926–13937.
68. Berger T, Larkum ME, Luscher HR. High I_h channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. *J Neurophysiol*. 2001;85:855–868.
69. Williams SR, Stuart GJ. Site independence of EPSP time course is mediated by dendritic I_h in neocortical pyramidal neurons. *J Neurophysiol*. 2000;83:3177–3182.
70. Berger T, Senn W, Luscher HR. Hyperpolarization-activated current I_h disconnects somatic and dendritic spike initiation zones in layer V pyramidal neurons. *J Neurophysiol*. 2003;90:2428–2437.
71. Rosenkranz JA, Johnston D. Dopaminergic regulation of neuronal excitability through modulation of I_h in layer V entorhinal cortex. *J Neurosci*. 2006;26:3229–3244.
72. Wang M, Ramos BP, Paspalas CD, Shu Y, Simen A, Duque A, Vijayraghavan S, Brennan A, Dudley A, Nou E, Mazer JA, McCormick DA, Arnsten AF. Alpha2A-adrenoceptors strengthen working memory networks by inhibiting cAMP-HCN channel signaling in prefrontal cortex. *Cell*. 2007;129:397–410.
73. Barth AM, Vizi ES, Zelles T, Lendvai B. Alpha2-adrenergic receptors modify dendritic spike generation via HCN channels in the prefrontal cortex. *J Neurophysiol*. 2008;99:394–401.
74. Carr DB, Andrews GD, Glen WB, Lavin A. alpha2-Noradrenergic receptor activation enhances excitability and synaptic integration in rat prefrontal cortex pyramidal neurons via inhibition of HCN currents. *J Physiol*. 2007;584:437–450.

Chapter 8

Phasic $GABA_A$-Mediated Inhibition

Enrico Cherubini

γ-Aminobutiric acid (GABA) is the main inhibitory neurotransmitter in the adult mammalian central nervous system (CNS). It inhibits neuronal firing by activating two different classes of receptors, $GABA_A$ and $GABA_B$. Unlike $GABA_A$ receptors, which form integral ion channels, $GABA_B$ receptors are coupled to ion channels via guanine nucleotide-binding proteins and second messengers. $GABA_A$ receptors mediate two distinct forms of inhibition, phasic and tonic. The first consists of fast inhibitory postsynaptic potentials (IPSPs) regulating point-to-point communication between neurons. The second consists of a persistent inhibitory conductance that plays a crucial role in regulating membrane potential and network excitability.[1] In the case of phasic inhibition, synaptic $GABA_A$ receptors, facing presynaptic release sites, are activated by brief exposure to a high concentration of GABA released by exocytosis of presynaptic vesicles. Once released, GABA diffuses throughout the neuropil before being taken up by selective plasma membrane transporters, which contribute to the clearance of the neurotransmitter.[2] In the case of tonic inhibition, extrasynaptic $GABA_A$ receptors, localized away from the synapses, are persistently exposed to low concentration of "ambient" GABA.

This review will focus on $GABA_A$-mediated phasic inhibition which, in physiological conditions, exerts a powerful control on cell excitability and network oscillations thought to be associated with higher cognitive functions.[3] An impairment of fast GABAergic signaling is involved in various psychiatric and neurological disorders including epilepsy.[4]

$GABA_A$-MEDIATED IPSCS: PRESYNAPTIC REGULATION

Source of GABA

GABA is released from GABAergic interneurons which comprise ~15% of the total neuronal population. It is synthesized from glutamate by the enzyme glutamic acid decarboxylase (GAD65 and GAD67) and, in particular, by GAD65, which is preferentially localized at GABAergic nerve terminals. GABAergic interneurons constitute a heterogeneous group of cells that have been characterized on the basis of their firing patterns, molecular expression profiles, and selective innervation of different domains of principal cells.[5] They provide feedback and feedforward inhibition.[6,7] In addition, the spatiotemporal dynamics between the activity of interneurons, with extensive axonal arborizations, and that of pyramidal cells leads to coherent network oscillations crucial for information processing.[5,8]

In the majority of cases, the release of GABA from GABAergic interneurons occurs via vesicle exocytosis, the process by which a single vesicle fuses with the presynaptic plasma membrane and releases its content into the synaptic cleft. However, the release of GABA may also occur in a nonvesicular way through the reversal of GABA transporters.[9] Thus, in pathological conditions (e.g., temporal lobe epilepsy), bursting-induced membrane depolarization and an increase in intracellular $[Na^+]$ would enhance GAT-1 reversal (GAT-1 is the isoform of GABA transporters localized almost exclusively on axon terminals) and GAT-1-mediated GABA release.[10] Recently, using a heterologous expression system, Wu et al.[9] have provided evidence that the membrane potential at which the GABA transporter GAT-1 reverses is close to the normal resting potential of neurons and that the reversal can occur rapidly enough to release GABA during action potentials. This is consistent with the hypothesis that transporter-mediated GABA release can also contribute to the maintenance of phasic $GABA_A$-mediated inhibition in physiological conditions, as in the case of vesicular transmitter failure during high-frequency firing, thus acting as a brake to prevent runaway excitation.[10]

The release of GABA from presynaptic nerve terminals is influenced by the probability of release *P* and by the number of release sites *N*. Large *P* and/or large *N* can be used to distinguish strong from weak GABAergic synapses. Strong connections produce large IPSPs with few transmitter failures. Weak synapses with high failure rates exhibit paired-pulse facilitation (facilitation of a second response evoked at a short interval from the first), while strong synapses with low failures rates exhibit paired-pulse depression (depression of a second response occurring at a short interval from the first).

Regulation of GABA Release by Presynaptic Intracellular Calcium Stores

Interestingly, *P* can be affected by spontaneous calcium signals (reminiscent of "calcium sparks"),[11] such as those detected at cerebellar interneuron–Purkinje cell synapses.[12] These events (sensitive to ryanodine) depend on the release of calcium from intracellular calcium stores following an action potential-dependent increase in intracellular calcium rise.[13] Ryanodine-sensitive calcium stores are likely to mediate large-amplitude miniature inhibitory postsynaptic currents (mIPSCs) due to the synchronous release of GABA from several release sites. At the same synapses, presynaptic calcium stores also regulate the evoked release of GABA, as demonstrated by the ryanodine-induced decrease in the paired-pulse ratio and the increase in the coefficient of variation,[14] defined as the ratio between the standard deviation and the mean. *P* can also be also modified in a synapse-selective manner by receptors localized on presynaptic terminals, whose activation up- or downregulates the release of GABA. In addition, synapses can differ in the number of functional release sites, *N*, which can vary from 1 to 10s. For instance, at individual synaptic contacts among stellate cells in the cerebellum, presynaptic action potentials generate synaptic currents presumably arising from single or multiple release sites.[15] In case of multiple release sites, decreasing the probability of GABA release (by modifying the calcium/magnesium ratio) decreases the average number of release sites and shifts the amplitude distribution toward smaller values. In contrast, at single release sites, the amplitude distribution of the evoked currents whose potency

(successes without failures) is unaffected by decreasing the release probability can be fitted with a Gaussian function.[16,17] Some GABAergic synapses are also characterized by multivesicular release. This occurs when the content of several vesicles is released simultaneously by an action potential at a presynaptic active zone, leading to fluctuations of inhibitory synaptic events that vary in magnitude according to the degree of receptor saturation. For instance, at cerebellar stellate-basket cell synapses, more than one containing vesicle, released slightly asynchronously, leads to a very small increase in the peak amplitude of the synaptic currents because, at these synapses, receptor occupancy is high and close to saturation.[6,17,18] It is noteworthy that the functionally determined number of release sites is usually larger than those structurally defined at the electron microscopic level, because a single presynaptic active zone is often made by a large number of functional release sites.[19]

Regulation of GABA Release by Presynaptic $GABA_A$ and $GABA_B$ Receptors

The release of GABA is up- or downregulated by a variety of receptors localized on GABAergic terminals in proximity to the release sites. Of particular interest are $GABA_A$ and $GABA_B$ autoreceptors, which can be activated by spillover of GABA from axon terminals or by "ambient" GABA present in the extracellular medium. While high-*P* synapses more likely undergo activity-dependent depression of synaptic transmission, low-*P* synapses undergo activity-dependent facilitation. A decrease in *P* may result from the shunting effect of GABA due to the increase in conductance produced by presynaptic receptor activation. This, in turn, would produce a decrease in the amplitude of presynaptic spikes or prevent them for invading axon terminals.[20] Alternatively activation of $GABA_A$ receptors may reduce *P* via inactivation of Na^+ or Ca^{2+} channels. An increase in *P* following activation of axonal $GABA_A$ receptors may result from depolarization of presynaptic terminals (due to the high chloride concentration in the terminals and the reversal of GABA, which would be more depolarized with respect to the resting membrane potential), with consequent activation of voltage-dependent calcium channels and elevation of intracellular calcium.[21] In the immature hippocampus, activation of $GABA_A$ receptors localized on mossy fiber terminals (which in the immediate postnatal period are GABAergic)[22] by spillover of GABA from neighboring GABAergic interneurons or mossy fibers themselves has been shown to enhance mossy fibers excitability, thus contributing to activity-dependent synaptic facilitation.[23] In cerebellar interneurons, activation of $GABA_A$ autoreceptors[24] leads to an enhancement of GABA release which is developmentally regulated [25] (see Fig. 8–1).

In contrast to $GABA_A$ receptors, activation of presynaptic $GABA_B$ receptors always limits GABA release via G-protein-dependent inhibition of P/Q and N types of voltage-dependent calcium channels.[26] For instance, at GABAergic synapses between Golgi cells and granule cells in the cerebellar glomerulus, activation of presynaptic $GABA_B$ receptors leads to a reduction of *P*.[27] In the neonatal hippocampus, spillover of GABA from mossy fiber terminals downregulates its own release, leading sometimes to synapse silencing.[28]

DYNAMICS OF GABA TRANSIENTS IN THE SYNAPTIC CLEFT

Once released from presynaptic nerve terminals, GABA crosses the synaptic cleft and binds to postsynaptic receptors. Fast synaptic transmission requires that GABA be released at high concentration for a very short period of time. The time course of the agonist in the cleft is therefore a key factor in shaping synaptic currents. Allosteric modifiers of receptor gating have allowed quantitative estimation of the peak concentration of the agonist in the cleft and the time course of its clearance. Using this method, the synaptic GABA transient was estimated to be ~3 mM, while the speed of GABA clearance was estimated to follow a single exponential function of ~100 μs.[29,30] Such brief exposure of $GABA_A$ receptors to GABA indicates that activation of postsynaptic receptors occurs in nonequilibrium conditions. Similar results have been found with fast-off competitive $GABA_A$ receptor antagonists. With this approach, synaptic GABA has been proposed to peak at 3–5 mM and to be cleared out within

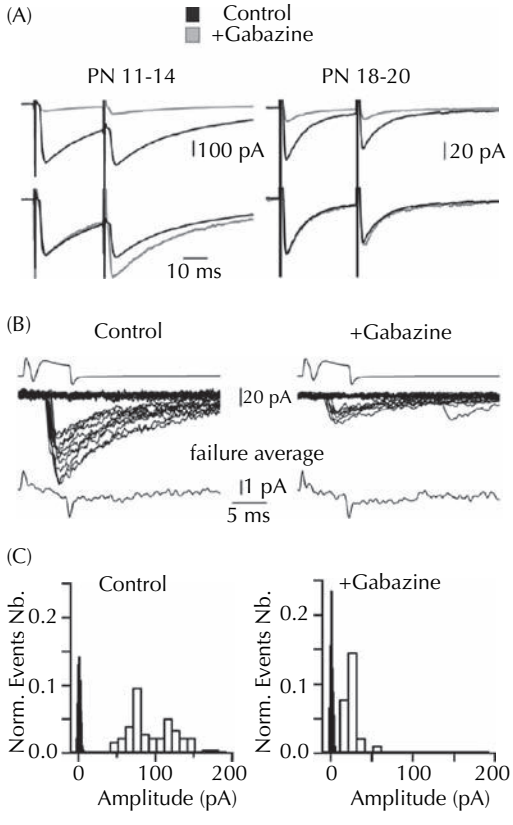

Figure 8–1. Endogenous activation of presynaptic $GABA_A$ receptors enhances GABA release from juvenile cerebellar basket cells. **A.** Gabazine (300 nM) reduced much more potently the amplitude of evoked synaptic currents in the younger than in the older age group (black traces: control; gray traces: + gabazine). However, after scaling to the amplitude of the first response, an increase in the paired-pulse ratio was apparent following gabazine application in the P11–P14 group but not in the P18–P20 group (bottom panels). **B.** Traces from two connected molecular layer interneurons showing depolarization-driven presynaptic action currents (top) and postsynaptic responses (middle traces) during control conditions (left) and in the presence of gabazine (right). Failure averages (bottom traces) fail to reveal any synaptic currents. Positive and negative current transients mark the onset and offset of the presynaptic depolarizing voltage step, reflecting capacitive coupling between presynaptic and postsynaptic pipettes. In this cell, gabazine reduced the peak current amplitude to 14% of the control level. **C.** Amplitude histograms for successful events (open bins) and for failures (closed bins), from the data in **A.** Note the increase in failure rate with gabazine. Reproduced with permission from ref. 25.

300–500 μs.[31] Although $GABA_A$ receptors are exposed to GABA for a very short period of time, synaptic currents outlast the duration of the GABA transient. This is because the activated channels have a high probability of entering into long-lived desensitized states, which precede channel reopening and agonist unbinding.[32]

GABA Transients May Regulate Synaptic Variability

It is noteworthy that, in cultured hippocampal neurons, fast-off competitive antagonists have been shown to differentially block small and large mIPSCs, suggesting that, at nonsaturated synapses, changes in the peak concentration and the speed of GABA clearance in the cleft may be important sources of presynaptic variability.[33] Interestingly, in the neocortex, slow $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs), originating from neurogliaform cells, are generated by a GABA transient with unusual characteristics, as indicated by the large inhibitory effect of the low-affinity competitive $GABA_A$ receptor antagonist (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA)[34] (see Fig. 8–2).

It has been proposed that the high density of neurogliaform cell axons could counteract transmitter reuptake mechanisms and that GABA released from these terminals could act as a volume transmitter to reach receptors at synaptic and extrasynaptic sites in the tissue with which the axons intermingle.[35]

Once released into the cleft, the agonist is cleared by the combined action of diffusion and buffering systems. Diffusion is influenced by the geometry of the cleft and by the viscosity of the extracellular liquid,[36] which is composed of glycoproteins and fibrils. Buffering systems consist of membrane transporters, receptors, and other GABA binding sites.

GABA Transporters

GABA transporters not only passively bind GABA but also actively remove it from the extracellular space. High-affinity Na^+/Cl^-coupled GABA transporters are expressed on the plasma membrane in close proximity to the synapses. Of the four GABA transporters identified to date (GAT-1, GAT-2, GAT-3, and BGT-1), GAT-1 prevails at GABAergic synapses and is almost exclusively localized on axon terminals (forming symmetric synaptic contacts)

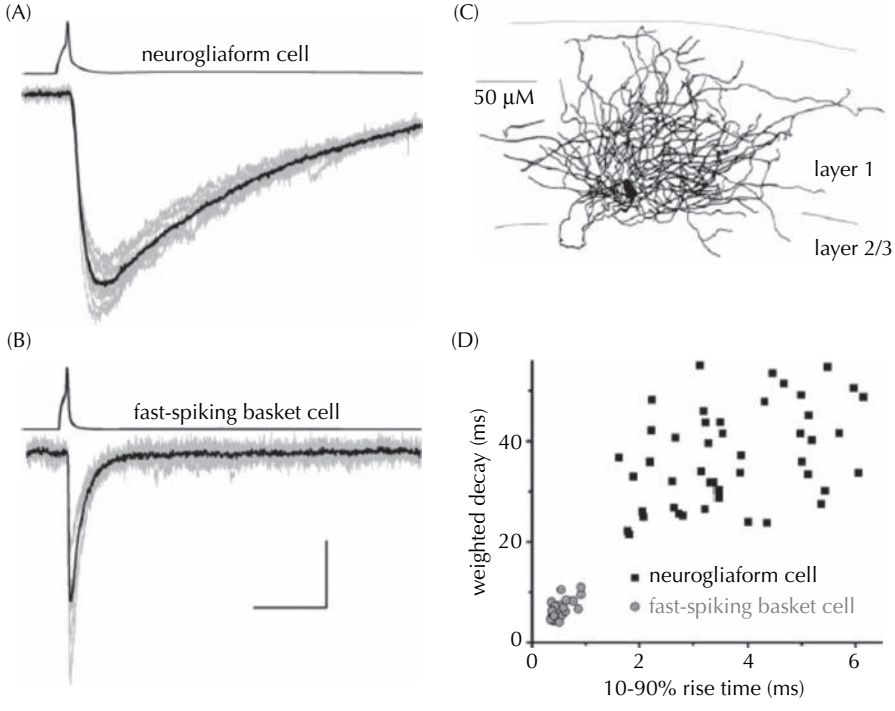

Figure 8–2. Slow $GABA_A$-mediated IPSCs originating from neurogliaform cells. **A.** Ten consecutive slow IPSCs (gray, lower trace) and their average (black) evoked in a layer 2/3 pyramidal cell after single action potentials in a layer 1 neurogliaform cell (upper trace). **B.** Inhibitory postsynaptic currents evoked by a layer 2 fast-spiking basket cell. **C.** Camera lucida reconstruction of the soma and axons of a neurogliaform cell. Gray lines indicate the borders of cortical layers. **D.** Comparison of the kinetics of postsynaptic responses evoked by neurogliaform cells (black squares) and fast-spiking basket cells (gray circles). Each point represents an individual connection. Reproduced with permission from ref. 34.

and on distal astrocytic processes.[37] The use of selective GAT-1 inhibitors has allowed investigation of the functional role of this transporter in $GABA_A$-mediated inhibition. Thus, in rat neocortex and hippocampus, blockers of GAT-1 have been shown to affect not only tonic but also phasic $GABA_A$-mediated inhibition, as indicated by the increase in the decay time of evoked IPSCs.[38–42] GAT-1 seems to play a crucial role during sustained neuronal activity such as that observed during seizures.[43]

Recent experiments with neocortical neurons in slices obtained from GAT-1 knockout (KO) mice have demonstrated that high-frequency stimulation of GABAergic terminals induces slowly developing bicuculline-sensitive inward currents that last for several seconds and are larger in GAT-1 KO than in WT mice, indicating a severe impairment of GABA clearance from the extracellular space in the former. In addition, in GAT-1 KO mice, stimuli delivered to the same fibers immediately after the high-frequency train evokes synaptic currents smaller in amplitude and exhibiting slower decay kinetics and a slower recovery process[43] (see Fig. 8–3).

Interestingly, GAT-1 KO mice exhibited increased expression of GABA-synthesizing enzymes GAD65 and GAD67 as an adaptive process for compensating for the loss of GAT-1.

$GABA_A$-MEDIATED IPSCS: POSTSYNAPTIC REGULATION

$GABA_A$ Receptors

The speed and reliability of inhibitory synaptic transmission require the presence of clustered

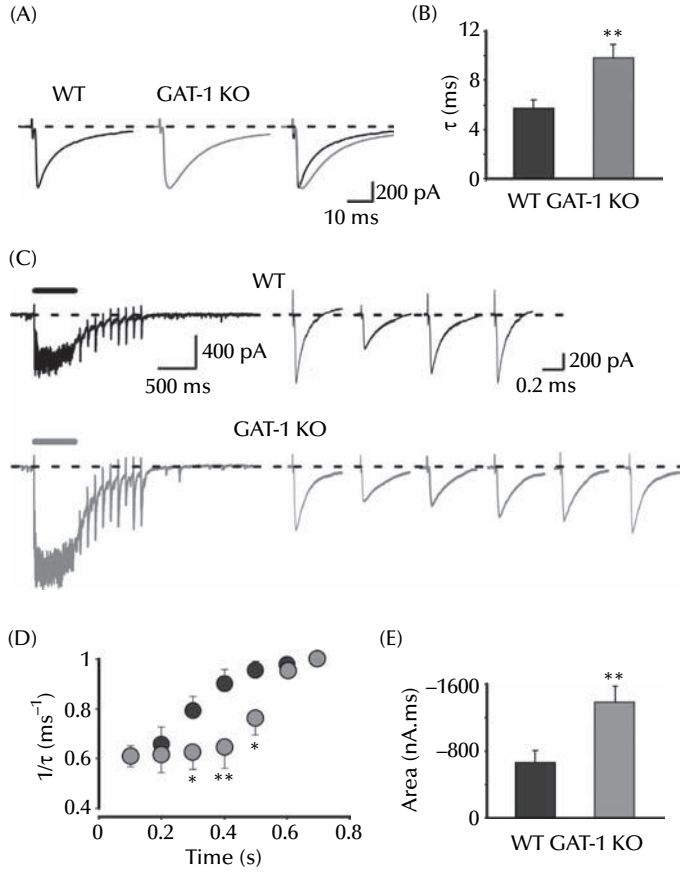

Figure 8–3. GABA transporters 1 play a crucial role during sustained synaptic activity. **A.** Prolonged decay of IPSCs evoked from layer II/III pyramidal neurons by local stimulation of GABAergic fibers (in the presence of 20 µM 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 1 µM (2*S*)-3-[[(1*S*)-1-(3,4-Dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid hydrochloride (CGP 55845) in neocortical slices from GAT-1 KO mice compared to wild-type (WT) mice. **B.** Each column represents the mean decay time constant of IPSCs obtained from WT (n = 7) and GAT-1 KO mice (n = 7). $^{**}p < 0.01$. **C.** Representative traces recorded at –60 mV from WT and GAT-1 KO mice during and after repetitive stimulation of GABAergic fibers (50 stimuli at 100 Hz, horizontal bars) in WT (black) GAT-1 KO mice (gray). Note the larger inward current and the longer recovery time of individual IPSCs in GAT-1 KO mice. **D.** Recovery of the decay of individual IPSCs evoked at 200 ms intervals after the high-frequency stimulation train in WT (black) and GAT-1 KO mice (gray). Each point is the average of six experiments. **E.** Charge transfers associated with $GABA_A$-mediated slow inward currents induced by repetitive activation of presynaptic GABAergic terminals in WT (n = 6) and GAT-1 KO mice (n = 5). $^{*}p < 0.05$; $^{**}p < 0.01$. Adapted from ref. 43.

$GABA_A$ receptors at the plasma membrane in precise apposition to presynaptic releasing sites. The postsynaptic organization comprises a large number of proteins that ensure the correct targeting, clustering, and stabilization of neurotransmitter receptors. Among them, the tubulin-binding protein gephyrin plays a crucial role in the functional organization of inhibitory synapses.[44] Through its self-oligomerizing properties, gephyrin forms hexagonal lattices that trap glycine and $GABA_A$ receptors[45,46] and link them to the cytoskeleton. Disruption of gephyrin function leads to reduced $GABA_A$ receptor clustering,[47] an effect associated with loss of GABAergic innervation.[48] This observation suggests the existence of cross-talk between the post- and presynaptic sites of the synapse. The backward control of presynaptic signaling may occur via neuroligin 2, a postsynaptic cell adhesion molecule that directly binds gephyrin

through a conserved cytoplasmatic domain[49] and interacts transynaptically with neurexins.[50]

$GABA_A$ receptors belong to the Cys-loop ligand-gated ion channels family, which includes nicotine acetylcholine receptors (nAChRs), glycine receptors, and ionotropic 5-hydroxytryptamine ($5\text{-}HT_3$) receptors. Nicotine acetylcholine receptors and $5\text{-}HT_3$ receptors are cation-selective channels, while $GABA_A$ and glycine receptors are anionic-selective channels. All the subunit members of this family show 30% sequence homology but have greater similarity at the level of their secondary and tertiary structure. These receptors are organized as pentameric membrane-spanning proteins surrounding a central pore forming the ion channel. Each subunit has a large extracellular N terminus (containing a signal peptide, a cysteine bridge, and glycosylation sites), four hydrophobic transmembrane domains (M1–M4), an intracellular loop between M3 and M4 (containing protein kinase A, protein kinase C, and tyrosine kinase phosphorylation sites), and a short C terminus. M2 is crucial for receptor gating and selectivity. This region lines the channel pore and constitutes the most conserved part of the receptor. Introduction of site-direct mutagenesis of three amino acids from the M2 domain of glycine or $GABA_A$ receptors into the M2 segment of α7 nAChRs is sufficient to convert a cation-selective channel into an anion-selective channel.[51] Channel opening would result from the concerted tilting of the subunits that rotate around an axis parallel to the membrane plane.

Permeability of $GABA_A$ Receptor Channels

Activation of $GABA_A$ receptors opens channels largely permeable to Cl^-. However, a significant inwardly directed component of GABA-gated anionic current is carried by HCO_3^-.[52,53] The passive redistribution of H^+ and HCO_3^- following activation of $GABA_A$ receptors by GABA would lead to a more acidic intraneuronal pH.[54] Hence, the electrochemical equilibrium potential for HCO_3^- would be less negative (~15 mV) than the resting membrane potential, and since HCO_3^- can also act as a carrier of a depolarizing current, the GABA reversal potential (E_{GABA}) will not be identical to the equilibrium potential for Cl^- but it will shift toward more positive values.[55] Taking into account an HCO_3^- and Cl^- permeability ratio of 0.2–0.4, the quantitative influence of HCO_3^- on E_{GABA} can be estimated using the Goldman-Hodgkin-Katz equation, which shows that $[HCO_3^-]_i$ (~15 mM at a pH of 7.1–7.2) influences E_{GABA} in the same way as 3–5 mM of $[Cl^-]_i$. Thus, the depolarizing influence of HCO_3^- on E_{GABA} will be significant in neurons with low $[Cl^-]_i$, as in adults, but it will be negligible in immature neurons in which $[Cl^-]_i$ is relatively high. High $[Cl^-]_i$ in immature neurons would make the equilibrium potential for Cl^- less negative than the resting membrane potential, providing the driving force that accounts for the depolarizing and excitatory action of GABA.[56] $[Cl^-]_i$ is under the control of two main cation-chloride cotransporters, NKCC1 and KCC2, which increase and lower $[Cl^-]_i$, respectively.[55] Due to the low expression of the KCC2 extruder at birth, chloride accumulates inside the neuron via NKCC1. The developmentally upregulated expression of KCC2 toward the end of the first postnatal week results in the extrusion of chloride, causing the shift of GABA from the depolarizing to the hyperpolarizing direction.[57] KCC2 extrudes K^+ and Cl^- using the electrochemical gradient for K^+. Genetically encoded chloride probes have allowed investigators to directly monitor $[Cl^-]_i$ in a noninvasive way.[58] Using this tool, it has been clearly demonstrated that $[Cl^-]_i$ decreases during development in hippocampal neurons. It is worth mentioning that GABA-induced depolarization may still result in inhibition of cell firing if the positive shift in the membrane potential is associated with a strong "shunting inhibition."[59]

GABA may still have a depolarizing and excitatory action in mature CNS neurons when the equilibrium potential of this neurotransmitter (E_{GABA}) is more positive that the resting membrane potential.[60] Shifts of E_{GABA} have been suggested to underline modifications of $[Cl^-]_i$ observed in the suprachiasmatic nucleus during the circadian cycle of adult mammal neurons[61] (but see ref. 62). In addition, evidence has been provided that in adult animals, GABA excites layer V cortical cells through an action that critically depends on the resting membrane potential of the cell and on the temporal and spatial (dendritic and somatic) profile of GABAergic synaptic inputs of targeted neurons.[63]

Heterogeneity of $GABA_A$ Receptors

In mammals, $GABA_A$ receptors are highly heterogeneous. Twenty different subunits encoded by different genes have been cloned to date. They have a molecular weight ranging between 48,000 (γ2) and 64,000 (α4) kDa, and can be subdivided into seven structurally related subfamilies (α1–6, β1–4, γ1–3, ε, δ, θ, π, and ρ1–3) with a high degree of homology. The most common native receptor stoichiometry is two α subunits, two β subunits, and one γ/δ/ε subunit.[64] However, the θ subunit, cloned from the rat striatum, can also coassemble with α2, β1, and γ1.[65] In addition, alternative splicing further increases the diversity. Thus, the gene encoding the γ2 subunit has two splice variants, designed short (γ2S) and long (γ2L), that differ only in eight amino acids inserted into the large intracellular loop.[66] The γ2 splice variants show differential abundance in different brain regions and different age-related changes in their level of expression.[67] A recent study has demonstrated that the γ2S variant not only serves as an integral subunit of $GABA_A$ receptors but also as an accessory protein acting as an external modulator of fully formed receptors.[68] The ρ subunits, identified in the retina and possibly present also in other brain structures, initially described as $GABA_C$ receptors based on their different pharmacological properties (they are relatively insensitive to bicuculline but sensitive to the GABA analog *cis*-aminocrotonic acid), are closely related in sequence, structure, and function to other $GABA_A$ receptors, and therefore they should be fully considered $GABA_A$ receptors[69, 70] (see Fig. 8–4).

The multiplicity of subunit combinations has crucial consequences for the function of native receptors, as indicated by recombinant studies.[71] In addition, receptor heterogeneity constitutes the major determinant of receptor pharmacology. The kinetics and pharmacological diversity generated by the interaction of synaptically released GABA with different native receptors are particularly evident during development, when changes in the time course of GABA-mediated transmission are essential for synaptic integration. During the third postnatal week, in granule cells of the dentate gyrus, the acceleration of mIPSCs kinetics is related to the enhanced expression of the α1 subunit.[72] Similarly, the age-dependent decrease in deactivation kinetics of cerebellar granule cell IPSCs and their increased sensitivity to furosemide can be attributed to an enhanced expression of the α6 subunits.[73]

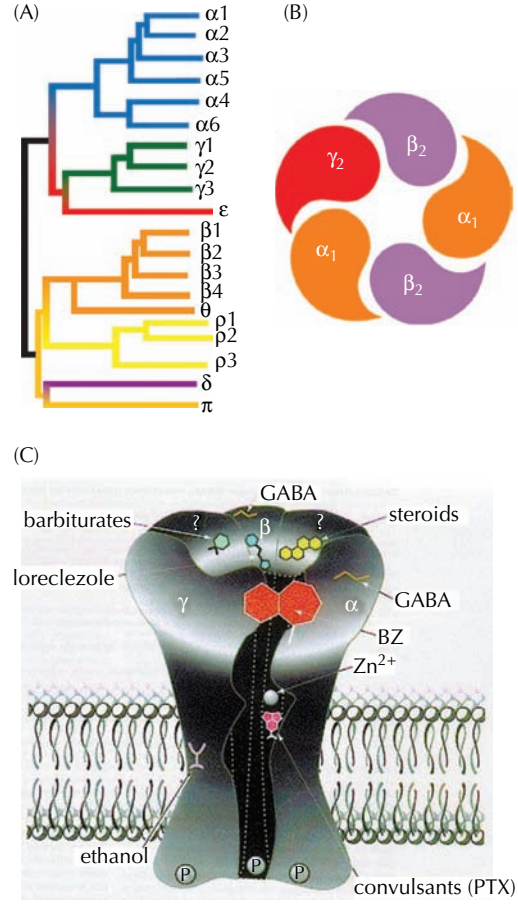

Figure 8–4. $GABA_A$ receptors. **A.** Dendrogram of the known 20 genes for $GABA_A$ receptors. The distances along each line are proportional to the degree of sequence identity between different homologous subunits. The Greek letters represent subunit families of high (>70%) identity. **B.** Schematic representation of the most common $GABA_A$ receptor assembly found in the CNS, α1β2γ2 with its appropriate stoichiometry of 2:2:1. **C.** Schematic representation of agonist binding sites and extracellular modulatory domains within the $GABA_A$ receptor. Adapted from refs. 2 and 70.

$GABA_A$ Receptor Distribution

In situ hybridization of mRNA and immunostaining experiments have revealed that the vast majority of $GABA_A$ receptors in the CNS contain the γ2 subunit.[74] Among the α subunits, α1 is the most abundant, and is often coexpressed

with the β2 and γ2 subunits. The α5 subunit is relatively rare except in the olfactory bulb, hypothalamus, and hippocampus, where it is located mainly at extrasynaptic sites. At synaptic sites, α5-containing $GABA_A$ receptors contribute to the slow decay of IPSCs evoked in the hippocampus by Schaffer collateral or stratum lacunosum-moleculare activation.[75,76] The α6 subunits, which are highly expressed in the forebrain and cerebellum, are extrasynaptic. The β subunits exist in functional receptors usually with only one type per pentamer (β2 is widespread, while β1 is less common). The δ subunit, localized at extrasynaptic dendritic and somatic membranes, is thought to be able to replace the γ subunit in the pentamer.[77] However, the α4 and α6 subunits are just as often combined with the δ subunit as with the γ2 subunit. In cerebellar granule cells the δ subunit is an obligatory partner of the α6 subunit, whereas in the forebrain it is primarily associated with the α4 subunit. Interestingly, some glutamatergic mossy fiber synapses in the cerebellum express abundant α6, γ2, and β2/3 but not δ subunits.[78] Although the functional role of these receptor subunits has not been fully elucidated, it is reasonable to assume that their activation by spillover of GABA from adjacent synapses may exert a modulatory effect on excitatory inputs. The ε subunit is rare, but in some areas, such as the hypothalamus, it can substitute for the γ or δ subunits.[79] The function of the θ and π subunits has not yet been fully characterized.

Modulation of $GABA_A$ Receptors

$GABA_A$ receptors are the targets for a variety of endogenous modulators or exogenous ligands generally used for the treatment of neurological and psychiatric disorders. Their pharmacological effect depends strictly on the subunit combinations. The use of transgenic mice in which different $GABA_A$ receptor subunits have been either deleted (due to gene ablation) or mutated has allowed better characterization of the functional properties of various receptor subunits.

MODULATION OF $GABA_A$ RECEPTORS BY ENDOGENOUS MOLECULES

$GABA_A$ receptors are substrates for phosphorylation via protein kinases. The resulting covalent modification constitutes one of the major mechanisms regulating their trafficking, cell surface expression and function.[80] Phosphorylation largely depends on subunit composition. For example, in pyramidal cells, intracellular delivery of protein kinase A (PKA), but not of protein kinase C (PKC), reduces the amplitude of mIPSCs, whereas in the dentate gyrus, PKC but not PKA enhances the peak amplitude of mIPSCs.[81] Protein kinase A–dependent phosphorylation depends strongly on the β subunits: PKA increases mIPSC amplitude in olfactory granule cells that express the β3 subunit[82] but reduces it in CA1 pyramidal cells that express the β1 subunit.[81]

$GABA_A$ receptors can also be modulated by zinc, a divalent cation highly expressed in neurons at areas associated with transmitter release.[83] In the hippocampus, zinc is stored with glutamate in synaptic vesicles present on mossy fiber terminals from which it can be released upon nerve stimulation in a calcium-dependent way to regulate synaptic excitation.[83] However, during sustained activation of mossy fibers, zinc may spill over to reach synaptic and/or extrasynaptic $GABA_A$ receptors. Zinc acts as an inhibitor of $GABA_A$ receptors lacking the γ subunits and composed mainly of αβ or αβδ subunits.[83] Therefore, it affects mainly tonic inhibition. However, at higher concentrations, zinc can also alter phasic inhibition by interfering with receptor gating.[84] Compelling evidence that endogenously released zinc can modulate IPSCs has been provided by Ruiz et al.,[85] who demonstrated that chelating zinc with either calcium-saturated EDTA or *N*,*N*,*N*′,*N*′-tetrakis (2-pyridylmethyl)ethylenediamine enhances the amplitude of IPSCs evoked by stimulation of the dentate gyrus.

Neuroactive steroids are potent endogenous modulators of $GABA_A$ receptors[86] and play a crucial role in neuronal excitability and brain function. Neuroactive steroids such as allopregnanolone and allotetrahydrodeoxycorticosterone (THDOC) at nanomolar concentrations have been shown to enhance GABA action by prolonging the channel open time, thus producing sedative-hypnotic, anxiolytic, and anticonvulsant effects.[87] Natural fluctuations in their level during the menstrual cycle and stress could affect several neuropsychiatric disorders including catamenial epilepsy, anxiety, and depression. At higher concentrations (>1 μM), neurosteroids produce a direct opening of

$GABA_A$ receptor–associated chloride channels.[88] This suggests the existence of at least two different binding sites on $GABA_A$ receptors, although their identification has remained elusive until now. Most of the behavioral effects of neurosteroids are mediated by extrasynaptic receptors containing δ and ε subunits. However, these compounds can also modulate synaptic receptors, as demonstrated by their ability to prolong IPSC decay.[89] However, the concentrations needed to produce such an effect vary considerably among different neurons.[86] Studies designed to mimic synaptic events using rapid application of saturated concentrations of GABA have suggested that neurosteroids produce the observed effect by slowing the recovery of $GABA_A$ receptor channels from the desensitized state.[90] Interestingly, modulation of synaptic α3β3γ2 $GABA_A$ receptors by neurosteroids may mediate the putative endogenous analgesic action of these compounds in the spinal cord.[91]

MODULATION OF $GABA_A$ RECEPTORS BY EXOGENOUS MOLECULES

$GABA_A$ receptors can be allosterically modulated by clinically important drugs including benzodiazepines, barbiturates, anesthetics, and so on. The pharmacological action of these compounds on $GABA_A$ receptor function has been extensively reviewed.[92–94] In this chapter, I will focus only on benzodiazepenes (BZs), which are widely used in clinical practice as anxiolytics, sedative-hypnotics, and anticonvulsants. By enhancing the apparent affinity of $GABA_A$ receptors for GABA, BZs potentiate the action of GABA via an increased probability of channel opening. Benzodiazepines bind at the interface between α and γ subunits and their pharmacology is influenced by these subunits, whereas β subunits, although necessary to construct a channel, do not affect the sensitivity of $GABA_A$ receptors to these drugs. Since most of the actions of diazepam are mediated by receptors composed of α1βγ2, α2βγ2, α3βγ2, and α5βγ2, a point mutation in the genes encoding for α subunits (a histidine to arginine substitution) is able to render these receptors insensitive to BZs.[95] Knockout mice expressing BZ-insensitive α1 subunits fail to exhibit the sedative effects of diazepam while retaining its anxiolytic response,[96] which seems to depend on α2, α3,[97] and/or γ2 subunits.[98] Interestingly, by positively modulating $GABA_A$ receptors, BZs can cause addiction in vulnerable individuals. A recent study has provided evidence that the addictive properties of these drugs depend on the increased firing of dopaminergic neurons in the ventral tegmental area following disinhibition.[99] This would rely on the potentiation of α1-containing $GABA_A$ receptors, usually absent on midbrain dopaminergic neurons, which in turn would selectively inhibit nearby GABAergic interneurons, leading to disinhibition of dopaminergic cells.

FUTURE PERSPECTIVES

The evidence reviewed here shows that $GABA_A$-mediated synaptic currents can be tuned by a variety of different factors acting at the presynaptic, cleft, and postsynaptic levels. The multitude of $GABA_A$ receptor subunits can assemble in a restricted and nonrandom way to generate distinct receptor subtypes with different physiological and pharmacological properties.

Although much remains to be learned about the mechanisms controlling GABA release and clearance, neuron–glia interaction, trafficking, and anchoring of $GABA_A$ receptors and associated proteins, a thorough assessment of $GABA_A$ receptor subtype-specific functions will provide the rational basis for developing new therapeutic tools for the treatment of neuropsychiatric disorders. In addition, using optogenetic techniques[100] to target specific neurons expressing different $GABA_A$ receptor subtypes will allow elucidation of the functional role of various subunits in network activity and animal behavior.

Finally, a better understanding of the mechanisms by which excitatory GABAergic signaling (phasic and tonic) controls adult neurogenesis and the integration of newborn neurons into existing neuronal circuits[101] will allow highlighting of the plasticity and regenerative capacities of the adult mammalian brain in response to experience.

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REFERENCES

1. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci*. 2005;6:215–229.
2. Cherubini E, Conti F. Generating diversity at GAB Aergic synapses. *Trends Neurosci*. 2001;24:155–162.
3. Buzsáki G, Draguhn A. Neuronal oscillations in cortical networks *Science*. 2004;304:1926–1929.
4. Olsen RW, DeLorey TM, Gordey M, Kang MH. GABA receptor function and epilepsy. *Adv Neurol*. 1999;79:499–510.
5. Somogyi P, Klausberger T. Defined types of cortical interneurone structure space and spike timing in the hippocampus. *J Physiol*. 2005;562:9–26.
6. Miles R, Tóth K, Gulyás AI, Hájos N, Freund TF. Differences between somatic and dendritic inhibition in the hippocampus. *Neuron*. 1996;16:815–823.
7. Freund TF, Buzsáki G. Interneurons of the hippocampus. *Hippocampus.* 1996;6:347–470.
8. Klausberger T, Somogyi P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science.* 2008;321:53–57.
9. Wu Y, Wang W, Díez-Sampedro A, Richerson GB. Nonvesicular inhibitory neurotransmission via reversal of the GABA transporter GAT-1. *Neuron*. 2007;56:851–865.
10. During MJ, Ryder KM, Spencer DD. Hippocampal GABA transporter function in temporal-lobe epilepsy. *Nature*. 1995;376:174–177.
11. Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science*. 1993;262:740–744.
12. Llano I, González J, Caputo C, Lai FA, Blayney LM, Tan YP, Marty A. Presynaptic calcium stores underlie large-amplitude miniature IPSCs and spontaneous calcium transients. *Nat Neurosci*. 2000;3:1256–1265.
13. Collin T, Marty A, Llano I. Presynaptic calcium stores and synaptic transmission. *Curr Opin Neurobiol*. 2005;15:275–281.
14. Galante M, Marty A. Presynaptic ryanodine-sensitive calcium stores contribute to evoked neurotransmitter release at the basket cell–Purkinje cell synapse. *J Neurosci*. 2003;23:11229–11234.
15. Kondo S, Marty A. Synaptic currents at individual connections among stellate cells in rat cerebellar slices. *J Physiol*. 1998;509:221–232.
16. Auger C, Marty A. Heterogeneity of functional synaptic parameters among single release sites. *Neuron*. 1997;19:139–150.
17. Auger C, Kondo S, Marty A. Multivesicular release at single functional synaptic sites in cerebellar stellate and basket cells. *J Neurosci*. 1998;8:4532–4547.
18. Nusser Z, Cull-Candy S, Farrant M. Differences in synaptic GABA(A) receptor number underlie variation in GABA mini amplitude. *Neuron*. 1997;19:697–709.
19. Biró AA, Holderith NB, Nusser Z. Release probability–dependent scaling of the postsynaptic responses at single hippocampal GABAergic synapses. *J Neurosci.* 2006;26:12487–12496.
20. Ruiz A, Fabian-Fine R, Scott R, Walker MC, Rusakov DA, Kullmann DM. $GABA_A$ receptors at hippocampal mossy fibers. *Neuron*. 2003;39:961–973.
21. Xiao C, Zhou C, Li K, Ye JH. Presynaptic GABAA receptors facilitate GABAergic transmission to dopaminergic neurons in the ventral tegmental area of young rats. *J Physiol*. 2007;580:731–743.
22. Safiulina VF, Fattorini G, Conti F, Cherubini E. GABAergic signaling at mossy fiber synapses in neonatal rat hippocampus. *J Neurosci*. 2006;26:597–608.
23. Nakamura M, Sekino Y, Manabe T. GABAergic interneurons facilitate mossy fiber excitability in the developing hippocampus. *J Neurosci*. 2007;27:1365–1373.
24. Pouzat C, Marty A. Somatic recording of GABAergic autoreceptor current in cerebellar stellate and basket cells. *J Neurosci*. 1999;19:1675–1690.
25. Trigo FF, Chat M, Marty A. Enhancement of GABA release through endogenous activation of axonal GABA(A) receptors in juvenile cerebellum. *J Neurosci*. 2007;27:12452–12463.
26. Poncer JC, McKinney RA, Gähwiler BH, Thompson SM. Either N- or P-type calcium channels mediate GABA release at distinct hippocampal inhibitory synapses. *Neuron*. 1997;18:463–472.
27. Mapelli L, Rossi P, Nieus T, D'Angelo E. Tonic activation of $GABA_B$ receptors reduces release probability at inhibitory connections in the cerebellar glomerulus. *J Neurophysiol*. 2009;101:3089–3099.
28. Safiulina VF, Cherubini E. At immature mossy fibers–CA3 connections, activation of presynaptic GABA(B) receptors by endogenously released GABA contributes to synapses silencing. *Front Cell Neurosci*. 2009;3:1–11.
29. Mozrzymas JW, Barberis A, Michalak K, Cherubini E. Chlorpromazine inhibits miniature GABAergic currents by reducing the binding and by increasing the unbinding rate of $GABA_A$ receptors. *J Neurosci*. 1999;19:2474–2488.
30. Mozrzymas JW, Zarnowska ED, Pytel M, Mercik K. Modulation of GABA(A) receptors by hydrogen ions reveals synaptic GABA transient and a crucial role of the desensitization process. *J Neurosci*. 2003;23:7981–7992.
31. Overstreet LS, Westbrook GL, Jones MV. Measuring and modeling the spatiotemporal profile of GABA at the synapse. In: Quick M, ed. *Transmembrane Transporters*. New York: Wiley; 2002:259–275.
32. Jones MV, Westbrook GL. Desensitized states prolong $GABA_A$ channel responses to brief agonist pulses. *Neuron.* 1995;15:181–191.
33. Barberis A, Petrini EM, Cherubini E. Presynaptic source of quantal size variability at GABAergic synapses in rat hippocampal neurons in culture. *Eur J Neurosci*. 2004;20:1803–1810.
34. Szabadics J, Tamás G, Soltesz I. Different transmitter transients underlie presynaptic cell type specificity

of $GABA_A$, slow and $GABA_A$, fast. *Proc Natl Acad Sci USA*. 2007;104:14831–14836.
35. Oláh S, Füle M, Komlósi G, Varga C, Báldi R, Barzó P, Tamás G. Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. *Nature*. 2009;461:1278–12781.
36. Rusakov DA, Kullmann DM. A tortuous and viscous route to understanding diffusion in the brain. *Trends Neurosci*. 1998;21:469–470.
37. Conti F, Minelli A, Melone M. GABA transporters in the mammalian cerebral cortex: localization, development and pathological implications. *Brain Res Brain Res Rev*. 2004;45:196–212.
38. Thompson SM, Gähwiler BH. Effects of the GABA uptake inhibitor tiagabine on inhibitory synaptic potentials in rat hippocampal slice cultures. *J Neurophysiol*. 1992;67:1698–1701.
39. Engel D, Schmitz D, Gloveli T, Frahm C, Heinemann U, Draguhn A. Laminar difference in GABA uptake and GAT-1 expression in rat CA1. *J Physiol*. 1998; 512:643–649.
40. Nusser Z, Mody I. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol*. 2002;87:2624–2628.
41. Semyanov A, Walker MC, Kullmann DM. GABA uptake regulates cortical excitability via cell type–specific tonic inhibition. *Nat Neurosci*. 2003;6:484–490.
42. Keros S, Hablitz JJ. Subtype-specific GABA transporter antagonists synergistically modulate phasic and tonic $GABA_A$ conductances in rat neocortex. *J Neurophysiol*. 2005;94:2073–2085.
43. Bragina L, Marchionni I, Omrani A, Cozzi A, Pellegrini-Giampietro DE, Cherubini E, Conti F. GAT-1 regulates both tonic and phasic GABA(A) receptor-mediated inhibition in the cerebral cortex. *J Neurochem*. 2008;105:1781–1793.
44. Renner M, Specht CG, Triller A. Molecular dynamics of postsynaptic receptors and scaffold proteins. *Curr Opin Neurobiol*. 2008;18:532–540.
45. Kneussel M, Loebrich S. Trafficking and synaptic anchoring of ionotropic inhibitory neurotransmitter receptors. *Biol Cell*. 2007;99:297–309.
46. Tretter V, Jacob TC, Mukherjee J, Fritschy JM, Pangalos MN, Moss SJ. The clustering of GABA(A) receptor subtypes at inhibitory synapses is facilitated via the direct binding of receptor alpha 2 subunits to gephyrin. *J Neurosci*. 2008;28:1356–1365.
47. Kneussel M, Brandstätter JH, Laube B, Stahl S, Müller U, Betz H. Loss of postsynaptic GABA(A) receptor clustering in gephyrin-deficient mice. *J Neurosci*. 1999;19:9289–9297.
48. Marchionni I, Kasap Z, Mozrzymas JW, Sieghart W, Cherubini E, Zacchi P. New insights on the role of gephyrin in regulating both phasic and tonic GABAergic inhibition in rat hippocampal neurons in culture. *Neuroscience*. 2009;164:552–562.
49. Poulopoulos A, Aramuni G, Meyer G, Soykan T, Hoon M, Papadopoulos T, Zhang M, Paarmann I, Fuchs C, Harvey K, Jedlicka P, Schwarzacher SW, Betz H, Harvey RJ, Brose N, Zhang W, Varoqueaux F. Neuroligin 2 drives postsynaptic assembly at perisomatic inhibitory synapses through gephyrin and collybistin. *Neuron*. 2009;63:628–642.
50. Südhof TC. Neuroligins and neurexins link synaptic function to cognitive disease. *Nature*. 2008;455:903–911.
51. Galzi JL, Devillers-Thiéry A, Hussy N, Bertrand S, Changeux JP, Bertrand D. Mutations in the channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. *Nature*. 1992;359:500–505.
52. Kaila K, Voipio J. Postsynaptic fall in intracellular pH induced by GABA-activated bicarbonate conductance. *Nature*. 1987;330:163–165.
53. Kaila K. Ionic basis of $GABA_A$ receptor channel function in the nervous system. *Prog Neurobiol*. 1994;42:489–537.
54. Lee J, Taira T, Pihlaja P, Ransom BR, Kaila K. Effects of CO_2 on excitatory transmission apparently caused by changes in intracellular pH in the rat hippocampal slice. *Brain Res*. 1996;706:210–216.
55. Blaesse P, Airaksinen MS, Rivera C, Kaila K. Cation-chloride cotransporters and neuronal function. *Neuron*. 2009;61:820–838.
56. Cherubini E, Gaiarsa JL, Ben-Ari Y. GABA: an excitatory transmitter in early postnatal life. *Trends Neurosci*. 1991;14:515–519.
57. Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. The K^+/Cl^- cotransporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*. 1999;397:251–255.
58. Bregestovski P, Waseem T, Mukhtarov M. Genetically encoded optical sensors for monitoring of intracellular chloride and chloride-selective channel activity. *Front Mol Neurosci*. 2009;2:1–15.
59. Mohajerani MH, Cherubini E. Spontaneous recurrent network activity in organotypic rat hippocampal slices. *Eur J Neurosci*. 2005;22:107–118.
60. Marty A, Llano I. Excitatory effects of GABA in established brain networks. *Trends Neurosci*. 2005;28:284–289.
61. Wagner S, Castel M, Gainer H, Yarom Y. GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. *Nature*. 1997;387:598–603.
62. Choi HJ, Lee CJ, Schroeder A, Kim YS, Jung SH, Kim JS, Kim do Y, Son EJ, Han HC, Hong SK, Colwell CS, Kim YI. Excitatory actions of GABA in the suprachiasmatic nucleus. *J Neurosci*. 2008;28: 5450–5459.
63. Gulledge AT, Stuart GJ. Excitatory actions of GABA in the cortex. *Neuron*. 2003;37:299–309.
64. Farrar SJ, Whiting PJ, Bonnert TP, McKernan RM. Stoichiometry of a ligand-gated ion channel determined by fluorescence energy transfer. *J Biol Chem*. 1999;274:10100–10104.
65. Bonnert TP, McKernan RM, Farrar S, le Bourdellès B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghji DJ, Brown N, Wafford KA, Whiting PJ. Theta, a novel gamma-aminobutyric acid type A receptor subunit. *Proc Natl Acad Sci USA*. 1999;96: 9891–9896.
66. Whiting P, McKernan RM, Iversen LL. Another mechanism for creating diversity in gamma-aminobutyrate type A receptors: RNA splicing directs expression of two forms of the gamma 2 phosphorylation site. *Proc Natl Acad Sci USA*. 1990;87:9966–9970.
67. Gutiérrez A, Khan ZU, De Blas AL. Immunocytochemical localization of gamma 2 short and gamma 2 long subunits of the $GABA_A$ receptor in the rat brain. *J Neurosci*. 1994;14:7168–7179.

68. Boileau AJ, Pearce RA, Czajkowski C. The short splice variant of the (gamma)2 subunit acts as an external modulator of $GABA_A$ receptor function. *J Neurosci*. 2010;30:4895–4903.
69. Olsen RW, Sieghart W. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev.* 2008;60:243–260.
70. Nutt D. $GABA_A$ receptors: subtypes, regional distribution, and function. *J Clin Sleep Med*. 2006;2:S7–S11.
71. Macdonald RL, Olsen RW. $GABA_A$ receptor channels. *Annu Rev Neurosci*. 1994;17:569–602.
72. Hollrigel GS, Soltesz I. Slow kinetics of miniature IPSCs during early postnatal development in granule cells of the dentate gyrus. *J Neurosci*. 1997;17: 5119–5128.
73. Tia S, Wang JF, Kotchabhakdi N, Vicini S. Developmental changes of inhibitory synaptic currents in cerebellar granule neurons: role of GABA(A) receptor alpha 6 subunit. *J Neurosci.* 1996;16: 3630–3640.
74. Sieghart W, Ernst M. Heterogeneity of $GABA_A$ receptors: revised interest in the development of subtype-selective drugs. *Curr Med Chem Cent Nerv Syst Agents*. 2005;5:217–242.
75. Zarnowska ED, Keist R, Rudolph U, Pearce RA. $GABA_A$ receptor alpha5 subunits contribute to $GABA_A$, slow synaptic inhibition in mouse hippocampus. *J Neurophysiol*. 2009;101:1179–1191.
76. Vargas-Caballero M, Martin LJ, Salter MW, Orser BA, Paulsen O. Alpha5 subunit–containing GABA(A) receptors mediate a slowly decaying inhibitory synaptic current in CA1 pyramidal neurons following Schaffer collateral activation. *Neuropharmacology*. 2010;58:668–675.
77. Sieghart W, Fuchs K, Tretter V, Ebert V, Jechlinger M, Höger H, Adamiker D. Structure and subunit composition of GABA(A) receptors. *Neurochem Int*. 1999;34:379–385.
78. Nusser Z, Sieghart W, Somogyi P. Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci.* 1998;18:1693–1703.
79. Whiting PJ, McAllister G, Vassilatis D, Bonnert TP, Heavens RP, Smith DW, Hewson L, O'Donnell R, Rigby MR, Sirinathsinghji DJ, Marshall G, Thompson SA, Wafford KA. Neuronally restricted RNA splicing regulates the expression of a novel $GABA_A$ receptor subunit conferring atypical functional properties. *J Neurosci*. 1997;17:5027–5037.
80. Jacob TC, Moss SJ, Jurd R. GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci*. 2008;9: 331–343.
81. Poisbeau P, Cheney MC, Browning MD, Mody I. Modulation of synaptic $GABA_A$ receptor function by PKA and PKC in adult hippocampal neurons. *J Neurosci*. 1999;19:674–683.
82. Nusser Z, Sieghart W, Mody I. Differential regulation of synaptic $GABA_A$ receptors by cAMP-dependent protein kinase in mouse cerebellar and olfactory bulb neurones. *J Physiol.* 1999;521:421–435.
83. Smart TG, Hosie AM, Miller PS. Zn^{2+} ions: modulators of excitatory and inhibitory synaptic activity. *Neuroscientist*. 2004;10:432–442.
84. Barberis A, Cherubini E, Mozrzymas JW. Zinc inhibits miniature GABAergic currents by allosteric modulation of $GABA_A$ receptor gating. *J Neurosci*. 2000:20:8618–8627.
85. Ruiz A, Walker MC, Fabian-Fine R, Kullmann DM. Endogenous zinc inhibits GABA(A) receptors in a hippocampal pathway. *J Neurophysiol*. 2004;91:1091–1096.
86. Herd MB, Belelli D, Lambert JJ. Neurosteroid modulation of synaptic and extrasynaptic GABA(A) receptors. *Pharmacol Ther.* 2007;116:20–34.
87. Barker JL, Harrison NL, Lange GD, Owen DG. Potentiation of gamma-aminobutyric-acid-activated chloride conductance by a steroid anaesthetic in cultured rat spinal neurones. *J Physiol.* 1987;386:485–501.
88. Callachan H, Cottrell GA, Hather NY, Lambert JJ, Nooney JM, Peters JA. Modulation of the $GABA_A$ receptor by progesterone metabolites. *Proc R Soc Lond B Biol Sci.* 1987;231:359–369.
89. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci*. 2005;6:565–575.
90. Zhu WJ, Vicini S. Neurosteroid prolongs $GABA_A$ channel deactivation by altering kinetics of desensitized states. *J Neurosci*. 1997;17:4022–4031.
91. Poisbeau P, Patte-Mensah C, Keller AF, Barrot M, Breton JD, Luis-Delgado OE, Freund-Mercier MJ, Mensah-Nyagan AG, Schlichter R. Inflammatory pain upregulates spinal inhibition via endogenous neurosteroid production. *J Neurosci*. 2005;25: 11768–11776.
92. Sieghart W. Structure and pharmacology of gamma-aminobutyric acid A receptor subtypes. *Pharmacol Rev.* 1995;47:181–234.
93. Korpi ER, Gründer G, Lüddens H. Drug interactions at GABA(A) receptors. *Prog Neurobiol*. 2002;67:113–159.
94. Rudolph U, Möhler H. GABA-based therapeutic approaches: $GABA_A$ receptor subtype functions. *Curr Opin Pharmacol*. 2006;6:18–23.
95. Rudolph U, Möhler H. Analysis of $GABA_A$ receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. *Annu Rev Pharmacol Toxicol.* 2004;44:475–498.
96. McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, Garrett L, Bristow L, Marshall G, Macaulay A, Brown N, Howell O, Moore KW, Carling RW, Street LJ, Castro JL, Ragan CI, Dawson GR, Whiting PJ. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. *Nat Neurosci.* 2000;3:587–592.
97. Löw K, Crestani F, Keist R, Benke D, Brünig I, Benson JA, Fritschy JM, Rülicke T, Bluethmann H, Möhler H, Rudolph U. Molecular and neuronal substrate for the selective attenuation of anxiety. *Science*. 2000;290:131–134.
98. Chandra D, Korpi ER, Miralles CP, De Blas AL, Homanics GE. $GABA_A$ receptor gamma 2 subunit knockdown mice have enhanced anxiety-like behavior but unaltered hypnotic response to benzodiazepines. *BMC Neurosci*. 2005;6:30–43.
99. Tan KR, Brown M, Labouèbe G, Yvon C, Creton C, Fritschy JM, Rudolph U, Lüscher C. Neural bases

for addictive properties of benzodiazepines. *Nature*. 2010;463:769–774.
100. Zhang F, Gradinaru V, Adamantidis AR, Durand R, Airan RD, de Lecea L, Deisseroth K. Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. *Nat Protoc*. 2010;5:439–456.
101. Ge S, Pradhan DA, Ming GL, Song H. GABA sets the tempo for activity-dependent adult neurogenesis. *Trends Neurosci*. 2007;30:1–8.

Chapter 9

Tonic $GABA_A$ Receptor–Mediated Signaling in Epilepsy

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Fast inhibitory signaling in the brain has conventionally been considered to be predominantly mediated by the vesicular release of GABA from presynaptic terminals onto postsynaptic $GABA_A$ receptors.[1] Transient opening of such receptors results in a brief increase in postsynaptic permeability to Cl^-, generating an inhibitory postsynaptic potential (IPSP) that reduces the probability of firing of the neuron. However, there is abundant evidence that GABA can also act relatively far from its site of release, and this, together with several other discoveries in the last two decades, has contributed to a reappraisal of the roles of $GABA_A$ receptors in modulating neuronal and circuit excitability.[1]

Much of the new understanding of GABAergic transmission comes from in vitro brain slice experiments, which allow direct postsynaptic recording of IPSPs or, by voltage-clamping the postsynaptic neuron, inhibitory postsynaptic currents (IPSCs). Importantly, during the synchronous activation of many synapses, sufficient GABA can "spill" out of the synapse to activate extrasynaptic $GABA_A$ and also metabotropic $GABA_B$ receptors, prolonging the IPSP.[2,3] This may serve to dampen excessive network activity. More recently, a slower form of signaling has been described in which high-affinity, slowly desensitizing $GABA_A$ receptors detect low concentrations of ambient GABA in the extracellular space even in the absence of evoked release. This has been termed *tonic $GABA_A$ receptor–mediated inhibition*[1,4] (see Fig. 9–1).

Tonic $GABA_A$ receptor activity was evident in some of the first patch-clamp studies of GABAergic inhibition in the rodent hippocampus[5] and was later described in layer III cells of somatosensory cortical slices.[6] In these and other studies, application of a $GABA_A$ receptor antagonist reduced the resting membrane conductance and produced a shift in the baseline "holding" current required to clamp neurons at a given membrane potential (Fig. 9–1). Shortly afterward, $GABA_A$ receptor–mediated tonic

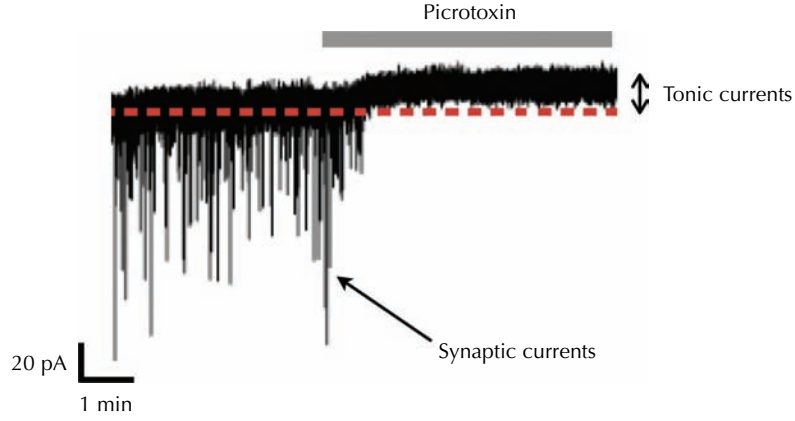

Figure 9–1. Voltage-clamp recording from a dentate gyrus granule cell in the presence of glutamate receptor blockers. Inhibitory postsynaptic currents (IPSCs) are represented by phasic inward currents (downward deflections) that are inhibited by the $GABA_A$ receptor blocker picrotoxin. Picrotoxin also blocks the tonic current, which results in an outward shift of the holding current.

currents were described in cerebellar granule cells.[7,8] Application of $GABA_A$ receptor antagonists in this preparation not only resulted in a shift in the baseline current but also decreased the background noise, consistent with a block of stochastic channel openings. These tonic currents are both developmentally regulated and cell-type specific.[1,4] Indeed, in some neurons, the tonic current represents a greater proportion of the total $GABA_A$ receptor–mediated current than that mediated by spontaneous synaptic activity.[1,4]

Although the tonic activity of $GABA_A$ receptors is often termed tonic *inhibition,* it is important to bear in mind that $GABA_A$ receptors do not always inhibit neurons. Opening of these receptors has two main effects: a membrane potential change and a decrease in membrane resistance. The membrane potential change caused by opening of $GABA_A$ receptors depends on the electrochemical gradients for Cl^- and, to a lesser extent, for $HCO3^-$ ions that also move through the open channel.[9] Irrespective of whether the receptors are activated phasically (by local presynaptic exocytosis) or tonically (by ambient GABA), these gradients determine if $GABA_A$ receptors de- or hyperpolarize the neuron. In mature neurons, $GABA_A$ receptors are generally hyperpolarizing, but early in development, they can be depolarizing because the intracellular Cl^- concentration is high.[9] These voltage changes further interact with subthreshold voltage-dependent conductances and propagate within dendrites. $GABA_A$ receptor opening also shunts excitatory inputs by decreasing the resistance of the membrane, and this provides an additional mechanism that reduces the excitability of the postsynaptic cell irrespective of whether GABAergic signaling is de- or hyperpolarizing.

$GABA_A$ RECEPTOR SUBTYPES UNDERLYING TONIC CONDUCTANCES

$GABA_A$ receptors are pentameric structures composed of 5 subunits from a possible choice of 19 subunits.[10] Other than those containing the ρ subunit (which form a separate class of $GABA_C$ receptors), $GABA_A$ receptors usually consist of two (from a repertoire of six) α subunits, two (from three) β subunits, and another subunit (usually a γ or δ subunit). There are thousands of possible subunit combinations; however, relatively few are expressed with any frequency in the mammalian central nervous system. The specific subunit combinations confer different channel conductances, affinities for GABA, and kinetics. Moreover, different subunits alter the response of the receptors to endogenous and exogenous modulators, including Zn^{2+} and neurosteroids. The subcellular location of $GABA_A$ receptors (synaptic versus extrasynaptic) is also determined

by subunit composition. As a general rule, γ subunits are required for synaptic expression; however, γ subunit–containing receptors are also found extrasynaptically.[11] δ Subunit–containing $GABA_A$ receptors, in contrast, are exclusively extrasynaptic. The β subunit may also play a role in determining the location, trafficking, and pharmacology of $GABA_A$ receptors.[12] Importantly, the receptors mediating tonic currents are different from those contributing to the peak synaptic currents and tend to have a high affinity for GABA, desensitize slowly and incompletely in the presence of the agonist, and are probably located extrasynaptically. However, as hinted above, the distinction between receptors mediating phasic and tonic inhibition is blurred because, during intense synaptic activity, exocytosed GABA can diffuse out of the synaptic cleft and overwhelm transporters to activate peri- and extrasynaptic receptors.[13,14]

Among the receptor subtypes that contribute to tonic signaling are $\alpha_4\beta_x\delta$ receptors, which have been proposed to mediate the tonic current in dentate granule cells[15–17] and thalamocortical neurons,[18–20] and $\alpha_6\beta_x\delta$ receptors that mediate the tonic current in cerebellar granule cells.[8,16,21] Other receptor subtypes may also mediate tonic currents, including $\alpha_5\beta_x\gamma_x$ receptors in CA1 pyramidal cells[22] and ε-containing receptors in hypothalamic neurons.[23] The ε-containing $GABA_A$ receptors, which occur not only in the hypothalamus but also in the amygdala and locus ceruleus, are of specific interest in this regard, as they can open spontaneously in the absence of GABA but desensitize with high concentrations of GABA.[23] This leads to the prediction that tonic $GABA_A$ receptor–mediated currents in these brain structures should be paradoxically "turned off" by increases in extracellular GABA. It is likely that other $GABA_A$ receptor subunit combinations are also expressed extrasynaptically and are able to mediate a tonic current. Some whole-cell patch-clamp data complemented by single-channel recordings support the presence of a zolpidem-sensitive $GABA_A$ receptor that can mediate a tonic current in the hippocampus, implying the presence of α_1, α_2, or α_3 with a γ subunit.[24,25] These subunit combinations are not usually associated with high-affinity extrasynaptic receptors, but rather with low-affinity synaptic receptors. The presence of receptors with unusual subunit composition, such as $\alpha_x\beta_3$ without either γ or δ, has also been inferred on the basis of sensitivity to GABA and Zn^{2+} as well as single-channel conductance.[26] These studies illustrate our lack of knowledge of the full range of $GABA_A$ receptor subtypes, a situation that is not helped by the poor availability of subtype-specific agonists and antagonists. Moreover, there is growing evidence that within one cell type (e.g., CA1 pyramidal cells, dentate gyrus granule cells and interneurons), more than one receptor subtype may contribute to the tonic current.[25,27–31] A heterogeneous population of receptors with different affinities mediating the tonic current increases the number of potential modulators and also extends the range of extracellular GABA concentrations that can modulate excitability of the neuron.

FUNCTIONS OF TONIC $GABA_A$ RECEPTOR CURRENTS

The physiological importance of tonic $GABA_A$ receptor–mediated currents is supported by the finding that genetic deletion of the α_6 subunit, resulting in the absence of tonic inhibition in cerebellar granule cells, leads to a compensatory upregulation of two-pore K^+ channels to restore the resting membrane conductance.[32] There is also considerable evidence that tonic currents mediate a qualitatively different form of inhibition from that generated by synaptic currents. Clearly, tonic currents provide a form of signaling over a different timescale than that mediated by phasic synaptic currents. Although the mechanisms that modulate tonic conductances are poorly understood, it is likely that they fluctuate relatively slowly, on a timescale of seconds to days. Among the mechanisms that have been reported to modulate tonic inhibition are alterations in receptor expression, variations in extracellular GABA concentrations, changes in endogenous neuromodulators (in particular neurosteroids), and a variety of neuroactive drugs, both therapeutic and recreational.[4] Thus, tonic $GABA_A$ receptor–mediated signaling is far more than a background leak conductance, and the developmental and computational roles of tonic signaling in different neurons are beginning to attract attention.

Tonically active $GABA_A$ receptors have been detected in embryonic neocortical cells in situ in the ventricular zone[33] prior to synapse

formation in hippocampal principal cells.[34] Currents mediated by these receptors have, therefore, been hypothesized to play a part in neuronal development. This hypothesis has received further support from the observation of tonic currents in developing dentate gyrus granule cells; these currents are depolarizing because of the high intracellular chloride concentration and have been suggested to have a trophic effect, promoting dendritic development.[35]

In neurons where the Cl^- reversal potential is close to the resting membrane potential, the major effect of tonically active $GABA_A$ receptors is to increase membrane conductance. This will reduce the membrane time constant[4] and, at the same time, attenuate rapid fluctuations in membrane voltage arising from the action of excitatory synapses. The consequences for the computational properties of individual neurons are difficult to predict. In particular, spontaneous fluctuations in membrane potential play an important role in setting the slope or gain of the input–output relationship of a neuron.[36–39] Information is encoded in very different ways in different brain circuits, and the effect of the tonic conductance on the input–output relationship is likely to depend on whether information is represented as changes in the rate of firing of individual neurons (*rate coding*) or as changes in the recruitment of neurons to firing threshold (*sparse coding*).[39] For small neurons that use rate coding (such as cerebellar granule cells), there is convincing evidence that the gain of the input–output slope is sensitive to tonic inhibition.[36,39] In contrast, tonic inhibition has less effect on the gain of the input–output function of a complex neuron that uses sparse coding (such as a hippocampal pyramidal cell) and instead has a larger effect on the offset of the input–output curve[37,39] (Fig. 9–2). Such an effect on offset is further promoted by a strong outward rectification of the tonic current in hippocampal pyramidal cells.[37] This effect on excitability may contribute to increasing the threshold for induction of long-term potentiation.[40] These considerations provide a possible explanation for the finding that genetic ablation or pharmacological reduction of tonic currents mediated by α_5 subunits, which contribute to tonic currents in hippocampal pyramidal cells, increases the rate of spatial learning.[41–43] However, this increased rate of learning may be at the expense of a general increase in the probability that a neuron will fire, loss of sparse network activity, and consequently a decrease in the ability to discriminate different input patterns.

Tonically active $GABA_A$ receptors not only shunt excitatory currents, but also affect the membrane potential. In many neurons in the adult brain $GABA_A$ receptors hyperpolarize

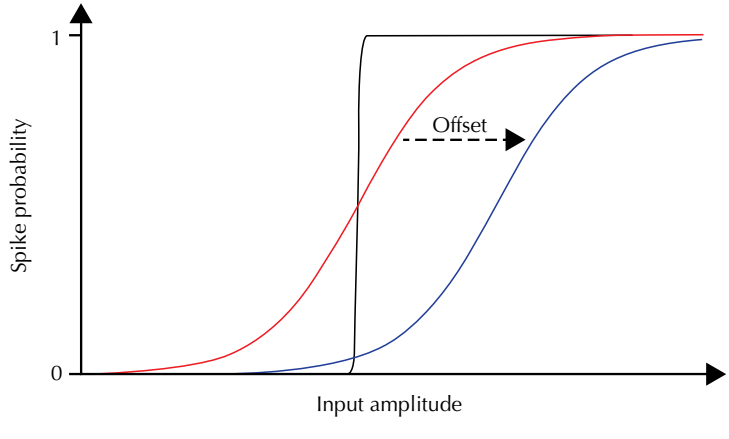

Figure 9–2. The input–output function of a pyramidal cell can be represented by the probability of neuronal firing plotted against the amplitude of the input (black curve). Synaptic noise reduces the gain (slope) of the input–output function because inputs that would otherwise be subthreshold occasionally summate with positive membrane potential fluctuations, thus reaching the firing threshold (red curve). Tonic currents, in contrast, predominantly affect offset, shifting the curve to the right (blue curve).

the membrane, and this aspect of tonic currents further reduces neuronal firing. However, in situations in which $GABA_A$ receptor–mediated currents are strongly depolarizing, tonic current can have a paradoxical excitatory effect by bringing neurons closer to the action potential threshold. Tonic activation of presynaptic $GABA_A$ receptors on mossy fiber terminals has such a depolarizing effect, promoting glutamate release and long-term potentiation.[44] Furthermore, in the thalamus, the hyperpolarizing effect of tonic currents can change the firing pattern of thalamocortical neurons from a regular firing to a burst-firing pattern,[19] which is important for the generation of sleep rhythms (such as slow waves during deep sleep) and also for absence seizures (see below).

To understand the network effects of tonic $GABA_A$ signaling, it is also necessary to consider its relative magnitude in different cell types.[4,25] Under baseline conditions in vitro, the $GABA_A$ receptor–mediated tonic current in hippocampal interneurons is considerably larger than the current mediated by spontaneous IPSCs and thus is an important determinant of interneuron excitability.[25] In keeping with this situation, a low concentration of picrotoxin that relatively selectively inhibits tonic currents significantly increases the frequency of spontaneous IPSCs in hippocampal pyramidal cells. These findings suggest that tonic conductances in interneurons act as a homeostatic regulator of synaptic inhibition of principal cells: if the ambient GABA concentration decreases, interneurons become more excitable, resulting in an increase in the frequency of GABA receptor–mediated IPSCs in pyramidal cells.[25] Conversely, an increase in ambient GABA concentration would be expected to render interneurons relatively less excitable, leading to a decrease in synaptic inhibition, while tonic inhibition of pyramidal neurons increases (Fig.9–3). What are the likely network consequences of a shift from synaptic to tonic inhibition in pyramidal cells? The decrease in synaptic inhibition would be expected to decrease membrane voltage fluctuations and therefore to increase the gain of the neuron's input amplitude-firing probability curve, and at the same time to shift it to the left (decrease offset). However, this shift due to the decrease in synaptic inhibition may be more than compensated for by the increase in tonic inhibition.[37] The net effect would therefore be to increase the neuronal gain while maintaining or increasing the offset.[37] Interestingly, extracellular GABA has been shown to increase in the hippocampus when an animal is stressed or exposed to a new environment,[45,46] and by increasing the neuronal gain, such an effect is likely to promote neuronal firing and long-term potentiation, while the increase in neuronal offset prevents indiscriminate neuronal firing.

Cell-type specificity has also been reported in the thalamus; tonic $GABA_A$ receptor–mediated currents are present in thalamocortical neurons but not in reticular thalamic neurons.[18,19] The thalamocortical neurons gate sensory input into the neocortex, while the reticular thalamic neurons are inhibitory and provide the main synaptic inhibitory drive onto thalamocortical neurons. Therefore, in contrast to the hippocampus, the synaptic inhibitory currents onto thalamocortical neurons are likely to remain unaltered by a local increase in extracellular GABA.

Tonic inhibition likely plays a role in numerous other brain functions. For example, in the striatum, tonic $GABA_A$ receptor–mediated signaling has been shown to be larger in D2

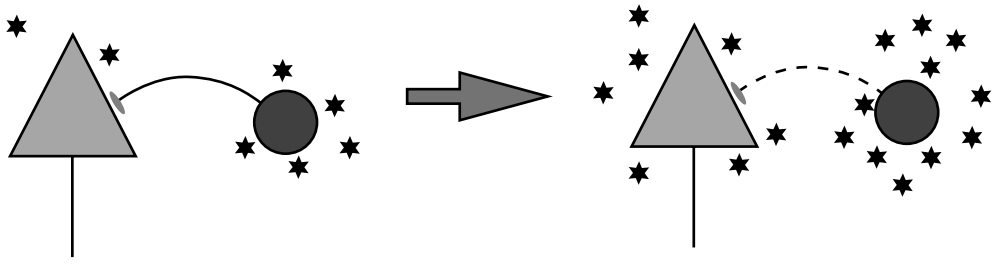

Figure 9–3. Tonic $GABA_A$ receptor–mediated currents in interneurons of the hippocampus are relatively larger and more sensitive to low ambient GABA concentrations than are currents in pyramidal cells. As the GABA concentration increases, interneurons become less excitable, so phasic inhibition of pyramidal cells is "switched off," while tonic inhibition of pyramidal cells increases.

dopamine receptor-expressing spiny stellate cells that project to the globus pallidum (the *indirect* pathway) than in D1-expressing cells in the *direct* pathway that project to the substantia nigra.[47] This difference appears to result both from a complex interplay of expression of α_5 subunits and β_3 phosphorylation by protein kinase A, which itself is activated or inhibited by D1 and D2 receptors, respectively.[48] These findings provide a mechanism for dopamine release to shift the balance of strength of signaling via the direct and indirect pathways.

TONIC INHIBITION IN THE HIPPOCAMPUS AND TEMPORAL LOBE EPILEPSY

Tonic $GABA_A$ receptor currents have been described in dentate granule cells,[15,49] hippocampal pyramidal cells,[50] and hippocampal interneurons.[25] The receptors predominantly mediating tonic current in these three cell types are different. In CA1 pyramidal cells, the receptors are predominantly those containing α_5 and γ subunits,[22] while in dentate granule cells, the tonic current is predominantly mediated by receptors containing α_4 and δ subunits.[16] δ Subunits also contribute to tonic currents in interneurons.[51]

Neurosteroids increase the efficacy of $GABA_A$ receptors containing the δ subunit[52] and thus increase tonic conductances.[16,53] Indeed, the synthetic neurosteroid ganaxolone has entered a clinical trial for the treatment of epilepsy.[54] In addition, neurosteroids alter δ and α_4 subunit expression.[55,56] This effect appears to depend upon the length of exposure, with short-term exposure to allopregnalone resulting in increased δ subunit expression and increased tonic currents in dentate granule cells,[57] while longer-term exposure can result in δ subunit and $GABA_A$ receptor downregulation.[58] Short-term increases in neurosteroids occur with acute stress and during the menstrual cycle, and it is likely that these effects contribute to changes in seizure threshold under these conditions.[56,57,59] Indeed, withdrawal of neurosteroids can result in a lowered seizure threshold,[60] and an effect on tonic inhibition provides an attractive candidate mechanism to explain the menstrual variation in seizure frequency (catamenial epilepsy).[61] In some conditions of chronic stress or during pregnancy, the effect of downregulation of receptors mediating tonic inhibition is probably compensated for by the increase in circulating neurosteroids directly enhancing the efficacy of remaining receptors.

Seizures themselves and epilepsy can also affect $GABA_A$ receptor–mediated inhibition. In particular, prolonged acute seizures alter $GABA_A$ receptor expression, and the epileptogenic process is accompanied by differential changes in subunits. During status epilepticus, there is an internalization of synaptic $GABA_A$ receptors,[62,63] providing a candidate mechanism for loss of benzodiazepine potency.[64] However, extrasynaptic δ-containing receptors are preserved.[65] The efficacy of drugs that act on tonically active $GABA_A$ receptors, such as some anesthetic agents,[66,67] may therefore be maintained during status epilepticus. With the development of chronic temporal lobe epilepsy, animal models indicate that δ subunit expression decreases in dentate granule cells[68] and α_5 subunit expression decreases in CA1 pyramidal cells.[69] Since these subunits contribute to tonic $GABA_A$ receptor currents in dentate granule cells and CA1 pyramidal cells, respectively, tonic inhibition in these cell types would be expected to decrease. Surprisingly, this is not the case: tonic currents are maintained or increased during epileptogenesis.[28,30,70] This is not secondary to a decrease in GABA uptake, but is rather the result of the substitution of one set of extrasynaptic $GABA_A$ receptors by another and/or translocation of synaptic receptors to extrasynaptic sites. Although the tonic currents are preserved, their pharmacology changes because of the subunit alterations.[28,30] What is perhaps more surprising is the maintenance of these tonic currents, even in the face of quite marked decreases in synaptic inhibition. The preservation of tonic currents is also evident in resected tissue from patients with refractory temporal lobe epilepsy.[71]

The network implications of the relative preservation of tonic, but loss of phasic, inhibition can only be speculated on but are likely be similar to the effects observed when extracellular GABA increases: an increase in neuronal gain but a maintenance or increase in the offset of the input–output curve.[37] The tonic current probably provides an adequate inhibitory restraint under conditions of low network activity, but as activity increases there may be

two consequences: first, inadequate fast compensatory changes through feedforward and feedback inhibition, and second, increased neuronal gain resulting in larger numbers of firing neurons. This may provide the ideal conditions for seizure generation. This hypothesis could also explain why seizures are rare, intermittent events in people with even severe epilepsy.

TONIC INHIBITION IN ABSENCE SEIZURES AND ABSENCE EPILEPSY

Tonic currents are expressed in thalamocortical cells but not in reticular thalamic neurons.[18,19] Such currents hyperpolarize thalamocortical neurons, and thus predispose to burst firing by deinactivating T-type Ca^{2+} channels and/or activating hyperpolarization-activated cation conductance. Tonic $GABA_A$ receptor signaling has therefore been proposed to be intimately involved in both the generation and pharmacology of absence seizures. Specifically, an increase in tonic currents in thalamocortical neurons results in neuronal hyperpolarization, and therefore a change of thalamocortical neuron firing from regular to burst patterns, promoting spike-wave generation. Several lines of evidence support this hypothesis.[72,73] First, drugs that increase tonic currents in thalamocortical neurons promote absence seizures. This has been observed in animal models treated with GABA uptake inhibitors or with 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP), a drug that acts specifically on δ-containing receptors.[74,75] Thalamocortical seizures, or their spike-wave electroencephalographic (EEG) signature, have also been observed in humans in whom extracellular GABA concentrations have been increased by the administration of tiagabine, a GABA uptake inhibitor, or vigabatrin, a GABA transaminase inhibitor (both drugs used in the therapy of partial epilepsy).[76] Second, gamma-hydroxybutyrate (GHB), a drug that is well recognized to induce absence seizures, increases extracellular GABA in the thalamus.[72,77] Third, δ subunit knockout mice are resistant to the pro-absence effects of such drugs.[72] Fourth, increased tonic currents in thalamocortical neurons, and reduced GABA transporter activity, have been reported in animal models of spontaneous absence seizures (e.g., the Genetic Absence Epilepsy Rat from Strasbourg [GAERS], *lethargic* mice, *stargazer*).[72,73,78] Lastly, knockdown of the δ subunit in thalamocortical neurons in GAERS using antisense oligodeoxynucleotides inhibits absence seizures.[72] It is also noteworthy that polymorphisms in δ subunits in humans have been associated with familial generalized epilepsies.[79] However, interestingly, these genetic variants tend to decrease $GABA_A$ receptor function; therefore, how this change in tonic $GABA_A$ current contributes to the generation of absence seizures remains unclear.

TONIC INHIBITION AS A TARGET FOR ANTIEPILEPTIC DRUGS

Two main approaches can, in principle, be used to alter tonic inhibition: target the specific $GABA_A$ receptor subtypes that mediate tonic inhibition and increase the extracellular GABA concentration.

$\alpha_4\delta$-Containing receptors, which underlie tonic currents in dentate gyrus granule cells, are positively modulated by neurosteroids.[16,53] In addition, they are particularly sensitive to anesthetic agents (such as propofol) and to THIP.[20,66,67,80] Since these receptors are maintained in status epilepticus despite the internalization of γ-containing $GABA_A$ receptors, they could be a useful target in the later stages of status epilepticus when there is increasing benzodiazepine resistance. This may explain the usefulness of propofol as an anesthetic and anticonvulsant agent in the later stages of status epilepticus.[81] Similarly, the preservation of tonic currents in partial epilepsy argues for the use of drugs that target these receptors. Ganaxalone, a synthetic neurosteroid, is one such drug;[54] however, because the δ subunit is downregulated by chronic exposure to neurosteroids, this approach may not be effective in the long term. δ Subunit–containing receptors can also be directly activated by drugs such as THIP. A problem with this approach is lack of spatial specificity, in that such drugs target not only dentate granule cells but also thalamocortical neurons, thus inducing sleep and, at higher concentrations, anesthesia. In addition, as mentioned above, such drugs can have a pro-absence effect. Indeed, these drugs have been disappointing in animal seizure models.[82]

A further confounding factor when using $GABA_A$ receptor subtype–specific drugs is that

epileptogenesis results in subunit alterations that may decrease the efficacy of these compounds. This may be the case for the δ subunit in dentate gyrus granule cells (but see ref. 71) and also for the expression of the α_5 subunit in hippocampal pyramidal cells.[28]

As for an increase in extracellular GABA concentration, this can be achieved by inhibiting GABA transporters (tiagabine) or by decreasing GABA breakdown by GABA transaminase (vigabatrin). Another approach would be to increase GABA synthesis, and it has been argued that some presently available antiepileptic drugs (e.g., valproate) may work partly via this mechanism.[83] Decreasing GABA uptake would seem to be an ideal approach to decreasing network excitability, but this suffers from four important problems. First, although a specific inhibitor (for instance, one that targets GABA transporter 1[GAT1]) may be effective in increasing the tonic current in the hippocampus, this may not be the case in the neocortex, where GAT3 can compensate for GAT1 inhibition.[84] Second, as discussed above, increasing extracellular GABA will also have an effect on interneurons, thus decreasing synaptic inhibition and paradoxically increasing network excitability.[25] Third, increasing tonic inhibition in the thalamus can promote the generation of absence seizures.[72] Fourth, increasing extracellular GABA also activates pre- and postsynaptic $GABA_B$ receptors, and this can have complex network effects, reducing both excitability and synaptic inhibition (the latter by inhibiting vesicular GABA release).[85] Lastly, reversal of GABA transporters during neuronal activity may even be a mechanism by which extracellular GABA can be increased during excessive neuronal activity.[86] Thus, although tiagabine is an effective antiepileptic drug, it has been described to have, in some circumstances, pro-absence effects and its efficacy in humans is, overall, disappointing. Use of vigabatrin is also hampered by many of the same problems; its clinical use is also limited by concentric visual field restriction, which most likely results from GABA accumulation in the retina.[87]

CONCLUSION

Tonic currents have a profound effect on network excitability and are not lost in focal epilepsy. They therefore represent an attractive target for antiepileptic drug therapy. However, the hyperpolarization mediated by tonic currents in the thalamus may contribute to the generation of absence seizures. A promising area for future development is to target the modulation of tonic currents in principal neurons, especially if the $GABA_A$ receptors subunits mediating them differ pharmacologically in the epileptic and healthy brain.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci*. 2005;6(3):215–229.
2. Rossi DJ, Hamann M. Spillover-mediated transmission at inhibitory synapses promoted by high affinity alpha6 subunit GABA(A) receptors and glomerular geometry. *Neuron*. 1998;20(4):783–795.
3. Isaacson JS, Solís JM, Nicoll RA. Local and diffuse synaptic actions of GABA in the hippocampus. *Neuron*. 1993;10(2):165–175.
4. Semyanov A, Walker MC, Kullmann DM, Silver RA. Tonically active GABA A receptors: modulating gain and maintaining the tone. *Trends Neurosci*. 2004;27(5): 262–269.
5. Otis TS, Staley KJ, Mody I. Perpetual inhibitory activity in mammalian brain slices generated by spontaneous GABA release. *Brain Res*. 1991;545(1–2):142–150.
6. Salin PA, Prince DA. Spontaneous $GABA_A$ receptor–mediated inhibitory currents in adult rat somatosensory cortex. *J Neurophysiol*. 1996;75(4):1573–1588.
7. Wall MJ, Usowicz MM. Development of action potential–dependent and independent spontaneous $GABA_A$ receptor–mediated currents in granule cells of postnatal rat cerebellum. *Eur J Neurosci*. 1997;9(3):533–548.
8. Brickley SG, Cull-Candy SG, Farrant M. Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABAA receptors. *J Physiol* (*Lond*). 1996;497 (pt 3):753–759.
9. Rivera C, Voipio J, Kaila K. Two developmental switches in GABAergic signalling: the K^+-Cl^- cotransporter KCC2 and carbonic anhydrase CAVII. *J Physiol* (*Lond*). 2005;562(pt 1):27–36.
10. Mehta AK, Ticku MK. An update on $GABA_A$ receptors. *Brain Res Brain Res Rev*. 1999;29(2–3):196–217.
11. Lüscher B, Keller CA. Regulation of $GABA_A$ receptor trafficking, channel activity, and functional plasticity of inhibitory synapses. *Pharmacol Ther*. 2004;102(3):195–221.
12. Walker MC. $GABA_A$ receptor subunit specificity: a tonic for the excited brain. *J Physiol* (*Lond*). 2008;586(4):921–922.

13. Wei W, Zhang N, Peng Z, Houser CR, Mody I. Perisynaptic localization of delta subunit–containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. *J Neurosci*. 2003;23(33):10650–10661.
14. Overstreet LS, Westbrook GL. Synapse density regulates independence at unitary inhibitory synapses. *J Neurosci*. 2003;23(7):2618–2626.
15. Nusser Z, Mody I. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol*. 2002;87(5):2624–2628.
16. Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit–containing $GABA_A$ receptors. *Proc Natl Acad Sci USA*. 2003;100(24):14439–14444.
17. Stell BM, Mody I. Receptors with different affinities mediate phasic and tonic GABA(A) conductances in hippocampal neurons. *J Neurosci*. 2002;22(10): RC223.
18. Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ. Extrasynaptic $GABA_A$ receptors of thalamocortical neurons: a molecular target for hypnotics. *J Neurosci*. 2005;25(50):11513–11520.
19. Cope DW, Hughes SW, Crunelli V. $GABA_A$ receptor–mediated tonic inhibition in thalamic neurons. *J Neurosci*. 2005;25(50):11553–11563.
20. Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, Spigelman I, Houser CR, Olsen RW, Harrison NL, Homanics GE. $GABA_A$ receptor alpha 4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. *Proc Natl Acad Sci USA*. 2006;103(41):15230–15235.
21. Hamann M, Rossi DJ, Attwell D. Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. *Neuron*. 2002;33(4):625–633.
22. Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit–containing gamma-aminobutyric acid type A receptors. *Proc Natl Acad Sci USA*. 2004;101(10):3662–3667.
23. Wagner DA, Goldschen-Ohm MP, Hales TG, Jones MV. Kinetics and spontaneous open probability conferred by the epsilon subunit of the $GABA_A$ receptor. *J Neurosci*. 2005;25(45):10462–10468.
24. Lindquist CEL, Birnir B. Graded response to GABA by native extrasynaptic GABA receptors. *J Neurochem*. 2006;97(5):1349–1356.
25. Semyanov A, Walker MC, Kullmann DM. GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. *Nat Neurosci*. 2003;6(5):484–490.
26. Mortensen M, Smart TG. Extrasynaptic alphabeta subunit $GABA_A$ receptors on rat hippocampal pyramidal neurons. *J Physiol* (*Lond*). 2006;577(pt 3): 841–856.
27. Glykys J, Mody I. Hippocampal network hyperactivity after selective reduction of tonic inhibition in GABA A receptor alpha5 subunit-deficient mice. *J Neurophysiol*. 2006;95(5):2796–2807.
28. Scimemi A, Semyanov A, Sperk G, Kullmann DM, Walker MC. Multiple and plastic receptors mediate tonic $GABA_A$ receptor currents in the hippocampus. *J Neurosci*. 2005;25(43):10016–10024.
29. Prenosil GA, Schneider Gasser EM, Rudolph U, Keist R, Fritschy JM, Vogt KE.Specific subtypes of $GABA_A$ receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. *J Neurophysiol*. 2006;96(2):846–857.
30. Zhang N, Wei W, Mody I, Houser CR. Altered localization of GABA(A) receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *J Neurosci*. 2007;27(28):7520–7531.
31. Herd MB, Haythornthwaite AR, Rosahl TW, Wafford KA, Homanics GE, Lambert JJ, Belelli D. The expression of $GABA_A$ beta subunit isoforms in synaptic and extrasynaptic receptor populations of mouse dentate gyrus granule cells. *J Physiol* (*Lond*). 2008;586(4):989–1004.
32. Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M. Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature*. 2001;409(6816):88–92.
33. LoTurco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron*. 1995;15(6):1287–1298.
34. Demarque M, Represa A, Becq H, Khalilov I, Ben-Ari Y, Aniksztejn L. Paracrine intercellular communication by a Ca^{2+}- and SNARE-independent release of GABA and glutamate prior to synapse formation. *Neuron.* 2002;36(6):1051–1061.
35. Ge S, Goh ELK, Sailor KA, et al. GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature.* 2006;439(7076):589–593.
36. Mitchell SJ, Silver RA. Shunting inhibition modulates neuronal gain during synaptic excitation. *Neuron*. 2003;38(3):433–445.
37. Pavlov I, Savtchenko LP, Kullmann DM, Semyanov A, Walker MC. Outwardly rectifying tonically active $GABA_A$ receptors in pyramidal cells modulate neuronal offset, not gain. *J Neurosci*. 2009;29(48): 15341–15350.
38. Chance FS, Abbott LF, Reyes AD. Gain modulation from background synaptic input. *Neuron.* 2002;35(4):773–782.
39. Silver RA. Neuronal arithmetic. *Nat Rev Neurosci*. 2010;11(7):474–489.
40. Martin LJ, Zurek AA, MacDonald JF, Roder JC, Jackson MF, Orser BA.Alpha5$GABA_A$ receptor activity sets the threshold for long-term potentiation and constrains hippocampus-dependent memory. *J Neurosci*. 2010;30(15):5269–5282.
41. Dawson GR, Maubach KA, Collinson N, et al. An inverse agonist selective for alpha5 subunit–containing $GABA_A$ receptors enhances cognition. *J Pharmacol Exp Ther*. 2006;316(3):1335–1345.
42. Collinson N, Kuenzi FM, Jarolimek W, et al. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. *J Neurosci*. 2002;22(13): 5572–5580.
43. Atack JR, Bayley PJ, Seabrook GR, et al. L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for alpha5-containing $GABA_A$ receptors. *Neuropharmacology.* 2006;51(6):1023–1029.
44. Ruiz A, Campanac E, Scott RS, Rusakov DA, Kullmann DM. Presynaptic $GABA_A$ receptors enhance

transmission and LTP induction at hippocampal mossy fiber synapses. *Nat Neurosci*. 2010;13(4):431–438.
45. Bianchi L, Ballini C, Colivicchi MA, Della Corte L, Giovannini MG, Pepeu G. Investigation on acetylcholine, aspartate, glutamate and GABA extracellular levels from ventral hippocampus during repeated exploratory activity in the rat. *Neurochem Res*. 2003;28(3–4):565–573.
46. de Groote L, Linthorst ACE. Exposure to novelty and forced swimming evoke stressor-dependent changes in extracellular GABA in the rat hippocampus. *Neuroscience*. 2007;148(3):794–805.
47. Ade KK, Janssen MJ, Ortinski PI, Vicini S. Differential tonic GABA conductances in striatal medium spiny neurons. *J Neurosci*. 2008;28(5):1185–1197.
48. Janssen MJ, Ade KK, Fu Z, Vicini S. Dopamine modulation of GABA tonic conductance in striatal output neurons. *J Neurosci*. 2009;29(16):5116–5126.
49. Overstreet LS, Westbrook GL. Paradoxical reduction of synaptic inhibition by vigabatrin. *J Neurophysiol*. 2001;86(2):596–603.
50. Bai D, Zhu G, Pennefather P, Jackson MF, MacDonald JF, Orser BA. Distinct functional and pharmacological properties of tonic and quantal inhibitory postsynaptic currents mediated by gamma-aminobutyricacid(A) receptors in hippocampal neurons. *Mol Pharmacol.* 2001;59(4):814–824.
51. Mann EO, Mody I. Control of hippocampal gamma oscillation frequency by tonic inhibition and excitation of interneurons. *Nat Neurosci*. 2010;13(2):205–212
52. Bianchi MT, Macdonald RL. Neurosteroids shift partial agonist activation of GABA(A) receptor channels from low- to high-efficacy gating patterns. *J Neurosci*. 2003;23(34):10934–10943.
53. Belelli D, Herd MB. The contraceptive agent Provera enhances GABA(A) receptor-mediated inhibitory neurotransmission in the rat hippocampus: evidence for endogenous neurosteroids? *J Neurosci*. 2003;23(31):10013–10020.
54. Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS. Progress report on new antiepileptic drugs: a summary of the Tenth Eilat Conference (EILAT X). *Epilepsy Res*. 2010;92(2–3):89–124.
55. Smith SS, Shen H, Gong QH, Zhou X. Neurosteroid regulation of $GABA_A$ receptors: focus on the [alpha]4 and [delta] subunits. *Pharmacol Ther.* 2007;116(1):58–76.
56. Maguire J, Mody I. Steroid hormone fluctuations and GABA(A)R plasticity. *Psychoneuroendocrinology*. 2009;34(suppl 1):S84–S90.
57. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci*. 2005;8(6):797–804.
58. Maguire J, Mody I. GABA(A)R plasticity during pregnancy: relevance to postpartum depression. *Neuron*. 2008;59(2):207–213.
59. Maguire J, Mody I. Neurosteroid synthesis-mediated regulation of GABA(A) receptors: relevance to the ovarian cycle and stress. *J Neurosci*. 2007;27(9):2155–2162.
60. Moran MH, Smith SS. Progesterone withdrawal I: proconvulsant effects. *Brain Res.* 1998;807(1–2):84–90.
61. Rogawski MA. Progesterone, neurosteroids, and the hormonal basis of catamenial epilepsy. *Ann Neurol*. 2003;53(3):288–291.
62. Goodkin HP, Yeh J, Kapur J. Status epilepticus increases the intracellular accumulation of $GABA_A$ receptors. *J Neurosci*. 2005;25(23):5511–5520.
63. Naylor DE, Liu H, Wasterlain CG. Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci*. 2005;25(34):7724–7733.
64. Kapur J, Macdonald RL. Rapid seizure-induced reduction of benzodiazepine and Zn^{2+} sensitivity of hippocampal dentate granule cell $GABA_A$ receptors. *J Neurosci*. 1997;17(19):7532–7540.
65. Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J. Subunit-specific trafficking of GABA(A) receptors during status epilepticus. *J Neurosci*. 2008;28(10):2527–2538.
66. McCartney MR, Deeb TZ, Henderson TN, Hales TG. Tonically active $GABA_A$ receptors in hippocampal pyramidal neurons exhibit constitutive GABA-independent gating. *Mol Pharmacol.* 2007; 71(2):539–548.
67. Caraiscos VB, Newell JG, You-Ten KE, Elliott EM, Rosahl TW, Wafford KA, MacDonald JF, Orser BA. Selective enhancement of tonic GABAergic inhibition in murine hippocampal neurons by low concentrations of the volatile anesthetic isoflurane. *J Neurosci*. 2004;24(39):8454–8458.
68. Peng Z, Huang CS, Stell BM, Mody I, Houser CR. Altered expression of the delta subunit of the $GABA_A$ receptor in a mouse model of temporal lobe epilepsy. *J Neurosci*. 2004;24(39):8629–8639.
69. Houser CR, Esclapez M. Downregulation of the alpha5 subunit of the GABA(A) receptor in the pilocarpine model of temporal lobe epilepsy. *Hippocampus*. 2003;13(5):633–645.
70. Zhan R, Nadler JV. Enhanced tonic GABA current in normotopic and hilar ectopic dentate granule cells after pilocarpine-induced status epilepticus. *J Neurophysiol*. 2009;102(2):670–681.
71. Scimemi A, Andersson A, Heeroma JH, Strandberg J, Rydenhag B, McEvoy AW, Thom M, Asztely F, Walker MC. Tonic GABA(A) receptor-mediated currents in human brain. *Eur J Neurosci*. 2006;24(4):1157–1160.
72. Cope DW, Di Giovanni G, Fyson SJ, et al. Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. *Nat Med*. 2009;15(12):1392–1398.
73. Belelli D, Harrison NL, Maguire J, et al. Extrasynaptic $GABA_A$ receptors: form, pharmacology, and function. *J Neurosci.* 2009;29(41):12757–12763.
74. Coenen AML, Blezer EHM, van Luijtelaar ELJM. Effects of the GABA-uptake inhibitor tiagabine on electroencephalogram, spike-wave discharges and behaviour of rats. *Epilepsy Res*. 1995;21(2):89–94.
75. Vergnes M, Marescaux C, Micheletti G, Depaulis A, Rumbach L, Warter JM.Enhancement of spike and wave discharges by GABAmimetic drugs in rats with spontaneous petit-mal-like epilepsy. *Neurosci Lett*. 1984;44(1):91–94.
76. Genton P. When antiepileptic drugs aggravate epilepsy. *Brain Dev*. 2000;22(2):75–80.
77. Williams SR, Turner JP, Crunelli V. Gamma-hydroxybutyrate promotes oscillatory activity of rat and cat thalamocortical neurons by a tonic $GABA_B$, receptor-mediated hyperpolarization. *Neuroscience*. 1995;66(1):133–141.

78. Richards DA, Lemos T, Whitton PS, Bowery NG. Extracellular GABA in the ventrolateral thalamus of rats exhibiting spontaneous absence epilepsy: a microdialysis study. *J Neurochem*. 1995;65(4):1674–1680.
79. Dibbens LM, Feng HJ, Richards MC, Harkin LA, Hodgson BL, Scott D, Jenkins M, Petrou S, Sutherland GR, Scheffer IE, Berkovic SF, Macdonald RL, Mulley JC. GABRD encoding a protein for extra- or peri-synaptic $GABA_A$ receptors is a susceptibility locus for generalized epilepsies. *Hum Mol Genet*. 2004;13(13):1315–1319.
80. Bonin RP, Orser BA. GABA(A) receptor subtypes underlying general anesthesia. *Pharmacol Biochem Behav*. 2008;90(1):105–112.
81. Holtkamp M, Tong X, Walker MC. Propofol in subanesthetic doses terminates status epilepticus in a rodent model. *Ann Neurol*. 2001;49(2):260–263.
82. Löscher W, Schwark WS. Evaluation of different GABA receptor agonists in the kindled amygdala seizure model in rats. *Exp Neurol*. 1985;89(2):454–460.
83. Kwan P, Sills GJ, Brodie MJ. The mechanisms of action of commonly used antiepileptic drugs. *Pharmacol Ther*. 2001;90(1):21–34.
84. Keros S, Hablitz JJ. Subtype-specific GABA transporter antagonists synergistically modulate phasic and tonic $GABA_A$ conductances in rat neocortex. *J Neurophysiol*. 2005;94(3):2073–2085.
85. Davies CH, Collingridge GL. The physiological regulation of synaptic inhibition by $GABA_B$ autoreceptors in rat hippocampus. *J Physiol* (*Lond*). 1993;472:245–265.
86. Richerson GB, Wu Y. Dynamic equilibrium of neurotransmitter transporters: not just for reuptake anymore. *J Neurophysiol*. 2003;90(3):1363–1374.
87. Sills GJ, Patsalos PN, Butler E, Forrest G, Ratnaraj N, Brodie MJ. Visual field constriction: accumulation of vigabatrin but not tiagabine in the retina. *Neurology*. 2001;57(2):196–200.

Chapter 10

Glutamatergic Mechanisms Related to Epilepsy

Ionotropic Receptors

Raymond Dingledine

The ionotropic glutamate receptors are ligand-gated ion channels that mediate the vast majority of excitatory neurotransmission in the brain. The past 20 years have been a golden age for glutamate receptor research. Even before that time, in the early 1980s the invention of the first selective antagonists for what would come to be known as *N-methyl-D-aspartate* (*NMDA*) *receptors*[1] triggered a flood of investigations as the realization grew that NMDA receptors were critically involved in synaptic plasticity, learning, creation of the proper wiring diagram of the brain during development, excitotoxicity, and a host of neurological disorders involving aberrant circuitry organization, including epilepsy (reviewed in refs. 2 and 3). Cloning of the first glutamate receptor subunit was reported in December 1989,[4] and within the next 2 years an additional 15 subunits were cloned.[3,5] The subsequent application of molecular and gene ablation technologies has revealed a wealth of subtlety regarding control of synaptic transmission highlighted, perhaps, by a resurgence of interest in how excitatory input patterns to GABAergic interneurons regulate synchronous firing throughout the brain. Over the past decade, our understanding of how these receptors work has been brought to the structural level by successful crystallization of numerous glutamate receptor subunits (see ref. 6). The mechanisms (transcriptional, translational, and post-translational) underlying seizure-induced changes in expression of glutamate receptors have been elucidated. A wealth of new pharmacologic reagents, particularly allosteric receptor modulators, have been introduced that can facilitate study of the roles of specific glutamate receptors in epilepsy. The proposal that reactive astrocytes release glutamate, which then acts to synchronize neuron firing within local microdomains, has been developed. Here I review the functional properties of glutamate

Table 10–1 **Glutamate Receptor Nomenclature**

IUPHAR Name	HUGO Symbol	Common Names	Receptor Family
GluA1	GRIA1	GluR1, GluRA	AMPA
GluA2	GRIA2	GluR2, GluRB	AMPA
GluA3	GRIA3	GluR3, GluRC	AMPA
GluA4	GRIA4	GluR4, GluRD	AMPA
GluK1	GRIK1	GluR5	Kainate
GluK2	GRIK2	GluR6	Kainate
GluK3	GRIK3	GluR7	Kainate
GluK4	GRIK4	KA1	Kainate
GluK5	GRIK5	KA2	Kainate
GluN1	GRIN1	NMDAR1, NR1, GluRξ1	NMDA
GluN2A	GRIN2A	NMDAR2A, NR2A, GluRε1	NMDA
GluN2B	GRIN2B	NMDAR2B, NR2B, GluRε2	NMDA
GluN2C	GRIN2C	NMDAR2C, NR2C, GluRε3	NMDA
GluN2D	GRIN2D	NMDAR2D, NR2D, GluRε4	NMDA
GluN3A	GRIN3A	NR3A	NMDA
GluN3B	GRIN3B	NR3B	NMDA
GluD1	GRID1	δ1, GluRdelta-1	Delta
GluD2	GRID2	δ2, GluRdelta-2	Delta

receptors and discuss recent data pointing to their potential roles in epilepsy. But first, a word on nomenclature.

NOMENCLATURE

The rapid growth of literature on glutamate receptors throughout the 1990s predictably spawned multiple names for the same subunit in different species, or sometimes in the same species but cloned nearly simultaneously in different laboratories. The resulting confusion has abated slowly, but a strong effort has been made recently to replace the older names for glutamate receptor subunits by International Union of Basic and Clinical Pharmacology (IUPHAR) names[7] (see http://www.iuphar-db.org/LGICNomenclature.jsp). The IUPHAR nomenclature, which was designed to harmonize with the gene names and which will be used throughout this chapter, is presented in Table 10–1. Several excellent reviews have appeared in recent years that complement and extend the next three sections.[6,8–13]

GLUTAMATE RECEPTOR STRUCTURE

Ionotropic glutamate receptors are tetrameric protein complexes made up of four subunits surrounding an ion channel pore. Each protein complex draws subunits from the same receptor subfamily (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate [AMPA], kainate, NMDA), with subunit assembly between subfamilies apparently strictly prohibited. Each subunit sports three transmembrane domains and a membrane reentrant loop (M2) that results in a cytoplasmically located C terminus (Fig. 10–1A). The cytoplasmic location of the Cterminus is important because residues near this terminus interact with numerous

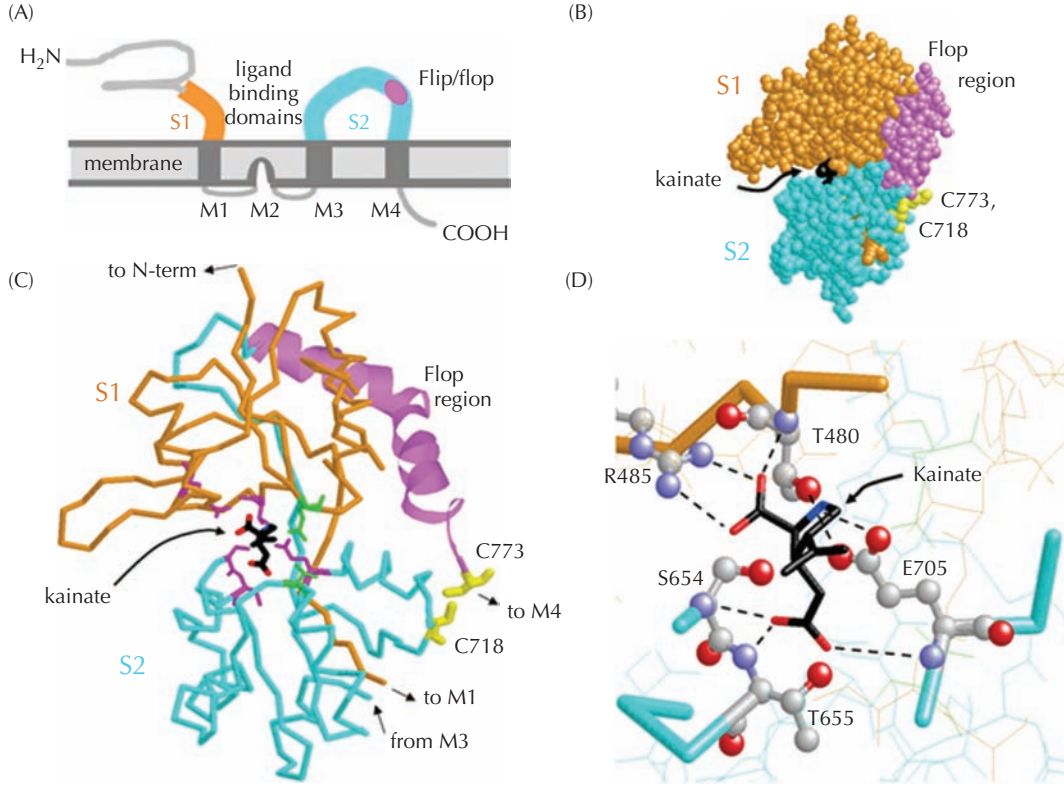

Figure 10–1. Transmembrane topology (**A**) and crystal structure of the agonist-binding domain (**B–D**) of the GluA2 subunit protein. The two domains that contain agonist-binding residues are colored in gold (S1) and turquoise (S2). The flip/flop region is indicated in violet. **B.** Space-filled representation of the kainate-bound S1 and S2 domains joined by an 11-residue linker peptide, with coloration the same as in **A**. The position of a single-kainate agonist molecule (black) within a deep gorge of the protein is indicated; the two disulfide-bonded cysteines (C718 and C773) are shown in yellow. **C.** Backbone representation of the subunit, with kainate (black) docked into its binding site. The kainate-binding residues are shown as stick figures in magenta, the two cysteines in yellow, and the flop helix structure in violet. The two green residues (E402 and T686) do not directly bind to kainate but instead interact with each other, helping to hold the clamshell in the closed conformation. **D.** Close-up view of the ligand-binding pocket. The binding residues are in space-filled representation, with atoms colored conventionally (gray carbon, cyan nitrogen, red oxygen). Modified from ref. 3.

intracellular scaffolding and trafficking proteins that regulate both insertion of the receptors into the postsynaptic membrane and activity-triggered recycling.[6]

How structure drives molecular function of the ionotropic glutamate receptors is discussed in great detail in ref. 6. For all ionotropic glutamate receptors studied, the ligand-binding domain is in the form of a hinged clamshell consisting of two lobes that close around the agonist molecule (depicted as gold and blue sections in Fig. 10–1A,B). Agonists bind deep within a gorge and make atomic contacts with both gold and blue lobes of the clamshell (Fig. 10–1C,D). Agonist binding causes partial closure of the clamshell, trapping the agonist in the receptor and putting a torque on the transmembrane segments that apparently induces a twist in the transmembrane helices, thereby opening the channel. For AMPA receptors, "full" agonists cause a more complete clamshell closure than partial agonists.[14] Each subunit in a tetrameric structure typically binds the same agonist; however, NMDA receptors are unique among all neurotransmitter receptors in requiring the binding of both glutamate (to GluN2 subunits) and glycine or D-serine (to GluN1 subunits) for channel opening.[15]

The amino-terminal domain of glutamate receptors also forms a clamshell,[16–18] but in this

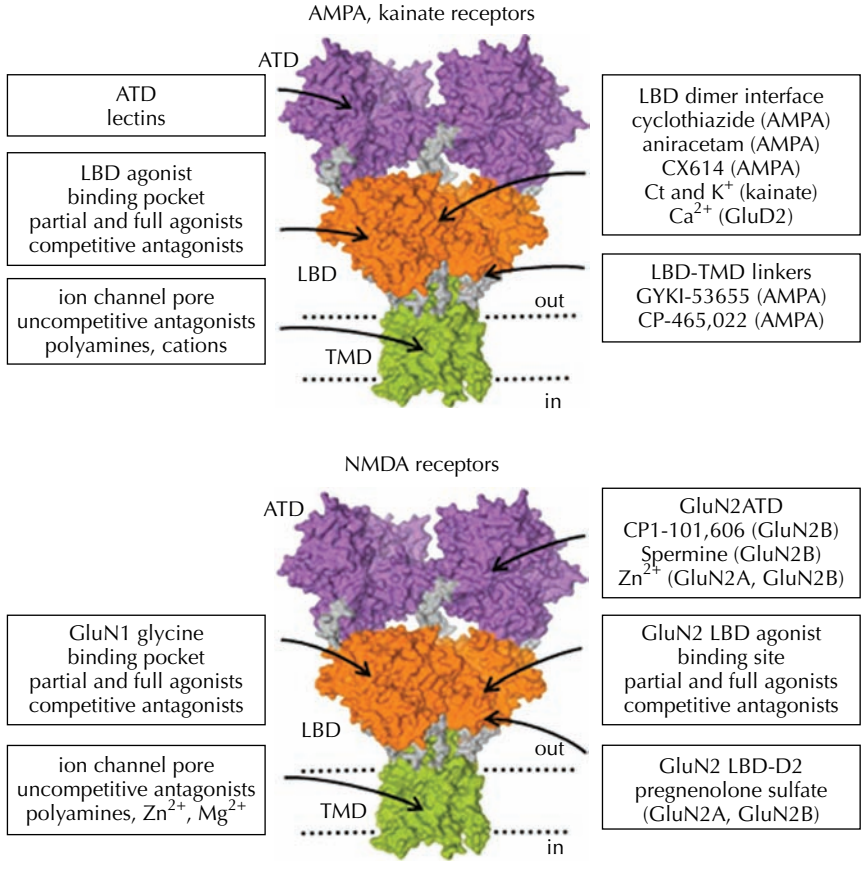

Figure 10–2. Binding sites for agonists, antagonists, and modulators in the ligand binding domain (LBD), amino terminal domain (ATD), and transmembrane domain (TMD). The receptor targets of ligands selective for one or several subunits are listed in parenthesis. "AMPA" and "kainate" indicates that the ligand selectively targets GluA or GluK receptor subunits, respectively. The ATDs, LBDs, TMDs, and linkers are shown in purple, orange, green, and gray, respectively. Modified from ref. 6.

case modulators bind at the interface between GluN1 and GluN2 rather than within the cleft (18a). The structure of the extracellular and transmembrane domains of one of the subunit dimers in a tetrameric glutamate receptor is shown in Fig. 10–2, along with the location of binding sites for agonists and allosteric modulators. The pharmacology of ionotropic glutamate receptors has become quite rich in the past decade, with the creation or discovery of numerous antagonists and allosteric modulators that are selective for particular receptors or even particular subunit combinations.[6,8] Many of the highly selective allosteric compounds target the amino-terminal domain.[10,13] For example, cyclothiazide allosterically potentiates AMPA but not kainate receptors, whereas concanavalin A potentiates kainate but not AMPA receptors; in both cases, potentiation occurs by relief from desensitization. Similarly, CP-101,606 selectively inhibits GluN2B-containing NMDA receptors, whereas zinc provides a high-affinity (10–30 nM IC50) block only of GluN2A-containing NMDA receptors.

CONTROL OF RECEPTOR PROPERTIES

Splice variants that control function or subcellular location have been identified for most of the subunits. The first example was the flip and

flop variants of each of the four AMPA receptor subunits. This cassette of 38 amino acids, which is located in an extracellular loop shown in Fig. 10–1A–C, influences desensitization rates of the receptors. C-terminal splice variants exist for most of the subunits and regulate coupling of the subunits to cytoplasmic proteins. For example, alternative exon 5 incorporation into GluN1 strongly determines responsiveness to allosteric modulation by protons and polyamines.

A very interesting regulation is determined by editing of the primary RNA transcript for AMPA GluA2 and kainateGluK1-GluK2 subunits. This editing involves enzymatic conversion of a glutamine codon to an arginine codon in the prespliced RNA,[19] which influences a wide range of functional properties including calcium permeability, single-channel conductance, voltage-dependent block by cytoplasmic polyamines, and, in the case of AMPA receptors, assembly efficiency.

The particular subunits that each neuron chooses to express are strong determinants of the synaptic phenotype, and subunit expression, in turn, is controlled both transcriptionally and translationally. The most intensively studied ionotropic glutamate receptor in this respect is GluA2, which features a neuron-restrictive silencer element (NRSE) in its promoter that is responsible for downregulation of GluA2 after status epilepticus.[20–22] Seizures cause rapid (within hours) induction of the transcriptional repressor, REST, which recruits histone deacetylases to the GluA2 promoter via the corepressor, Sin3A (Fig. 10–3A). The mechanism of repression likely involves deacetylation of histones that are physically bound to the *Gria2* gene because a histone deacetylase inhibitor prevents both deacetylation of *Gria2*-bound histones and GluA2 downregulation after status epilepticus.[22] Genes encoding GluN1 and GluN2C also have an NRSE, but less is known about the conditions under which the REST repression system is brought into play for the NMDA receptors. Currently, more than 1300 genes are known to have confirmed NRSE sequences, making the REST/NRSE system broadly seizure-responsive.

The 5′-untranslated region (UTR) of many ionotropic glutamate receptor mRNAs is unusually long. These long 5'-UTRs often exhibit high GC content and contain out-of-frame AUG codons that could act as decoys for scanning ribosomes, reducing or preventing translation initiation at the true glutamate receptor AUG. At least two major transcriptional start sites exist for the *Gria2* gene, as depicted by the pair of bent arrows in Fig. 10–3A. Interestingly, the longer transcripts contain an imperfect GU repeat that serves as a translational repressor, is conserved between rodents and humans, and is polymorphic in length in humans.[23] Additionally, GluA2 transcripts have alternative 3' UTRs that control the rate of initiation of protein synthesis. Translational suppression by the longer

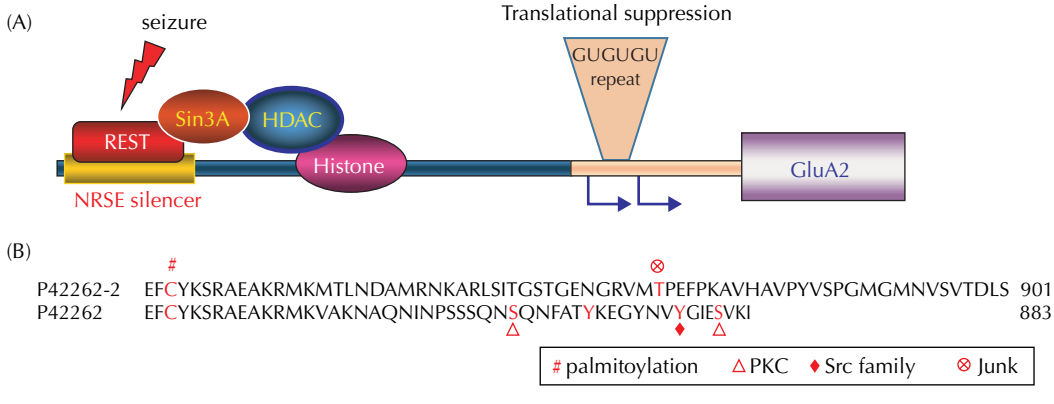

Figure 10–3. Control of GluA2 expression by transcriptional repression mediated by the REST/NRSE system (**A**), translational repression mediated by alternative 5'UTRs (**A**) and posttranslational processing on alternative C-terminal domains (**B**). To the left in **B** is the UniProt-SwissProt human accession number of the two splice variants. The length of each subunit, including the signal peptide, is shown at the right, with residue numbering beginning with the initiating methionine.

3'UTR seems to be mediated in part by binding of CPEB3[24] and in the rat hippocampus is relieved by seizures.[25,26] Thus, both transcription and translation of GluA2 are regulated by seizure-induced signaling systems. The GluN1 and GluN2A subunits are also under translational control by the 5'-UTR and 3'-UTR, respectively.[27,28]

The combination of specific phosphoprotein antibodies, site-directed mutagenesis, chromophore-tagged receptors, and, in some cases, fragmentation followed by mass spectrometry, has in the past decade led to the secure identification of phosphorylation sites on the C-terminal domains of ionotropic glutamate receptors, and in some cases to an understanding of the functional consequences of phosphorylation. The most intensively studied subunits are GluN1 and GluA1. The C-terminal domain of GluA1 exhibits four protein kinase C (PKC) targets, plus one protein kinase A (PKA) and one calmodulin kinase II (CAMKII) site. Phosphorylation at each of these sites has been shown to regulate activity-dependent receptor trafficking, open probability or conductance of the channel.[6] GluA2 has alternative C-terminal domains that are differentially regulated by phosphorylation, as shown in Fig. 10–3B. GluN1 has four C-terminal splice variants, only the longest of which appears to harbor phosphorylation sites. Status epilepticus causes rapid dephosphorylation of serines 890 and 897 on GluN1, then slowly developing hyperphosphorylation of these serines by PKCγ and PKA, respectively.[29,30] Phosphorylation of S890 disrupts surface clusters of NMDA receptors,[31] whereas phosphorylation of S897 promotes insertion of receptors into the synaptic membrane.[32]

GLUTAMATERGIC MECHANISMS IN EPILEPSY

Currently available glutamate receptor antagonists, with the possible exception of those directed toward kainate receptors, are as a rule poor anticonvulsants due to meager selectivity toward rapidly firing neurons, yet activation of glutamate receptors on neurons and probably on astrocytes contributes to the initiation and propagation of seizures. Given this conundrum, I will consider here several related topics that might be developed therapeutically.

Astrocytic Release of Glutamate

It is now clear that astrocytes in vitro can respond to chemicals (e.g., glutamate, prostaglandins, tumor necrosis factor alpha) released from surrounding cells during periods of repetitive firing or inflammatory stimuli. The astrocytic response in vitro involves generation of a cytoplasmic calcium signal and subsequent release of glutamate and/or D-serine.[33] The consequence of astrocytic glutamate release has been the subject of numerous studies. One of the most convincing was that of Jourdain et al.,[34] who showed that repetitive depolarization of an astrocyte through a patch pipette in hippocampal slices increased the frequency of spontaneous miniature excitatory postsynaptic currents (EPSCs) recorded in nearby dentate granule cells. A number of internal controls ruled out neuronal depolarization as the culprit. Following such astrocytic stimulation, GluN2B-containing receptors on presynaptic terminals of the perforant path were activated, which in turn potentiated synaptic transmission by elevating the probability of transmitter release. GluN2B activation could be prevented by direct astrocytic infusion of the calcium chelator 1,2-*b*is(o-*a*minop*h*enoxy)e*t*hane-N,N,N',N'-*t*etra*a*cetic acid (BAPTA) or a tetanus toxin protease. The precise conditions under which such strong, prolonged depolarization of astrocytes would occur was not addressed by their study, but it can be supposed that status epilepticus would serve this purpose. Moreover, the key astrocytic event—release of glutamate or a similar compound, or perhaps adjustment of the extracellular space to allow neuron-released glutamate to feed back onto presynaptic receptors—could not be definitively determined.

Although evidence is mounting for a specific role of astrocytic calcium waves in neurodegeneration during status epilepticus[35] and ischemia,[36] it is proving frustratingly difficult to determine exactly how and under what conditions the calcium wave contributes to neuropathology.[33,37] One clue comes from the studies of Bezzi et al.,[38] who showed that the tumor necrosis factor-α (TNFα) produced

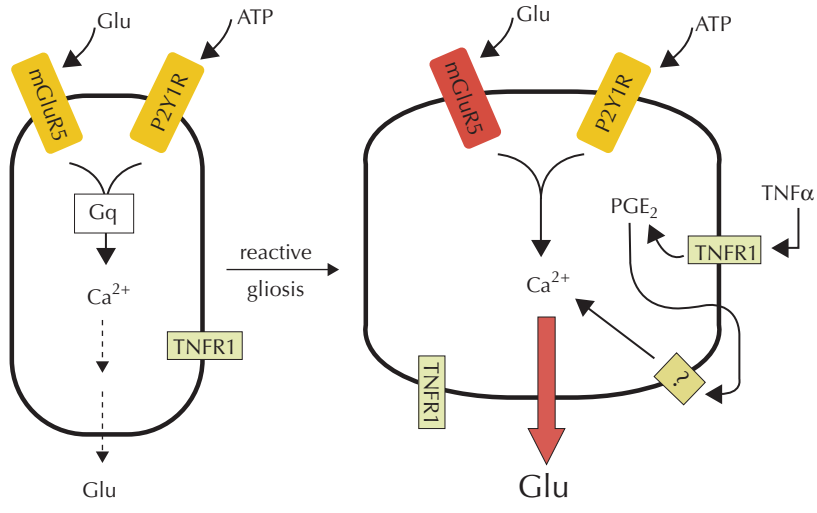

Figure 10–4. Potential mechanism by which inflammation could boost astrocytic glutamate release. *Left*: Scheme for control of astrocytic glutamate release by two G protein–coupled receptors. Right: In the epileptic brain, mGluR5 is upregulated, and activated microglia as well as reactive astrocytes release TNFα, which acts on TNFR1 receptors in a pathway that promotes prostaglandin formation. Prostaglandin, in turn, activates a Gq-coupled prostanoid receptor that boosts intraastrocyte Ca^{2+} release and thus astrocytic glutamate release. From ref. 33.

by reactive astrocytes and activated microglia boosts glutamate release from astrocytes through a cyclooxygenase-mediated pathway. Thus, it is possible that astrocytic glutamate release is minimal normally but enhanced in inflamed tissue (Fig. 10–4).

Impaired Glutamine Cycle in Sclerotic Tissue

Much of the glutamate released from excitatory nerve terminals is transported by active membrane pumps from the extrasynaptic space into surrounding astrocytes. Glutamate is then largely converted to glutamine by an enzyme, glutamine synthase, that is expressed by astrocytes and oligodendrocytes but not by neurons. Glutamine, in turn, is transported to extracellular fluid and is taken up by both inhibitory and excitatory neurons, where it is converted back to glutamate by phosphate-activated glutaminase. The movement of glutamate and glutamine between neurons and astrocytes is called the *glutamine cycle* and is an important feature of the close metabolic relationship that exists between neurons and glia in the brain. The glutamine cycle supplies this glutamate precursor to both glutamateric and GABAergic neurons. The role of the glutamine cycle in epilepsy is somewhat controversial. Inhibition of glutamine transport or glutamine synthase reduces the amplitude of GABAergic evoked or spontaneous inhibitory postsynaptic currents (IPSCs),[39,40] resulting in disinhibition and suggesting a major function of the glutamine cycle in the dynamic regulation of inhibitory synaptic strength. The notion that disruption of the glutamine cycle should promote seizures is reinforced by the observations that astrocytic glutamine synthase is downregulated in sclerotic tissue resected from epilepsy patients,[41] that selective induction of astrogliosis causes reduced GABAergic inhibition in the hippocampus that could be reversed by exogenous glutamine,[42] and that recurrent seizures develop in rats after local inhibition of glutamine synthase in the hippocampus.[43] However, when GABAergic inhibition is blocked, the resulting epileptiform activity in hippocampal[44] and cortical[45] slices maintained in vitro is dramatically attenuated by inhibition of glutamine transport or glutamine synthase. This finding suggests that a continual supply of glutamine is needed to fuel glutamate synthesis for excitatory synaptic transmission in rapidly

firing neurons. Taken together, these studies indicate that inhibition of the glutamine cycle can reduce both GABAergic inhibition and synaptic excitation mediated by glutamatergic synapses. To develop this notion further, it will be important to explore the anticonvulsant potential of potentiating residual glutamine synthase in reactive astrocytes under the more realistic conditions of chronic epilepsy. For a more thorough discussion of the potential roles for disruption of the glutamine cycle in epilepsy, see Chapter 12 in this volume.

Special Role for Kainate Receptors in Epilepsy

The year 1974 brought the first demonstration that a newly developed antihelminthic, kainic acid, caused convulsions in mice.[46]Much has been learned about the pharmacology and biology of kainate receptors in the last decade (reviewed in refs. 8 and 47). Because kainate is a powerful agonist at AMPA receptors as well as at kainate receptors, consideration of the roles of kainate receptors in epileptiform activity awaited the development of selective drugs and genetically modified mice. Genetically engineered mice lacking GluK2 showed an interesting phenotype.[48,49] First, the potency with which kainate induced an inward current in CA3 pyramidal cells from these mice was reduced about sixfold. Kainate-induced inward currents on CA3 interneurons, and kainate-induced increase in the frequency of spontaneous IPSCs on pyramidal cells, were also absent in mice lacking GluK2. Second, kainate was unable to induce gamma oscillations (38 Hz) in the CA3 region of mice lacking GluK2. Finally, mice injected with a moderate (20 mg/kg ip) but not a higher (30 mg/kg ip) dose of kainate were protected from seizure development. These results point to an important role for GluK2 in mediating epileptiform activity produced by kainic acid.

The role of GluK1 in seizure development, on the other hand, is more controversial, with superficially opposing results of pharmacological and genetic experiments. Genetic ablation of GluK1 *increased* the potency with which kainate induced gamma oscillations and epileptiform activity in CA3,[49] and yet a first-generation GluK1-selective antagonist prevented pilocarpine-induced seizures in rats as well as seizure activity produced by 6 Hz corneal stimulation.[50] This antagonist, LY377770, was 10- to 100-fold selective for GluK1 versus the four AMPA receptors in ligand binding assays, and was >40-fold selective for GluK2 or GluK2/GluK5 receptors. Kainate was still able to elicit seizure activity in GluK1 knockouts (unpublished data reported in ref. 47), but the *sensitivity* to kainate in the GluK1 nulls was not reported. It would be simple to dismiss the pharmacological results based on insufficient selectivity, but one must also consider the consequence of homeostatic adjustments in circuitry or receptor expression in the GluK2 knockouts (as shown by Christensen et al.[51]).

CHALLENGES AND OPPORTUNITIES

In the past decade, we have seen the first fruits of understanding the molecular function of ionotropic glutamate receptors based on their protein structure and posttranslational processing. In addition, roles for reactive astrocytes in the buildup of extracellular glutamate during seizures have been proposed. A major challenge for using this information to develop new anticonvulsants is the near-ubiquitous distribution of ionotropic glutamate receptors, which mediate the vast majority of excitatory neurotransmission in virtually every brain region. However, the tremendous strides made in developing subunit-selective antagonists of kainate receptors[8] represent a real opportunity to explore the potential for the use of kainate receptor antagonists in epilepsy. A related opportunity relies on the finding that activation of Gαq-coupled receptors such as M1 muscarinic receptors (by, e.g., pilocarpine) can potentiate the activation of heteromeric kainate receptors.[52] It might therefore be possible to use selective antagonists of group 1 metabotropic glutamate receptors, muscarinic receptors, or Gαq-coupled prostaglandin receptors to allosterically dampen kainate receptor activity. More gentle allosteric inhibition may be preferable to frank antagonism of the ionotropic glutamate receptors to minimize adverse effects.

Glutamine synthase represents another intriguing molecular target, even though this astrocytic enzyme supplies glutamate to both

inhibitory and excitatory neurons. A potentiator of residual glutamine synthase expressed by reactive astrocytes might restore inhibitory balance. No chemical potentiator exists yet, but the application of high-throughput screening of small molecules followed by medicinal chemistry would be the preferred route to developing an allosteric potentiator.

DISCLOSURE STATEMENT

The author is a cofounder of NeurOp, Inc., a preclinical pharmaceutical company that seeks to develop NMDA receptor antagonists for CNS indications.

REFERENCES

1. Davies J, Francis AA, Jones AW, Watkins JC. 2-Amino-5-phosphonovalerate (2APV), a potent and selective antagonist of amino acid-induced and synaptic excitation. *Neurosci Lett.* 1981;21:77–81.
2. Dingledine R, McBain CJ, McNamara JO. Excitatory amino acid receptors in epilepsy. *Trends Pharmacol*. 1990;11:334–338.
3. Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. *Pharmacol Rev*. 1999;51:7–62.
4. Hollmann M, O'Shea-Greenfield A, Rogers SW, Heinemann S. Cloning by functional expression of a member of the glutamate receptor family. *Nature*. 1989;342:643–648
5. Hollmann M, Heinemann S. Cloned glutamate receptors. *Annu Rev Neurosci*. 1994;17:31–108.
6. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev.* 2010;62:405–496.
7. Collingridge GL, Olsen RW, Peters J, Spedding M. A nomenclature for ligand-gated ion channels. *Neuropharmacology.* 2009;56:2–5.
8. Jane DE, Lodge D, Collingridge GL. Kainate receptors: pharmacology, function and therapeutic potential. *Neuropharmacology.* 2009;56:90–113.
9. Penn AC, Greger IH. Sculpting AMPA receptor formation and function by alternative RNA processing. *RNA Biol.* 2009;6:517–521.
10. MonyL, Kew JN, Gunthorpe MJ, Paoletti P. Allosteric modulators of NR2B-containing NMDA receptors: molecular mechanisms and therapeutic potential. *Br J Pharmacol.* 2009;157:1301–1317.
11. Swanson GT. Targeting AMPA and kainate receptors in neurological disease: therapies on the horizon? *Neuropsychopharmacology.* 2009;34:249–250.
12. Bassani S, Valnegri P, Beretta F, Passafaro M. The GLUR2 subunit of AMPA receptors: synaptic role. *Neuroscience.* 2009;158:55–61.
13. Hansen KB, Furukawa H, Traynelis SF. Control of assembly and function of glutamate receptors by the amino-terminal domain. *Mol Pharmacol.* 2010;78:535–549.
14. Jin R, Banke TG, Mayer ML, Traynelis SF, Gouaux E. Structural basis for partial agonist action at ionotropic glutamate receptors. *Nat Neurosci.* 2003;6: 803–810.
15. Kleckner NW, Dingledine R. Requirement for glycine in activation of NMDA receptors expressed in *Xenopus*oocytes. *Science*.1988;241:835–837.
16. Kumar J, Schuck P, Jin R, Mayer ML. The N-terminal domain of GluR6-subtype glutamate receptor ion channels. *Nat Struct Mol Biol.* 2009;16:631–638.
17. Jin R, Singh SK, Gu S, Furukawa H, Sobolevsky AI, Zhou J, Jin Y, Gouaux E.Crystal structure and association behaviour of the GluR2 amino-terminal domain. *EMBO J.* 2009;28:1812–1823.
18. Karakas E, Simorowski N, Furukawa H. Structure of the zinc-bound amino-terminal domain of the NMDA receptor NR2B subunit. *EMBO J.* 2009; 28:3910–3920.

18a. Karakas E, Simorowski N, Furukawa H. Subunit arrangement and phenylethanolamine binding in GluN1/GluN2B NMDA receptors. *Nature* 2011; 475: 249–253.

19. Higuchi M, Single FN, Köhler M, Sommer B, Sprengel R, Seeburg PH. RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. *Cell*. 1993;75:1361–1370.
20. Myers SJ, Peters J, Comer M, Barthel F, Dingledine R. Transcriptional regulation of the GluR2 gene: neural-specific expression, multiple promoters, and regulatory elements. *J Neurosci*. 1998;18:6723–6739.
21. Huang, Y-F, Myers SJ, Dingledine R. Transcriptional repression by REST: recruitment of Sin3A and histone deacetylase to neuronal genes. *Nat Neurosci*. 1999;2:867–872.
22. Huang Y, Doherty JJ, Dingledine R. Altered histone acetylation at glutamate receptor 2 and brain-derived neurotrophic factor genes is an early event triggered by status epilepticus. *J Neurosci.* 2002;22: 8422–8428.
23. Myers SJ, Huang Y, Genetta T, Dingledine R. Inhibition of glutamate receptor 2 translation by a polymorphic repeat sequence in the 5′-untranslated leaders. *J Neurosci.* 2004;24:3489–3499.
24. Huang YS, Kan MC, Lin CL, Richter JD.CPEB3 and CPEB4 in neurons: analysis of RNA-binding specificity and translational control of AMPA receptor GluR2 mRNA. *EMBO J.* 2006;25:4865–4876.
25. Irier HA, Quan Y, Yoo J, Dingledine R. Control of glutamate receptor 2 (GluR2) translational initiation by its alternative 3′ untranslated regions. *Mol Pharmacol.* 2009;76:1145–1149.
26. Irier HA, Shaw R, Lau A, Feng Y, Dingledine R. Translational regulation of GluR2 mRNAs in rat hippocampus by alternative 3′ untranslated regions. *J Neurochem.* 2009;109:584–594.
27. Wood MW, Van Dongen HMA, Van Dongen AMJ. The 59-untranslated region of the *N*-methyl-D-aspartate receptor NR2A subunit controls efficiency of translation. *J Biol Chem.* 1996;271:8115–8120.
28. Wells DG, Dong X, Quinlan EM, Huang YS, Bear MF, Richter JD, Fallon JR. A role for the cytoplasmic polyadenylation element in NMDA receptor–regulated

mRNA translation in neurons. *J Neurosci.* 2001;21:9541–9548.
29. Sanchez-Perez AM, Felipo V. Serines 890 and 896 of the NMDA receptor subunit NR1 are differentially phosphorylated by protein kinase C isoforms. *Neurochem Int.* 2005;47:84–91.
30. Niimura M, Moussa R, Bissoon N, Ikeda-Douglas C, Milgram NW, Gurd JW. Changes in phosphorylation of the NMDA receptor in the rat hippocampus induced by status epilepticus. *J Neurochem.* 2005;92: 1377–1385.
31. Tingley WG, Ehlers MD, Kameyama K, Doherty C, Ptak JB, Riley CT, Huganir RL. Characterization of protein kinase A and protein kinase C phosphorylation of the *N*-methyl-D-aspartate receptor NR1 subunit using phosphorylation site-specific antibodies. *J Biol Chem.* 1997;272:5157–5166.
32. Scott DB, Blanpied TA, Ehlers MD. (2003) Coordinated PKA and PKC phosphorylation suppresses RXR-mediated ER retention and regulates the surface delivery of NMDA receptors. *Neuropharmacology.* 2003;45:755–767.
33. Wetherington J, Serrano G, Dingledine R. Astrocytes in the epileptic brain. *Neuron*. 2008;58:168–178.
34. Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, Domercq M, Matute C, Tonello F, Gundersen V, Volterra A. Glutamate exocytosis from astrocytes controls synaptic strength. *Nat Neurosci.* 2007;10:331–339.
35. Ding S, Fellin T, Zhu Y, Lee SY, Auberson YP, Meaney DF, Coulter DA, Carmignoto G, Haydon PG. Enhanced astrocytic Ca^{2+} signals contribute to neuronal excitotoxicity after status epilepticus. *J Neurosci.* 2007;27:10674–10684.
36. Ding S, Wang T, Cui W, Haydon PG. Photothrombosis ischemia stimulates a sustained astrocytic Ca^{2+} signaling in vivo. *Glia.* 2009;57:767–776.
37. Hamilton NB, Attwell D. Do astrocytes really exocytose neurotransmitters? *Nat Rev Neurosci.* 2010;11:227–238.
38. Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, Vescovi A, Bagetta G, Kollias G, Meldolesi J, Volterra A. CXCR4- activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity. *Nat Neurosci.* 2001;4:702–710.
39. Liang SL, Carlson GC, Coulter DA. Dynamic regulation of synaptic GABA release by the glutamate-glutamine cycle in hippocampal area CA1. *J Neurosci.* 2006;26:8537–8548.
40. Fricke MN, Jones-Davis DM, Mathews GC. Glutamine uptake by System A transporters maintains neurotransmitter GABA synthesis and inhibitory synaptic transmission. *J Neurochem.* 2007;102: 1895–1904.
41. Eid T, Thomas MJ, Spencer DD, Rundén-Pran E, Lai JC, Malthankar GV, Kim JH, Danbolt NC, Ottersen OP, de Lanerolle NC. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet.* 2004;363: 28–37.
42. Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ, Haydon PG, Coulter DA. Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nat Neurosci.* 2010;13:584–591.
43. Eid T, Ghosh A, Wang Y, Beckström H, Zaveri HP, Lee TS, Lai JC, Malthankar-Phatak GH, de Lanerolle NC. Recurrent seizures and brain pathology after inhibition of glutamine synthetase in the hippocampus in rats. *Brain.*2008;131:2061–2070.
44. Bacci A, Sancini G, Verderio C, Armano S, Pravettoni E, Fesce R, Franceschetti S, Matteoli M. Block of glutamate-glutamine cycle between astrocytes and neurons inhibits epileptiform activity in hippocampus. *J Neurophysiol* .2002;88:2302–2310.
45. Tani H, Dulla CG, Huguenard JR, Reimer RJ. Glutamine is required for persistent epileptiform activity in the disinhibited neocortical brain slice. *J Neurosci.*2010;30:1288–1300.
46. Olney JW, Rhee V, Ho OL. Kainic acid: a powerful neurotoxic analogue of glutamate. *Brain Res*. 1974;77:507–512.
47. Vincent P, Mulle C. Kainate receptors in epilepsy and excitotoxicity. *Neuroscience*. 2009;158: 309–323.
48. Mulle C, Sailer A, Pérez-Otaño I, Dickinson-Anson H, Castillo PE, Bureau I, Maron C, Gage FH, Mann JR, Bettler B, Heinemann SF. Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. *Nature.*1998;392:601–605.
49. Fisahn A, Contractor A, Traub RD, Buhl EH, Heinemann SF, McBain CJ. Distinct roles for the kainate receptor subunits GluR5 and GluR6 in kainate-induced hippocampal gamma oscillations. *J Neurosci*. 2004;24:9658–9668.
50. Smolders I, Bortolotto ZA, Clarke VR, Warre R, Khan GM, O'Neill MJ, Ornstein PL, Bleakman D, Ogden A, Weiss B, Stables JP, Ho KH, Ebinger G, Collingridge GL, Lodge D, Michotte Y. Antagonists of GLU(K5)-containing kainate receptors prevent pilocarpine-induced limbic seizures. *Nat Neurosci.* 2002;5: 796–804.
51. Christensen JK, Paternain AV, Selak S, Ahring PK, Lerma J.A mosaic of functional kainate receptors in hippocampal interneurons. *J Neurosci*. 2004;24:8986–8993.
52. Benveniste M, Wilhelm J, Dingledine R, Mott DD. Subunit-dependent modulation of kainate receptors by muscarinic acetylcholine receptors. *Brain Res.* 2010;1352:61–69.

Chapter 11

Glutamate Receptors in Epilepsy

Group I mGluR-Mediated Epileptogenesis

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In the early 1990s, the epileptogenic potential of metabotropic glutamate receptor (mGluR) activation in the hippocampus was first suggested by data using the then newly developed broad- spectrum mGluR agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD).[1] These studies revealed that mGluR activation had the potent ability to recruit the hippocampal network to express robust synchronized discharges. These synchronized bursts had features suggestive of typical seizure discharges in that (1) their length was on the order of seconds and (2) they were comprised of an intrinsic oscillatory series of discharges that began at a high frequency and gradually slowed. And indeed, work in other labs confirmed that ACPD application does elicit seizures in the intact organism.[2] A hypothesis was developed proposing that the group I mGluRs, which are predominantly localized to the edges of synapses (*perisynaptic*),[3] were likely to be activated at times of intense glutamate release,

and this could result in the expression of acute seizures such as the impact seizure that occurs acutely in the setting of head trauma. However, subsequent studies using the selective group I mGluR agonist (*S*)-3,5-dihydroxyphenylglycine (DHPG) revealed a potential additional consequence of group I mGluR activation: long-lasting changes in network excitability.[4]

THE mGluR MODEL OF EPILEPTOGENESIS

The impact of selective group I mGluR activation on the in vitro hippocampal network was profound. Not only did such activation result in the expression of ictal-like discharges, but removal of the mGluR agonist was insufficient to restore baseline network excitability; instead, ictal-like discharges continued to be expressed unabated for hours following agonist exposure and removal (Fig. 11–1).[4] This network plasticity was strongly suggestive of the initiation of an epileptogenic process, one in which normal neuronal cortex is converted into a persistently hyperexcitable state with a lowered threshold for the production of seizure discharges. It was proposed that this form of epileptogenesis was likely to be clinically relevant because the instigating agent was acting at glutamate receptors, and glutamate was long recognized as the key excitatory transmitter in the central nervous system (CNS) underlying the expression of seizure discharges.

Numerous studies revealed that group I mGluR activation can induce long-lasting changes in cellular and synaptic properties (plasticity) in the CNS, including long-term potentiation and depression.[5,6] As with all metabotropic receptors, a G-protein-coupled second-messenger signaling cascade is recruited upon stimulation, and the intracellular enzymes activated can have long-lasting consequences for cellular excitability. In the case of the group I mGluRs—receptors coupled to the $G_{q/11}$ protein and phospholipase C signaling pathway—two direct effects were well characterized early on: production of diacylglycerol, which would result in protein kinase C activation, and inositol triphosphate production, which would stimulate release of calcium from intracellular stores.[7] Early studies attributed the synaptic long-lasting effects of group I mGluR activation to either or both of these intracellular pathways, and it was initially assumed that the network plasticity we observed was similarly mediated, but later studies revealed the additional involvement of a more complex set of proteins and enzymes, to be described later in this chapter.

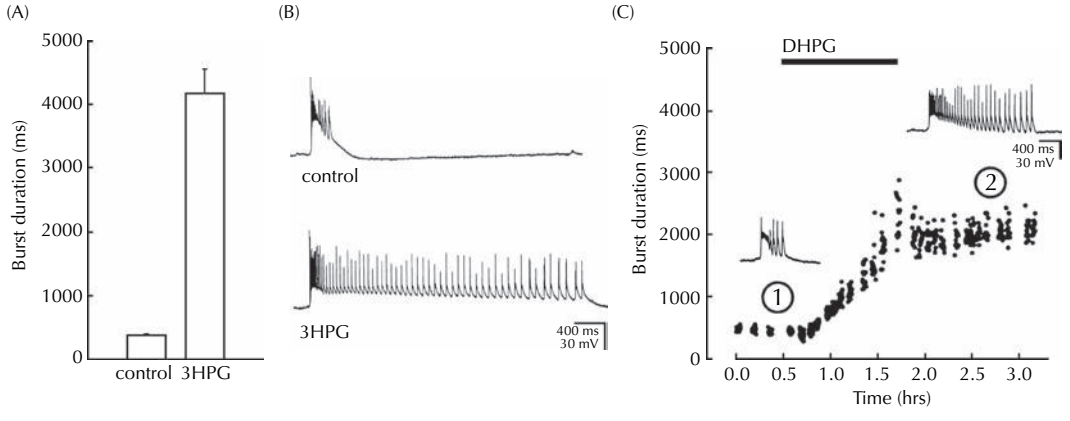


Figure 11–1. Group I metabotropic glutamate receptor (mGluR) activation induces persistent ictal-like discharges in the hippocampus. **A.** Summary histogram of mean duration of synchronized bursts elicited by picrotoxin (*control*) and maximum duration of prolonged bursts during application of the selective group I mGluR agonist (*S*)-3-hydroxyphenylglycine ((*S*)3HPG, 250–500 μM, $n = 7$ slices). Error bars: SEM. **B.** Intracellular recording from a CA3 pyramidal cell in a hippocampal slice before and during exposure to 250 μM (*S*)3HPG. **C.** Time course of synchronized burst prolongation; burst duration displayed before (*1*), during (*bar*), and after (*2*) group I mGluR stimulation with (*R,S*)-3,5-dihydroxyphenylglycine ((*R,S*)DHPG; 100 μM). Ictal-like discharges induced by (*R,S*)DHPG persisted for hours after washout of the agonist. Insets: Records before (*1*) and after (*2*) transient exposure to DHPG. Modified from ref. 4.

Plasticity of any type has two critical components: the initiating factors necessary for induction of the modification, and the downstream effectors that underlie the sustained expression of the enhanced response. Experiments described below have delved into understanding the necessary contributors to each of these components.

KEY FEATURES RELEVANT TO INDUCTION OF GROUP I mGluR-DEPENDENT EPILEPTOGENESIS

Most studies examining the in vitro model of mGluR-induced epileptogenesis have been performed on the hippocampal slice preparation. In many instances, slices were initially exposed to picrotoxin, an antagonist of $GABA_A$ receptor-mediated inhibition, to elicit baseline interictal activity in the CA3 network. While there were data to indicate that this additional agent was not necessary for the induction of persistent ictal-length activity, the picrotoxin model was useful for the following reasons: (1) It helped to confirm the baseline health of the slice by establishing the initial responsiveness of the network. (2) It reduced the interslice variability of the mGluR-induced ictaform activity. (3) It allowed easier interpretation of the site of action of the mGluR agonist: numerous studies had indicated that group I mGluRs are localized to principal neurons, interneurons, and glial cells,[8] and mGluR activation could therefore potentially have direct suppressive effects on inhibitory responses. Nevertheless, elimination of $GABA_A$ receptor-mediated inhibition from the network activity in the mature hippocampal slice never elicited ictaform discharges; only interictal-length bursts (generally <650 ms in duration) were produced. Furthermore, additional suppression of $GABA_B$ receptor-mediated responses also failed to elicit ictal-length discharges.[9]

Under conditions of suppressed GABA-mediated inhibition, exposure to selective group I mGluR agonists invariably induced ictaform discharges.[4] It was therefore suggested that group I mGluR activation participated in the interictal-to-ictal transition, eliciting ictaform discharges via a direct excitatory effect on the hippocampal network. But the persistence of the ictal discharges following transient agonist exposure was the finding of greatest interest due to its relevance to epileptogenesis. A series of experiments using coapplied antagonists indicated that the agonist was truly washing out of the chamber, suggesting that the persistence of the effect was due to a true network modification and not a pharmacological artifact.[4] But was group I mGluR activation truly necessary to induce this modification?

Silent Induction

When hippocampal slices are perfused with the $GABA_A$ antagonist picrotoxin, interictal bursts recur spontaneously and rhythmically approximately every 10 s for as long as the slice is exposed to the agent. When group I mGluR agonist is subsequently introduced, the burst frequency promptly increases to two to three times its original rate, then returns close to baseline frequency as the length of the bursts gradually increase to ictal length.[10] In light of this, it was hypothesized that perhaps the persistence of the effect was merely a kindling-like phenomenon resulting from the bombardment of recurrent bursting rather than a direct effect of the mGluR activation itself. To address this question, experiments were performed in which, after demonstration of normal interictal activity in the presence of picrotoxin, all bursting activity in the slice was suppressed via the perfusion of ionotropic glutamate receptor antagonists. Then, in the presence of this pharmacological blockade, the slice was transiently exposed to 20–40 min of group I mGluR agonist (DHPG), an experimental protocol referred to as *silent induction*.[11] Upon removal of all agents except picrotoxin, the synchronized discharges that emerged were markedly potentiated to ictal length. The data demonstrated that (1) induction of ictaform events in this model is independent of ionotropic glutamatergic activation, and (2) group I mGluR activation itself has a powerful, direct, long-lasting effect of enhancing hippocampal network responsiveness.

Group I mGluR Subtypes: mGluR1 and mGluR5

Experiments in which the group I mGluR agonist was applied in the presence of antagonist

confirmed that the epileptogenic effect was indeed driven by activation of group I mGluRs. The group I mGluR family is comprised of two receptor members, mGluR1 and mGluR5. Were both receptor subtypes necessary for induction of this epileptogenic effect? Utilizing selective antagonists against either mGluR1 or mGluR5, it was demonstrated that mGluR5 activation alone was often both necessary and sufficient to induce a persistent ictogenic effect.[12] This was evident from two sets of data: (1) If group I mGluR agonist was transiently applied in the presence of mGluR1-selective antagonist, persistent ictal-length discharges often appeared following subsequent removal of the mGluR1 antagonist, uncovering the epileptogenic effect that was induced by activation of mGluR5 alone. (2) If group I mGluR agonist was applied in the presence of mGluR5-selective antagonist, persistent ictal-length discharges were generally not elicited, revealing that mGluR5 activation was a necessary component of the induction process.

NMDA Receptors

N-methyl-D-aspartate (NMDA) receptor activation has frequently been associated with the induction of long-term potentiation and/or depression of synaptic responses.[13] It was therefore natural to consider the possibility that NMDA receptor activation may be necessary for the induction of the mGluR-driven long-lasting network effect. Nevertheless, experiments performed in the presence of NMDA receptor antagonist demonstrated that the group I mGluR agonist could still elicit its full epileptogenic effect, in regards to both enhanced burst length and persistence, following agonist removal.[14]

Protein Synthesis

Protein synthesis has been shown to play a key role in various forms of long-lasting synaptic modifications.[15] Experiments were performed to examine the protein synthesis dependence of the group I mGluR-induced epileptogenic effect. Utilizing anisomycin and cycloheximide, agents that suppress mRNA translation, it was shown that even prolonged group I mGluR agonist application failed to elicit burst prolongation in the presence of either protein synthesis inhibitor. By contrast, protein synthesis inhibitor had no effect on the expression of picrotoxin-induced interictal discharges.[10] The dependence of group I mGluR-mediated induction of ictaform events on mRNA translation would turn out to be highly relevant to understanding the pathophysiology of seizures and other clinical features of fragile X syndrome, as will be discussed later in this chapter.

Protein synthesis inhibitors had no effect on the expression of ictal-length discharges elicited with the broad-spectrum mGluR agonist ACPD, even though these discharges were shown to be group I mGluR-dependent.[16] Consistent with this finding, these ACPD-induced group I mGluR-driven but protein synthesis-independent ictal discharges were not persistent; rather, they readily disappeared upon removal of the broad-spectrum agonist.[4,16] Encouragingly, these data suggest that group I mGluR-mediated seizure discharges can be elicited without inducing an epileptogenic process, and further investigations clarifying the distinction between these two forms of mGluR activation may allow for the development of unique antiepileptogenic agents.

Phospholipase C

Group I mGluRs are coupled to intracellular signaling pathways via activation of phospholipase C (PLC);[7] this PLC activation was shown to be a critical component of the epileptogenic process. Pharmacological inhibition of PLC prevented induction of ictal-like discharges by DHPG, and similarly, in slices prepared from PLCβ1 knockout mice (mice lacking the major isoform of PLC expressed in the hippocampus), group I mGluR activation failed to elicit ictal-like discharges.[17] These data revealed that PLC activation is necessary for group I mGluR-induced epileptogenesis.

Activation of PLC stimulates two parallel signaling pathways: diacylglycerol-mediated protein kinase C (PKC) activation and inositol triphosphate–driven increases of intracellular Ca^{2+} via release from intracellular stores.[7] Although PKC is a mediator of many group I mGluR-induced effects, experiments performed in the presence of chelerythrine, a PKC inhibitor, revealed no significant suppressive effect on the induction of persistent ictaform activity by group I mGluR agonist.[18]

On the other hand, agents that interfere with Ca^{2+} release from intracellular stores did prevent the expression of ictaform activity elicited by either DHPG[19] or ACPD,[20] suggesting that mGluR-induced epileptogenesis is dependent on intracellular Ca^{2+} mobilization.

Extracellular-Signal-Regulated Kinase 1/2

Group I mGluR agonist elicits two phases of extracellular-signal-regulated kinase (ERK) 1/2 activation in hippocampal slices: an early response elicited directly by receptor activation and signaled via tyrosine kinase, and a later response driven by both neuronal firing and PKC activation. As indicated above, neither neuronal firing[11] nor PKC activation[18] is necessary for the mGluR-mediated induction of long-lasting ictaform activity. However, inhibition of either tyrosine kinase or ERK 1/2 phosphorylation prevented DHPG from inducing ictal-like discharges, suggesting that the induction process is dependent on the early receptor-mediated ERK 1/2 response.[21]

Phospholipase D

Cysteine sulfinic acid (CSA) is an amino acid endogenous to the nervous system that is released in the hippocampus during periods of high-intensity activity[22] and, among its many actions, activates phospholipase D (PLD)-coupled metabotropic receptors.[23] Cysteine sulfinic acid-mediated activation of PLD can be antagonized with the agent PCCG-13.[24] Through the use of these two agents, it was shown that PLD activation impedes the epileptogenic process induced by selective group I mGluR activation, while it has no suppressive effect on baseline interictal bursting or on fully induced persistent ictal activity.[25] Activation of PLD also had no effect on reversible ictal activity elicited with the broad-spectrum mGluR agonist ACPD.[16] As the CSA-mediated suppression of epileptogenesis was blocked by chelerythrine,[18] it was proposed that CSA's antiepileptogenic effect may be mediated by PLD-driven PKC activation, which could then feed back to inhibit group I mGluR-mediated responses.

The role of PKC in the antiepileptogenic effect of CSA was tested with experiments in which phorbol esters were used to activate PKC. Phorbol esters failed to inhibit mGluR-driven epileptogenesis; in fact, the ictal activity induced in the presence of phorbol esters was more robust than that elicited by DHPG alone. Additional experiments revealed that phorbol esters elicited a powerful epileptogenic effect of their own, accelerating the epileptogenic process and potentiating the ictal discharge duration.[26] It is possible that the PKC activation responsible for PLD-mediated suppression of epileptogenesis may need to be subtype-specific and/or localized to specific synapses on the activated neurons to evoke its suppressive effect.

WHAT SUSTAINS THE ONGOING EXPRESSION OF THE GROUP I mGluR-INDUCED ICTAL DISCHARGES?

As NMDA receptor–mediated responses can be enhanced by group I mGluR activation,[27] and as potentiated NMDA responses can sustain enhanced synchronized activity in hippocampal networks,[28] it was hypothesized that mGluR-induced NMDA potentiation may underlie the sustained expression of the ictal bursts in the hippocampus. However, experiments revealed that this was not the case; the ictal discharges continued unabated in the presence of NMDA antagonist.[1,14] What, then, was responsible for the sustained expression of the prolonged ictal discharges?

Autopotentiation

The ictal-length persistent discharges that are expressed following the transient activation of group I mGluRs proved to be quite resistant to most of the agents described above that impede induction of the discharges: neither protein synthesis inhibitor[10] nor CSA[25] could suppress the ongoing ictal activity once it had been induced. However, there was one agent that could effectively suppress the sustained expression: group I mGluR antagonist.[1,4,11] If introduced after induction had occurred and prolonged bursts were fully expressed, the group I mGluR antagonist shortened the synchronized discharges to interictal length, but ictal bursts promptly reappeared upon removal

of the antagonist. These findings suggested that group I mGluRs undergo *autopotentiation*; that is, transient agonist-mediated selective activation results in long-lasting enhanced responsiveness of group I mGluRs, allowing endogenous glutamate to sustain the activation of the receptors. Experiments utilizing selective mGluR1 and mGluR5 antagonists revealed that both receptors participate in the expression of the prolonged bursts: either antagonist alone elicited significant reversible shortening of the sustained ictal-length bursts, although the mGluR1 antagonist was more effective, almost completely restoring the discharges to their interictal length prior to agonist exposure.[12]

Although presynaptic enhancement of glutamate release remains a possible contributor to this observed potentiation of mGluR responses, it appears more likely that the primary site of modification is postsynaptic. In recent studies, postsynaptic long-lasting effects on intrinsic properties of CA3 pyramidal cells induced by group I mGluR activation have been identified.[29,30] These cellular modifications may underlie epileptogenesis by inducing persistent changes in the cell-firing pattern, thereby sustaining prolonged ictal-like discharges.

Persistent Effects of Group I mGluR Activation

There are numerous reported cellular effects mediated by group I mGluR activation. Most have not been established as long-lasting, but even those that do persist (e.g., enhancement of NMDA responses[27]) do not necessarily contribute to the enduring nature of the epileptogenic effect.[14] The following are two excitatory effects of group I mGluR activation that have been established as long-lasting and likely contribute to the ongoing expression of the persistent ictal discharges.

INDUCTION OF A VOLTAGE-DEPENDENT CATIONIC CURRENT $I_{MGLUR(V)}$

A major response to group I mGluR stimulation in CA3 pyramidal cells is the appearance of a depolarization-activated, voltage-dependent ionic current ($I_{mGluR(V)}$) that has an activation threshold at around –60 mV, and a reversal potential of about –10 mV, and shows no inactivation[17,29,31] (Fig. 11–2). $I_{mGluR(V)}$ induces plateau potentials that sustain rhythmic periods of prolonged action potential firing of 2–7 s with intervals of similar durations.[17] $I_{mGluR(V)}$ also promotes repetitive firing by eliciting spike afterdepolarizations (ADPs).[32] This intrinsic pattern of neuronal firing could drive synchronized ictaform activity via the recurrent glutamatergic collateral connections in the CA3 cell population.[33] Indeed, pharmacological blockade of $I_{mGluR(V)}$[17,29] or its abolition in transgenic preparations[17,34] prevents the expression of group I mGluR-dependent ictal-like discharges, suggesting that $I_{mGluR(V)}$ underlies this mGluR-induced synchronized activity.

SUPPRESSION OF ACTION POTENTIAL AFTERHYPERPOLARIZATIONS

DHPG-mediated activation of group I mGluRs elicits suppression of action potential afterhyperpolarizations (AHPs), an effect that enhances repetitive firing in CA3 pyramidal cells.[32,35] This AHP suppression can be long-lasting,[30] suggesting that it may contribute to the prolonged neuronal firing during synchronized group I mGluR-mediated ictal activity.

Consistent with the persistent nature of these two cellular effects, DHPG cannot elicit $I_{mGluR(V)}$ or AHP suppression in the presence of protein synthesis inhibitors,[29,30] lending support to the hypothesis that these cellular effects may underlie the sustained expression of the protein synthesis-dependent persistent ictal bursts.[10]

ENDOGENOUS REGULATION OF GROUP I mGluR-DEPENDENT EPILEPTOGENESIS

Studies on the endogenous control of group I mGluR-dependent epileptogenesis were prompted by the observation that persistent ictaform discharges are elicited by selective activation of group I mGluRs with DHPG[4,10] but not by the broad-spectrum mGluR agonist ACPD[4,16] or by synaptic stimulation of group I mGluRs,[29,36,37] suggesting that group I mGluR-mediated epileptogenesis is normally prevented. Concurrent activation of group II mGluRs does not account for the reversibility of the ACPD-induced ictogenesis.[4,16] Active protein synthesis

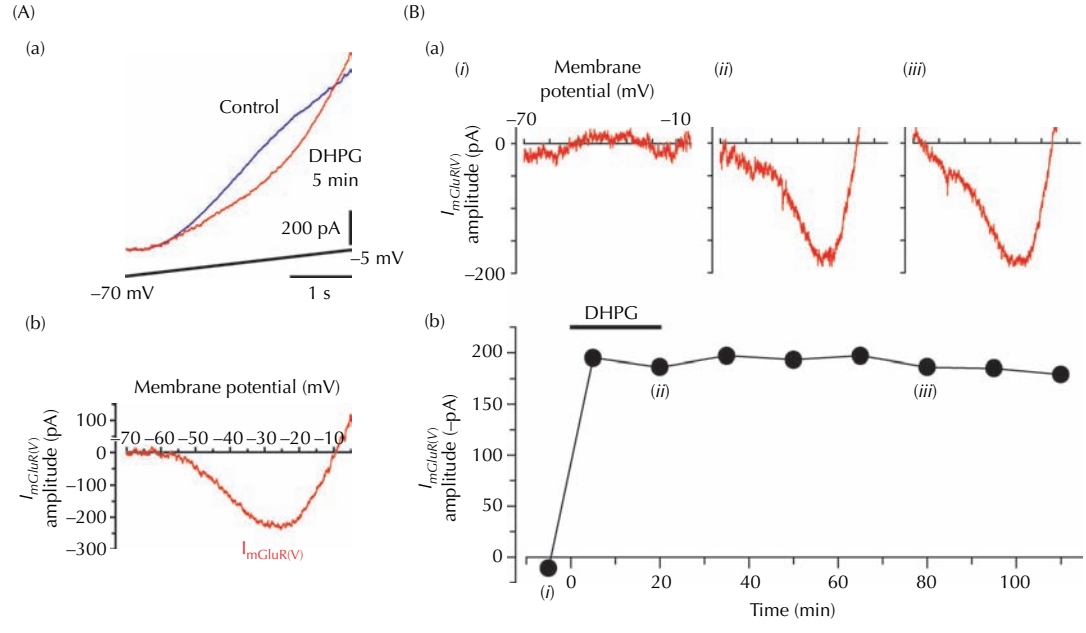


Figure 11–2. Group I mGluR activation induces a long-lasting voltage-dependent cationic current: $I_{mGluR(V)}$. **Aa.** Current response to a depolarizing ramp from –70 mV to –5 mV obtained from a CA3 pyramidal cell in a solution containing low Ca^{2+} (0.2 mM), Mn^{2+} (1 mM), TTX (1 µM), and Cs^{+} (5 mM) to suppress Ca^{2+}, Na^{+}, and K^{+} currents (*Control*) superimposed on current response in the same cell after addition of (*S*)DHPG (50 µM). **Ab.** *I–V* plot showing the current induced by DHPG, $I_{mGluR(V)}$, obtained by subtracting the control current response from the response in DHPG. **B**. *I–V* plots (***a***) of currents recorded in a CA3 pyramidal cell before (*i*), during (*ii*), and after (*iii*) DHPG application, and time course of $I_{mGluR(V)}$ development and maintenance relative to DHPG exposure (***b***). Modified from ref. 29.

is critical to the induction process underlying the mGluR-driven epileptogenic effect,[10,16,21] suggesting this to be a potential site of regulation.

Recent data reveal that there are multiple endogenous regulators of protein synthesis. Brain cytoplasmic 1 (BC1) RNA is a small non-protein-coding RNA expressed in neuronal dendrites,[38] where it inhibits activity-dependent protein synthesis.[39–41] Mice lacking BC1 RNA are susceptible to audiogenic seizures, and their hippocampal CA3 neuronal network displays synaptic hyperexcitability leading to ictal discharges.[37] These epileptogenic responses were shown to be protein synthesis dependent, and driven by enhanced activation of mGluR5 and ERK 1/2, as antagonism of either of these could suppress the epileptic effect.[37] These data suggest that, in wild-type mice, BC1 RNA is an endogenous translational repressor of group I mGluR-mediated epileptogenesis.[37,42]

The finding that group I mGluR-dependent long-term depression (LTD) is abnormal in preparations lacking functional fragile X mental retardation protein (FMRP),[43] an intracellular RNA-binding protein that represses mRNA translation,[44] pointed to this protein as another candidate for control of protein synthesis-dependent DHPG-induced epileptogenesis. Indeed, in experiments carried out on hippocampal slices from transgenic mice lacking FMRP, synaptic stimulation of group I mGluRs was able to elicit the DHPG-like persistent cellular and network excitatory effects previously seen only with DHPG application: both $I_{mGluR(V)}$ and persistent ictaform discharges were readily elicited, and these effects could be blocked with protein synthesis inhibitors.[29,36] These data are consistent with an inhibitory role of FMRP on group I mGluR-induced, protein synthesis-dependent plasticity in the normal condition, and with the hypothesis that absence of FMRP-mediated inhibition results in exaggerated group I mGluR-dependent protein synthesis-dependent responses responsible for the seizures seen in patients with fragile X syndrome[45] (Fig. 11–3).

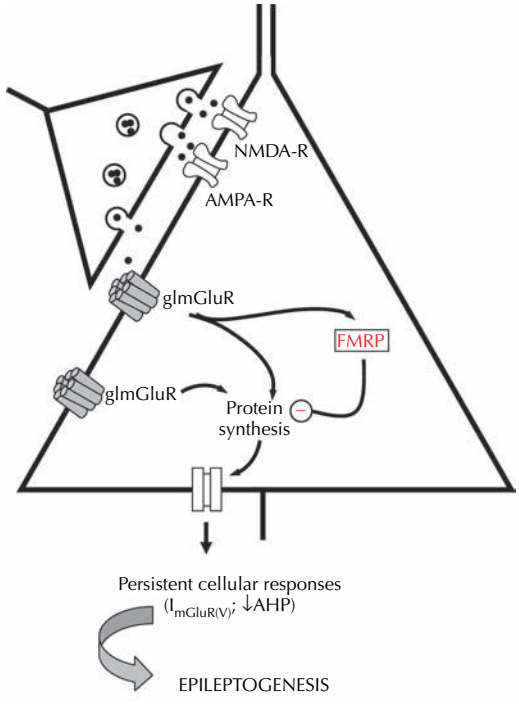


Figure 11–3. Group I mGluR model of epileptogenesis. Activation of group I mGluRs induces local translation eliciting cellular plasticity. Cellular plasticity includes long-lasting $I_{mGluR(V)}$ and AHP suppression. Integrative expression of cellular plasticity causes long-lasting ictal-like discharges. This epileptogenic process is normally controlled by a number of translation repressors, such as FMRP and BC1 RNA. Derepression of translation occurs in FXS, which is characterized by a single genetic defect resulting in loss of FMRP function. In the absence of FMRP, synaptic activation of group I mGluRs elicits exaggerated translation causing neurodevelopmental disorders and epilepsy.

FRAGILE X SYNDROME: A CLINICAL CONDITION IN WHICH HYPEREXCITABLE GROUP I mGluRS UNDERLIE A PHENOTYPE THAT INCLUDES SEIZURES

Group I mGluRs appear to play a central role in the pathophysiology of fragile X syndrome (FXS) in humans.[45] Fragile X syndrome is caused by a mutation that results in lack of expression of functional FMRP. The *Fmr1* knockout mouse expresses the full phenotype associated with FXS: memory deficits, learning disabilities, autism, epilepsy, altered body growth, and macroorchidism are features common to both the knockout mouse and the human condition, and thus this mouse has become a useful model for studying the underlying pathophysiology of the disease.[46] Studies have shown the efficacy of mGluR5 antagonist in suppressing audiogenic seizures in this model[47] (Fig. 11–3), and the results of clinical trials involving patients with FXS have been encouraging as well.[48] But even more remarkably, in *Fmr1* knockout mice crossbred to express 50% fewer mGluR5 receptors, a startling observation was made: all phenotypic anatomical and behavioral abnormalities usually seen in the fragile X mouse were normalized except for the macroorchidism.[46] The implications are profound: excessive mGluR5-driven protein synthesis is responsible for much more than just epileptogenesis; it has a broad impact on memory, behavior, and even prepubescent body growth in FXS. These data lead us to believe that the clinical importance of the group I mGluR system may be even more widespread than is currently realized.

ADDITIONAL CLINICAL CONDITIONS IN WHICH GROUP I mGluR HYPEREXCITABILITY MAY PLAY A KEY ROLE

The involvement of group I mGluR activation in epileptogenesis may well extend beyond FXS. The following are some examples of neurological conditions that are associated with enhanced susceptibility to seizures and for which recent data raise the issue of possible roles of group I mGluRs in their pathogenesis.

Alzheimer's Disease and Down Syndrome

Common features of Alzheimer's disease (AD) and Down syndrome (DS) are overexpression of amyloid precursor protein (APP) and accumulation of β-amyloid (Aβ) in brain plaques, with associated dementia.[49,50] Both AD and DS are associated with an increased incidence of seizures,[51] and recent data suggest that Aβ may be epileptogenic.[52] Studies on AD and DS mouse models reveal increased susceptibility to audiogenic seizures[53,54] and remarkably, mGluR5 antagonists reduce both Aβ production and the expression of seizures.[54,55] These

data suggest that mGluR5 activation may underlie epileptogenesis in AD and DS, and one may speculate that mGluR5 hyperexcitability contributes to the cognitive deficits and growth abnormalities as well. Interestingly, it appears that FMRP normally inhibits mGluR5-stimulated translation of APP and that Aβ is overexpressed in FXS,[56] indicating exaggerated mGluR5-mediated translation as a common pathogenic initiator in these distinct neurological diseases.

Posttraumatic and Poststroke Epilepsy

In patients who have sustained head trauma or stroke, there is an increased risk of developing epilepsy over the ensuing years.[57] Although the mechanisms for this epileptogenic process have not been fully elucidated and are likely multifactorial, the earliest inciting factor in both trauma and stroke is acute massive glutamate release, which may initiate an injurious process similar to kindling and culminating in the expression of recurrent unprovoked seizures. During the latent period, one can envision subclinical synchronized activity percolating in the network, contributing to a kindling-like phenomenon. The glutamate that instigates this cascade likely activates perisynaptic group I mGluRs, which may be key contributors to the epileptogenic process.

Traumatic brain injury (TBI) has been reproduced with animal models to study the mechanisms of epileptogenesis.[58] Chronic cellular modifications induced by TBI include loss of inhibitory interneurons, sprouting of excitatory connections, and increased excitability of pyramidal neurons.[59,60] Interestingly, at least some of these changes are activity-dependent because they are prevented by tetrodotoxin.[61] Contribution of group I mGluRs to posttraumatic neuronal injury is suggested by the findings in rat models that their activation exacerbates neuronal death in vitro, while their pharmacological blockade decreases the neuronal loss produced by TBI in vivo.[62,63] However, whether group I mGluR-dependent neuronal injury or activation of these receptors takes any part in epileptogenic processes following TBI is unknown. To assess the therapeutic potential of group I mGluR antagonists in this context is of particular interest since most of the classical antiepileptic drugs tested so far are rather ineffective against posttraumatic epilepsy.[64]

It is clinically recognized that only a subpopulation of patients with strokes or head trauma develop epilepsy, and at present we do not have the means to predict accurately which patients will have this outcome. We can speculate, however, that any patient with hyperexcitable group I mGluRs, either due to direct mutations or lack of sufficient endogenous repression, will be more prone to the epileptogenic process and may benefit from treatments targeting this pathway during the latent period, before their first clinical seizure is experienced. Advances in genetic testing may someday allow us to identify these patients. It is interesting to note that animals deficient in the expression of group I mGluRs have been shown to be particularly resistant to developing epilepsy following an episode of status epilepticus.[65] Although we are a long way from fully understanding the complex role of group I mGluRs in seizure expression and epileptogenesis, there are clear indications that such studies may have broad implications for the development of new therapeutic strategies for a wide variety of neurological conditions associated with epilepsy.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Taylor GW, Merlin LR, Wong RKS. Synchronized oscillations in hippocampal CA3 neurons induced by metabotropic glutamate receptor activation. *J Neurosci*. 1995;15:8039–8052.
2. McDonald JW, Fix AS, Tizzano JP, Schoepp DD. Seizures and brain injury in neonatal rats induced by 1S,3R-ACPD, a metabotropic glutamate receptor agonist. *J Neurosci*. 1993;13:4445–4455.
3. Baude A, Nusser Z, Roberts JDB, Mulvihill E, McIlhinney RAJ, Somogyi P. The metabotropic glutamate receptor (mGluR1α) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron*. 1993;11:771–787.
4. Merlin LR, Wong RKS. Role of group I metabotropic glutamate receptors in the patterning of epileptiform activities in vitro. *J Neurophysiol*. 1997;78:539–544.
5. Anwyl R. Metabotropic glutamate receptor–dependent long-term potentiation. *Neuropharmacology*. 2009;56:735–740.

6. Lüscher C, Huber KM. Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. *Neuron*. 2010;65:4 45–459.
7. Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol*. 2010;50:295–322.
8. Ferraguti F, Crepaldi L, Nicoletti F. Metabotropic glutamate 1 receptor: current concepts and perspectives. *Pharmacol Rev*. 2008;60:536–581.
9. Huszár P, Merlin LR. Contribution of $GABA_B$ receptor–mediated inhibition to the expression and termination of group I mGluR-induced ictaform bursts. *Epilepsy Res*. 2004;61:161–165.
10. Merlin LR, Bergold PJ, Wong RKS. Requirement of protein synthesis for group I mGluR-mediated induction of epileptiform discharges. *J Neurophysiol*. 1998;80:989–993.
11. Merlin LR. Group I mGluR-mediated silent induction of long-lasting epileptiform discharges. *J Neurophysiol*. 1999;82:1078–1081.
12. Merlin LR. Differential roles for mGluR1 and mGluR5 in the persistent prolongation of epileptiform bursts. *J Neurophysiol*. 2002;87:621–625.
13. Malenka RC, Nicoll RA. NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. *Trends Neurosci*. 1993;16:521–527.
14. Galoyan S, Merlin LR. Long-lasting potentiation of epileptiform bursts by group I mGluRs is NMDA receptor independent. *J Neurophysiol*. 2000;83:2463–2467.
15. Costa-Mattioli M, Sossin WS, Klann E, Sonenberg N. Translational control of long-lasting synaptic plasticity and memory. *Neuron*. 2009;61:10–26.
16. Fuortes MG, Rico MJ, Merlin LR. Distinctions between persistent and reversible group I mGluR-induced epileptiform burst prolongation. *Epilepsia*. 2010;51:1633–1637.
17. Chuang S-C, Bianchi R, Kim D, Shin H-S, Wong RKS. Group I metabotropic glutamate receptors elicit epileptiform discharges in the hippocampus through PLCβ1 signaling. *J Neurosci.* 2001;21:6387–6394.
18. Cuellar JC, Griffith EL, Merlin LR. Contrasting roles of protein kinase C in induction versus suppression of group I mGluR-mediated epileptogenesis in vitro. *J Neurophysiol*. 2005;94:3643–3647.
19. Zhao W, Bianchi R, Wong RKS. Ca^{2+} store-dependent and -independent population activity induced by the activation of group I mGluRs in the hippocampus. Program #559.14, 2001 Neuroscience Meeting Planner, San Diego, CA. Society for Neuroscience. http://www.sfn.org/absarchive/abstract.aspx. Accessed XXX
20. McDonald JW, Fix AS, Tizzano JP, Schoepp DD. Seizures and brain injury in neonatal rats induced by 1S,3R-ACPD, a metabotropic glutamate receptor agonist. *J Neurosci.* 1993;13:4445–4455.
21. Zhao W, Bianchi R, Wang M, Wong RKS. Extracellular signal-regulated kinase 1/2 is required for the induction of group I metabotropic glutamate receptor-mediated epileptiform discharges. *J Neurosci*. 2004;24:76–84.
22. Klancnik JM, Cuénod M, Gähwiler BH, Jiang ZP, Do KQ. Release of endogenous amino acids, including homocysteic acid and cysteine sulphinic acid, from rat hippocampal slices evoked by electrical stimulation of Schaffer collateral-commissural fibres. *Neuroscience*. 1992;49:557–570.
23. Boss V, Nutt KM, Conn PJ. L-cysteine sulfinic acid as an endogenous agonist of a novel metabotropic receptor coupled to stimulation of phospholipase D activity. *Mol Pharmacol*. 1994;45:1177–1182.
24. Albani-Torregrossa S, Attucci S, Marinozzi M, Pellicciari R, Moroni F, Pellegrini-Giampietro DE. Antagonist pharmacology of metabotropic glutamate receptors coupled to phospholipase D activation in adult rat hippocampus: focus on (2R,1′S,2′R,3′S)-2-(2′-carboxy-3′-phenylcyclopropyl)glycine versus 3,5-dihydroxyphenylglycine. *Mol Pharmacol.* 1999;55: 699–707.
25. Rico MJ, Merlin LR. Evidence that phospholipase D activation prevents group I mGluR-induced persistent prolongation of epileptiform bursts. *J Neurophysiol*. 2004;91:2385–2388.
26. Fuortes MG, Faria LC, Merlin LR. Impact of protein kinase C activation on epileptiform activity in the hippocampal slice. *Epilepsy Res*. 2008;82:38–45.
27. O'Connor JJ, Rowan MJ, Anwyl R. Long-lasting enhancement of NMDA receptor–mediated synaptic transmission by metabotropic glutamate receptor activation. *Nature*. 1994;367:557–559.
28. Traub RD, Jefferys JGR, Whittington MA. Enhanced NMDA conductance can account for epileptiform activity induced by low Mg^{2+} in the rat hippocampal slice. *J Physiol* (*Lond*). 1994;478:379–393.
29. Bianchi R, Chuang S-C, Zhao W, Young SR, Wong RKS. Cellular plasticity for group I mGluR-mediated epileptogenesis. *J Neurosci*. 2009;29:3497–3507.
30. Young SR, Bianchi R, Wong RKS. Signaling mechanisms underlying group I mGluR-induced persistent AHP suppression in CA3 hippocampal neurons. *J Neurophysiol*. 2008;99:1105–1118.
31. Chuang S-C, Bianchi R, Wong RKS. Group I mGluR activation turns on a voltage-gated inward current in hippocampal pyramidal cells. *J Neurophysiol*. 2000;83:2844–2853.
32. Young SR, Chuang S-C, Wong RKS. Modulation of afterpotentials and firing pattern in guinea pig CA3 neurones by group I metabotropic glutamate receptors. *J Physiol*. 2004;554:371–385.
33. Wong RKS, Chuang S-C, Bianchi R. Plasticity mechanisms underlying mGluR-induced epileptogenesis. *Adv Exp Med Biol.* 2004;548:69–75.
34. Chuang S-C, Zhao W, Young SR, Conquet F, Bianchi R, Wong RKS. Activation of group I mGluRs elicits different responses in murine CA1 and CA3 pyramidal cells. *J Physiol*. 2002;541:113–121.
35. Charpak S, Gähwiler BH, Do KQ, Knöpfel T. Potassium conductances in hippocampal neurons blocked by excitatory amino-acid transmitters. *Nature*. 1990;347:765–767.
36. Chuang S-C, Zhao W, Bauchwitz R, Yan Q, Bianchi R, Wong RKS. Prolonged epileptiform discharges induced by altered group I metabotropic glutamate receptor–mediated synaptic responses in hippocampal slices of a fragile X mouse model. *J Neurosci.* 2005;25:8048–8055.
37. Zhong J, Chuang S-C, Bianchi R, Zhao W, Lee H, Fenton AA, Wong RKS, Tiedge H. BC1 regulation of metabotropic glutamate receptor–mediated neuronal excitability. *J Neurosci*. 2009;29:9977–9986.
38. Tiedge H, Fremeau RT Jr, Weinstock PH, Arancio O, Brosius J. Dendritic location of neural BC1 RNA. *Proc Natl Acad Sci USA*. 1991;88:2093–2097.

39. Muslimov IA, Banker G, Brosius J, Tiedge H. Activity-dependent regulation of dendritic BC1 RNA in hippocampal neurons in culture. *J Cell Biol*. 1998;141:1601–1611.
40. Wang H, Iacoangeli A, Lin D, Williams K, Denman RB, Hellen CUT, Tiedge H. Dendritic BC1 RNA in translational control mechanisms. *J Cell Biol*. 2005;171:811–821.
41. Wang H, Iacoangeli A, Popp S, Muslimov IA, Imataka H, Sonenberg N, Lomakin IB, Tiedge H. Dendritic BC1 RNA: functional role in regulation of translation initiation. *J Neurosci*. 2002;22:10232–10241.
42. Iacoangeli A, Bianchi R, Tiedge H. Regulatory RNAs in brain function and disorders. *Brain Res*. 2010;1338:36–47.
43. Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci USA*. 2002;99:7746–7750.
44. Aschrafi A, Cunningham BA, Edelman GM, Vanderklish PW. The fragile X mental retardation protein and group I metabotropic glutamate receptors regulate levels of mRNA granules in brain. *Proc Natl Acad Sci USA*. 2005;102:2180–2185.
45. Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. *Trends Neurosci*. 2004;27:370–377.
46. Dölen G, Osterweil E, Rao BSS, Smith GB, Auerbach BD, Chattarji S, Bear MF. Correction of fragile X syndrome in mice. *Neuron*. 2007;56:955–962.
47. Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. *Neuropharmacology*. 2005;49:1053–1066.
48. Berry-Kravis E, Hessl D, Coffey S, Hervey C, Schneider A, Yuhas J, Hutchison J, Snape M, Tranfaglia M, Nguyen DV, Hagerman R. A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *J Med Genet*. 2009;46:266–271.
49. Isacson O, Seo H, Lin L, Albeck D, Granholm AC. Alzheimer's disease and Down's syndrome: roles of APP, trophic factors and ACh. *Trends Neurosci*. 2002;25:79–84.
50. Lott IT, Head E, Doran E, Busciglio J. Beta-amyloid, oxidative stress and Down syndrome. *Curr Alzheimer Res*. 2006;3:521–528.
51. Menéndez M. Down syndrome, Alzheimer's disease and seizures. *Brain Dev*. 2005;27:246–252.
52. Minkeviciene R, Rheims S, Dobszay MB, Zilberter M, Hartikainen J, Fülöp L, Penke B, Zilberter Y, Harkany T, Pitkänen A, Tanila H. Amyloid β–induced neuronal hyperexcitability triggers progressive epilepsy. *J Neurosci*. 2009;29:3453–3462.
53. Westmark CJ, Westmark PR, Beard AM, Hildebrandt SM, Malter JS. Seizure susceptibility and mortality in mice that over-express amyloid precursor protein. *Int J Clin Exp Pathol*. 2008;1:157–168.
54. Westmark CJ, Westmark PR, Malter JS. Alzheimer's disease and Down syndrome rodent models exhibit audiogenic seizures. *J Alzheimers Dis*. 2010;20:1009–1013.
55. Westmark CJ, Westmark PR, Malter JS. MPEP reduces seizure severity in Fmr-1 KO mice over expressing human Aβ. *Int J Clin Exp Pathol*. 2010;3:56–68.
56. Westmark CJ, Malter JS. FMRP mediates $mGluR_5$-dependent translation of amyloid precursor protein. *PLoS Biol*. 2007;5:e52.
57. Hauser WA, Annegers JF, Kurland LT. Prevalence of epilepsy in Rochester, Minnesota: 1940–1980. *Epilepsia*. 1991;32:429–445.
58. Pitkänen A, McIntosh TK. Animal models of posttraumatic epilepsy. *J Neurotrauma*. 2006;23:241–261.
59. Garga N, Lowenstein DH. Posttraumatic epilepsy: a major problem in desperate need of major advances. *Epilepsy Curr*. 2006;6:1–5.
60. Prince DA, Parada I, Scalise K, Graber K, Jin X, Shen F. Epilepsy following cortical injury: cellular and molecular mechanisms as targets for potential prophylaxis. *Epilepsia*. 2009;50(suppl 2):30–40.
61. Graber KD, Prince DA. Tetrodotoxin prevents posttraumatic epileptogenesis in rats. *Ann Neurol*. 1999;46:234–242.
62. Mukhin A, Fan L, Faden AI. Activation of metabotropic glutamate receptor subtype mGluR1 contributes to post-traumatic neuronal injury. *J Neurosci*. 1996;16:6012–6020.
63. Movsesyan VA, O'Leary DM, Fan L, Bao W, Mullins PGM, Knoblach SM, Faden AI. mGluR5 antagonists 2-methyl-6-(phenylethynyl)-pyridine and (E)-2-methyl-6-(2-phenylethenyl)-pyridine reduce traumatic neuronal injury in vitro and in vivo by antagonizing *N*-methyl-D-aspartate receptors. *J Pharmacol Exp Ther*. 2001;296:41–47.
64. Temkin NR. Preventing and treating posttraumatic seizures: the human experience. *Epilepsia*. 2009;50(suppl 2):10–13.
65. Chen J, Larionov S, Pitsch J, Hoerold N, Ullmann C, Elger CE, Schramm J, Becker AJ. Expression analysis of metabotropic glutamate receptors I and III in mouse strains with different susceptibility to experimental temporal lobe epilepsy. *Neurosci Lett*. 2005;375:192–197.

Chapter 12

Plasticity of Glutamate Synaptic Mechanisms

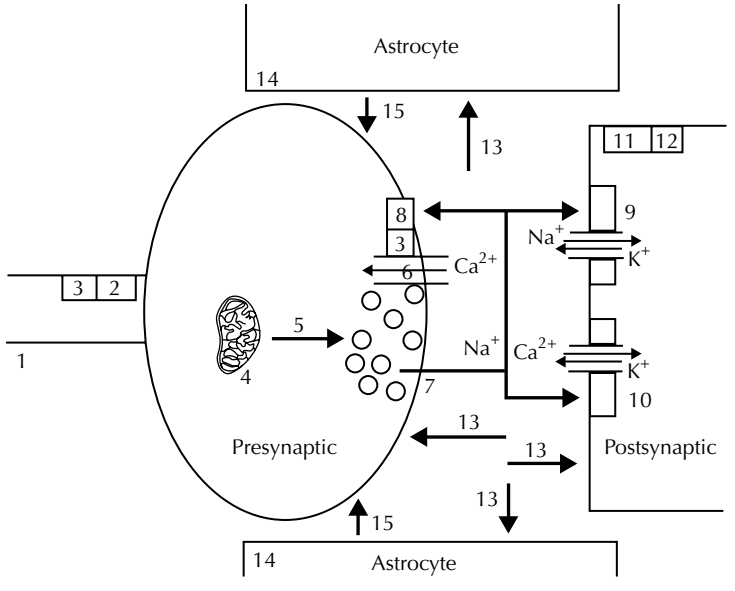
J. Victor Nadler

Epilepsy may be defined as a disorder of brain function characterized by the repeated and unpredictable occurrence of seizures. Seizures involve the disordered, rhythmic, and synchronous firing of central nervous system (CNS) neuron populations. Seizures originate in neuronal populations capable of bursting, develop because of an imbalance between neuronal excitation and inhibition, and are characterized by high-frequency firing associated with membrane depolarization. Neuronal excitation and inhibition may become unbalanced in many different ways. This chapter focuses on the contribution to seizures of glutamate synaptic plasticity, both anatomical plasticity that creates new excitatory synapses and functional plasticity that enhances the efficacy either of excitatory synapses or of glutamate itself. Observations made with human tissue are emphasized.

Glutamate is the principal excitatory neurotransmitter in mammals. About 60%–70% of all synapses in the CNS appear to be glutamate synapses (see Fig. 12–1). Glutamate also serves as the principal neurotransmitter utilized by sensory neurons. Autonomic neurons and motoneurons are about the only excitatory neurons in mammals that utilize a transmitter other than glutamate. Thus, the formation of enhanced or novel glutamate circuits, enhanced excitatory transmission, and/or an excess of glutamate itself could disrupt the balance of excitation and inhibition leading to the occurrence of seizures.

STRENGTHENING OF RECURRENT EXCITATION

Many cases of epilepsy arise from lesions of the brain. The consequences of brain injury depend not only on the degeneration of neurons and their axonal projections but also on reactive growth triggered by the injury. Degeneration of axonal projections stimulates the growth of collateral fibers from uninjured axons followed by a



1 Presynaptic action potential
2 Type II metabotropic receptor
3 G_o, G_i
4 Glutaminase
5 Vesicular transport
6 Voltage-dependent Ca^{2+} channel
7 Exocytotic release
8 Type III metabotropic receptor
9 AMPA receptor
10 NMDA receptor
11 Type I metabotropic receptor
12 G_q
13 Plasma membrane transport
14 Glutamine synthetase
15 Diffusion and terminal uptake of glutamine

Figure 12–1. Diagrammatic representation of a glutamate synapse. (1) Presynaptic action potential. (2) Type II metabotropic glutamate receptor. (3) G_o, G_i. (4) Mitochondrial phosphate-activated glutaminase. (5) Vesicular transport. (6) Voltage-dependent Ca^{2+} channel. (7) Exocytotic release. (8) Type III metabotropic glutamate receptor. (9) AMPA receptor. (10) NMDA receptor. (11) Type I metabotropic glutamate receptor. (12) G_q. (13) Plasma membrane transport. (14) Glutamine synthetase. (15) Diffusion and terminal uptake of glutamine. Reproduced with permission from *Encyclopedia of Stress*. 2nd ed. Vol. 1. Fink G, ed. Oxford: Academic Press; 2007:971.

process termed *reactive synaptogenesis*.[1] In many instances, axon sprouting and synaptogenesis appear to serve as mechanisms of repair. That is, the degenerated axons are replaced by new projections of the same type. The end result may be the restoration of at least some normal function. In contrast, the focal lesions often associated with epileptogenesis initiate the strengthening of recurrent excitatory circuitry. In humans with epilepsy, this phenomenon is most clearly observed in the dentate gyrus. Reorganization of the dentate gyrus is of particular interest because this region normally imposes high resistance to the propagation of seizures from the highly excitable entorhinal cortex to the equally excitable CA3 area of the hippocampus. The principal neurons of the dentate gyrus, the granule cells, are normally difficult to activate, both individually and as a population. The difficulty of activating synchronous population discharge can be explained, in part, by the scarcity of recurrent excitatory connections among dentate granule cells. The relative independence of granule cell activity is required to support the function of these cells in mediating pattern separation during the formation of new memories. Most persons with temporal lobe epilepsy exhibit a type of CNS pathology known as *hippocampal sclerosis*. Within the hippocampus, the most consistent region of neuronal loss is the hilus of the dentate gyrus. Loss of the hilar mossy cells opens synaptic territory on the proximal part of the granule cell apical dendrite. For reasons that remain unclear, denervation of this dendritic region attracts neoinnervation primarily from the axons of other granule cells. Granule cell

axons, the mossy fibers, sprout new collaterals and form recurrent excitatory connections. Because mossy fiber terminals contain numerous densely packed synaptic vesicles that contain zinc, mossy fiber sprouting can be detected by the Timm stain, which visualizes vesicle-bound zinc. Several investigators used this approach to demonstrate mossy fiber sprouting in hippocampal tissue resected for medically intractable temporal lobe epilepsy.[2–5] In addition, the new mossy fiber terminals were visualized with an antibody to dynorphin, a neuropeptide contained in those terminals.[6] Formation of recurrent excitatory circuitry in the human dentate gyrus is associated with a reduced threshold for granule cell synchronization.[7,8] The formation or strengthening of recurrent excitatory circuitry is one mechanism that could synchronize neuronal firing and thus support the initiation or propagation of seizures.

Robust mossy fiber sprouting has been observed in all animal models of epilepsy characterized by the loss of hilar mossy cells.[9,10] Studies of these models have generally confirmed the role of recurrent mossy fibers in synchronizing granule cell firing and supporting population bursts. Dentate granule cells are unusual in that they continue to be born and differentiate throughout life. Strong seizures increase the rate of granule cell neurogenesis markedly, and postseizure-generated granule cells possess properties distinct from those of preexisting granule cells. Many retain the hilar basal dendrite and burst potential characteristic of immature granule cells, and they serve as prominent sources and recipients of recurrent mossy fiber innervation. Granule cell basal dendrites provide a target for recurrent mossy fiber synapses in addition to the proximal apical dendrite. In humans, unlike rodents, about 25% of dentate granule cells normally have a basal dendrite. Thus, recurrent excitation of granule cells may be a normal feature of human brain, which is then enhanced by mossy fiber sprouting during epileptogenesis. About 95% of recurrent mossy fiber synapses are made with other granule cells and only 5% with inhibitory neurons. Frequency facilitation at mossy fiber–granule cell synapses operates over a rather narrow dynamic range (0.8–2 Hz), but even small changes in excitatory transmission could substantially increase synchronized granule cell discharge, especially if some granule cells generate a burst of action potentials. Thus, the normally rather quiescent dentate gyrus may be driven into reverberating epileptiform activity under conditions that strongly engage the novel recurrent dentate gyrus circuitry.

Recurrent axon sprouting is not confined to the dentate gyrus. In animal models of epilepsy, the number of monosynaptic recurrent excitatory synapses also increases dramatically in area CA1 of the hippocampus and the neocortex. Some seizures appear to propagate directly from the entorhinal cortex to area CA1, bypassing the dentate gyrus.[11] In these instances, the enhancement of recurrent excitation in area CA1 would appear to be of greater importance than changes in the dentate gyrus. Further studies are needed to determine how widespread the strengthening of recurrent excitation is in different regions of the brain, how large a role this type of plasticity plays compared to other changes that take place in the epileptic brain, and, importantly, the extent to which this type of plasticity outside the dentate gyrus occurs in the brain of humans with epilepsy.

EXTRACELLULAR GLUTAMATE CONCENTRATION

Measurements of extracellular glutamate concentration by microdialysis have revealed, as expected, an increase immediately before and during seizures in humans with epilepsy.[12,13] Perhaps less expected is the finding that the extracellular glutamate concentration is also elevated during the interictal period. This occurs despite neuronal loss and glial proliferation. Normally, the extracellular glutamate concentration is maintained in the low micromolar range. In one study, the extracellular glutamate concentration of the hippocampus was found to be four- to fivefold greater in patients with temporal lobe epilepsy than in controls.[14] This difference was only observed in brain regions from which seizures are thought to originate. A high ambient extracellular glutamate concentration could, among other actions, evoke a tonic *N*-methyl-D-aspartate (NMDA) current in neurons nearby, chronically depolarize those neurons, enhance neuronal excitability by activating Type I metabotropic receptors, and in extreme situations initiate an apoptotic/necrotic cascade. Mechanisms potentially responsible

for increasing the extracellular glutamate concentration include enhanced biosynthesis, enhanced release, impaired metabolic inactivation, and impaired clearance from the extracellular space.

Glutamate Biosynthesis

Glutamate can be synthesized by at least six different metabolic routes. However, perhaps 80% of the glutamate used as a transmitter by CNS neurons is synthesized from glutamine by phosphate-activated glutaminase. The mitochondria of glutamate terminals express this enzyme in abundance. Glutamine produced within astrocytes diffuses into the extracellular space and is taken up by nerve terminals. It is then hydrolyzed to glutamate by phosphate-activated glutaminase. Cytoplasmic glutamate is then made available for release through transport into synaptic vesicles by a specific vesicular transporter.

One study of human hippocampi resected for medically intractable temporal lobe epilepsy indicated enhanced expression and activity of phosphate-activated glutaminase per neuron in some regions.[15] Hippocampal enzyme activity was greater in the damaged hippocampus of lesional temporal lobe epilepsy than in the intact hippocampus of other forms of temporal lobe epilepsy. Thus enhanced production of glutamate may contribute to the increase in its ambient extracellular concentration.

Glutamate Release

Glutamate produced by intraterminal mitochondria is taken up into a population of synaptic vesicles that is specialized for this purpose. The expression of vesicular glutamate transport determines that a particular synaptic terminal will release glutamate.[16] Four vesicular transporters have been identified. Two of these, designated VGLUT1 and VGLUT2, are expressed by synaptic vesicles of glutamate pathways. VGLUT1 is the type most frequently expressed in forebrain regions. A third vesicular transporter, VGLUT3, is expressed by synaptic vesicles of some nonglutamate pathways, including pathways that release serotonin or dopamine. Synaptic terminals of these pathways contain largely or completely separate populations of glutamate and nonglutamate vesicles. Expression of VGLUT3 in some synaptic vesicles endows these pathways with the ability to release some glutamate along with their predominant transmitter. The physiological role of glutamate corelease is unclear. Finally, sialin, a lysosomal H^+/sialic acid cotransporter, accumulates glutamate and aspartate in synaptic vesicles by a mechanism independent of sialic acid transport.[17] Sialin is the only vesicular transporter identified thus far that can transport aspartate; the other excitatory amino acid vesicular transporters are highly specific for glutamate. Transport is driven by an inside-positive vesicular membrane potential that is created by an electrogenic proton pump. Glutamate is then released upon nerve terminal depolarization by Ca^{2+}-dependent exocytosis. In most instances, when the action potential invades the synaptic terminal, Ca^{2+} entry releases the contents of either one or zero glutamate vesicles, and the quantity of glutamate released is normally sufficient to saturate the postsynaptic receptors. However, both multiquantal release and lack of receptor saturation have been reported at some glutamate synapses. Glutamate regulates its further release by feeding back onto terminal autoreceptors. Glutamate release is also regulated by the previous activity of that terminal, other transmitters present locally in the extracellular fluid, and products of cell metabolism, most notably adenosine and arachidonic acid.

No studies of synaptic glutamate release from epileptic human tissue have been reported. However, three studies that utilized either an electrophysiological approach[18] or depolarization by elevated K^+[19,20] in animal models of temporal lobe epilepsy indicated an enhancement of glutamate release. Both approaches evoked predominantly the exocytotic release of glutamate from nerve terminals. Astrocytes can also release glutamate by both exocytotic and nonexocytotic mechanisms. Indirect evidence suggests that epileptogenesis may increase the capacity of astrocytes to release glutamate.[21] Enhanced expression of the flip splice variants of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors by astrocytes has been reported in lesional temporal lobe epilepsy.[22] Activation of these receptors by extracellular glutamate, in conjunction with the activation of Type I metabotropic receptors, would be expected to evoke intracellular Ca^{2+} oscillations that could potentially drive astrocytic glutamate release. However, enhanced astrocytic release has not been demonstrated in either human

tissue or animal models. Although enhanced neuronal release clearly could account, at least partly, for the increased ambient extracellular glutamate concentration in epileptic brain, it is not clear to what extent glial release of glutamate contributes to the extracellular pool.

Conversion to Glutamine

As described above, glutamine is the primary substrate for the biosynthesis of the glutamate transmitter pool. The biosynthesis of glutamine involves cooperativity between glutamate nerve terminals and the adjacent astrocytes. Glutamate released from nerve terminals is transported into astrocytes and converted to glutamine by glutamine synthetase, an enzyme expressed by astrocytes but not by neurons. The glutamine synthetase reaction requires adenosine triphosphate (ATP). Astrocytic end-feet, enriched in glucose transporters, cover virtually all capillary walls in the brain. Glutamate transport into astrocytes stimulates glucose uptake by these cells. Glutamine produced by the astrocytes diffuses into the extracellular space, where it is available for uptake by glutamate terminals and subsequent conversion to glutamate. This cooperative metabolic pathway involving two distinct cell types is referred to as the *glutamate-glutamine cycle*. The glutamate-glutamine cycle provides a mechanism for coupling glutamate transmission with glucose utilization, one of the signals detected by positron emission tomography (PET) imaging. The energy demands of this cycle account for ~85% of total brain glucose utilization.[23]

Glial proliferation (gliosis) is a prominent feature of damaged brain regions in lesional epilepsies. Reactive astrocytes have a reduced expression of glutamine synthetase. Thus, the immunoreactivity and total enzymatic activity of glutamine synthetase are reduced markedly in regions of neuronal loss, despite glial proliferation.[24–26] Similar results have been reported in animal models.[27–31] Congenital loss of glutamine synthetase results in seizures and death within days after birth.[32] Moreover, reducing the expression or activity of glutamine synthetase in animals either causes seizures or increases seizure susceptibility.[26,33,34] Thus, the conversion of glutamate to glutamine may be viewed as a neuroprotective measure intended, in part, to prevent the hyperactivation of glutamate receptors by extracellular glutamate. Failure of this mechanism in lesional epilepsies may contribute to the increase in extracellular glutamate concentration, leading in turn to seizure generation and excitotoxic cell death.

Plasma Membrane Transport

High-affinity (1–10 μM K_T) excitatory amino acid transporters expressed on the cell surface bind glutamate after its synaptic release, thus clearing the synaptic cleft in preparation for the next presynaptic action potential and limiting the spillover of glutamate from the cleft. The latter effect inhibits glutamate from activating extrasynaptic receptors or receptors located at nearby synapses. Because NMDA receptors bind glutamate with high affinity, distant NMDA receptors would be activated inappropriately if much glutamate were to escape the synaptic cleft routinely. In addition, transporters limit activation of the postsynaptic Group I and presynaptic Group II metabotropic glutamate receptors that lie in proximity to, but outside, the synaptic cleft. Maintenance of a low extracellular glutamate concentration is also necessary to prevent excitotoxicity. Indeed, knockout or knockdown of excitatory amino acid transporters in animals results in seizures and neurodegeneration.[35–37] In addition to limiting the extracellular concentration of glutamate, presynaptic excitatory amino acid transporters recover transmitter for reuse and astrocyte transporters accumulate glutamate for use as the metabolic precursor for glutamine. Transport activity requires energy, and both the direction and rate of transport depend on the extracellular Na^+ and intracellular K^+ concentrations.

Cloning studies identified five excitatory amino acid transporters (EAATs), two mainly glial and three neuronal. EAAT1 (GLAST) and EAAT2 (GLT-1) are expressed mainly by astrocytes, with EAAT2 predominating. In fact, EAAT2 accounts for 1% of total astrocyte protein and for ~90% of glutamate removal from the extracellular space. EAAT2 is also expressed to some degree by most glutamate neurons and has been localized to the plasma membrane of the synaptic terminal. Synaptic terminal expression of EAAT2 presumably explains the robust glutamate transport found in synaptosome preparations. EAAT3 (EAAC1) is expressed on the postsynaptic surface at glutamate synapses. EAAT4 is expressed largely

Table 12–1 **Localization of Excitatory Amino Acid Transporters**

EAAT1 (GLAST)	Astrocytes, Bergmann glia, Muller cells	
EAAT2 (GLT-1)	Astrocytes, glutamate nerve terminals	Accounts for ~90% of glutamate removal from the extracellular space
EAAT3 (EAAC1)	Postsynaptic membrane of glutamate synapses	
EAAT4	Cerebellar neurons (perisynaptic region)	
EAAT5	Retinal photoreceptors and bipolar cells	

by cerebellar neurons and EAAT5 by photoreceptors and bipolar cells of the retina (see Table 12–1).

In lesional epilepsies, one might expect increased expression of glial excitatory amino acid transporters because of the increase in glial membrane surface. In contrast, most reports have indicated reduced EAAT2 immunoreactivity and protein in regions of neuronal loss.[38–42] Similar to glutamine synthetase, expression of EAAT2 may be downregulated in reactive astrocytes. Alternative splicing of EAAT2 mRNA in patients with temporal lobe epilepsy may lead to reduced protein levels.[43] Because glutamate is cleared from the extracellular space predominantly by the action of EAAT2, downregulation of this transporter might well account for the high ambient extracellular glutamate concentration observed in the same brain regions. In addition, a chronic deficiency in glutamate transport might raise the extracellular glutamate concentration sufficiently to promote additional neuronal injury and death. However, two studies found no change in the expression of EAAT2.[44,45] The reason for this discrepancy is unclear. Aside from possible differences in technique, it is also possible that the different laboratories studied patients having different seizure etiologies, with a different treatment history, and/or having suffered brain damage at different times before the tissue was excised. None of the human studies included measurement of transport activity, only transporter protein. Therefore, the functional status of EAAT2 in human epilepsy remains uncertain. Studies of animal models are similarly conflicted; both reduced[20,46,47] and unchanged[48,49] expression of EAAT2 have been reported.

In contrast to EAAT2 expression, studies of both excised human tissue[38–40,44] and animal models[20,48,50,51] revealed the enhanced expression of EAAT3 by surviving neurons in damaged regions of epileptic brain. Upregulation of EAAT3 at postsynaptic sites may represent an attempt by surviving neurons to protect themselves from the deleterious effects of an increased extracellular glutamate concentration. However, the functional significance of this change has not been investigated.

GLUTAMATE RECEPTORS

Glutamate acts upon several structurally and pharmacologically different types of membrane receptor. The *ionotropic receptors* are themselves cation channels composed of multiple subunits, whereas the *metabotropic receptors* operate through G-protein coupling. For historical reasons, the ionotropic glutamate receptors are named for structural analogues of glutamate that activate receptors of that particular class selectively.

The vast majority of glutamate synapses operate through postsynaptic AMPA and NMDA receptors. Kainate receptors are expressed prominently on the presynaptic membrane, the postsynaptic membrane, or both at some glutamate synapses. AMPA receptors are expressed by astrocytes as well as neurons. Cloning studies justified the original separation of ionotropic glutamate receptors into classes based on agonist pharmacology. Glycopolypeptide subunits that participate in receptor assembly within one class exhibit much greater sequence homology than subunits that contribute to receptors of the other two classes (see Table 12–2).

The metabotropic glutamate receptors have been subdivided into three groups based on their agonist affinities and their preferred signal transduction pathways. Group I metabotropic glutamate receptors signal mainly through the activation of phospholipase C (mGluR1, mGluR5). Group I receptors are located postsynaptically but outside the synaptic cleft. They are also expressed by astrocytes. Neuronal

Table 12–2 **Ionotropic Glutamate Receptor Subunit Composition**

AMPA[a]	NMDA[b]	Kainate[c]
GluR1 (GluA1, GluRA)	NR1 (GluN1)	GluR5 (GluK1)
GluR2 (GluA2, GluRB)	NR2A (GluN2A)	GluR6 (GluK2)
GluR3 (GluA3, GluRC)	NR2B (GluN2B)	GluR7 (GluK3)
GluR4 (GluA4, GluRD)	NR2C (GluN2C)	KA1 (GluK4)
	NR2D (GluN2D)	KA2 (GluK5)
	NR3A (GluN3A)	
	NR3B (GluN3B)	

[a]AMPA receptor subunits can form a functional channel either by themselves or combined with other subunits. The channel is permeable to Na^+, K^+, and Ca^{2+} when a GluR2 subunit is absent, but only to Na^+ and K^+ when GluR2 is present.
[b]All NMDA receptors contain two glycine-binding NR1 subunits, and the channel is permeable to Na^+, K^+, and Ca^{2+}. Incorporation of two glutamate-binding NR2 subunits allows channel opening upon the binding of glutamate. NR3 subunits bind glycine and, if combined with NR1, may create a novel type of glycine receptor. NR1/NR2/NR3 combinations are also possible. The role of NR3 subunits in glutamate synaptic function is unclear.
[c]GluR5, GluR6, or GluR7 subunits or any combination of the three can form a functional channel with low agonist affinity. Incorporation of KA1 or KA2 into the complex forms a channel with high agonist affinity. Neither KA1 nor KA2 can form a homomeric channel.

postsynaptic Group I receptors are activated mainly during high-frequency activity when glutamate overflows the synapse. Group I receptors on astrocytes respond to changes in the ambient extracellular glutamate concentration. Receptor activation enhances excitability and causes release of Ca^{2+} from intracellular stores. One type of Group II metabotropic glutamate receptor, mGluR2, is located typically on the preterminal portion of the axon. This receptor is activated only when glutamate overflows the synapse. Activation of these receptors reduces subsequent glutamate release. The other type of Group II metabotropic glutamate receptor, mGluR3, appears to be expressed predominantly by glial cells. Group III metabotropic glutamate receptors are particularly sensitive to the glutamate analogue L-AP4. There are four receptors in this class, designated mGluR4, mGluR6, mGluR7, and mGluR8. mGluR6 serves as the postsynaptic receptor for glutamate at the synapse in the retina between the photoreceptor cell and the bipolar cell. mGluR4, mGluR7, and mGluR8 are expressed in a largely nonoverlapping pattern on terminals of glutamate pathways in the brain. They are located at the release sites; receptor activation reduces subsequent glutamate release. It appears that Group III receptors regulate glutamate release regardless of presynaptic action potential frequency, whereas mGluR2 functions in this way only when a high presynaptic firing frequency results in overflow of glutamate from the synaptic cleft. Firing frequencies as low as 3 Hz have been associated with glutamate overflow onto mGluR2 receptors (see Table 12–3).

AMPA Receptors

Nearly all glutamate-activated fast excitatory postsynaptic potentials (EPSPs) depend on the activation of AMPA receptors. For AMPA receptors in particular and ionotropic glutamate receptors in general, the receptor is composed of four glycopolypeptide chains (subunits) arranged like the staves of a barrel with a channel in the middle through which cations can flow. AMPA receptors bind glutamate with low affinity (K_D ~100 μM) and in most instances desensitize rapidly. All AMPA channels are permeable to both Na^+ and K^+. Because the inward Na^+ flux exceeds the outward K^+ flux ($E_{Na,K} \approx 0$ mV), the net effect is a depolarization: the fast EPSP. The underlying synaptic current is the fast excitatory postsynaptic current (EPSC).

Table 12–3 **Metabotropic Glutamate Receptors**

	Group I	Group II	Group III
Members	mGluR1 (mGlu1) mGluR5 (mGlu5)	mGluR2 (mGlu2) mGluR3 (mGlu3)	mGluR4 (mGlu4)[a] mGluR6 (mGlu6)[c] mGluR7 (mGlu7)[b] mGluR8 (mGlu8)[a]
Selective agonist	3,5-DHPG	DCG-IV	L-AP4
Signal transduction	Activation of PLC	Inhibition of adenylate cyclase K^+ channel opening Ca^{2+} channel inactivation	Inhibition of adenylate cyclase K^+ channel opening Ca^{2+} channel inactivation Phosphodiesterase activation (mGluR6)

[a]High agonist affinity.
[b]Low agonist affinity.
[c]Localized to retina.

Four subunits, designated GluR1–GluR4 (or GluA1–GluA4 or GluRA–GluRD), contribute to AMPA receptors. Each of these subunits can exist in *flip* and *flop* versions dependent on alternative splicing. The involvement of the flip versus flop version of the subunit in channel formation affects the extent of receptor desensitization, with flip subunits promoting less desensitization and thus more prolonged channel opening. Flip subunits are more commonly expressed during ontogenic development than in the adult brain. AMPA receptor subunits can form a channel either by themselves (homomeric channels) or with other subunits (heteromeric channels). The great majority of AMPA receptors expressed by excitatory neurons and many AMPA receptors expressed by inhibitory neurons include one or two GluR2 subunits. In this instance, the channel lacks permeability to Ca^{2+}. However, AMPA receptors expressed by astrocytes and many other inhibitory neurons lack a GluR2 subunit. Therefore, the channel is permeable to Ca^{2+}, in addition to Na^+ and K^+. Finally, the subunit composition of postsynaptic AMPA receptors is influenced by previous synaptic activity at that site. For example, in the basal state, postsynaptic AMPA receptors expressed by excitatory neurons are composed mainly of GluR2 and GluR3 subunits. Calcium/calmodulin-dependent (CaM) kinase II becomes activated during high-frequency activity as a result of NMDA channel opening (see below). CaM kinase II phosphorylates cytoplasmic GluR1 subunits, causing GluR1-GluR2 receptors to be inserted into the postsynaptic membrane. There is thus a net increase in the number of synaptic AMPA receptors, and the new receptors differ in subunit composition from the preexisting receptors. This is part of the molecular basis for the important biological process known as *NMDA receptor-dependent long-term potentiation* (LTP). Long-term potentiation is thought to be the elemental process that underlies declarative memory and types of learning that depend on formation of these memories.

Studies of excised tissue from epileptic human brain have indicated upregulation of neuronal AMPA receptors in certain brain regions. AMPA receptor binding associated with the dendrites of dentate granule cells was increased in persons with lesional temporal lobe epilepsy,[52] as was GluR1 immunoreactivity of CA3 hippocampal cells and hilar mossy cells.[53] These results are consistent with hyperexcitability driven by enhanced AMPA receptor activation. In some animal models, expression of GluR2 mRNA and protein is reportedly downregulated.[40,54,55] This change would be expected to increase the proportion of Ca^{2+}-permeable AMPA receptors and thus total Ca^{2+} influx in postsynaptic neurons. It has also been reported in animals that seizure-induced neuronal degeneration is preceded by downregulated expression of GluR2.[56] Thus, loss of GluR2 may lead to excitotoxic cell death.

Reactive astrocytes in sclerotic hippocampi of persons with temporal lobe epilepsy exhibit enhanced expression of flip splice variants, which may cause prolonged astrocyte depolarization and Ca^{2+} influx.[22] No such change was found in the undamaged hippocampi of persons with

nonlesional temporal lobe epilepsy. Increased cytoplasmic Ca^{2+} in astrocytes could lead to astrocytic Ca^{2+} waves that trigger glutamate release from those cells. Thus, AMPA receptor plasticity in astrocytes may contribute to the increased extracellular glutamate concentration in lesional forms of temporal lobe epilepsy.

NMDA Receptors

NMDA receptors normally coexist with AMPA receptors in the postsynaptic membrane. The extent to which NMDA receptor activation contributes to the EPSP depends on the resting membrane potential of the postsynaptic cell. Its contribution increases as the membrane potential becomes less negative. At most synapses, the NMDA receptor plays a negligible role in the EPSP evoked by a single impulse because the channel is blocked by Mg^{2+} ions. The binding of Mg^{2+} to the open channel is voltage-dependent; Mg^{2+} binds much less strongly when the membrane is depolarized. Thus, NMDA receptors are activated significantly only when glutamate release is paired with postsynaptic depolarization. In response to a single impulse, glutamate released from the synaptic terminal produces a depolarization by activating AMPA receptors. However, this EPSP is too short-lived to relieve much of the Mg^{2+} block. The EPSP is short-lived because (1) the AMPA receptor desensitizes rapidly when activated by glutamate, (2) the AMPA receptor has a low affinity for glutamate, causing bound glutamate to dissociate from the receptor rapidly, and (3) the following inhibitory postsynaptic potential (IPSP) returns the membrane potential toward the resting value. During repetitive activity, however, the postsynaptic depolarization is more prolonged and the Mg^{2+} block is effectively relieved. In addition, repetitive activity reduces GABA inhibition. Under these conditions, the NMDA receptor contributes substantially to the EPSP. In comparison to the AMPA receptor-mediated component, the NMDA component is of longer duration and exhibits slow kinetics. In general, the NMDA receptor serves as a mechanism by which experience alters synaptic transmission and properties of the postsynaptic cell for a period of hours to years. High-frequency activity is associated with experiences that result in memory and learning. High-frequency activity can also be pathological, as in seizures.

Both the long-lasting changes in synaptic function and NMDA receptor-induced pathology depend on the permeability of NMDA channels to Ca^{2+}. Like the AMPA receptor, it is the large fluxes of Na^+ and K^+ through the open channel that account for the NMDA component of the EPSC and EPSP. In contrast, the smaller Ca^{2+} influx is responsible for altering the properties of the synapse and the postsynaptic cell. Because the intracellular Ca^{2+} concentration at rest is very low, even a small influx of Ca^{2+} can increase the local intracellular concentration substantially. At this higher concentration, Ca^{2+} activates a number of key enzymes and transcription factors whose concerted action leads to a long-lasting enhancement of the EPSP and, in the extreme, to cell death.

Forebrain NMDA receptors are composed primarily of three subunits designated NR1, NR2A, and NR2B. Each receptor consists of two NR1 subunits and two NR2 subunits. Glutamate binds to the NR2 subunit, whereas the NR1 subunit binds the essential coactivator glycine. NR1-NR2B receptors exhibit much slower decay kinetics than NR1-NR2A receptors. Thus, the charge transferred per channel opening is much greater. Glutamate synapses in developing forebrain utilize predominantly NR1-NR2B receptors. During the maturation of the forebrain, NR1-NR2A receptors are inserted into the postsynaptic membrane and NR1-NR2B receptors are relegated largely to extrasynaptic sites. However, both NR1-NR2A and NR1-NR2B receptors can be found in both locations.

With respect to NMDA receptors, the most interesting finding reported in studies of resected human tissue is the often increased expression of NR2B mRNA and the increased function of NR1-NR2B receptors in damaged regions of epileptic brain,[57–59] even though total NMDA receptor expression may be downregulated.[52] Because responses mediated by NR1-NR2B receptors decay more slowly than those mediated by NR1-NR2A receptors, the associated Ca^{2+} influx would be enhanced. A large influx of Ca^{2+} through NMDA channels may facilitate seizure generation, and influx through extrasynaptic NR1-NR2B receptors can precipitate excitoxicity.[60] Of particular interest is the finding of increased NR2A and NR2B mRNA expression in dentate granule cells of persons with lesional temporal lobe epilepsy.[57] Studies of animal models suggest enhanced NMDA receptor function at perforant path

synapses on these cells.[18,61] In addition, NMDA receptors are expressed on some populations of glutamate synaptic terminals. In this location, tonic activation of these receptors by extracellular glutamate facilitates glutamate release. In an animal model of temporal lobe epilepsy, presynaptic NMDA receptors of the entorhinal cortex were found to be upregulated.[62] Because the entorhinal cortex is thought to be essential for the propagation of limbic seizures and of neocortical seizures into the limbic system, NMDA receptor-induced potentiation of glutamate release in that region may be significant.

Kainate Receptors

The kainate receptor resembles the AMPA receptor with respect to structure, regulation, and ionic selectivity. Kainate activates both AMPA and kainate receptors; there is considerable overlap in agonist action on the two receptors. Kainate receptors tend to be most abundant in those brain regions where NMDA receptors are least abundant. Their kinetic properties are intermediate between those of AMPA and NMDA receptors. Thus, simultaneous activation of postsynaptic AMPA and kainate receptors prolongs the response to glutamate and may result in repetitive firing of the postsynaptic cell. Kainate receptors are present on some glutamate preterminal axons and on portions of the synaptic terminal outside the synaptic cleft. Some glutamate terminals, including the mossy fiber terminals of the hippocampus, express both high- and low-affinity kainate receptors. They are activated when glutamate overflows the synaptic cleft during repetitive activation of the presynaptic neuron. Activation of low-affinity presynaptic kainate receptors depolarizes the terminal, reducing subsequent glutamate release by limiting action potential-evoked Ca^{2+} influx. Activation of high-affinity presynaptic kainate receptors prolongs the action potential-induced depolarization and enhances Ca^{2+} entry into the terminal. Thus, Ca^{2+} influx may be enhanced or reduced by kainate receptor activation, depending on the magnitude and duration of presynaptic depolarization and on the local extracellular glutamate concentration. By enhancing Ca^{2+} influx, kainate receptor activation mediates the dramatic frequency facilitation observed at mossy fiber synapses on CA3 hippocampal pyramidal cells. Kainate receptors are also expressed by inhibitory neurons, where their activation can both stimulate and depress GABA release.

Three subunits that bind agonist with low affinity (GluR5–7) and two subunits that bind agonist with high affinity (KA1–2) contribute to kainate receptors. Native receptors are composed of either a combination of low- and high-affinity subunits or exclusively of low-affinity subunits. Kainate receptors normally form a channel permeable just to Na^+ and K^+. However, some kainate receptors that contain GluR5 or GluR6 subunits are also permeable to Ca^{2+} (due to lack of RNA editing).

Compared to AMPA and NMDA receptors, the status of kainate receptors in epileptic brain has been little studied. Expression of GluR5 and GluR6 mRNA was reportedly decreased per CA2/CA3 pyramidal cell in the hippocampus of persons with temporal lobe epilepsy, whether or not the hippocampus was damaged.[63] In contrast, KA2 and GluR5 mRNAs were upregulated in dentate granule cells of patients with sclerotic hippocampi. The latter finding suggests the production of additional pre- and postsynaptic kainate receptors needed to regulate and mediate, respectively, transmission at newly formed recurrent mossy fiber synapses. In the temporal neocortex and hippocampus of humans with epilepsy, the editing efficiency of GluR5 and GluR6 subunits is reportedly enhanced.[64,65] This may be regarded as a compensatory response to seizures, reducing Ca^{2+} influx through kainate channels. Studies of animal models have not yielded consistent changes in kainate receptor expression, and possible changes in kainate receptor function have not been assessed.

Metabotropic Glutamate Receptors

Because metabotropic glutamate receptors are quite heterogeneous with respect to signal transduction and localization, their activation may alter seizure generation and propagation in diverse ways. Stimulation of Group I metabotropic receptors in the hippocampus transforms normal neuronal activity into a prolonged epileptiform discharge. This may come about through the metabotropic receptor-related activation of a voltage-gated cation current expressed in CA3 pyramidal cells.[66] In addition, the liberation of Ca^{2+} from intracellular stores that is driven by activation of Group I metabotropic receptors in

astrocytes, combined with Ca^{2+} influx through AMPA channels, triggers Ca^{2+} waves and subsequent astroglial glutamate release. Strong coactivation of AMPA and Group I metabotropic glutamate receptors in neurons can raise the intracellular Ca^{2+} concentration by enough to provoke long-lasting potentiation of the EPSP. Activation of Group II or Group III metabotropic glutamate receptors reduces transmitter release. The effect on seizures depends on whether the receptor is expressed by excitatory or inhibitory presynaptic elements and on the role of those particular synapses in circuit excitability. However, the overall effect of activating these receptors is likely to be inhibitory toward seizure generation and propagation.

Little is known about the role of metabotropic glutamate receptors in human epilepsy, although excessive activation of Group I receptors may account for seizures in persons with fragile X syndrome.[67] Studies of animal models, however, suggest downregulation of Group II and Group III metabotropic receptors.[68–72] In pilocarpine-treated mice, for example, feedback regulation of glutamate release from medial perforant path terminals in the dentate gyrus was found to be compromised.[69] Regulation of lateral perforant path transmission by feedback activation of mGluR8 was unchanged. In pilocarpine-treated rats, however, autoregulation through mGluR8 was reduced.[68] In reactive astrocytes of the hippocampus, the immunostaining of mGluR3 and mGluR5 was reportedly increased in an electrical stimulation model of temporal lobe epilepsy.[73] The combination of enhanced AMPA (see above) and Group I metabotropic receptor expression by astrocytes would be expected to increase the cytoplasmic Ca^{2+} concentration in the presence of glutamate. Although much work on human tissue remains to be done, studies of animal models suggest that both enhanced activation of Group I metabotropic glutamate receptors and reduced autoregulation of glutamate release by Group II and Group III metabotropic receptors could facilitate spontaneous recurrent seizures.

SUMMARY AND FUTURE DIRECTIONS

Studies of glutamate pathways and synaptic mechanisms in human epilepsy have been limited by the restricted availability of tissue. This is especially true of biochemical and electrophysiological functions whose evaluation require fresh tissue. Nevertheless, studies to date suggest that epileptogenesis involves substantial plasticity of both glutamate circuitry and glutamate synaptic mechanisms, at least in regions of neuronal loss. These changes are predominantly in a direction that would be expected to promote hyperexcitability. An increased density of recurrent excitatory pathways may promote the synchronous discharge of principal neuron populations. A high ambient extracellular glutamate concentration may depolarize nearby neurons, evoke a tonic Ca^{2+} current by activation of extrasynaptic NMDA receptors, enhance neuronal excitability by activating Type I metabotropic glutamate receptors, and possibly initiate excitotoxicity. Several changes in glutamate mechanisms may act in concert to raise extracellular glutamate: enhanced biosynthesis from glutamine in nerve terminals, reduced conversion to glutamine by astrocytes, and downregulation of the glial plasma membrane transporter EAAT2. Studies of animal models suggest that enhanced glutamate release from both nerve terminals and astrocytes may also contribute. Glutamate receptor plasticity may also enhance neuronal excitability. Some evidence suggests enhanced AMPA receptor activation in both neurons and astrocytes, as well as enhanced expression and function of NR1-NR2B NMDA receptors. Although little is known about the status of glutamate metabotropic receptors in humans with epilepsy, studies of animal models suggest downregulation of inhibitory metabotropic receptors. Thus, coincident sprouting of recurrent excitatory connections and altered pre- and postsynaptic glutamate mechanisms could potentially promote circuit hyperexcitability.

Given the exuberant sprouting of glutamate axons and apparently upregulated glutamate synaptic function in lesional epilepsy, there would appear to be great potential for the development of drugs that interfere with glutamate transmission to prevent seizures and drugs that interfere with glutamate plasticity to prevent the development of epilepsy after neuronal loss. However, the epileptogenic potential of these changes depends on where the change is expressed —excitatory versus inhibitory circuits, excitatory versus inhibitory neurons, and neurons versus glia. Although studies of animal

models generally favor promotion of hyperexcitability, there has been no confirmation of this from human studies, apart from a few that focused on recurrent excitatory circuitry in the dentate gyrus. A major drawback to the development of glutamate-based drugs is that glutamate serves as the predominant excitatory transmitter throughout the CNS. Thus, it is difficult to target therapy to hyperexcitable brain tissue without causing adverse effects, even disturbing vital functions. Although mechanisms that regulate glutamate transmission differ in different excitatory pathways, nearly every form of regulation is repeated at many sites in the brain. The best way around this dilemma would be the development of agents that target glutamate circuits or mechanisms when they are overexpressed or overactive, but not when they are functioning normally. Conditional negative receptor modulators would be one example of such agents. The utility of memantine, a blocker of overactivated NMDA channels, as a treatment for Alzheimer's disease serves as a precedent for the type of agent that might be clinically useful.

DISCLOSURE STATEMENT

The author has no conflicts of interest to disclose.

REFERENCES

1. Cotman CW, Nadler JV. Reactive synaptogenesis in the hippocampus. In: Cotman CW, ed. *Neuronal Plasticity*. New York: Raven Press; 1978:227–271.
2. Represa A, Robain O, Tremblay E, Ben-Ari Y. Hippocampal plasticity in childhood epilepsy. *Neurosci Lett*. 1989;99:351–355.
3. Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol*. 1989;26:321–330.
4. Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF. Synaptic reorganization by mossy fibers in human epileptic fascia dentata. *Neuroscience*. 1991;42:351–363.
5. Franck JE, Pokorny J, Kunkel DD, Schwartzkroin PA. Physiologic and morphologic characteristics of granule cell circuitry in human epileptic hippocampus. *Epilepsia*. 1995;36:543–558.
6. Zhang N, Houser CR. Ultrastructural localization of dynorphin in the dentate gyrus in human temporal lobe epilepsy: a study of reorganized mossy fiber synapses. *J Comp Neurol*. 1999;405:472–490.
7. Masukawa LM, Uruno K, Sperling M, O'Connor MJ, Burdette LJ. The functional relationship between antidromically evoked field responses of the dentate gyrus and mossy fiber reorganization in temporal lobe epileptic patients. *Brain Res*. 1992;579:119–127.
8. Gabriel S, Njunting M, Pomper JK, Merschhemke M, Sanabria ERG, Eilers AQ, Kivi A, Zeller M, Meencke H-J, Cavalheiro EA, Heinemann U, Lehmann T-N. Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. *J Neurosci*. 2004;24:10416–10430.
9. Nadler JV. The recurrent mossy fiber pathway of the epileptic brain. *Neurochem Res*. 2003;28:1649–1658.
10. Nadler JV. Axon sprouting in epilepsy. In: Schwartzkroin PA, ed. *Encyclopedia of Basic Epilepsy Research*. Vol 3. Oxford: Academic Press; 2009:1143–1148.
11. Ang CW, Carlson GC, Coulter DA. Massive and specific dysregulation of direct cortical input to the hippocampus in temporal lobe epilepsy. *J Neurosci*. 2006;26:11850–11856.
12. Carlson H, Ronne-Engström E, Ungerstedt U, Hillered L. Seizure related elevations of extracellular amino acids in human focal epilepsy. *Neurosci Lett*. 1992;140:30–32.
13. During MJ, Spencer DD. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet*. 1993;341:1607–1610.
14. Cavus I, Kasoff WS, Cassaday MP, Jacob R, Gueorguieva R, Sherwin RS, Krystal JH, Spencer DD, Abi-Saab WM. Extracellular metabolites in the cortex and hippocampus of epileptic patients. *Ann Neurol*. 2005;57:226–235.
15. Eid T, Hammer J, Rundén-Pran E, Roberg B, Thomas MJ, Osen K, Davanger S, Laake P, Torgner IA, Lee T-SW, Kim JH, Spencer DD, Ottersen OP, de Lanerolle NC. Increased expression of phosphate-activated glutaminase in hippocampal neurons in human mesial temporal lobe epilepsy. *Acta Neuropathol*. 2007;113:137–152.
16. Fremeau RT, Voglmaier S, Seal RP, Edwards RH. VGLUTs define subsets of excitatory neurons and suggest novel roles for glutamate. *Trends Neurosci*. 2004;27:98–103.
17. Miyaji T, Echigo N, Hiasa M, Senoh S, Omote H, Moriyama Y. Identification of a vesicular aspartate transporter. *Proc Natl Acad Sci USA*. 2008;105:11720–11724.
18. Scimeni A, Schorge S, Kullmann DM, Walker MC. Epileptogenesis is associated with enhanced glutamatergic transmission in the perforant path. *J Neurophysiol*. 2006;95:1213–1220.
19. Costa MS, Rocha JBT, Perosa SR, Cavalheiro EA, Naffah-Mazzacoratti MdG. Pilocarpine-induced status epilepticus increases glutamate release in rat hippocampal synaptosomes. *Neurosci Lett*. 2004;356:41–44.
20. Ueda Y, Doi T, Tokumaru J, Yokoyama H, Nakajima A, Mitsuyama Y, Ohya-Nishiguchi H, Kamada H, Willmore LJ. Collapse of extracellular glutamate regulation during epileptogenesis: down-regulation and functional failure of glutamate transporter function in rats with chronic seizures induced by kainic acid. *J Neurochem*. 2001;76:892–900.
21. Lee T-S, Mane S, Eid T, Zhao H, Lin A, Guan Z, Kim JH, Schweitzer J, King-Stevens D, Weber P, Spencer SS, Spencer DD, de Lanerolle NC. Gene expression in temporal lobe epilepsy is consistent with increased release of glutamate by astrocytes. *Mol Med*. 2007;13:1–13.

22. Seifert G, Hüttmann K, Schramm J, Steinhäuser C. Enhanced relative expression of glutamate receptor 1 flip AMPA receptor subunits in hippocampal astrocytes of epilepsy patients with Ammon's horn sclerosis. *J Neurosci*. 2004;24:1996–2003.
23. Shulman RG, Rothman DL, Behar KL, Hyder F. Energetic basis of brain activity: implications for neuroimaging. *Trends Neurosci*. 2004;27:489–495.
24. Eid T, Thomas MJ, Spencer DD, Rundén-Pran E, Lai JCK, Malthankar GV, Kim JH, Danbolt NC, Ottersen OP, de Lanerolle NC. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular gluamate in mesial temporal lobe epilepsy. *Lancet*. 2004;363:28–37.
25. van der Hel WS, Notenboom RGE, Bos IWM, van Rijen PC, van Neelen CWM, de Graan PNE. Reduced glutamine synthetase in hippocampal areas with neuron loss in temporal lobe epilepsy. *Neurology*. 2005;64:326–333.
26. Eid T, Williamson A, Lee T-SW, Petroff OA, de Lanerolle NC. Glutamate and astrocytes —key players in human mesial temporal lobe epilepsy? *Epilepsia*. 2008;49(suppl 2):42–52.
27. Dutuit M, Didier-Bazès M, Vergnes M, Mutin M, Conjard A, Akaoka H, Belin M-F, Touret M. Specific alteration in the expression of glial fibrillary acidic protein, glutamate dehydrogenase, and glutamine synthetase in rats with genetic absence epilepsy. *Glia*. 2000;32:15–24.
28. Kang T-C, Kim D-S, Kwak S-E, Kim J-E, Won MH, Kim D-W, Choi S-Y, Kwon O-S. Epileptogenic roles of astroglial death and regeneration in the dentate gyrus of experimental temporal lobe epilepsy. *Glia*. 2006;54:258–271.
29. Alvestad S, Hammer J, Eyjolfsson E, Qu H, Ottersen OP, Sonnewald U. Limbic structures show altered glial-neuronal metabolism in the chronic phase of kainate induced epilepsy. *Neurochem Res*. 2008;33: 257–266.
30. Bidmon H-J, Görg B, Palomero-Gallagher N, Schleicher A, Häussinger D, Speckmann EJ, Zilles K. Glutamine synthetase becomes nitrated and its activity is reduced during repetitive seizure activity in the pentylenetetrazole model of epilepsy. *Epilepsia*. 2008;49:1733–1748.
31. Hammer J, Alvestad S, Osen KK, Skare Ø, Sonnewald U, Ottersen OP. Expression of glutamine synthetase and glutamate dehydrogenase in the latent phase and chronic phase in the kainate model of temporal lobe epilepsy. *Glia*. 2008;56:856–868.
32. Häberle J, Görg B, Rutsch F, Schmidt E, Toutain A, Benoist J-F, Gelot A, Suc A-L, Höhne W, Schliess F, Häussinger D, Koch HG. Congenital glutamine deficiency with glutamine synthetase mutations. *N Engl J Med*. 2005;353:1926–1933.
33. van Gassen KL, van der Hel WS, Hakvoort TBM, Lamers WH, de Graan PNE. Haplo insufficiency of glutamine synthetase increases susceptibility to experimental febrile seizures. *Genes Brain Behav*. 2009;8:290–295.
34. Wang Y, Zaveri HP, Lee T-SW, Eid T. The development of recurrent seizures after continuous intrahippocampal infusion of methionine sulfoximine in rats. A video-intracranial electroencephalographic study. *Exp Neurol*. 2009;220:293–302.
35. Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron*. 1996;16:675–686.
36. Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science*. 1997;276:1699–1702.
37. Sepkuty JP, Cohen AS, Eccles C, Rafiq A, Behar K, Ganel R, Coulter DA, Rothstein JD. A neuronal glutamate transporter contributes to neurotransmitter GABA synthesis and epilepsy. *J Neurosci*. 2002;22:6372–6379.
38. Mathern GW, Mendoza D, Lozada A, Pretorius JK, Dehnes Y, Danbolt NC, Nelson N, Leite JP, Chimelli L, Born DE, Sakamoto AC, Assirati JA, Fried I, Peacock WJ, Ojemann GA, Adelson PD. Hippocampal GABA and glutamate transporter immunoreactivity in patients with temporal lobe epilepsy. *Neurology*. 1999;52:453–472.
39. Proper EA, Hoogland G, Kappen SM, Jansen GH, Rensen MGA, Schrama LH, van Veelen CWM, van Rijen PC, van Nieuwenhuizen O, Gispen WH, de Graan PNE. Distribution of glutamate transporters in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. *Brain*. 2002;125:32–43.
40. Zhang G, Raol YSH, Hsu F-C, Brooks-Kayal AR. Long-term alterations in glutamate receptor and transporter expression following early-life seizures are associated with increased seizure susceptibility. *J Neurochem*. 2004;88:91–101.
41. Rakhade SN, Loeb JA. Focal reduction of neuronal glutamate transporters in human neocortical epilepsy. *Epilepsia*. 2008;49:226–236.
42. Sarac S, Afzal S, Broholm H, Madsen FF, Ploug T, Laursen H. Excitatory amino acid transporters EAAT-1 and EAAT-2 in temporal lobe and hippocampus in intractable temporal lobe epilepsy. *APMIS*. 2009;117:291–301.
43. Hoogland G, van Oort RJ, Proper EA, Jansen GH, van Rijen PC, van Veelen CWM, van Nieuwenhuizen O, Troost D, de Graan PNE. Alternative splicing of glutamate transporter EAAT2 RNA in neocortex and hippocampus of temporal lobe epilepsy patients. *Epilepsy Res*. 2004;59:75–82.
44. Crino PB, Jin H, Shumate MD, Robinson MB, Coulter DA, Brooks-Kayal AR. Increased expression of the neuronal glutamate transporter (EAAT3/EAAC1) in hippocampal and neocortical epilepsy. *Epilepsia*. 2002;43:211–218.
45. Bjørnsen LP, Eid T, Holmseth S, Danbolt NC, Spencer DD, de Lanerolle NC. Changes in glial glutamate transporters in human epileptogenic hippocampus: inadequate explanation for high extracellular glutamate during seizures. *Neurobiol Dis*. 2007;25:319–330.
46. Dutuit M, Touret M, Szymocha R, Nehlig A, Belin M-F, Didier-Bazès M. Decreased expression of glutamate transporters in genetic absence epilepsy rats before seizure occurrence. *J Neurochem*. 2002;80:1029–1038.
47. Wong M, Ess KC, Uhlmann EJ, Jansen LA, Li W, Crino PB, Mennerick S, Yamada KA, Gutmann

DH. Impaired glial glutamate transport in a mouse tuberous sclerosis epilepsy model. *Ann Neurol*. 2003;54:251–256.
48. Miller HP, Levey AI, Rothstein JD, Tzingounis AV, Conn PJ. Alterations in glutamate transporter protein levels in kindling-induced epilepsy. *J Neurochem*. 1997;68:1564–1570.
49. Akbar MT, Rattray M, Williams RJ, Chong NWS, Meldrum BS. Reduction of GABA and glutamate transporter messenger RNAs in the severe-seizure genetically epilepsy-prone rat. *Neuroscience.* 1998;85:1235–1251.
50. Gorter JA, van Vliet EA, Proper EA, de Graan PNE, Ghijsen WEJM, Lopes da Silva F, Aronica E. Glutamate transporters alterations in the reorganizing dentate gyrus are associated with progressive seizure activity in chronic epileptic rats. *J Comp Neurol*. 2002;442:365–377.
51. Voutsinos-Porche B, Koning E, Clément Y, Kaplan H, Ferrandon A, Motte J, Nehlig A. EAAC1 glutamate transporter expression in the rat lithium-pilocarpine model of temporal lobe epilepsy. *J Cereb Blood Flow Metab*. 2006;26:1419–1430.
52. Hosford DA, Crain BJ, Cao Z, Bonhaus DW, Friedman AH, Okazaki MM, Nadler JV, McNamara JO. Increased AMPA-sensitive quisqualate receptor binding and reduced NMDA receptor binding in epileptic human hippocampus. *J Neurosci*. 1991;11:428–434.
53. Eid T, Kovacs I, Spencer DD, de Lanerolle NC. Novel expression of AMPA-receptor subunit GluR1 on mossy cells and CA3 pyramidal neurons in the human epileptogenic hippocampus. *Eur J Neurosci*. 2002;15:517–527.
54. Kharazia VN, Prince DA. Changes of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors in layer V of epileptogenic, chronically isolated rat neocortex. *Neuroscience*. 2001;102:23–34.
55. Sanchez RM, Koh S, Rio C, Wang C, Lamperti ED, Sharma D, Corfas G, Jensen FE. Decreased glutamate receptor 2 expression and enhanced epileptogenesis in immature rat hippocampus after perinatal hypoxia-induced seizures. *J Neurochem*. 2001;21:8154–8163.
56. Friedman LK, Pellegrini-Giampietro DE, Sperber EF, Bennett MV, Moshe SL, Zukin RS. Kainate-induced status epilepticus alters glutamate and $GABA_A$ receptor gene expression in adult rat hippocampus: an in situ hybridization study. *J Neurosci*. 1994;14:2697–2707.
57. Mathern GW, Pretorius JK, Mendoza D, Leite JP, Chimelli L, Born DE, Fried I, Assirati JA, Ojemann GA, Adelson PD, Cahan LD, Kornblum HI. Hippocampal *N*-methyl-D-aspartate receptor subunit mRNA levels in temporal lobe epilepsy patients. *Ann Neurol*. 1999; 46:343–358.
58. Möddel G, Jacobson B, Ying Z, Janigro D, Bingaman W, González-Martínez J, Kellinghaus C, Prayson RA, Najm IM. The NMDA receptor NR2B subunit contributes to epileptogenesis in human cortical dysplasia. *Brain Res*. 2005;1046:10–23.
59. Liu FY, Wang XF, Li MW, Li JM, Xi ZQ, Luan GM, Zhang JG, Wang YP, Sun JJ, Li YL. Upregulated expression of postsynaptic density-93 and *N*-methyl-D-aspartate receptors subunits 2B mRNA in temporal lobe tissue of epilepsy. *Biochem Biophys Res Commun.* 2007;358:825–830.
60. Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat Neurosci*. 2002;5:405–414.
61. Lynch M, Sayin Ü, Golarai G, Sutula T. NMDA receptor-dependent plasticity of granule cell spiking in the dentate gyrus of normal and epileptic rats. *J Neurophysiol*. 2000;84:2868–2879.
62. Yang J, Woodhall GL, Jones RSG. Tonic facilitation of glutamate release by presynaptic NR2B-containing NMDA receptors is increased in the entorhinal cortex of chronically epileptic rats. *J Neurosci*. 2006;26:406–410.
63. Mathern GW, Pretorius JK, Kornblum HI, Mendoza D, Lozada A, Leite JP, Chimelli L, Born DE, Fried I, Sakamoto AC, Assirati JA, Peacock WJ, Ojemann GA, Adelson PD. Altered hippocampal kainate-receptor mRNA levels in temporal lobe epilepsy patients. *Neurobiol Dis*. 1998;5:151–176.
64. Grigorenko EV, Bell WL, Glazier S, Pons T, Deadwyler S. Editing status at the Q/R site of the GluR2 and GluR6 glutamate receptor subunits in the surgically excised hippocampus of patients with refractory epilepsy. *Neuroreport.* 1998;9:2219–2224.
65. Kortenbruck G, Berger E, Speckmann EJ, Musshoff U. RNA editing at the Q/R site for the glutamate receptor subunits GLUR2, GLUR5, and GLUR6 in hippocampus and temporal cortex from epileptic patients. *Neurobiol Dis.* 2001;8:459–468.
66. Bianchi R, Chuang S-C, Zhao W, Young SR, Wong RKS. Cellular plasticity for Group I mGluR-mediated epileptogenesis. *J Neurosci*. 2009;29:3497–3507.
67. Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. *Neuropharmacology*. 2005;49:1053–1066.
68. Kral T, Erdmann E, Sochivko D, Clusmann H, Schramm J, Dietrich D. Down-regulation of mGluR8 in pilocarpine epileptic rats. *Synapse*. 2003;47: 278–284.
69. Bough KJ, Mott DD, Dingledine RJ. Medial perforant path inhibition mediated by mGluR7 is reduced after status epilepticus. *J Neurophysiol*. 2004;92:1549–1557.
70. Pacheco Otalora LF, Couoh J, Shigamoto R, Zarei MM, Garrido Sanabria ER. Abnormal mGluR2/3 expression in the perforant path termination zones and mossy fibers of chronically epileptic rats. *Brain Res*. 2006;1098:170–185.
71. Ermolinsky B, Pacheco Otalora LF, Arshadmansab MF, Zarei MM, Garrido-Sanabria ER. Differential changes in mGluR2 and mGluR3 gene expression following pilocarpine-induced status epilepticus: a comparative real-time PCR analysis. *Brain Res*. 2008;1226: 173–180.
72. Garrido-Sanabria ER, Pacheco Otalora LF, Arshadmansab MF, Herrera B, Francisco S, Ermolinsky BS. Impaired expression and function of group II metabotropic glutamate receptors in pilocarpine-treated chronically epileptic rats. *Brain Res*. 2008;1240:165–176.
73. Aronica E, van Vliet EA, Mayboroda OA, Troost D, Lopes da Silva FH, Gorter JA. Upregulation of metabotropic glutamate receptor subtype mGluR3 and mGluR5 in reactive astrocytes in a rat model of mesial temporal lobe epilepsy. *Eur J Neurosci*. 2000;12:2333–2344.

Chapter 13

Neuronal Synchronization and Thalamocortical Rhythms during Sleep, Wake, and Epilepsy

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Neuronal synchronization can be divided into long-range and local synchrony. *Long-range synchrony* is usually detected with two or more electrodes placed some distance apart. It leads to brain activity that is correlated at long distances and may be seen using both local field potential (LFP) and electroencephalogram (EEG) recordings. The first tool (i.e., the LFP) provides a microscopic measure of brain activity summarizing electrical activities of possibly thousands of neurons [1–4]. The second type of recording (i.e., the EEG) is a result of changes in electrical activity of multiple sources and ultimately represents activity patterns of large populations of neurons and glial cells in the brain. *Local or short-range synchrony* can be detected either with one relatively large field potential electrode or with two or more small [intracellular or extracellular unit (action potential) recording] electrodes located at short (less than 1 mm) distances from each other. Synchronous activity of a few neurons does not necessarily lead to measurable EEG signals, but this can be seen using LFP recordings. Because of the low-pass filtering properties of the extracellular media,[5] high-frequency electric fields associated with action potentials steeply attenuate and large-amplitude slow

LFP and EEG potentials are mainly generated from nearly simultaneously occurring de- and hyperpolarizing events in a large number of neighboring cells with a major contribution from large pyramidal neurons.[6]

In general, synchronization of electrical activity in the brain occurs as the result of interaction among sets of neurons. The following major types of interaction between brain cells (neurons and glia) are possible: (1) chemical synaptic transmission, which takes place when an action potential that is fired by the presynaptic neuron invades the nerve terminals, allowing Ca^{2+} entry, triggering neurotransmitter release, and finally causing de- or hyperpolarization of the postsynaptic neuron[7,8]; (2) interactions between electrically coupled cells via *gap junctions*[9]; these interactions are bidirectional and work as a low-pass filter, that is, fast processes (e.g., action potentials) are filtered out, but slow processes (e.g., slow synaptic potentials) are transmitted with minor attenuation[10]; (3) ephaptic interactions, which are mediated by large extracellular currents and field potentials generated by cortical structures in the absence of specialized contacts[11,12] (it should be noted that some researchers separate ephaptic transmission from field effect interactions[13]); (4) nonspecific interactions, commonly achieved by alteration in the extracellular ionic balance caused by the activity of brain cells.[14–17]

MECHANISMS OF NEURONAL SYNCHRONIZATION

Neuronal synchronization is defined as the correlated appearance in time of two or more events associated with various aspects of neuronal activity at different levels, from single cell to the whole brain.

A common scenario involves adjustment or phase locking of the rhythms of two or more neurons leading to the stable phase differenceof membrane voltage oscillations (periodic or not)—for example, coincidence of action potentials. Such coincidence of electrical activities of many neurons would lead to coordinated changes in extracellular ionic currents in close proximity to these neurons, which can then be detected as changes of the LFP, which are reflected on the scalp EEG. If individual neurons fire action potentials periodically and these events are synchronized among many cells, periodic LFP oscillations would be observed. Finally, if long-range synchronization takes place among distinct brain areas, it would lead to global changes in electrical activity usually associated with EEG rhythms.

Chemical Synaptic Mechanisms of Synchronization

Synaptic interaction is a common mechanism of communication between neurons. Transmitter release by presynaptic terminal leads to receptor activation on the postsynaptic cell and the generation of inward or outward currents, which depolarize or hyperpolarize the postsynaptic cell. Summation of activities from synchronized inputs enhances transmission of information more effectively than raising discharge rates.[18] Generally, both excitatory and inhibitory synaptic interaction may contribute to synchronization of neuronal activity. For example, in a minimal circuit including synaptic connection from cell A to cell B, the action potential in cell A would trigger an excitatory postsynaptic potential (EPSP) in cell B. If this potential is ample enough, it can trigger an action potential in cell B**,** which may occur nearly simultaneously (after a small delay) with the action potential in cell A.

This process is more complex in real cortical networks. The amplitude of single-axon EPSPs is small and variable. In vitro, it ranges from 0.1 to 2 mV (but sometimes up to 9 mV), with a mean of about 1 mV.[19–23] In vivo, due to multiple factors associated with network activities (e.g., shunting or frequency-dependent attenuation), the amplitude of successful single-axon EPSPs is about 0.5 mV.[15] Given the fact that the EPSP amplitude depends linearly on the number of synaptic contacts formed by all the presynaptic neurons,[21,23] only 40 to 50 synaptic contacts are needed to bring the postsynaptic neuron membrane potential from a resting level of –75 mV to –80 mV to a firing threshold of about –55 mV in vivo. On the other hand, the actual rate of EPSPs received by a neocortical neuron in vivo may be much higher. Indeed, spontaneous firing rates in vivo are on the order of 10 Hz.[24,25] Large neocortical neurons possess between 10,000 and 50,000 synapses.[26] Assuming 25,000 synapses on average and considering that 80% of these synapses are excitatory,[27] about 200,000

synapses may be activated each second, or 200 synapses each millisecond (80% from 25,000 synapses × 10 Hz = 200,000 per s or 200 per ms). The half-width of EPSPs in vivo is about 10 ms, and this time might be assumed as a period for efficient integration of presynaptic inputs. Thus, a neocortical neuron can receive up to 2000 inputs during a 10 ms period for efficient integration. Because failure rates in vivo are very high (70%–80%),[15] the neuron receives about 400–800 EPSPs within the integrating period. This is still 10–20 times more than the needed number of simultaneously firing presynaptic neurons to fire postsynaptic neuron (40–50 synapses [see above]). Therefore, the mechanisms of cortical synchronization based exclusively on excitatory intracortical connectivity would lead to overexcitation of the network and cannot be considered as an efficient mechanism of cortical synchronization.

An efficient way to synchronize a population of excitatory cells involves inhibition. Imagine a group of excitatory neurons $A_1, \ldots, A_n$ that generate action potentials, which are not synchronized across cells. Imagine now that all these neurons receive common inhibitory input from inhibitory cell B. The action potential in cell B would provide synchronous inhibitory postsynaptic potentials (IPSPs) in neurons $A_1, \ldots, A_n$, so, when the IPSP terminates, all these neurons $A_1, \ldots, A_n$ can spike synchronously. If some of these excitatory cells now project back to inhibitory neuron B, synchronous spiking between $A_1, \ldots, A_n$ would trigger a new spike in cell B, starting a new cycle of oscillation (Fig. 13–1). This mechanism of synchronization, commonly referred to as *feedback inhibition* (see ref. 28 for a review of this topic), is involved in many brain rhythms in different brain systems including, for example, some types of gamma oscillations,[29,30] thalamic spindles,[1,31] and others.

Gap Junctions and Synchronization

Gap junctions (i.e., electrical synapses) allow direct flux of ions between connected cells, providing a mechanism of communication that is action potential independent.[32,33] If few cells are connected through electrical synapses, any change of membrane voltage in one neuron would trigger current flow between coupled neurons leading to corresponding changes of membrane voltage in the latter cells, providing a synchronizing effect. Because of the relatively high resistance of electrical synapses, they act as low-pass filters.[10] In cortical networks, groups of GABA-releasing interneurons[34,35] and glial cells[36] are interconnected via gap junctions. There is a set of indirect evidence suggesting that electrical coupling may occur between axons of pyramidal neurons.[37,38] The role of axonal gap junctions in the generation of electrical activities is unclear. Intraaxonal recordings did not reveal the presence of afterhyperpolarizing potentials (AHPs)

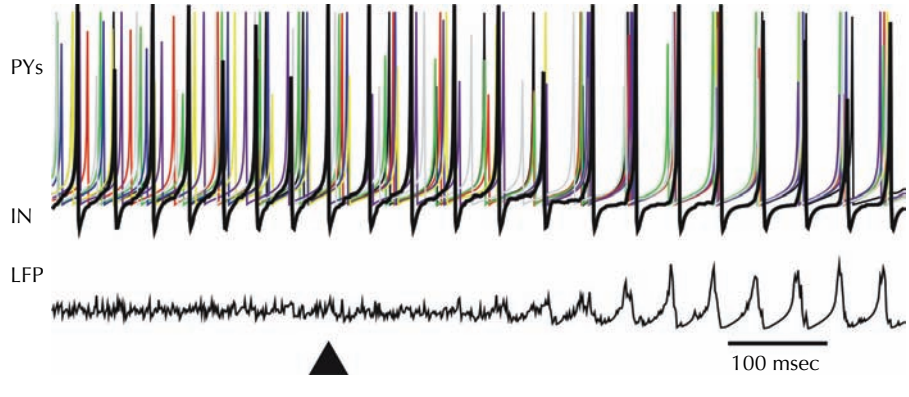

Figure 13–1. Effect of feedback inhibition in spike synchronization**.** Top: eight pyramidal neurons (PYs, thin lines of different color) oscillate asynchronously in the 20–25 Hz frequency range with no inhibitory feedback. About 200 ms after an inhibitory GABAergic synapse from the interneuron (IN, black thick line) to PYs was activated (arrowhead), spikes in the pyramidal neurons became synchronized by IN-mediated IPSPs. Bottom: increase of PY synchronization was reflected in large-amplitude LFP oscillations (M Bazhenov, I Timofeev, unpublished observation).

in axons.[39,40] Low-pass filter properties of gap junctions prevent efficient transmission of fast spikes to coupled neurons, and absence of AHPs prevents slower synchronization. Because of this and because of the extremely short range of electrical coupling, while axo-axonal gap junctions may enhance already existing synchronization manifested by slow membrane voltage oscillations, it is unlikely that they play a primary role in establishing neocortical synchronization.

Ephaptic Interactions

Extracellular currents produced by electrical activity of neurons and constituting local field potentials may directly influence electrical properties of neurons.[13] This effect may include depolarization or hyperpolarization of the cell membrane and therefore a change in excitability. Although they are relatively weak, these effects have a global influence and may provide a significant impact when neuronal activity is already synchronized by means of other mechanisms. Examples include slow-wave sleep oscillations or epileptic activity when a certain degree of synchrony of electrical activity between neurons is already achieved through chemical or electrical interactions. In that case, the weak but global effect of ephaptic interaction further enhances the synchrony. Not only internally generated fields can affect neuronal excitability. The application of an external electric field with an amplitude within the range of in vivo endogenous fields modulates cortical slow oscillation[12,41] and potentiates memory.[41]

Changes in Extracellular Ionic Concentrations

Electrical activity of neurons is associated with opening and closing of different ionic channels and therefore may lead to changes in extracellular ion concentrations. These effects include primarily ions whose extracellular concentrations are low, such as K^+ and Ca^{2+}. Opening of K^+ channels (such as delayed rectifier voltage-gated channels mediating the hyperpolarizing phase of the action potential) or Ca^{2+} channels increases the extracellular K^+ concentration and decreases the extracellular Ca^{2+} concentration. While these local changes are normally compensated for by ionic pumps and interaction with glia, significant alternations in extracellular ion concentrations triggered by electrical activity in one cell may diffuse to neighboring neurons, leading to changes in their excitability and, therefore, contributing to synchronization of electrical activity between neighboring cells. Experimental evidence suggests that a variety of ions undergo activity-dependent changes in concentration.[14,16] During active states of cortical slow oscillation, due to activation of synaptic currents (primarily mediated by the *N*-methyl-D-aspartate [NMDA] receptor) and Ca^{2+}-gated intrinsic channels, the extracellular concentration of Ca^{2+} decreases from 1.2 mM to 1.0 mM.[15,42] This leads to a dramatic increase in synaptic failure rates (up to 80%).[15] Therefore, chemical synaptic interactions contributing to synchronization become largely impaired. On the other hand, lower concentrations of extracellular Ca^{2+} promote opening of hemichannels,[43] which increases the effectiveness of gap junctions and, therefore, electrical coupling. The most dramatic changes in extracellular concentrations of K^+ and Ca^{2+} occur during paroxysmal discharges. The extracellular K^+ concentration reaches 7–18 mM during spontaneous electrographic seizures in neocortex[44–46] and the extracellular Ca^{2+} concentration decreases to 0.4–0.7 mM.[44,47,48] These changes dramatically reduce synaptic excitability, basically abolishing synaptic responses.[49] Changes in ionic concentrations affect the reversal potential of a variety of intrinsic currents that contribute to neuronal excitability, synchronization, and generation of oscillatory activities, potentially promoting synchronized oscillations during epileptic seizures.[17,50] For example, hyperpolarization-activated depolarizing current (I_h) in neocortical neurons is relatively weak, and in normal conditions it is unlikely to play any significant role in the generation of cortical oscillations. However, during seizure activity, when the extracellular K^+ concentration increases, the I_h reversal potential shifts to a more depolarized value, allowing this current to depolarize a set of cortical neurons to the firing threshold, thus leading to the generation of spikes and onset of the next cycles of spike-wave discharges.[51]

THALAMOCORTICAL OSCILLATIONS

Periodic oscillatory activity is a common result of neuronal synchronization and it is an emerging property of the thalamocortical system. Neuronal oscillations enable selective and dynamic control of distributed functional cell assemblies.[52] The patterns and the dominant frequencies of these oscillations are defined by the specific mechanisms involved and depend on the functional state of the brain. Normal oscillatory activities include slow (0.1–15 Hz, present mainly during slow-wave sleep or anesthesia) and fast (20–600 Hz) activities. The fast activities may be present in various states of vigilance and frequently coexist with slower rhythms. Each type of oscillation is generated by a particular set of intrinsic neuronal currents, synaptic interactions, and extracellular factors. Spontaneous brain rhythms during different states of vigilance may lead to increased responsiveness and plastic changes in the strength of connections among neurons, thus affecting information flow in the thalamocortical system.

The various oscillatory rhythms generated in the thalamocortical system may be divided into two main classes: intrinsic**,** generated by a single neuron as a result of an interplay between specific intrinsic currents (e.g., thalamic delta oscillation), and extrinsic, or network oscillations, which require the interaction of excitatory and inhibitory cells within a neuronal population (e.g., spindle oscillation, reviewed in ref. 53). Intrinsic neuronal currents contribute to the generation of network oscillations. Oscillations may also be generated in a population of nonpacemaker neurons coupled through gap junctions**.**

Near-Steady or Infra-Slow Oscillation (<0.1 Hz)

This type of oscillatory activity has a period ranging from tens of seconds to 1 min.[54] Prolonged metabolic disturbance, irritation of brain structures, or afferent stimulation intensifies these activities, and phenobarbital or diethyl ether anesthesia as well as strong electrical stimulation of cerebral cortex dramatically decreases or abolishes this brain activity.[54] Very little is known about the underlying mechanisms of these oscillations. Hypercapnia induced a negative scalp direct current (DC) shifts (~–0.3 mV/CO_2%) and hypocapnia induced a positive scalp DC shifts (~0.3 mV/CO_2%)[55] Infra-slow activities likely have a cortical origin given that they can be recorded from neocortical slabs.[56] Indirect evidence suggests that infra-slow oscillations (0.02–0.2 Hz) synchronize faster activities, modulate cortical excitability and contribute to the increase of epileptic activity during sleep.[57]

Slow Oscillation

During slow-wave sleep and some types of anesthesia, the dominant activity pattern is a slow oscillation occurring with a low frequency (0.3–1 Hz).[1,25,58–60] During slow oscillation the entire cortical network alternates between silent (hyperpolarizing, or Down) and active (depolarizing, or Up) states, each lasting for 0.2–1 s (Fig. 13–2). Silent periods are periods of disfacilitation, that is, periods of absence of synaptic activity, while active periods are periods of intensive synaptic activity leading to the generation of fast oscillations within the thalamocortical system.[25,59,61–64] The survival of slow oscillations after extensive thalamic lesions in vivo[65] and in cortical in vitro preparations,[66] and the absence of slow oscillation in the thalamus of decorticated cats,[67] point to an intracortical origin of this rhythm.

A particular feature of slow oscillation is that each cortical slow wave originates in a particular location and propagates to involve other cortical regions. According to Massimini et al.,[68] in young adult human males (20–25 years old) the preferential sites of origin of slow waves are in frontal cortical regions. However, in this high-density EEG study, it was unclear whether it was active or silent states that propagated. Multisite intracellular experiments on cats demonstrated that it was the onset of the active state that propagated, but the onset of silent states occurred more synchronously.[69] In contrast to the previous study, the active states in cats more commonly start at the border between areas 5 and 7 of parietal cortex.[69] A very recent study[70] demonstrates that propagation of slow activity in the human brain undergoes progressive age-dependent changes. In preschool-age children (2–3 years old), the propagating waves start from the occipital cortex. From early

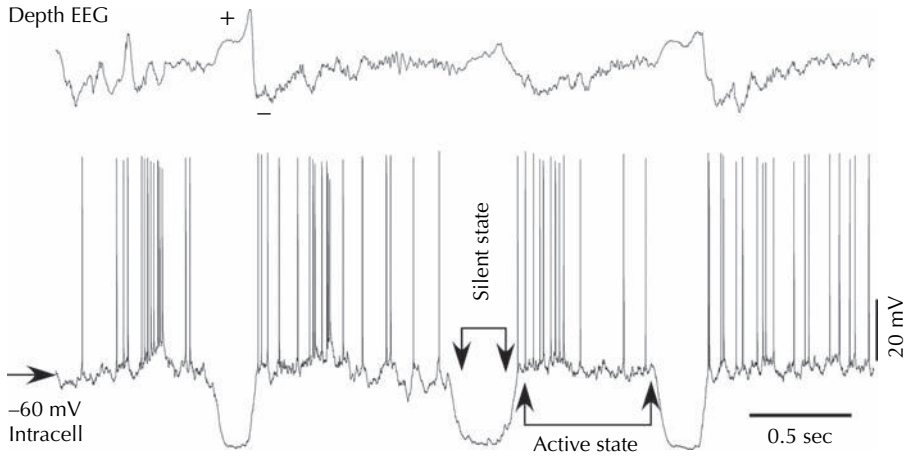

Figure 13–2. Cortical sleep slow oscillation**.** At the EEG level, the slow oscillation appears as periodic alterations of positive and negative waves (indicated by + and – signs). During EEG depth-positivity, cortical neurons remain in the hyperpolarized silent state. During EEG depth-negativity, cortical neurons move to active states, reveal barrages of synaptic events, and fire action potentials. Modified from ref. 53.

childhood to late adolescence, the location on the scalp showing maximal slow wave activity undergoes a shift from posterior to anterior regions. This suggests a progressive change in excitability of various cortical areas and that slow oscillation in the brain of adult cat is generated in areas homotypical to the human adolescent brain. Propagation of activity suggests (1) the presence of cortical regions with different levels of excitability that start the active states and (2) the presence of synaptic chains that mediates slow oscillation with some delays.

At least three distinct mechanisms for the origin of slow cortical oscillations were proposed. The first depends on spontaneous miniature synaptic activities (miniature postsynaptic potentials, mPSP)[71] caused by the action potential-independent release of transmitter vesicles and regulated at the level of single synapses.[72,73] Occasional summation of miniature EPSPs during the hyperpolarized (silent) phase of slow-sleep oscillations activates the persistent sodium current and depolarizes the membrane of cortical pyramidal cells, which is sufficient for spike generation.[74–76] This triggers the active phase of the slow oscillation, which is maintained by synaptic activities and the persistent sodium current. Modeling experiments suggest that short-term synaptic depression and activation of the Ca^{2+}-dependent K^+ current were sufficient to terminate the active state.[75] More recent electrophysiological experiments demonstrated that the active states terminate with high levels of synchrony.[69] Because the short-term synaptic plasticity and activity of intrinsic currents are very specific for each synapse or neuron, the occurrence of nearly simultaneous termination of the active widespread states over large distances suggests the presence of an active (inhibitory) mechanism for the termination of active states. Indeed, about 40% of inhibitory fast-spiking interneurons increase their firing rates at the end of spontaneous active states during slow oscillation.[77] These cells can contribute to the synchronous termination of active states.

Another possible mechanism accounting for the generation of active states during slow-sleep oscillation may be the generation of spontaneous activity by layer V cortical neurons.[66,78] Using a cortical slice preparation, it was shown that oscillatory activity in the frequency range of slow-sleep oscillation could be generated at an extracellular K^+ concentration of 3.5 mM, which is slightly higher than that in vivo (i.e., 3.2 mM).[66] This activity was usually initiated in layer V and propagated over the whole slice.

The third hypothesis attributes transitions from silent to active states to the selective synchronization of spatially structured neuronal ensembles involving a small number of cells.[79] The *selective synchronization* hypothesis predicts that even during silent states, some neurons of the network still generate irregular spontaneous firing.

A group of recent in vitro studies suggests that thalamocortical neurons encompass an

intrinsic mechanism that may act as a secondary oscillator supporting slow rhythm.[80] Cortical activity can recruit, through corticothalamic synapses, intrinsic oscillatory mechanisms in thalamocortical neurons,[81] which could then increase synchrony among cortical inhibitory interneurons. Our current studies indicate that thalamic mechanisms may be responsible for the maintenance of active states with properties observed in vivo.[82,83]

A recent in vivo study on anesthetized or sleeping cats demonstrated that spontaneous active states can originate from any cortical layer, but most often they start from deep layers and propagate toward more superficial layers.[76] It was shown that numerous spontaneous spike-independent synaptic events detected during network silent states summate prior to the onset of active states in all recorded neurons. The pyramidal layer V neurons are the biggest cortical neurons[26]; they possess the largest number of synapses, making it most likely that these neurons will summate spontaneous synaptic events to a threshold level for action potential generation and initiate the active network states. During silent network states, the levels of extracellular Ca^{2+} are increased and contribute to the increase in synaptic release of neurotransmitter,[15] facilitating the onset of the active network states.

Regardless of the specific mechanisms involved in sleep slow oscillation generation, long-range excitatory and inhibitory synaptic connectivity likely plays a major role in synchronization of transitions between active and silent states of slow oscillation leading to the periodic LFP and EEG oscillations observed in animals and humans during deep sleep.

Delta Oscillation

Field potential recordings from neocortex in human and animal models during sleep reveal the presence of delta oscillations (1–4 Hz). The delta oscillation likely has two different components, one of which originates in the neocortex and the other in the thalamus. Neocortical delta activity was significantly enhanced after surgical removal of thalamus[84] and in chronically isolated neocortical slabs.[85] Little is known about the cellular mechanisms mediating cortical delta oscillation. One hypothesis suggests that cortical delta activity could be driven by the discharge of intrinsically bursting neurons.[86] On the other hand, the thalamic delta rhythm is a well-known example of rhythmic activity generated intrinsically by thalamic relay neurons as a result of an interplay between low-threshold Ca^{2+} current (I_T) and hyperpolarization-activated cation current (I_h).[86a] As such, the delta oscillation may be observed during deep sleep when thalamic relay neurons are hyperpolarized sufficiently to deinactivate I_T.[87–90] It was also shown that at a certain level of leak current (I_{leak}), the "window" component of I_T in thalamocortical neurons may create oscillations similar in frequency to the intrinsic thalamic delta oscillation.[91] While specific intrinsic mechanisms are responsible for the generation of oscillatory activity in individual thalamic neurons, these oscillations would not be synchronized and would not lead to the observed LFP and EEG rhythms unless the spiking/bursting activity of individual neurons becomes synchronized by chemical and electrical synapses, as described earlier in this chapter.

Slow-wave sleep may be essential for memory consolidation and memory formation.[92–95] It has been proposed that synaptic plasticity associated with slow and delta oscillations could contribute to the consolidation of memory traces acquired during wakefulness.[96]

Sleep Spindle Oscillations

Sleep spindle oscillations consist of waxing-and-waning field potentials at 7–14 Hz, which last for 1–3 s and recur every 5–15 s. In vivo, spindle oscillations are typically observed during the early stages of sleep or during active phases of sleep slow oscillation. In vivo, in vitro, and in silico studies suggest that the minimal substrate accounting for spindle oscillations consists of the interaction between thalamic reticular and relay cells[31,97–100] (Fig. 13–3). Burst firing of reticular thalamic neurons induces IPSPs in thalamocortical neurons. This deinactivates I_T, inducing rebound burst firing in thalamocortical neurons, which, in turn, excite reticular thalamic neurons, allowing the cycle to start again. During the early phase of spindles, the reticular nucleus singlehandedly drives spindle oscillation via intrinsic mechanisms.[100–103] The second component of spindles, on the other hand, develops primarily as a result of interactions between reticular

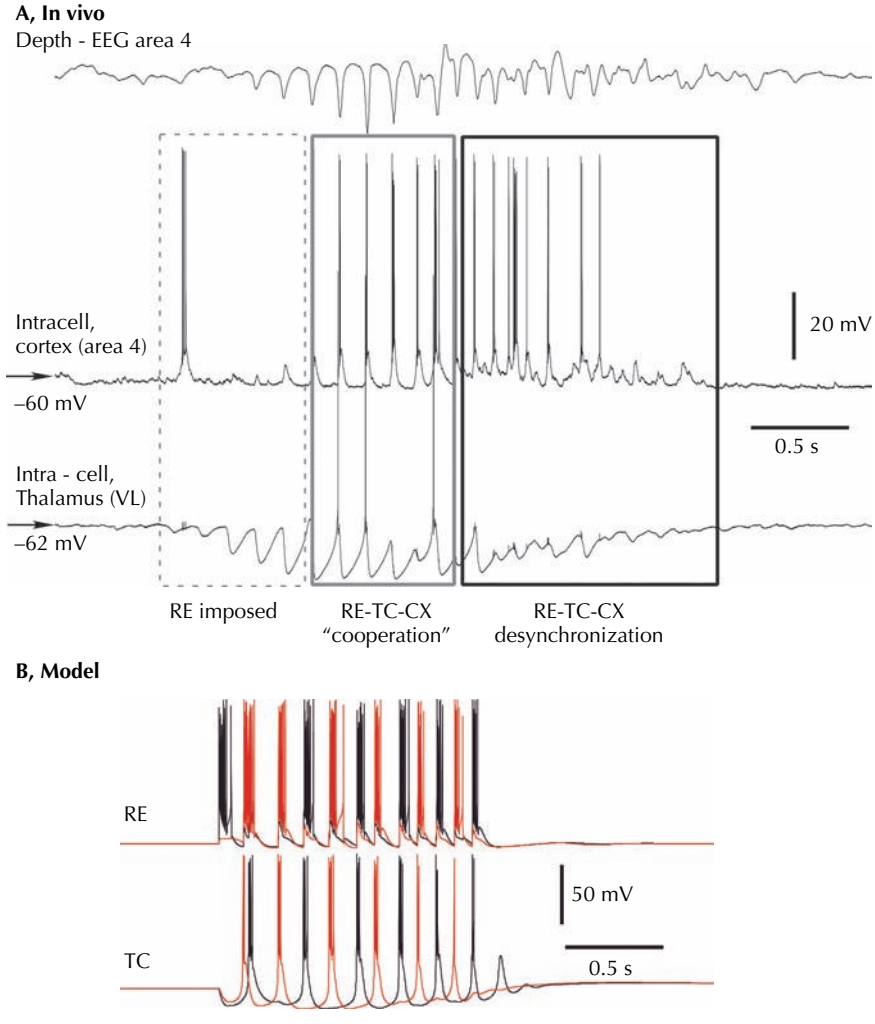

Figure 13–3. Cellular basis of spindle activity**. A.** In vivo recordings. Three phases of a spindle sequence. Dual intracellular recording of cortical (area 4) and thalamocortical (TC) neurons from the ventrolateral (VL) nucleus of the thalamus. **B.** Computational model. Spindle oscillations in the circuit of two reticular thalamic (RE) and two TC cells. RE cells fire in every cycle of oscillations, while TC cells skip every other cycle. Black and red colors are used to distinguish two different neurons. Modified from ref. 53.

and relay neurons.[104,105] Additionally, cortical firing contributes to spindle synchronization through corticothalamic feedback connections, thereby producing simultaneous excitation of reticular and relay neurons.[106,107] The waning phase of spindles occurs as a result of network desynchronization[103,107a] and Ca^{2+}-induced, cyclic adenosine monophosphate (cAMP)-dependent upregulation of the hyperpolarization-activated cation current, I_h, in relay cells.[108–110] It is important to mention that reticular thalamic neurons are interconnected via gap junctions, which aid in synchronizing spindles.[111,112]

While spindle oscillations propagate in thalamic slices,[113] thalamocortical synaptic interactions promote large-scale synchrony of spindle oscillations in vivo.[114] Human sleep spindles are highly synchronous across the scalp when measured by EEG but not when measured simultaneously with a magnetoencephalogram (MEG)[115]. The signals obtained with MEG technique show low correlation and low coherence with each other or with EEG signals. Principal components analysis shows that the MEG field pattern is more complex than the EEG pattern, implying that MEG signals are produced from

multiple partially independent cortical generators, whereas EEG signals may instead be dominated by a weak but widespread spindle generator. It was proposed that the differential activity patterns in the core (thalamocortical neurons forming specific, focused projections to cortical layers IV and VI) and matrix (thalamocortical neurons forming diffuse projections to layer I) subsystems may explain the discrepancies between the temporal patterns of spindles simultaneously observed in EEG and MEG recordings.[116]

Recent studies show that sleep-related spindle oscillations are essential for memory formation[92] and demonstrate short- and middle-term synaptic plasticity (reviewed in ref. 96). Spindling may activate the protein kinase A molecular "gate," thus opening the door for gene expression[117] and allowing long-term changes to take place following subsequent inputs.

Beta-Gamma Oscillation

The waking state of the brain is characterized by the predominance of frequencies in the beta (15–30 Hz) and gamma (30–80 Hz) ranges.[118,119] These fast rhythms are also synchronized between neighboring cortical sites during some forms of anesthesia, natural slow-wave sleep, and rapid-eye-movement (REM) sleep,[62,120a] when consciousness is suspended and thoughts are either absent or bizarre. During slow-wave sleep the fast rhythms follow the onset of depth-negative EEG waves (Fig. 13–4). In vitro experiments and computational models demonstrated that gamma activity generation depends on the activity of inhibitory interneurons.[121–126] Gamma activity can exist in transient and persistent forms. Experimentally, transient (hundreds of milliseconds) gamma oscillations can be induced by

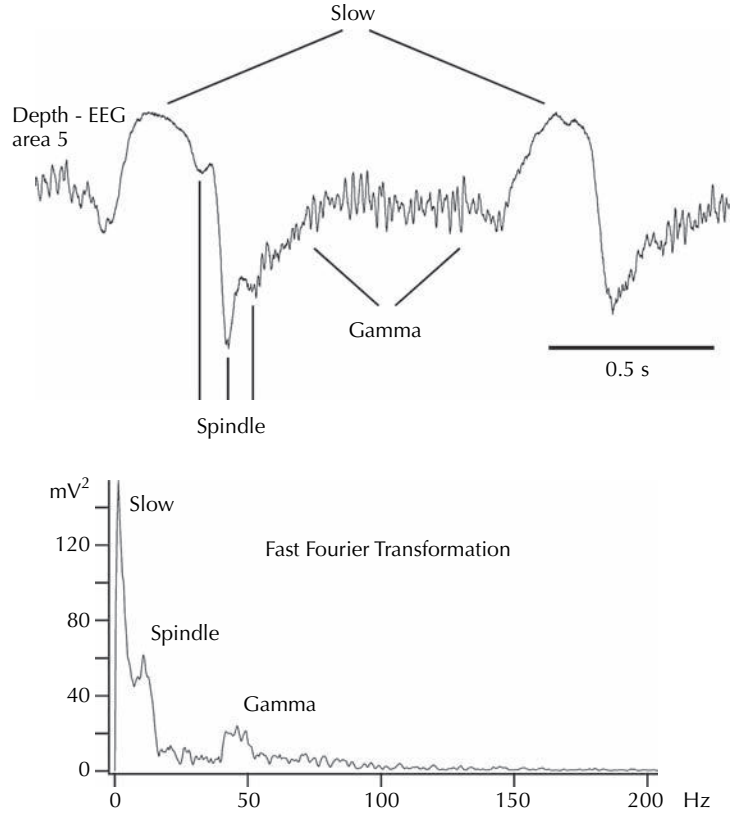

Figure 13–4. Gamma oscillation is an important component of slow oscillation of sleep. Depth- EEG recoding from area 5. Slow oscillation, spindles, and gamma activities are indicated. Below, fast Fourier transformation of the recoding shown above. Modified from ref. 53.

tetanic stimulation of the hippocampus.[124,127] Persistent gamma activity is found in CA3[128] and neocortex[129]; this form of gamma oscillation can be induced by bath application of carbachol or kainate, and it can last for minutes to hours. During persistent gamma activity, the interneurons fire on every cycle or every two cycles and pyramidal cells fire at much lower frequencies, suggesting that active inhibition is involved in the generation of this particular rhythm. Finally, it was found that GABAergic interaction in isolated interneuron networks may lead to network oscillation in the gamma frequency range.[130,131] In both computational models and in vitro experiments, it was shown that the frequency of these oscillations depends on the conductance and decay time of $GABA_A$ currents.[130] Large-scale network simulations revealed that coherent gamma range oscillations may appear through occasional increases in spiking synchrony within local groups of cortical neurons.[132] It was shown that even local synaptic connectivity may explain long-range synchrony of gamma oscillations.[133]

During waking, gamma activity is associated with attentiveness,[134,135] focused arousal,[136] sensory perception,[137] movement,[138,139] and prediction.[140] It has been proposed that synchronization in the gamma frequency range is related to cognitive processing and is important for temporal binding of sensory stimuli.[18,141,142] Gamma activity with low levels of coherence is present within active phases of slow-wave sleep.[62,64,143,144] However, the role of this activity is unknown. During REM sleep, highly coherent gamma activity[143] may contribute to the generation of dreams.

Ripples

Fast oscillations (>100 Hz), termed *ripples*, were initially described in the CA1 hippocampal area and in the perirhinal cortex, where they were associated with bursts of sharp potentials during anesthesia, behavioral immobility, and natural sleep.[145–149] In the neocortex, fast oscillations (>200 Hz, up to 600 Hz) have been found in sensory-evoked potentials in the rat barrel cortex[150,151] during high-voltage spike-and-wave patterns.[152] The neocortical network seems to be sufficient for the generation of ripples, as demonstrated using isolated cortical slab preparations.[153] In addition to active inhibition,[149,153] the electrical coupling mediated by gap junctions contributes to ripple synchronization.[154–156] The electrical coupling may occur between axons of principal cells[157] as well as via a network of inhibitory interneurons.[10,34,35,158,159] Since ripples are also recorded in glial cells, the electrical coupling between gial cells could also play a role in the synchronization of ripples.[155] The field potentials increase neuronal excitability, and by a positive feedback loop they could also be involved in the generation of neocortical ripples.[160] In nonanesthetized brain, the highest amplitude of ripples in neocortex occurs during active phases of slow-wave sleep, it is lower during both REM sleep and the waking state, and it reaches the lowest values during the silent phases of slow-wave sleep.[153]

Neocortical ripples are generated during large-amplitude spontaneous or evoked field potential deflections. These ample changes in the field potential are associated with synchronous activity of many neurons. This suggests that ripples may "alert" the brain network to the presence of a large firing neuronal constellation. The danger of such a focal synchronous excitation of a neuronal pool is that it may overcome a certain threshold of excitability, leading to the onset of seizures.[155,160]

Short- and Long-Range Synchrony during Experimental Epilepsy

Cortically generated electrographic seizures arise without discontinuity from sleep oscillations.[161] These seizures are accompanied by the generation of large-amplitude field potentials, suggesting the presence of enhanced local synchrony,[161] and are characterized by spike-wave (SW) or spike-wave/polyspike-wave (SW/PSW) complexes at 1–2.5 Hz, intermingled with episodes of fast runs at ~7–16 Hz.[162–165] The evolvement of these seizures from the cortically generated slow oscillations may be shaped by the thalamus.[81,163,166–169] The electrographic pattern of SW/PSW seizures, as well as their occurrence during slow-wave sleep, resembles the seizures accompanying the Lennox-Gastaut syndrome in humans.[170–173]

These seizures could be classified as primarily focal and secondarily generalized (see Fig. 3

in ref. 174); visual inspection suggests that on most occasions they start and stop almost simultaneously in all recorded areas. Precise measurements of synchrony during SW/PSW seizures suggest that long-range synchrony recorded on a wave-by-wave basis is rather loose.[161,168,174,175] The coefficient of correlation of field potentials recorded during SW complexes fluctuates between 0.3 and 0.8.[176] However, the field potentials recorded during these seizures are of large amplitude, often exceeding the amplitude of slow waves recorded during natural slow-wave sleep[176] or during anesthesia-induced slow oscillation,[161, 163] suggesting the existence of high local synchrony. Within the fast runs, the patterns of synchronization recorded in different electrodes are as follows: (1) synchronous, in phase, (2) synchronous, with phase shift, (3) patchy, repeated in phase/phase shift transitions and (4) nonsynchronous, with slightly different frequencies in different recording sites or absence of oscillatory activity in one of the recording sites; the synchronous patterns (in phase or with phase shifts) are most common.[174] All these patterns could be recorded in the same pair of electrodes during different seizures or even during different periods of fast runs within the same seizure. An example of such recording is shown in Fig. 13–5. Here, during the first period of fast runs, which started and terminated almost simultaneously at both the intracellular and EEG levels, the field potential and the neuron recorded 2 mm apart oscillated with different frequencies, and thus there was no synchronization. During the next period of fast runs, both the neuron and the field potential signals oscillated in perfect synchrony, and the neuron fired constantly during the descending phase of the field potential deflection.

Why is the local synchrony during seizures enhanced, as indicated by large-amplitude field potentials, and why is the long-range synchrony impaired, as shown by a variety of synchrony patterns (Fig. 13–5)? We propose the following scenario that explains both enhanced local and decreased long-range synchrony. Seizures are associated with activation of a large variety of neuronal currents.[161,177] During seizures, the extracellular levels of K^+ increase and the extracellular levels of Ca^{2+} decrease. While diffusion potentially reduces ion gradients in the extracellular space, a limited diffusion rate, further reduced by cell swelling during a seizure, allows formation of local areas of abnormal ion concentrations. These changes are commonly found in different structures, including neocortex and hippocampus, and in both in vivo and in vitro recordings.[44,47,178,179] The changes in extracellular concentration of K^+ and Ca^{2+} occur simultaneously.[47,180,181] Low levels of extracellular Ca^{2+} significantly dampen synaptic transmission.[8,182] Thus, chemical synaptic transmission cannot play a leading role in synaptic interactions during seizures. In addition, our recent data show that in high (12 mM) extracellular K^+ conditions, action potential generation is impaired and action potentials fail to propagate via axons (Fig. 13–6), probably due to a depolarizing block and decreased input resistance.[49] Therefore, action potentials do not reach target structures. All these factors lead to an impairment of long-range synchronization during seizures because the neurotransmitter mediating these interactions is not released. On the other hand, low Ca^{2+} conditions could favor local synchronization via gap junctions. Indeed, when extracellular levels of Ca^{2+} drop to 1 mM or lower, as it is observed during seizures, the hemichannels that form gap junctions remain open,[43] while they are half closed in physiological Ca^{2+} conditions (1.2 mM). This strengthens the electrical coupling between the glial cells[183] and cortical interneurons[34,35] that mediate local synchrony. Furthermore, elevated extracellular levels of K^+ enhance cell excitability and promote bursting activity[17,50,184] that is transmitted better through gap junctions than through single-action potentials. Potentially, electrical coupling between axons of pyramidal cells[37] could also contribute to local synchronization, although the major types of activities recorded in axons are action potentials, which cannot be efficiently transferred via gap junctions because they constitute a low-pass filter. The use of halothane, a gap junction blocker, effectively blocks seizure generation.[155]

Hence, the decrease in extracellular levels of Ca^{2+} and the increase in extracellular levels of K^+ to those levels that occur during seizures significantly impair long-range neuronal synchronization due to a decrease in neuronal firing ability and a diminution of transmitter release. However, the same changes may increase local synchrony, mediated via gap junctions and possibly by ephaptic interactions.

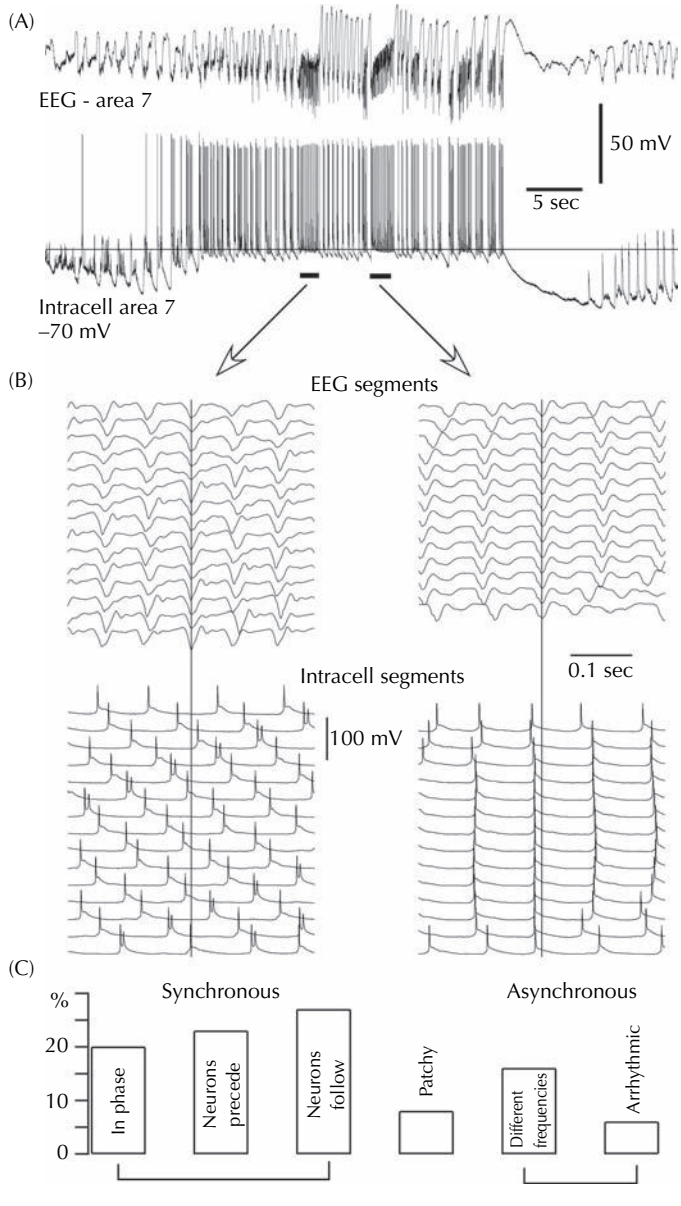

Figure 13–5. Variability in neuron–field synchronization during fast runs. **A.** Depth-EEG and simultaneous intracellular recordings during an electrographic seizure. The distance between the field potential electrode and the intracellularly recorded neuron was 2 mm. **B.** A superposition of field potential (upper panels) and intracellular recordings (lower panels) during fast runs for the two consecutive periods of fast runs. Note the different frequencies of oscillations in the EEG and intracellularly recorded neuron during the first period and in phase synchronization during the second period. **C.** The distribution of patterns of synchronization for 312 periods of fast runs. The coherent patterns (zero time lag or with phase shift) constituted 70% of the cases. "Arrhythmic" stands for periods of fast runs recorded at one electrode, while the activity in another electrode was not rhythmic. Adapted from ref. 174.

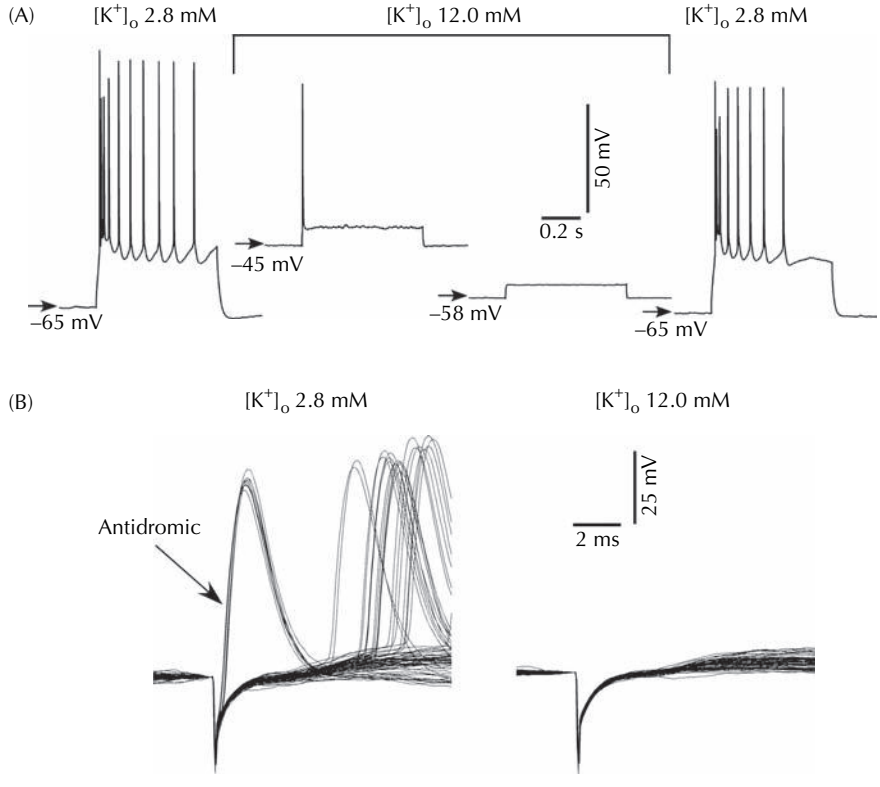

Figure 13–6. High K^+ concentration impairs generation of action potentials in neocortical neurons. **A.** Responses of a cortical neuron to depolarizing current pulses in control (2.8 mM) and "paroxysmal" (12 mM) K^+ conditions. When 12 mM K^+ was used, the neuron became depolarized and generated a maximum one spike in response to an intracellularly applied current pulse. When the membrane potential was returned to near-control values (–58 mV) by injection of a negative holding current, action potentials were not generated. **B.** Electrical stimulation of neighboring cortical tissue induced antidromic and orthodromic responses in recorded neurons. Both anti- and orthodromic spikes were abolished when 12 mM K^+ was used. J Seigneur, I Timofeev, unpublished observations.

CONCLUSIONS

We propose that neocortical synchronization can be achieved via intracortical and thalamocortical mechanisms. Four leading mechanisms of synchronization play a distinct and important role: (1) chemical synaptic transmission, (2) electrical coupling, (3) ephaptic interactions, and (4) activity-dependent changes in ionic concentrations. Major types of cortical rhythmic activities (slow oscillation, delta, spindles, beta-gamma, ripples, paroxysmal spike waves, and fast runs) are generated using these mechanisms. It is difficult, however, to identify the exact contribution of each mechanism to a specific type of oscillation. It is clear that long-range synchronization is achieved using chemical synaptic interactions. Due to the low-pass filtering properties of gap junctions, electrical coupling should play a smaller role in synchronization of fast rhythmic activities compared to slower activities. Nevertheless, the gamma rhythm depends on the activity of interneurons, which are known to be interconnected via gap junctions; therefore, gap junctions should play a critical role in the generation of these oscillations. Weak electrical fields in the EEG range effectively synchronize cortical activities at low but not at high frequencies. Finally, activity-dependent ionic changes may influence local synchronization by modulating neuronal excitability; however, since ionic changes also affect synaptic transmitter release, this could result in impairment of long-range synchrony.

DISCLOSURE STATEMENT

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REFERENCES

1. Steriade M, McCormick DA, Sejnowski TJ. Thalamocortical oscillations in the sleeping and aroused brain. *Science*. 1993;262:679–685.
2. Bazhenov M, Timofeev I. Thalamocortical oscillations. *Scholarpedia*. 2006;1:1319.
3. Niedermeyer E, Lopes da Silva F. *Electroencephalography: Basic Principles, Clinical Applications and Related Fields*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005:1309.
4. Katzner S, Nauhaus I, Benucci A, Bonin V, Ringach DL, Carandini M. Local origin of field potentials in visual cortex. *Neuron*. 2009;61:35–41.
5. Bedard C, Kroger H, Destexhe A. Modeling extracellular field potentials and the frequency-filtering properties of extracellular space. *Biophys J*. 2004;86:1829–1842.
6. Lopes da Silva F, Van Rotterdam A. Biophysical aspects of EEG and magnetoencephalogram generation. In: Niedermeyer E, Lopes da Silva F, eds. *Electroencephalography: Basic Principles, Clinical Applications, and Related Fields*. Philadelphia: Lippincott Williams & Wilkins; 2005;107–125.
7. Eccles JC. *The Physiology of Synapses*. Berlin: Springer; 1964:316.
8. Katz B, Miledi R. The role of calcium in neuromuscular facilitation. *J Physiol*. 1968;195:481–492.
9. Bennett MV. Gap junctions as electrical synapses. *J Neurocytol*. 1997;26:349–366.
10. Galarreta M, Hestrin S. Electrical synapses between GABA-releasing interneurons. *Nat Rev Neurosci*. 2001;2:425–433.
11. Taylor CP, Dudek FE. Synchronous neural afterdischarges in rat hippocampal slices without active chemical synapses. *Science*. 1982;218:810–812.
12. Frohlich F, McCormick DA. Endogenous electric fields may guide neocortical network activity. *Neuron*. 2010;67:129–143.
13. Jefferys JG. Nonsynaptic modulation of neuronal activity in the brain: electric currents and extracellular ions. *Physiol Rev*. 1995;75:689–723.
14. Somjen GG. Ion regulation in the brain: implications for pathophysiology. *Neuroscientist*. 2002;8:254–267.
15. Crochet S, Chauvette S, Boucetta S, Timofeev I. Modulation of synaptic transmission in neocortex by network activities. *Eur J Neurosci*. 2005;21:1030–1044.
16. Somjen GC. *Ions in the Brain: Normal Function, Seizures, and Stroke*. New York: Oxford University Press; 2004:470.
17. Frohlich F, Bazhenov M, Iragui-Madoz V, Sejnowski TJ. Potassium dynamics in the epileptic cortex: new insights on an old topic. *Neuroscientist*. 2008;422–433.
18. Singer W, Gray CM. Visual feature integration and the temporal correlation hypothesis. *Annu Rev Neurosci*. 1995;18:555–586.
19. Thomson AM, West DC, Deuchars J. Properties of single axon excitatory postsynaptic potentials elicited in spiny interneurons by action potentials in pyramidal neurons in slices of rat neocortex. *Neuroscience*. 1995;727–738.
20. Stratford KJ, Tarczy-Hornoch K, Martin KA, Bannister NJ, Jack JJ. Excitatory synaptic inputs to spiny stellate cells in cat visual cortex. *Nature*. 1996;382:258–261.
21. Markram H, Lubke J, Frotscher M, Roth A, Sakmann B. Physiology and anatomy of synaptic connections between thick tufted pyramidal neurones in the developing rat neocortex. *J Physiol*. 1997;500:409–440.
22. Buhl EH, Tamās G, Szilāgyi T, Stricker C, Paulsen O, Somogyi P. Effect, number and location of synapses made by single pyramidal cells onto aspiny interneurones of cat visual cortex. *J Physiol*. 1997;500:689–713.
23. Krimer LS, Goldman-Rakic PS. Prefrontal microcircuits: membrane properties and excitatory input of local, medium, and wide arbor interneurons. *J Neurosci*. 2001;21:3788–3796.
24. Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, Cirelli C, Tononi G. Cortical firing and sleep homeostasis. *Neuron*. 2009;63:865–878.
25. Steriade M, Timofeev I, Grenier F. Natural waking and sleep states: a view from inside neocortical neurons. *J Neurophysiol*. 2001;85:1969–1985.
26. DeFelipe J, Farinas I. The pyramidal neuron of the cerebral cortex: morphological and chemical characteristics of the synaptic inputs. *Prog Neurobiol*. 1992;39:563–607.
27. Gabbott PL, Somogyi J, Stewart MG, Hamori J. GABA-immunoreactive neurons in the rat cerebellum: a light and electron microscope study. *J Comp Neurol*. 1986;251:474–490.
28. Bazhenov M, Stopfer M. Forward and back: motifs of inhibition in olfactory processing. *Neuron*. 2010;67:357–358.
29. Whittington MA, Traub RD, Jefferys JG. Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. *Nature*. 1995;373:612–615.
30. Joho RH, Ho CS, Marks GA. Increased gamma- and decreased delta-oscillations in a mouse deficient for a potassium channel expressed in fast-spiking interneurons. *J Neurophysiol*. 1999;82:1855–1864.
31. von Krosigk M, Bal T, McCormick DA. Cellular mechanisms of a synchronized oscillation in the thalamus. *Science*. 1993;261:361–364.
32. Dermietzel R, Spray DC. Gap junctions in the brain: where, what type, how many and why? *Trends Neurosci*. 1993;16:186–192.
33. Perez Velazquez JL, Carlen PL. Gap junctions, synchrony and seizures. *Trends Neurosci*. 2000;23:68–74.
34. Galarreta M, Hestrin S. A network of fast-spiking cells in the neocortex connected by electrical synapses. *Nature*. 1999;402:72–75.

35. Gibson JR, Beierlein M, Connors BW. Two networks of electrically coupled inhibitory neurons in neocortex. *Nature*. 1999;402:75–79.
36. Mugnaini E. Cell junctions of astrocytes, ependyma, and related cells in the mammalian central nervous system, with emphasis on the hypothesis of a generalized functional syncytium of supporting cells. In: Fedoroff S, Vernadakis A, eds. *Astrocytes*. New York: Academic Press; 1986:329–371.
37. Schmitz D, Schuchmann S, Fisahn A, Draguhn A, Buhl EH, Petrasch-Parwez E, Dermietzel R, Heinemann U, Traub RD. Axo-axonal coupling, a novel mechanism for ultrafast neuronal communication. *Neuron*. 2001;31:831–840.
38. Vigmond EJ, Perez Velazquez JL, Valiante TA, Bardakjian BL, Carlen PL. Mechanisms of electrical coupling between pyramidal cells. *J Neurophysiol*. 1997;78:3107–3116.
39. Yu Y, Shu Y, McCormick DA. Cortical action potential backpropagation explains spike threshold variability and rapid-onset kinetics. *J Neurosci*. 2008;28:7260–7272.
40. Naundorf B, Wolf F, Volgushev M. Unique features of action potential initiation in cortical neurons. *Nature*. 2006;440:1060–1063.
41. Marshall L, Helgadottir H, Molle M, Born J. Boosting slow oscillations during sleep potentiates memory. *Nature*. 2006;444:610–613.
42. Massimini M, Amzica F. Extracellular calcium fluctuations and intracellular potentials in the cortex during the slow sleep oscillation. *J Neurophysiol*. 2001;85:1346–1350.
43. Thimm J, Mechler A, Lin H, Rhee S, Lal R. Calcium-dependent open/closed conformations and interfacial energy maps of reconstituted hemichannels. *J Biol Chem*. 2005;280:10646–10654.
44. Amzica F, Massimini M, Manfridi A. Spatial buffering during slow and paroxysmal sleep oscillations in cortical networks of glial cells in vivo. *J Neurosci*. 2002;22:1042–1053.
45. Moody WJ, Futamachi KJ, Prince DA. Extracellular potassium activity during epileptogenesis. *Exp Neurol*. 1974;42:248–263.
46. Prince DA, Lux HD, Neher E. Measurement of extracellular potassium activity in cat cortex. *Brain Res*. 1973;50:489–495.
47. Heinemann U, Lux HD, Gutnick MJ. Extracellular free calcium and potassium during paroxysmal activity in the cerebral cortex of the cat. *Exp Brain Res*. 1977;27:237–243.
48. Pumain R, Kurcewicz I, Louvel J. Fast extracellular calcium transients: involvement in epileptic processes. *Science*. 1983;222:177–179.
49. Seigneur J, Timofeev I. Synaptic impairment induced by paroxysmal ionic conditions in neocortex. *Epilepsia*. 2011;52(1):132–139.
50. Bazhenov M, Timofeev I, Frohlich F, Sejnowski TJ. Cellular and network mechanisms of electrographic seizures. *Drug Discov Today Dis Models*. 2008;5:45–57.
51. Timofeev I, Bazhenov M, Sejnowski T, Steriade M. Cortical hyperpolarization-activated depolarizing current takes part in the generation of focal paroxysmal activities. *Proc Natl Acad Sci USA*. 2002;99:9533–9537.
52. Canolty RT, Ganguly K, Kennerley SW, Cadieu CF, Koepsell K, Wallis JD, Carmena JM. Oscillatory phase coupling coordinates anatomically dispersed functional cell assemblies. *Proc Natl Acad Sci USA*. 2010; 107:17356–17361.
53. Timofeev I, Bazhenov M. Mechanisms and biological role of thalamocortical oscillations. In: Columbus F, ed. *Trends in Chronobiology Research*. Hauppauger: Nova Science Publishers; 2005:1–47.
54. Aladjalova NA. Infra-slow rhythmic oscillations of the steady potential of the cerebral cortex. *Nature*. 1957;4567:957–959.
55. Nita DA, Vanhatalo S, Lafortune FD, Voipio J, Kaila K, Amzica F. Nonneuronal origin of CO_2-related DC EEG shifts: an in vivo study in the cat. *J Neurophysiol*. 2004;92:1011–1022.
56. Aladjalova NA. *Slow Electrical Processes in the Brain*. Moscow: Academy of Science of USSR; 1962.
57. Vanhatalo S, Palva JM, Holmes MD, Miller JW, Voipio J, Kaila K. Infraslow oscillations modulate excitability and interictal epileptic activity in the human cortex during sleep. *Proc Natl Acad Sci USA*. 2004;101:5053–5057.
58. Steriade M, Nuñez A, Amzica F. A novel slow (<1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci*. 1993;13:3252–3265.
59. Timofeev I, Grenier F, Steriade M. Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. *Proc Natl Acad Sci USA*. 2001;98:1924–1929.
60. Achermann P, Borbely AA. Low-frequency (<1 Hz) oscillations in the human sleep electroencephalogram. *Neuroscience*. 1997;81:213–222.
61. Contreras D, Timofeev I, Steriade M. Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. *J Physiol*. 1996;494:251–264.
62. Steriade M, Contreras D, Amzica F, Timofeev I. Synchronization of fast (30–40 Hz) spontaneous oscillations in intrathalamic and thalamocortical networks. *J Neurosci*. 1996;16:2788–2808.
63. Timofeev I, Contreras D, Steriade M. Synaptic responsiveness of cortical and thalamic neurones during various phases of slow sleep oscillation in cat. *J Physiol*. 1996;494:265–278.
64. Mukovski M, Chauvette S, Timofeev I, Volgushev M. Detection of active and silent states in neocortical neurons from the field potential signal during slow-wave sleep. *Cereb Cortex*. 2007;17:400–414.
65. Steriade M, Nuñez A, Amzica F. Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillations and other sleep rhythms of electroencephalogram. *J Neurosci*. 1993;13:3266–3283.
66. Sanchez-Vives MV, McCormick DA. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat Neurosci*. 2000;3:1027–1034.
67. Timofeev I, Steriade M. Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. *J Neurophysiol*. 1996;76:4152–4168.
68. Massimini M, Huber R, Ferrarelli F, Hill S, Tononi G. The sleep slow oscillation as a traveling wave. *J Neurosci*. 2004;24:6862–6870.
69. Volgushev M, Chauvette S, Mukovski M, Timofeev I. Precise long-range synchronization of activity and silence in neocortical neurons during slow-wave sleep. *J Neurosci*. 2006;26:5665–5672.
70. Kurth S, Ringli M, Geiger A, LeBourgeois M, Jenni OG, Huber R. Mapping of cortical activity in the first

two decades of life: a high-density sleep electroencephalogram study. *J Neurosci*. 2010;30:13211–13219.
71. Fatt P, Katz B. Spontaneous sub-threshold activity at motor-nerve endings. *J Physiol*. 1952;117:109–128.
72. Paré D, Lebel E, Lang EJ. Differential impact of miniature synaptic potentials on the soma and dendrites of pyramidal neurons in vivo. *J Neurophysiol*. 1997;78:1735–1739.
73. Salin PA, Prince DA. Spontaneous $GABA_a$ receptor–mediated inhibitory currents in adult rat somatosensory cortex. *J Neurophysiol*. 1996;75:1573–1588.
74. Timofeev I, Grenier F, Bazhenov M, Sejnowski TJ, Steriade M. Origin of slow cortical oscillations in deafferented cortical slabs. *Cereb Cortex*. 2000;10:1185–1199.
75. Bazhenov M, Timofeev I, Steriade M, Sejnowski TJ. Model of thalamocortical slow-wave sleep oscillations and transitions to activated states. *J Neurosci*. 2002;22:8691–8704.
76. Chauvette S, Volgushev M, Timofeev I. Origin of active states in local neocortical networks during slow sleep oscillation. *Cereb Cortex*. 2010;20:2660–2674.
77. Puig MV, Ushimaru M, Kawaguchi Y. Two distinct activity patterns of fast-spiking interneurons during neocortical up states. *Proc Natl Acad Sci USA*. 2008;105:8428–8433.
78. Compte A, Sanchez-Vives MV, McCormick DA, Wang XJ. Cellular and network mechanisms of slow oscillatory activity (<1 Hz) and wave propagations in a cortical network model. *J Neurophysiol*. 2003;89:2707–2725.
79. Cossart R, Aronov D, Yuste R. Attractor dynamics of network up states in the neocortex. *Nature*. 2003;423:283–288.
80. Crunelli V, Hughes SW. The slow (<1 Hz) rhythm of non-rem sleep: a dialogue between three cardinal oscillators. *Nat Neurosci*. 2010;13:9–17.
81. Hughes SW, Cope DW, Blethyn KL, Crunelli V. Cellular mechanisms of the slow (<1 Hz) oscillation in thalamocortical neurons in vitro. *Neuron*. 2002;33:947–958.
82. Lemieux M, Timofeev I. Impact of thalamic inactivation on slow oscillation in the suprasylvian gyrus of cat. Paper presented at the 40th Annual Meeting of the Society for Neuroscience; November 2010; San Diego, CA.
83. Lonjers P, Timofeev I, Chen J-Y, Bazhenov M. The roles of thalamocortical input in generating slow sleep oscillations. Paper presented at the 40th Annual Meeting of the Society for Neuroscience; November 2010. San Diego, CA.
84. Villablanca J Role of the thalamus in sleep controle: sleep-wakefulness studies in chronic diencephalic and athalamic cats. In: Petre-Quadens O, Schlag J, eds. *Basic Sleep Mechanisms*. New York: Academic Press;1974:51–81.
85. Grafstein B, Sastry PB. Some preliminary electrophysiological studies on chronic neuronally isolated cerebral cortex. *Electroencephalogr Clin Neurophysiol*. 1957;9:723–725.
86. Amzica F, Steriade M. Electrophysiological correlates of sleep delta waves. *Electroencephalogr Clin Neurophysiol*. 1998;107:69–83.
86a. McCormick DA, Pape HC. Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurons. *J Physiol.* 1990;431:291–318.
87. Curró Dossi R, Nuñez A, Steriade M. Electrophysiology of slow (0.5–4 Hz) intrinsic oscillation of cat thalamocortical neurones in vivo. *J Physiol*. 1992;447:215–234.
88. Leresche N, Lightowler S, Soltesz I, Jassik-Gerschenfeld D, Crunelli V. Low-frequency oscillatory activities intrinsic to rat and cat thalamocortical cells. *J Physiol*. 1991;441:155–174.
89. McCormick DA, Pape HC. Noradrenergic and serotonergic modulation of a hyperpolarization-activated cation current in thalamic relay neurones. *J Physiol.* 1990;431:319–342.
90. Soltesz I, Lightowler S, Leresche N, Jassik-Gerschenfeld D, Pollard CE, Crunelli V. Two inward currents and the transformation of low-frequency oscillations of rat and cat thalamocortical cells. *J Physiol*. 1991;441:175–197.
91. Williams SR, Tóth TI, Turner JP, Hughes SW, Crunelli W. The "window" component of the low threshold Ca^{2+} current produces input signal amplification and bistability in cat and rat thalamocortical neurones. *J Physiol*. 1997;505:689–705.
92. Gais S, Plihal W, Wagner U, Born J. Early sleep triggers memory for early visual discrimination skills. *Nat Neurosci*. 2000;3:1335–1339.
93. Huber R, Ghilardi MF, Massimini M, Tononi G. Local sleep and learning. *Nature*. 2004;430:78–81.
94. Stickgold R, James L, Hobson JA. Visual discrimination learning requires sleep after training. *Nat Neurosci*. 2000;3:1237–1238.
95. Maquet P. The role of sleep in learning and memory. *Science*. 2001;294:1048–1052.
96. Steriade M, Timofeev I. Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. *Neuron*. 2003;37:563–576.
97. Steriade M, Deschenes M. The thalamus as a neuronal oscillator. *Brain Res Rev*. 1984;8:1–63.
98. Steriade M, Llinas R. The functional states of the thalamus and the associated neuronal interplay. *Physiol Rev.* 1988;68:649–742.
99. Steriade M, Jones EG, Llinas R. *Thalmic Oscillations and Signaling*. The Neuroscience Institute Publications. New York: John Wiley & Sons; 1990:431.
100. Steriade M, Deschenes M, Domich L, Mulle C. Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. *J Neurophysiol*. 1985;54:1473–1497.
101. Destexhe A, Contreras D, Sejnowski TJ, Steriade M. A model of spindle rhythmicity in the isolated thalamic reticular nucleus. *J Neurophysiol*. 1994;72:803–818.
102. Bazhenov M, Timofeev I, Steriade M, Sejnowski TJ. Self-sustained rhythmic activity in the thalamic reticular nucleus mediated by depolarizing $GABA_a$ receptor potentials. *Nat Neurosci*. 1999;2:168–174.
103. Timofeev I, Bazhenov M, Sejnowski T, Steriade M. Contribution of intrinsic and synaptic factors in the desynchronization of thalamic oscillatory activity. *Thalamus Related Syst.* 2001;1:53–69.
104. Destexhe A, Bal T, McCormick DA, Sejnowski TJ. Ionic mechanisms underlaying synchronized and propagating waves in a model of ferret thalamic slices. *J Neurophysiol*. 1996;76:2049–2070.

105. Bazhenov M, Timofeev I, Steriade M, Sejnowski T. Spiking-bursting activity in the thalamic reticular nucleus initiate sequences of spindle oscillations in thalamic network. *J Neurophysiol*. 2000;84: 1076–1087.
106. Contreras D, Destexhe A, Sejnowski TJ, Steriade M. Control of spatiotemporal coherence of a thalamic oscillation by corticothalamic feedback. *Science*. 1996;274:771–774.
107. Destexhe A, Contreras D, Steriade M. Mechanisms underlying the synchronizing action of corticothalamic feedback through inhibition of thalamic relay cells. *J Neurophysiol*. 1998;79:999–1016.
107a. Bonjean M, Baker T, Lemieux M, Timofeev I, Sejnowski T, Bazhenov M. Corticothalamic feedback controls sleep spindle duration in vivo. *J Neurosci.* 2011;31:9124–9134.
108. Bal T, McCormick DA. What stops synchronized thalamocortical oscillations? *Neuron.* 1996;17:297–308.
109. Budde T, Biella G, Munsch T, Pape H-C. Lack of regulation by intracellular Ca^{2+} of the hyperpolarization-activated cation current in rat thalamic neurones. *J Physiol* (*Lond*). 1997;503:79–85.
110. Luthi A, Bal T, McCormick DA. Periodicity of thalamic spindle waves is abolished by ZD7288, a blocker of ih. *J Neurophysiol*. 1998;79:3284–3289.
111. Fuentealba P, Crochet S, Timofeev I, Bazhenov M, Sejnowski TJ, Steriade M. Experimental evidence and modeling studies support a synchronizing role for electrical coupling in the cat thalamic reticular neurons in vivo. *Eur J Neurosci*. 2004;20:111–119.
112. Landisman CE, Long MA, Beierlein M, Deans MR, Paul DL, Connors BW. Electrical synapses in the thalamic reticular nucleus. *J Neurosci*. 2002;22: 1002–1009.
113. Kim U, Bal T, McCormick DA. Spindle waves are propagating synchronized oscillations in the ferret ligand in vitro. *J Neurophysiol*. 1995;74:1301–1323.
114. Contreras D, Destexhe A, Sejnowski TJ, Steriade M. Spatiotemporal patterns of spindle oscillations in cortex and thalamus. J Neurosci. 1997;17:1179–1196.
115. Dehghani N, Cash SS, Chen CC, Hagler DJ Jr, Huang M, Dale AM, Halgren E. Divergent cortical generators of meg and EEG during human sleep spindles suggested by distributed source modeling. *PLoS One*. 2010;5:e11454.
116. Baker T, Bonjean M, Bazhenov M, Cash SS, Halgren E, Sejnowski T. Role of thalamocortical matrix projections in sleep spindles synchronization. Paper presented at the 40th Annual Meeting of the Society for Neuroscience; November 2010; San Diego, CA.in SFN. 2010. San Diego, CA.
117. Sejnowski TJ, Destexhe A. Why do we sleep? *Brain Res*. 2000;886:208–223.
118. Bressler SL. The gamma wave: a cortical information carrier? *Trends Neurosci*. 1990;13:161–162.
119. Freeman WJ. The physiology of perception. *Sci Am*. 1991;264:78–85.
120. Steriade M, Amzica F, Contreras D. Synchronization of fast (30–40 Hz) spontaneous cortical rhythms during brain activation. *J Neurosci*. 1996;16:392–417.
120a. Chauvette S, Crochet S, Volgushev M, Timofeev I. Properties of slow oscillation during slow-wave sleep and anesthesia in cats. *J Neurosci.* 2011;31: 14998–15008.
121. Traub RD, Whittington MA, Buhl EH, Jefferys JG, Faulkner HJ. On the mechanism of the gamma —> beta frequency shift in neuronal oscillations induced in rat hippocampal slices by tetanic stimulation. *J Neurosci*. 1999;19:1088–1105.
122. Traub RD, Spruston N, Soltesz I, Konnerth A, Whittington MA, Jefferys GR. Gamma-frequency oscillations: a neuronal population phenomenon, regulated by synaptic and intrinsic cellular processes, and inducing synaptic plasticity. *Prog Neurobiol*. 1998;55:563–575.
123. Traub RD, Jefferys JG, Whittington MA. Simulation of gamma rhythms in networks of interneurons and pyramidal cells. *J Comput Neurosci*. 1997;4:141–150.
124. Traub RD, Whittington MA, Stanford IM, Jefferys JG. A mechanism for generation of long-range synchronous fast oscillations in the cortex. *Nature.* 1996;383:621–624.
125. Lytton WW, Sejnowski TJ. Simulations of cortical pyramidal neurons synchronized by inhibitory interneurons. *J Neurophysiol*. 1991;66:1059–1079.
126. Borgers C, Kopell N. Synchronization in networks of excitatory and inhibitory neurons with sparse, random connectivity. *Neural Comput*. 2003;15:509–538.
127. Whittington MA, Stanford IM, Colling SB, Jefferys JG, Traub RD. Spatiotemporal patterns of gamma frequency oscillations tetanically induced in the rat hippocampal slice. *J Physiol*. 1997;502(pt 3):591–607.
128. Fisahn A, Pike FG, Buhl EH, Paulsen O. Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro. Nature, 1998;394:186–189.
129. Buhl EH, Tamas G, Fisahn A, Cholinergic activation and tonic excitation induce persistent gamma oscillations in mouse somatosensory cortex in vitro. *J Physiol* (*Lond*), 1998;513:117–126.
130. Traub RD, Whittington MA, Colling SB, Buzsaki G, Jefferys JG, Analysis of gamma rhythms in the rat hippocampus in vitro and in vivo. *J Physiol*, 1996; 493(pt 2):471–484.
131. Wang XJ, Buzsaki G, Gamma oscillation by synaptic inhibition in a hippocampal interneuronal network model. *J Neurosci*, 1996;16:6402–6413.
132. Rulkov NF, Timofeev I, Bazhenov M. Oscillations in large-scale cortical networks: map-based model. *J Comput Neurosci*. 2004;17:203–223.
133. Bazhenov M, Rulkov NF, Timofeev I. Effect of synaptic connectivity on long-range synchronization of fast cortical oscillations. *J Neurophysiol*. 2008;100: 1562–1575.
134. Bouyer JJ, Montaron MF, Rougeul A. Fast fronto-parietal rhythms during combined focused attentive behaviour and immobility in cat: cortical and thalamic localozations. *Electroencephalogr Clin Neurophysiol*. 1981;51:244–252.
135. Rougeul-Buser A, Bouyer JJ, Buser P. From attentiveness to sleep. A topographical analysis of localized "synchronized" activities on the cortex of normal cat and monkey. *Acta Neurobiol Exp* (*Warsz*). 1975;35:805–819.
136. Sheer DE. Focused arousal and the cognitive 40-Hz event-related potentials: differential diagnosis of Alzheimer's disease. *Prog Clin Biol Res*. 1989;317: 79–94.
137. Gray CM, Konig P, Engel AK, Singer W. Oscillatory responses in cat visual cortex exhibit inter-columnar

synchronization which reflects global stimulus properties. *Nature*. 1989;338:334–337.
138. Murthy VN, Fetz EE. Coherent 25- to 35-Hz oscillations in the sensorimotor cortex of awake behaving monkeys. *Proc Natl Acad Sci USA*. 1992;89: 5670–5674.
139. Pfurtscheller G, Neuper C. Simultaneous eeg 10 Hz desynchronization and 40 Hz synchronization during finger movements. *Neuroreport*. 1992;3:1057–1060.
140. Womelsdorf T, Fries P, Mitra PP, Desimone R. Gamma-band synchronization in visual cortex predicts speed of change detection. *Nature*. 2006;439:733–736.
141. Joliot M, Ribary U, Llinas R. Human oscillatory brain activity near 40 Hz coexists with cognitive temporal binding. *Proc Natl Acad Sci USA*. 1994;91:11748–11751.
142. Llinas R, Ribary U. Coherent 40-Hz oscillation characterizes dream state in humans. *Proc Natl Acad Sci USA*. 1993;90:2078–2081.
143. Achermann P, Borbely AA. Coherence analysis of the human sleep electroencephalogram. *Neuroscience*. 1998;85:1195–1208.
144. Steriade M, Amzica F, Contreras D. Synchronization of fast (30–40 Hz) spontaneous cortical rhythms during brain activation. *J Neurosci*. 1996;16:392–417.
145. Chrobak JJ, Buzsáki G. High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. *J Neurosci*. 1996;16:3056–3066.
146. Collins DR, Lang EJ, Pare D. Spontaneous activity of the perirhinal cortex in behaving cats. *Neuroscience*. 1999;89:1025–1039.
147. Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsaki G. Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving rat. *J Neurosci*. 1999;19:274–287.
148. Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsaki G. Fast network oscillations in the hippocampal CA1 region of the behaving rat. *J Neurosci*. 1999;19:RC20 (1–4).
149. Ylinen A, Bragin A, Nadasdy Z, Jando G, Szabo I, Sik A, Buzsaki G. Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *J Neurosci*. 1995;15:30–46.
150. Jones MS, Barth DS. Spatiotemporal organization of fast (>200 Hz) electrical oscillations in rat vibrissa/barrel cortex. *J Neurophysiol*. 1999;82:1599–1609.
151. Jones MS, MacDonald KD, Choi B, Dudek FE, Barth DS. Intracellular correlates of fast (>200 Hz) electrical oscillations in rat somatosensory cortex. *J Neurophysiol*. 2000;84:1505–1518.
152. Kandel A, Buzsaki G. Cellular-synaptic generation of sleep spindles, spike-and-wave discharges, and evoked thalamocortical responses in the neocortex of the rat. *J Neurosci*. 1997;17:6783–6797.
153. Grenier F, Timofeev I, Steriade M. Focal synchronization of ripples (80–200 Hz) in neocortex and their neuronal correlates. *J Neurophysiol*. 2001;86: 1884–1898.
154. Draguhn A, Traub RD, Schmitz D, Jefferys JG. Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro. *Nature*. 1998;394: 189–192.
155. Grenier F, Timofeev I, Steriade M. Neocortical very fast oscillations (ripples, 80–200 Hz) during seizures: intracellular correlates. *J Neurophysiol*. 2003;89:841–852.
156. Traub RD, Schmitz D, Jefferys JG, Draguhn A. High-frequency population oscillations are predicted to occur in hippocampal pyramidal neuronal networks interconnected by axoaxonal gap junctions. *Neuroscience*. 1999;92:407–426.
157. Schmidt M, Sudkamp S, Wahle P. Characterization of pretectal-nuclear-complex afferents to the pulvinar in the cat. *Exp Brain Res*. 2001;138:509–519.
158. Galarreta M, Hestrin S. Spike transmission and synchrony detection in networks of GABAergic interneurons. *Science*. 2001;292:2295–2299.
159. Gibson JR, Beierlein M, Connors BW. Functional properties of electrical synapses between inhibitory interneurons of neocortical layer 4. *J Neurophysiol*. 2005;93:467–480.
160. Grenier F, Timofeev I, Crochet S, Steriade M. Spontaneous field potentials influence the activity of neocortical neurons during paroxysmal activities in vivo. *Neuroscience*. 2003;119:277–291.
161. Timofeev I, Steriade M. Neocortical seizures: initiation, development and cessation. *Neuroscience*. 2004;123:299–336.
162. Steriade M, Contreras D. Spike-wave complexes and fast components of cortically generated seizures. I. Role of neocortex and thalamus. *J Neurophysiol*. 1998;80:1439–1455.
163. Steriade M, Amzica F, Neckelmann D, Timofeev I. Spike-wave complexes and fast components of cortically generated seizures. II. Extra- and intracellular patterns. *J Neurophysiol*. 1998;80:1456–1479.
164. Neckelmann D, Amzica F, Steriade M. Spike-wave complexes and fast components of cortically generated seizures. III. Synchronizing mechanisms. *J Neurophysiol*. 1998;80:1480–1494.
165. Timofeev I, Grenier F, Steriade M. Spike-wave complexes and fast components of cortically generated seizures. IV. Paroxysmal fast runs in cortical and thalamic neurons. *J Neurophysiol*. 1998;80: 1495–1513.
166. Steriade M, Contreras D. Relations between cortical and thalamic cellular events during transition from sleep patterns to paroxysmal activity. *J Neurosci*. 1995;15:623–642.
167. Steriade M, Timofeev I. Corticothalamic operations through prevalent inhibition of thalamocortical neurons. Thalamus Related Syst. 2001;1:225–236.
168. Meeren HK, Pijn JP, Van Luijtelaar EL, Coenen AM, Lopes da Silva FH. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci*. 2002;22:1480–1495.
169. Nita D, Cisse Y, Frohlich F, Timofeev I. Cortical and thalamic components of neocortical kindling-induced epileptogenesis in behaving cats. *Exp Neurol*. 2008;211:518–528.
170. Halasz P. Runs of rapid spikes in sleep: a characteristic EEG expression of generalized malignant epileptic encephalopathies. A conceptual review with new pharmacological data. *Epilepsy Res Suppl*. 1991;2:49–71.
171. Kotagal P. Multifocal independent spike syndrome: relationship to hypsarrhythmia and the slow

spike-wave (Lennox-Gastaut) syndrome. *Clin Electroencephalogr*. 1995;26:23–29.
172. Niedermeyer E. Abnormal EEG patterns: epileptic and paroxysmal. In: Niedermeyer E, Lopes de Silva, eds. *Electroencephalography: Basic Principles, Clinical Applications, and Related Fields*. Philadelphia: Lippincott Williams & Wilkins; 2005: 255–280.
173. Niedermeyer E. Epileptic seizure disorders. In: Niedermeyer E, Lopes de Silva, eds. *Electroencephalography: Basic Principles, Clinical Applications, and Related Fields*. Philadelphia: Lippincott Williams & Wilkins; 2005:505–620.
174. Boucetta S, Chauvette S, Bazhenov M, Timofeev I. Focal generation of paroxysmal fast runs during electrographic seizures. *Epilepsia*. 2008;49:1925–1940.
175. Derchansky M, Rokni D, Rick JT, Wennberg R, Bardakjian BL, Zhang L, Yarom Y, Carlen PL. Bidirectional multisite seizure propagation in the intact isolated hippocampus: the multifocality of the seizure "focus." *Neurobiol Dis*. 2006;23:312–328.
176. Nita D, Cisse Y, Timofeev I. State-dependent slow outlasting activities following neocortical kindling in cats. *Exp Neurol*. 2008;211:456–468.
177. Timofeev I, Grenier F, Steriade M. Contribution of intrinsic neuronal factors in the generation of cortically driven electrographic seizures. *J Neurophysiol*. 2004;92:1133–1143.
178. Avoli M, Barbarosie M, Lucke A, Nagao T, Lopantsev V, Kohling R. Synchronous GABA-mediated potentials and epileptiform discharges in the rat limbic system in vitro. *J Neurosci*. 1996;16:3912–3924.
179. Jensen MS, Yaari Y. Role of intrinsic burst firing, potassium accumulation, and electrical coupling in the elevated potassium model of hippocampal epilepsy. *J Neurophysiol*. 1997;77:1224–1233.
180. Krnjevic K, Morris ME, Reiffenstein RJ. Stimulation-evoked changes in extracellular K^+ and CA^{2+} in pyramidal layers of the rat's hippocampus. *Can J Physiol Pharmacol*. 1982;60:1643–1657.
181. Nicholson C, Bruggencate GT, Steinberg R, Stockle H. Calcium modulation in brain extracellular microenvironment demonstrated with ion-selective micropipette. *Proc Natl Acad Sci USA*. 1977;74: 1287–1290.
182. Silver RA, Lubke J, Sakmann B, Feldmeyer D. High-probability uniquantal transmission at excitatory synapses in barrel cortex. *Science*. 2003;302: 1981–1984.
183. Giaume C, McCarthy KD. Control of gap-junctional communication in astrocytic networks. *Trends Neurosci*. 1996;19:319–325.
184. Frohlich F, Bazhenov M. Coexistence of tonic firing and bursting in cortical neurons. *Phys Rev E Stat Nonlin Soft Matter Phys*. 2006;74:031922.

Chapter 14

Limbic Network Synchronization and Temporal Lobe Epilepsy

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Cognitive function, including perception and the formation and recall of memories, depends on the integrated, often synchronous, activity of many neurons. The limbic system in general, and the hippocampal formation in particular, have well-developed anatomical and physiological mechanisms that promote neuronal synchronization. Examples of physiological synchronization include the theta rhythm, beta and gamma oscillations, and sharp-wave ripples. In general, the mechanisms of these synchronous activities depend on intrinsic neuronal properties and the interplay between populations of principal cells and interneurons.

Excessive neuronal synchronization is, however, the hallmark of epileptic discharges as well. Synchronization in epilepsy occurs at different levels: between cells, within local networks, and between limbic structures. It also occurs at different time scales, from milliseconds upward, using a range of modalities. In this chapter, we will discuss pathological synchronizing mechanisms involved in epilepsy of limbic origin, with an emphasis on seizures and epileptic high-frequency activity. We will also discuss potential methods of disrupting synchrony with a view to developing novel therapeutic approaches.

Limbic seizures typically last for a few tens of seconds to several minutes. However, they are not the only electroencephalographic (EEG) anomaly found in the epileptic brain. Interictal discharges are brief events, stopping within a couple of hundred milliseconds to a few seconds. They are discussed in detail in this book in Chapter 17 (see also ref. 1), but here we need to mention them briefly because they provide crucial clues to synchronizing mechanisms that

may also play a role in seizures. A large part of this early work relied on the hippocampal slice in vitro exposed to convulsant treatments. Under these conditions, the CA3 region usually generates interictal discharges and not seizures (although there are exceptions[2–4]). However, discharges lasting as long as seizures have been recorded in juvenile CA3 networks[3] or adult CA1[5–7] and/or entorhinal cortex[8,9] in rodents.

MECHANISMS OF SYNCHRONIZATION DURING LIMBIC SEIZURES

Perhaps the first detailed understanding of epileptic synchronization came from experiments on the actions of convulsants on normal brain tissue, and particularly on normal rodent hippocampus both in vivo and in brain slices in vitro. Many of these convulsants block GABAergic inhibition[10] (e.g., penicillin, picrotoxin, bicuculline), but some boost glutamatergic excitation[11] (e.g., low Mg^{2+}, which also compromises inhibition, albeit slowly), or intrinsic excitability[5,12] (high K^+, which also has some impact on inhibition, and low Ca^{2+}), while 4-aminopyridine has the unusual property of enhancing both excitatory and inhibitory synapses as a result of its blocking K^+ channels.[13] It is important to realize that these kinds of acute models really reflect symptomatic epileptiform activity in normal brain, with the convulsant treatment subverting normal physiological synchronizing mechanisms to produce abnormal hypersynchronous epileptiform discharges. In contrast, chronic epileptic foci depend on (usually multiple) changes in neuronal intrinsic and synaptic properties, connectivity, changes in glial cell properties, and in many cases neuronal death (see the section "Neurobiology of Temporal Lobe Epilepsy" below).

Several mechanisms have been implicated in the initiation and persistence of seizures. Failure of inhibition, loss of afterhyperpolarization, increase in $[K^+]_o$, enhanced excitatory synaptic transmission, paradoxical enhancement of inhibitory network activity, and depolarizing GABA all have been proposed, and all of these mechanisms may contribute, to varying degrees in different situations, to epileptiform synchronization. Synaptic transmission mediated by ligand-gated ion channels synchronizes over milliseconds to tens of milliseconds. Faster synchronization depends on electrical mechanisms, gap junctions, and ephaptic or field interactions, which can operate over fractions of a millisecond to a few milliseconds. Slower synchronization, hundreds of milliseconds upward, can depend on several mechanisms: G-protein coupled receptors (neuropeptides, amines, and other neuromodulators), fluctuations in the extracellular concentration of ions (most notably K^+), interactions between neurons and glia, and the dynamics of interaction between coupled hyperexcitable regions.

Synaptic Mechanisms

Early work with acute convulsants showed that the CA3 region of the hippocampus was particularly good at generating interictal discharges, which were soon related to abrupt "paroxysmal" depolarization shifts (PDSs) in pyramidal cells.[14,15] The PDS produced by most acute convulsants proved to be caused by a chain reaction of excitation.[10,16] The anatomical substrate for this chain reaction is the extensive axon collateral network of CA3 pyramidal cells, which connect with many other CA3 pyramidal cells (and even with themselves in *autapses*) in addition to their longer-range projections to CA1, septum, and contralateral CA3. These synaptic networks can play a role in the synchronization occurring during both seizures and interictal discharges, and indeed may be strengthened by sprouting of new connections and other synaptic changes in chronic epileptic foci.

Divergent connections of CA3 pyramidal cells to other local pyramidal cells allows for the expansion of the synchronous population discharge that is necessary for the chain reaction (Fig. 14–1). Physiologically, the unitary potentials at these synapses are relatively strong (~1 mV)[17] for excitatory synapses in the mammalian brain. The effect of these strong synapses is amplified by the presence of voltage-dependent inward currents, mainly through Ca^{2+} channels, which make CA3 pyramidal cells fire bursts of action potentials under appropriate conditions.[10] Of course, these anatomical and physiological properties play crucial roles; under normal condition, the sharp-wave ripple of consumatory behaviors and memory consolidation[18] resembles, and may be a more controlled version of, the interictal discharge. However, under

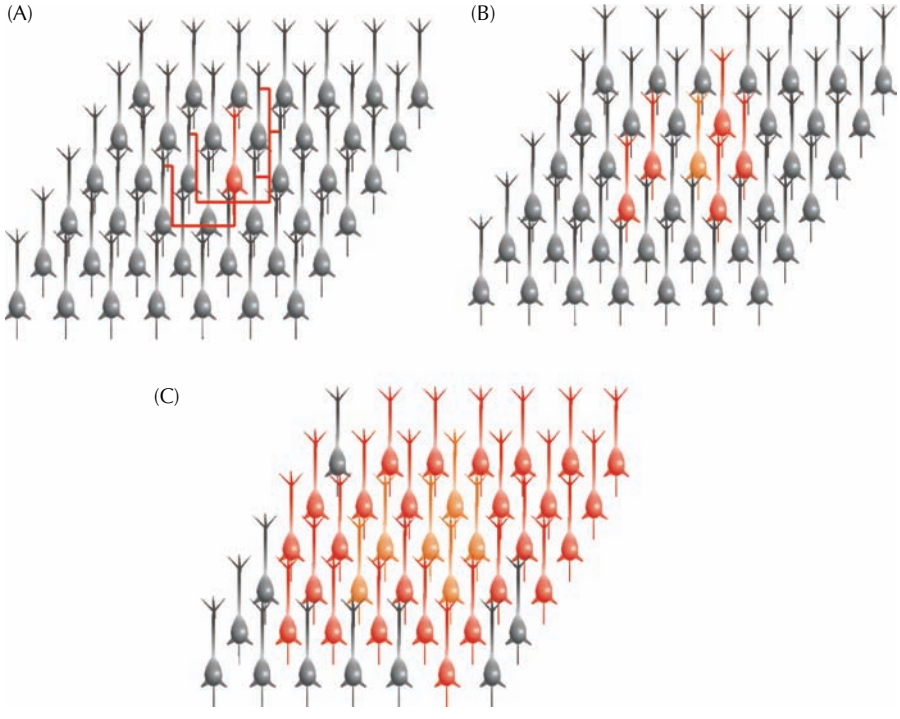

Figure 14–1. Chain reaction. **A.** CA3 pyramidal neuron has divergent connections with (in this case) five other pyramidal cells. **B.** Firing of the CA3 neuron will result in postsynaptic activation of the five pyramidal cells. **C.** Each of the five pyramidal cells then recruits its divergent postsynaptic targets, resulting in the recruitment of a large population of CA3 pyramidal neurons into synchronous firing within a small number of synaptic steps.

convulsant treatments, physiological mechanisms of synchronization get out of hand and produce the hypersynchronous depolarizations with burst firing that result in the large-amplitude field potential transient of the epileptic interictal discharge.

The chain reaction mechanism needs a critical mass or minimum aggregate to produce a hypersynchronous epileptic discharge: essentially, the population needs to be large enough to allow the majority of neurons to be recruited successfully. The concept of a minimum aggregate has had practical implications for the surgical treatment of refractory epilepsy in the development of multiple subpial transection,[19] which can be useful for seizures originating in eloquent cortex. This surgical procedure uses a series of cuts through the cortical thickness to disrupt horizontal axons that propagate and synchronize epileptic activity across the cortex. Cutting these associational fibers and leaving relatively small islands of cortical circuits can result in the disappearance of seizures and interictal discharges, both of which require critical numbers of neurons. The key point here is that the neocortex, where this procedure is applied, differs from the hippocampus in making its afferent and efferent connections vertically down through the underlying white matter. This means that the vertical longer range and extracortical connections are preserved and can sustain physiological function, while the horizontal intracortical connections necessary for horizontal spread and synchronization of neuronal activity during epileptic discharges are interrupted.

Inhibitory neurons also form interconnected networks, which play roles in physiological oscillations.[20–23] If the local neuronal population is tonically depolarized, then networks of particular classes of interneuron can sculpt coherent oscillations from the otherwise disorganized firing of the population. Feedback excitation from pyramidal cells onto inhibitory neurons is also capable of eliciting coherent oscillations because of the divergence of the

synaptic connections.[24] Inhibitory networks can play several distinct roles in epilepsy. Inhibitory networks can drive oscillations, for instance in the run-up to seizure onset, when in some situations they have the effect of entraining the excitatory population in ways that promotes hypersynchrony.[25] The dynamics of these networks can produce high-frequency activity (HFA) at frequencies seen preceding seizure onsets (see the section "High Frequency Oscillations" below).

Inhibitory networks can also lead to excessive inhibitory currents that can overload the ability of the adult neuronal Cl^- transporter (KCC2) to maintain a low intracellular Cl^- and thus allow HCO_3^- to dominate the postsynaptic current, resulting in depolarization and potentially excitation. Efflux of HCO_3^- is not the only mechanism by which excessive GABA release causes depolarization: GABAergic synchronous events are associated with increases in extracellular K^+, probably due to the activity of transporters responsible for reuptake of GABA and extrusion of Cl^-. This synchronizing mechanism is well typified when limbic cortical networks are treated with the K^+ channel blocker 4-aminopyridine.[8,26] As illustrated in Fig. 14–2A, slow interictal discharges continue to occur spontaneously, and to spread to other limbic structures during pharmacological blockade of ionotropic glutamatergic transmission. It should be emphasized that these

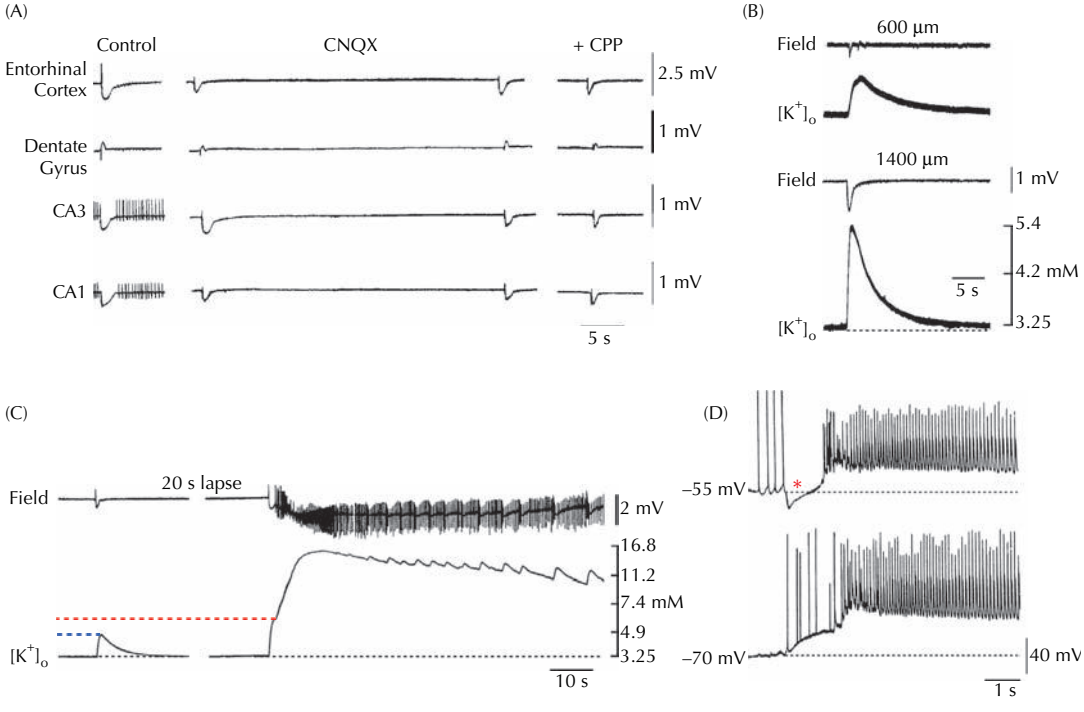

Figure 14–2. Epileptiform activity recorded in a rodent combined hippocampus-entorhinal cortex slice during continuous application of 4-aminopyridine. **A.** Field potential recordings from different hippocampal areas and the entorhinal cortex demonstrate the occurrence of fast CA3-driven interictal activity in the hippocampus proper and slow interictal events that are present in all recorded structures. Note that the latter type of activity continues to occur during application of glutamatergic receptor antagonists (CPP [(*RS*)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid] and CNQX [6-cyano-7-nitroquinoxaline-2,3-dione]). **B.** Slow interictal discharges analyzed with simultaneous field potential and $[K^+]_o$ recordings at two different depths of the entorhinal cortex. Note the transient elevations in $[K^+]_o$ that accompany the interictal discharges. **C.** Simultaneous field potential and $[K^+]_o$ recordings during interictal and ictal activity in the entorhinal cortex; note that the slow interictal event (left) is associated with a $[K^+]_o$ increase up to around 4 mM, while that seen at ictal discharge onset is accompanied by an increase up to 7 mM. **D.** Intracellular recording (sharp microelectrode filled with K-acetate) of the ictal discharge onset from an entorhinal cortex cell. Note that when the neuronal membrane is depolarized by intracellular injection of steady positive current (–55 mV), the amplitude of the sustained ictal depolarization decreases while the initial long-lasting depolarization becomes hyperpolarizing compared with the recording obtained at resting membrane potential (resting membrane potential = –70 mV). Panels A to C are modified from ref. 8; panel D is modified from ref. 27.

glutamatergic-independent events depend upon the activation of $GABA_A$ receptors since they disappear during application of picrotoxin or bicuculline (not illustrated). They are associated with transient increases in $[K^+]_o$ (Fig. 14–2B). Events of this sort can initiate seizures (Fig. 14–2C), and intracellular current injection shows reversal at membrane potentials consistent with predominant roles for $GABA_A$ receptors, and not with predominant roles for glutamate receptors (Fig. 14–2D).[8,27] Under some circumstances, $GABA_A$ receptor–mediated activity can be the sole synaptic mechanism in the generation of seizure-like events.[28]

Depolarizing GABA mechanisms have been implicated in the transition to seizure in several experimental models of epileptiform discharge.[7,29,30] Depolarizing GABAergic mechanisms may also play roles in clinical epilepsy: (1) at least one antiepileptic drug (AED), topiramate, has been shown to inhibit carbonic anhydrase,[31] thus reducing the driving force of the HCO_3^-, and (2) synchronous, predominantly depolarizing, GABAergic events occur in brain slices of resected epileptic foci.[32–35]

Interneurons can contribute to the transition to seizure through mechanisms other than GABA-mediated depolarization.[36] Intense and prolonged GABAergic activity preceding seizures has been associated with an increase in extracellular potassium, which may then depolarize principal cells and recruit them for seizure activity[7,37,38] characterized by fast oscillatory activity at 30–100 Hz, defined as beta-gamma activity.[39] Prolonged interneuronal depolarization may result in depolarization block of their action potential firing, which decreases the strength of inhibition onto principal cells, thus contributing to the transition to seizure.[37] Furthermore, interneurons can have a synchronizing role through conventional inhibitory, hyperpolarizing postsynaptic potentials.[25]

As we mentioned above, interneuronal networks play crucial roles in generating physiological rhythms,[23] and it has been suggested that interneurons may play similar functions in early stages of seizures with prominent activity in the beta, gamma, and slower ripple bands. In these cases, it is likely that synchronous activity of interneuronal networks connected by gap junctions and inhibitory synapses can constrain the firing of large populations of principal cells into specific time windows between synchronized inhibitory postsynaptic potentials (IPSPs), potentially strengthening their collective impact on convergent postsynaptic targets. Recovery from strong hyperpolarizing inhibition can cause rebound excitation by activating low-threshold currents, including the T-type calcium and I_h currents.[40] Single interneurons exert a widespread control over hundreds of synaptically connected neurons that is particularly remarkable in the hippocampus.[41] Therefore, rebound excitation of principal cells driven by interneuron network activity may result in strong synchronization of excitation[21] that can generate epileptiform discharges under pathological conditions.

Nonsynaptic Mechanisms

Nonsynaptic coupling has probably been underrecognized in the neuroscience literature. The ability of hippocampal slices to generate seizure-like events in the presence of low or zero extracellular Ca^{2+},[5,42] and in the presence of drugs blocking synaptic transmission,[43] shows that synaptic transmission is not necessary for epileptiform synchronization.[5,44] The fast population spikes that can initiate these *field bursts*, and that can occur throughout the discharge, appear to depend on electrical interactions, most likely field effects but possibly also gap junctions.[44] The prolonged duration of the field bursts can be attributed to the accumulation of extracellular K^+, which has a relatively slow clearance and is able to sustain depolarization over tens of seconds.[44] While the in vitro low-Ca^{2+} condition is distinctly artificial, these observations are relevant to epilepsy in vivo: low levels of extracellular Ca^{2+} do occur during seizures,[45,46] and similar nonsynaptic mechanisms operate under conditions of intact synaptic transmission and normal extracellular Ca^{2+} concentration.[47,48] In practice, synaptic and nonsynaptic mechanisms interact intimately both in promoting and in controlling epileptiform synchronization (see Fig. 14–3).

GAP JUNCTIONS

Gap junctions are sites of direct electrical communication between cells. Proteins known as *connexins* assemble into pores aligned in the two communicating cells. The pore allows relatively large molecules to pass, including dyes

and intracellular signaling molecules. Gap junctions switch between open and closed states and can be modulated by a range of intracellular signals, including pH, cyclic adenosine monophosphate (cAMP), and Ca^{2+}.[44]

Glia, especially astrocytes, are coupled by gap junctions and show prominent dye coupling, at least in cortical gray matter,[49] where they mediate the propagation of intracellular Ca^{2+} waves through the syncytium they form. These gap junctions are powerful enough to contribute ~30% of the input conductance of individual glia.[50] Astrocyte gap junctions play a role in promoting the clearance of extracellular K^+ following its accumulation. Hippocampal slices from mice lacking astrocyte gap junctions have lower seizure thresholds, supporting the idea that this mechanism helps control seizures.[50] Gap junctions between astrocytes are upregulated in patients with temporal lobe epilepsy (TLE), which could be seen as a protective response, but glutamatergic signaling between astrocytes and neurons may mean that this upregulation could be pro-epileptic.[51]

Interneurons have well-documented gap junctions both in hippocampus[52] and in neocortex.[53,54] These gap junctions are capable of synchronizing pairs of interneurons of the same subtype (e.g., fast spiking or low-threshold spiking) and promoting synchronous firing of interneuronal populations,[55] and of enhancing coherent oscillations emerging from their GABAergic synaptic network.[56] Seizure-like activity mediated by $GABA_A$ receptors with glutamatergic receptors blocked also depends on gap junctions, implicating gap junction coupling of interneurons in seizure generation.[28]

Pyramidal cells may have gap junctions too. Indirect evidence implicated electrotonic coupling between hippocampal pyramidal cells, and in particular between pyramidal cell axons,[57] but direct evidence for these gap junctions has proved elusive, suggesting that they are rather rare;[58,59] thus, only 10 out of 2000 pairs of neocortical pyramidal neurons were found to be electrotonically coupled in a recent study.[60]

EPHAPTIC (FIELD EFFECT) INTERACTIONS

Neuronal activity generates electric fields, which, of course, are responsible for the EEG. Neuronal excitability also can be modulated by electric fields; essentially, if a voltage gradient in the extracellular space produces a negative potential just outside the neuron, then its membrane will experience a *transmembrane* depolarization and it will become more excitable.[44] The details differ, depending on the neuron's membrane resistance and length constant, but the link between extracellular negativity and transmembrane depolarization is consistent.

A central question is whether the endogenous fields are strong enough to modulate neuronal activity. In hippocampal slices, exogenous fields of a few millivolts per millimeter DC can modulate evoked responses in vitro[61] and AC fields of a few hundred microvolts per millimeter can entrain activity when neurons are close to threshold so that small perturbations in transmembrane potential have significant effects.[62,63,63a] Stronger fields of up to 10–20 mV/mm occur during seizures, suggesting that they may participate in synchronization during the seizure itself.[44] Relatively strong fields can also occur during physiological activity, on the order of 1 mV/mm, in both hippocampus[44] and neocortex,[64] suggesting that they could play a (probably modest) role in normal brain function. The situation we have outlined here is for electric fields generated by populations of neurons. The fields generated by individual neurons or small groups of neurons are smaller and more complex. Such interactions depend on the proximity of the potentially coupled neurons and align more closely with the original use of the term *ephaptic*. Computer simulations suggest that they are strong enough to have a significant effect.[65]

EXTRACELLULAR POTASSIUM AND OTHER ION FLUCTUATIONS

The long duration (up to many tens of seconds) of the low-Ca^{2+} field bursts may be attributed to elevations in extracellular K^+ concentrations that should increase neuronal excitability. K^+ increases during seizures in models with intact synaptic transmission in vitro (e.g., Figs. 14–2C, 14–3) and in vivo, resulting in slow negative shifts of the extracellular potential, which have also been detected in humans.[66] In some situations, the extracellular K^+ concentration needs to reach a threshold value to trigger a seizure.[2,8] The clearance of extracellular K^+ occurs over many seconds and is slow enough to contribute significantly to the prolongation of

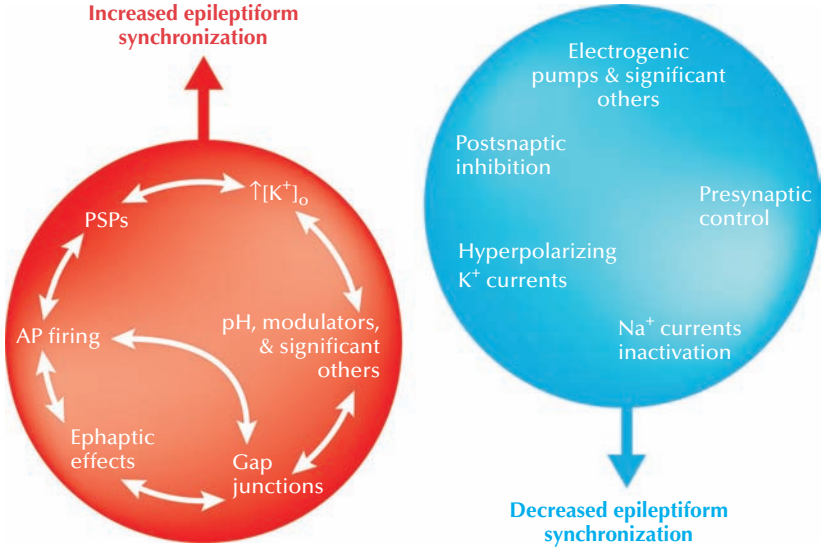

Figure 14–3. Schematic diagrams illustrating mechanisms that promote epileptiform synchronization and their broad relationships (left panel in red) and those that reduce epileptiform synchronization (right panel in blue).

seizures. Intense neuronal firing is superficially the most obvious source of extracellular K^+, but as mentioned above, inhibitory synaptic transmission is a particularly potent source because the transporters for GABA reuptake and Cl^- extrusion,[67] like many other ion and neurotransmitter transporters, depend in part on the efflux of K^+ down its concentration gradient. These increases in K^+, probably with contributions from changes in other ions,[45] tend to increase excitation synchronously for all the neurons exposed to the ionic change.

K^+ buffering is impaired in chronic epileptic foci with hippocampal sclerosis,[51] apparently because of decreased expression of astrocyte Kir channels. This loss of function will exacerbate the impact of K^+ transients on promoting and sustaining epileptic activity. The resulting impairment of glutamate uptake, along with the downregulation of glutamine synthetase found in both clinical and chronic experimental foci, will further promote epileptic activity.[51]

Glial cells play active roles in neuronal function beyond regulating extracellular K^+ and taking up neurotransmitters. It has become increasingly clear that glia can release glutamate and other neurotransmitters in response to elevations of intracellular Ca^{2+}, which may involve Ca^{2+}-dependent exocytosis. This can be triggered by activation of receptors on the glial surface, notably mGluRs, or by depolarization from increased extracellular K^+.[51] It is likely that they will contribute to seizure activity by providing pathways for the propagation of excitation that are not limited to classical synaptic pathways and by providing relatively slow forms of neuronal depolarization[51] (Fig. 14–3).

SEIZURE ONSET

Temporal lobe seizures, both clinical and experimental, have been divided into two groups on the basis of their onset: low-voltage fast (LVF) and hypersynchronous (HYP).[68,69] The clinical evidence argues that cases with LVF onsets may have more diffuse seizure onset zones that include extrahippocampal areas.[68] However, if the LVF onset reflects recording of a seizure onset at site/s remote from where the seizure initiates, then the mechanism of onset could be similar to that of HYP.[69] This issue is still controversial, because human data support the notion that LVF in the beta-gamma frequency range have a high localizing value for the identification of the seizure-onset zone[70–72] (for a review, see ref. 36). The advent of faster sample rates in clinical electrophysiology showed the presence of high-frequency activity (HFA) at seizure onset.[70,71] In conventional EEG this activity

appears as an electrodecremental pattern.[36,70,71] The presence of beta-gamma oscillations at seizure onset is also supported by the characterization of seizure patterns in chronic animal models of partial epilepsy.[73] Moreover, in an in vitro model of seizures induced by transient disinhibition of the whole guinea pig brain, LVF sustained by inhibitory postsynaptic activity coupled with interneuronal bursting were found in the entorhinal cortex at the very onset of limbic seizures.[39] A role for GABAergic reinforcement to either initiate or precede an ictal discharge was also reported in hippocampal slices treated with low $[Mg^{2+}]_o$ with[37] or without 4-aminopyridine.[74]

The existence of a preictal state has been postulated and may provide opportunities for seizure prediction[75] as well as providing clues on the mechanisms of the transition to seizure. Experimental investigations of the preseizure period in acute experimental epilepsy in brain slices revealed low-amplitude HFA at ~200 Hz some tens of seconds before seizure onset.[48,76,77] No individual neuron fired at this rate: each cycle of HFA was generated by synchronous activity of a small aggregate of neurons (within ~5 ms), with each neuron firing on a minority of cycles.[48] This synchronization was most likely due to ephaptic interactions[44] and not gap junctions.[48] Under other circumstances, HFA of up to 200 Hz (known as the *ripple band*) was associated with, and most likely entrained by, firing of fast-spiking interneurons.[78] Preictal HFA in models in vitro changed dynamically in the run-up to the next seizure, with progressive increase in power, expansion in the spatial extent of synchronization, and decrease in mean frequency.[48] This hypothesis seems to be supported by recent human findings[79] that demonstrate the coalescence of microseizures recorded with microelectrodes just ahead of a large-scale clinical seizure observed during diagnostic presurgical assessment in pharmacoresistant patients.

The role of preictal HFA in humans with epilepsy remains an open question. Some reports showed increased power in high-frequency bands up to 20 min preceding the seizures,[80] but others found no preictal HFA.[81] Where it has been reported, it tends to be highly localized,[70,82] which means that failure to record it could mean either that it is not present or that the electrodes are not in the right place.

SEIZURE TERMINATION

In most cases, limbic seizures stop within tens of seconds to a few minutes. Identifying what mechanisms make seizures stop would have a major impact on the treatment of epilepsy. Seizure termination is as complicated a process as seizure initiation (Fig. 14–3). Several mechanisms of seizure termination have been described, including: synaptic inhibition, afterhyperpolarizations due to K^+ channels, neuromodulators (adenosine, endogenous opioids, endocannibinoids), depletion of the readily releasable pools of synaptic vesicles, sodium channel inactivation, changes in extracellular ions (notably Ca^{2+}, K^+, and H^+), and activation of sodium-potassium pumps. Such mechanisms may well play roles, alone or in combination, in seizure termination. Intracellular acidification arising from seizure activity would tend to close gap junctions,[83] which may reduce electrotonic synchrony between neurons, particularly between classes of interneurons where gap junctions are well documented, although gap junctions between glia may also be important, particularly in light of the roles they play in neuronal activity. Acidosis can impact on seizures by other mechanisms, such as acid-sensing ion channels (ASIC): work on genetically modified mice revealed that ASIC1a has a major effect on seizure severity, contributing to seizure termination and being responsible for the modulation of seizures by modifications of extracellular pH.[83a]

While seizures are generally considered to be hypersynchronous events, recent work suggests that spatial synchrony may increase to a maximum towards the termination of the seizures, raising the possibility that increased synchrony may play role in terminating epileptic seizures, both clinical[84] and experimental.[47] One idea that is gaining ground is that seizures stop when cycles of activity become too synchronous throughout the seizure-generation zone at the same time that the frequency of the activity drops.[47,73] The end result is that the prolonged and synchronous hyperpolarization of the seizure-generation zone prevents excitation from resuming anywhere in the circuit.[47,84] One specific idea is that the slow afterhyperpolarization due to Ca^{2+}- activated K^+ channels builds progressively, as a result of intracellular accumulation of Ca^{2+} and Na^+, to such a degree that the hyperpolarization becomes so

prolonged that it blocks reexcitation by I_h[47] or by synaptic activation from asynchronously active regions.

ROLES AND INTERACTIONS OF SPECIFIC LIMBIC AREAS IN SEIZURES

Where limbic seizures originate may vary both between and within cases, whether patients or experimental animals. It has been shown experimentally in vitro and in vivo, and in human patients, that several limbic structures can initiate seizures: hippocampus, entorhinal cortex, perirhinal cortex, amygdala. In some cases one structure can dominate, while in other cases onset can be multifocal. During the course of individual seizures, activity propagates widely along synaptic pathways, and can become highly synchronized between limbic and other regions in both hemispheres.

Early theories argued that propagation through *reentrant loops* in the limbic system played a key role in sustaining and prolonging seizures beyond the few seconds of the interictal discharge.[85] However, more detailed measurements of the cycles of activity within each seizure show near-zero mean phase lags between the various limbic structures,[86,87] suggesting that they perhaps have more in common with coupled oscillators than with reentrant loops. In line with this view, left and right hippocampi in the intrahippocampal tetanus toxin model had a near-zero phase lag, due to the lead swapping sides repeatedly during each seizure, while on each side CA3 consistently led CA1, reflecting the predominantly unidirectional transmission through the Shaffer collaterals.[88] The long-range networks may be crucial for the persistence of seizures in vivo through the reexcitation of each region by others that are active as it recovers from periods of inhibition.

Some parts of the limbic system may make specific contributions to seizures. A classic example is the dentate gyrus, which has been considered a "gate" that controls the spread of seizures through the network. As mentioned above, evidence obtained from in vitro models of epileptiform synchronization indicates that the CA3 region is particularly effective at generating interictal discharges, and the entorhinal cortex is especially effective at generating prolonged seizure-like events. The relatively frequent and brief interictal discharges may control seizure generation, as shown in Fig. 14–4A, where lesioning slices between the hippocampus and entorhinal cortex revealed seizures in the latter. Moreover, stimulation at 0.5–1.0 Hz can block seizure initiation (Fig. 14–4B), providing further support for the idea that repetitive activity of the kind produced by interictal discharges can be anticonvulsant.[9,43,89] In the intact brain the circuitry is even more complex; for instance, midline thalamic nuclei, which are part of the limbic system, may play a key role in synchronizing limbic seizures through their diffuse and widespread connections.

NEUROBIOLOGY OF TEMPORAL LOBE EPILEPSY

Hippocampal Sclerosis and Other Pathologies

Hippocampal sclerosis is found in about two-thirds of cases of TLE. Prolonged status epilepticus does lead to neuronal death, so experimental models of TLE induced by an episode of status epilepticus are associated with substantial neuronal loss and gliosis. In cases without an initial status epilepticus, whether hippocampal sclerosis is a consequence of repeated seizures or of some initial precipitating injury remains an open question. More subtle and selective losses of neurons, particularly inhibitory interneurons, have been reported and may have functional consequences.[90,91]

Principal neuron damage associated with hippocampal sclerosis has been implicated in the mechanism for fast ripples[92] but now seems not to be necessary for them.[93] Damage of large numbers of principal neurons will have a major impact on their synaptic targets, notably by the loss of their synaptic drive, but also potentially by causing plastic changes in both connectivity and neuronal excitability. This is a process in which loss of function of the hippocampus proper could produce gain of function in parahippocampal structures, such as the entorhinal and perirhinal cortices or amygdala, thus leading to a

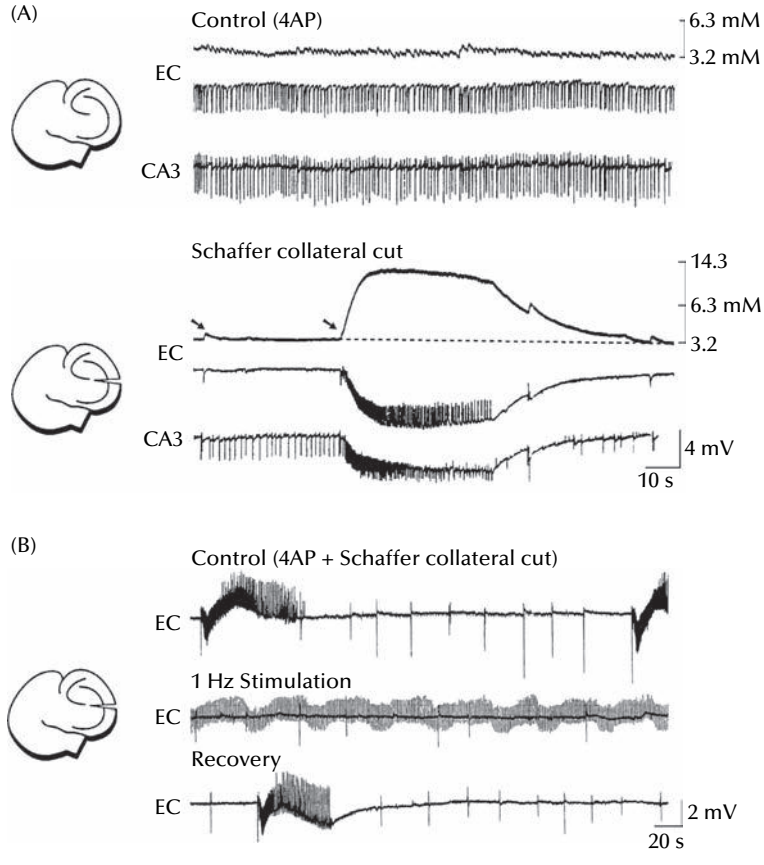

Figure 14–4. A. Effects induced by cutting the Schaffer collaterals on the epileptiform activity recorded from a rodent combined hippocampus–entorhinal cortex slice during continuous application of 4-aminopyridine (4AP). Note that under control conditions the pattern is characterized by fast CA3-driven interictal discharges, while after Schaffer collateral cut, ictal discharges are disclosed. (EC: entorhinal cortex.) **B.** Effect induced by extracellular stimuli delivered in the CA1-subiculum area subfield at 1 Hz on the ictal activity recorded after Schaffer collateral cut. Note that the low-frequency stimulation prevents the occurrence of ictal discharges and that ictal activity reappears after termination of the repetitive stimulation protocol.

chronic condition of poorly controlled seizures in epileptic animals and perhaps in TLE patients.

High-Frequency Oscillations

High-frequency oscillations (HFOs) associated with the epileptic state can be found during interictal periods, often, but not always, superimposed on interictal discharges. High-frequency oscillations in the band between 100 and 250 Hz, known as *ripples*, also occur during normal physiological activity, most notably in *sharp-wave ripples*, which may play key roles in memory formation and consolidation.[94] More promising in terms of pathophysiology are HFOs faster than 250 Hz, known as *fast ripples*, which appear to be selective for epileptic foci and have been proposed as biomarkers for the epileptogenic zone.[18,81,95,96] (The precise demarcation frequency between ripples and fast ripples varies among publications.)

The mechanisms of HFOs are starting to be clarified. Ripples may well be paced by fast rhythmic IPSPs[97] controlling the activity of pyramidal neurons induced by synchronous excitation, which produces the *sharp-wave* and is due to something akin to the glutamatergic chain reaction of the interictal discharge described above. The mechanisms of

fast ripples are more controversial. They are correlated with the presence and extent of hippocampal sclerosis.[92,98,99] This led to the hypothesis that the loss of neurons was a necessary requirement for the higher frequency of fast ripples, because it fragmented the ephaptic coupling of pyramidal cells.[92] Our work on the hippocampal tetanus toxin model of nonlesional TLE argues that neuronal loss is not a necessary requirement for the production of fast ripples.[93] The selective production of fast ripples by the hippocampus injected with tetanus toxin argues that they are good markers for the primary epileptogenic zone even in the absence of neuronal loss, and that they are better than interictal discharges or ripples, both of which occurred equally often in injected and contralateral hippocampi.[93]

TARGETING SYNCHRONY AS AN APPROACH TO THERAPY

As outlined above, epileptic seizures are essentially a form of neuronal hypersynchrony. Conventional AEDs generally target excitability rather than synchrony, although they may impact on it indirectly, for instance by retarding the chain reaction of excitation described above. In contrast, electrical stimulation of the epileptic focus can directly impact on neuronal synchrony. Strong depolarizing direct and high-frequency sinusoidal currents can block seizure activity in vitro, most likely because excessive depolarization results in inactivation of Na^+ channels so that neurons are no longer able to fire action potentials.[100,101] The depolarization will decay electrotonically along the axons, so that at some distance from the soma it will drive repetitive firing. While this may appear unhelpful, the resulting action potentials will be asynchronous between axons and may contribute to desynchronization of postsynaptic targets. Stimulation may also have the effect of releasing enough neurotransmitter to deplete the readily releasable pool of neurotransmitter vesicles, perhaps mimicking the effects of some classes of interictal activity.[9,43,89]

The termination of seizures by slow, repetitive, hypersynchronous activity (see the section "Seizure Termination" above)[47,73] suggests that stimulation at low frequencies could block seizures. In line with this view, low-frequency stimulation at ~1 Hz (Fig. 14–4B) reduces ictal-like discharges generated by the rodent brain in an in vitro slice preparation,[9,43,89] as well as in at least one model in vivo.[102]

CONCLUSIONS

Neuronal hypersynchronization is a key aspect of limbic seizures and interictal activity. In the case of symptomatic seizures or acute models of epilepsy, this hypersynchronization depends on the aberrant engagement of mechanisms normally responsible for physiological synchronization, which is prominent in the hippocampus and other limbic areas. In chronic epileptic foci, aberrant synchronizing mechanisms may contribute substantially to the generation of spontaneous epileptic discharges.

Neuronal synchronization depends in large part on the operation of synaptic networks, both excitatory and inhibitory. However, other mechanisms play significant roles, notably relatively slow fluctuations in $[K^+]_o$ and other ions and the faster actions of ephaptic and gap junction coupling. Evidence is accumulating for important roles for glia, both in $[K^+]_o$ and neurotransmitter buffering, and their ability to release glutamate and other neuroactive substances. The long-range connectivity of the limbic system is also important, both because interactions between its components appear to sustain and propagate seizures and because certain areas exert control over the epileptiform activity generated by others, such as the hippocampus controlling seizures in the entorhinal cortex. We conclude that investigating epileptic synchronization in the limbic system will help us understand the generation of epileptic discharges and may provide leads on more selective treatments to prevent or ameliorate epileptic seizures.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. De Curtis M, Avanzini G. Interictal spikes in focal epileptogenesis. *Prog Neurobiol*. 2001;63:541–567.

2. Borck C, Jefferys JGR. Seizure-like events in disinhibited ventral slices of adult rat hippocampus. *J Neurophysiol*. 1999;82:2130–2142.
3. Avoli M, Louvel J, Kurcewicz I, Pumain R, Barbarosie M. Extracellular free potassium and calcium during synchronous activity induced by 4-aminopyridine in the juvenile rat hippocampus. *J Physiol*. 1996;493: 707–717.
4. Avoli M, Psarropoulou C, Tancredi V, Fueta Y. On the synchronous activity induced by 4-aminopyridine in the CA3 subfield of juvenile rat hippocampus. *J Neurophysiol*. 1993;70:1018–1029.
5. Jefferys JGR, Haas HL. Synchronized bursting of CA1 pyramidal cells in the absence of synaptic transmission. *Nature*. 1982;300:448–450.
6. Traynelis SF, Dingledine R. Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. *J Neurophysiol*. 1988;59:259–276.
7. Köhling R, Vreugdenhil M, Bracci E, Jefferys JGR. Ictal epileptiform activity is facilitated by hippocampal $GABA_A$ receptor-mediated oscillations. *J Neurosci*. 2000;20:6820–6829.
8. Avoli M, Barbarosie M, Lucke A, Nagao T, Lopantsev V, Köhling R. Synchronous GABA-mediated potentials and epileptiform discharges in the rat limbic system in vitro. *J Neurosci*. 1996;16:3912–3924.
9. Barbarosie M, Avoli M. CA3-driven hippocampal-entorhinal loop controls rather than sustains in vitro limbic seizures. *J Neurosci*. 1997;17:9308–9314.
10. Traub RD, Miles R, Jefferys JGR. Synaptic and intrinsic conductances shape picrotoxin-induced synchronized after-discharges in the guinea-pig hippocampal slice. *J Physiol*. 1993;461:525–547.
11. Traub RD, Jefferys JGR, Whittington MA. Enhanced NMDA conductance can account for epileptiform activity induced by low Mg^{2+} in the rat hippocampal slice. *J Physiol*. 1994;478:379–393.
12. Traub RD, Dingledine R. Model of synchronized epileptiform bursts induced by high potassium in CA3 region of rat hippocampal slice. Role of spontaneous EPSPs in initiation. *J Neurophysiol*. 1990;64:1009–1018.
13. Rutecki PA, Lebeda FJ, Johnston D. 4-Aminopyridine produces epileptiform activity in hippocampus and enhances synaptic excitation and inhibition. *J Neurophysiol*. 1987;57:1911–1924.
14. Prince DA. Neurophysiology of epilepsy. *Annu Rev Neurosci*. 1978;1:395–415.
15. Johnston D, Brown TH. Giant synaptic potential hypothesis for epileptiform activity. *Science*. 1981;211:294–297.
16. Traub RD, Wong RKS. Cellular mechanism of neuronal synchronization in epilepsy. *Science*. 1982;216:745–747.
17. Miles R, Wong RKS. Unitary inhibitory synaptic potentials in the guinea-pig hippocampus in vitro. *J Physiol*. 1984;356:97–113.
18. Engel J, Bragin A, Staba R, Mody I. High-frequency oscillations: what is normal and what is not? *Epilepsia*. 2009;50:598–604.
19. Morrell F. Multiple subpial transection in treatment of focal epilepsy. Response. *J Neurosurg*. 1989;71:630.
20. Buzsáki G, Chrobak JJ. Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. *Curr Opin Neurobiol*. 1995;5:504–510.
21. Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P. Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature*. 1995;378:75–78.
22. Whittington MA, Traub RD, Jefferys JGR. Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. *Nature*. 1995; 373:612–615.
23. Bartos M, Vida I, Frotscher M, Meyer A, Monyer H, Geiger JRP, Jonas P. Fast synaptic inhibition promotes synchronized gamma oscillations in hippocampal interneuron networks. *Proc Natl Acad Sci USA*. 2002;99:13222–13227.
24. Bartos M, Vida I, Jonas P. Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat Rev Neurosci*. 2007;8:45–56.
25. Mann EO, Mody I. The multifaceted role of inhibition in epilepsy: seizure-genesis through excessive GABAergic inhibition in autosomal dominant nocturnal frontal lobe epilepsy. *Curr Opin Neurol*. 2008;21:155–160.
26. Avoli M, D'Antuono M, Louvel J, Kohling R, Biagini G, Pumain R, D'Arcangelo G, Tancredi V. Network and pharmacological mechanisms leading to epileptiform synchronization in the limbic system in vitro. *Prog Neurobiol*. 2002;68:167–207.
27. Lopantsev V, Avoli M. Participation of $GABA_A$-mediated inhibition in ictallike discharges in the rat entorhinal cortex. *J Neurophysiol*. 1998;79:352–360.
28. Uusisaari M, Smirnov S, Voipio J, Kaila K. Spontaneous epileptiform activity mediated by GABA(A) receptors and gap junctions in the rat hippocampal slice following long-term exposure to GABA(B) antagonists. *Neuropharmacology*. 2002;43:563–572.
29. Fujiwara-Tsukamoto Y, Isomura Y, Nambu A, Takada M. Excitatory GABA input directly drives seizure-like rhythmic synchronization in mature hippocampal CA1 pyramidal cells. *Neuroscience*. 2003;119: 265–275.
30. Id Bihi R, Jefferys JGR, Vreugdenhil M. The role of extracellular potassium in the epileptogenic transformation of recurrent GABAergic inhibition. *Epilepsia*. 2005;46:64–71.
31. Lyseng-Williamson KA, Yang LPH. Topiramate—a review of its use in the treatment of epilepsy. *Drugs*. 2007;67:2231–2256.
32. Köhling R, Lücke A, Straub H, Speckmann EJ, Tuxhorn I, Wolf P, Pannek H, Oppel F. Spontaneous sharp waves in human neocortical slices excised from epileptic patients. *Brain*. 1998;121:1073–1087.
33. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science*. 2002;298: 1418–1421.
34. Wozny C, Kivi A, Lehmann TN, Dehnicke C, Heinemann U, Behr J. Comment on "On the origin of interictal activity in human temporal lobe epilepsy in vitro." *Science*. 2003;301:463.
35. Huberfeld G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, Rivera C. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. *J Neurosci*. 2007;27:9866–9873.
36. De Curtis M, Gnatkovsky V. Reevaluating the mechanisms of focal ictogenesis: the role of low-voltage fast activity. *Epilepsia*. 2009;50:2514–2525.
37. Ziburkus J, Cressman JR, Barreto E, Schiff SJ. Interneuron and pyramidal cell interplay during in vitro seizure-like events. *J Neurophysiol*. 2006;95:3948–3954.

38. Velazquez JL, Lozano HK, Bardakjian BL, Carlen PL, Wennberg R. Type III intermittency in human partial epilepsy. *Eur J Neurosci*. 1999;11:2571–2576.
39. Gnatkovsky V, Librizzi L, Trombin F, De Curtis M. Fast activity at seizure onset is mediated by inhibitory circuits in the entorhinal cortex in vitro. *Ann Neurol*. 2008;64:674–686.
40. Surges R, Sarvari M, Stefens M, Els T. Characterization of rebound depolarization in hippocampal neurons. *Biochem Biophys Res Commun*. 2006;348:1343–1349.
41. Freund TF, Buzsaki G. Interneurons of the hippocampus. *Hippocampus.* 1996;6:347–470.
42. Konnerth A, Heinemann U, Yaari Y. Nonsynaptic epileptogenesis in the mammalian hippocampus in vitro. I. Development of seizurelike activity in low extracellular calcium. *J Neurophysiol*. 1986;56:409–423.
43. Jensen MS, Yaari Y. The relationship between interictal and ictal paroxysms in an in vitro model of focal hippocampal epilepsy. *Ann Neurol.* 1988;24:591–598.
44. Jefferys JGR. Non-synaptic modulation of neuronal activity in the brain: electric currents and extracellular ions. *Physiol Rev*. 1995;75:689–723.
45. Pumain R, Menini C, Heinemann U, Louvel J, Silva-Barrat C. Chemical synaptic transmission is not necessary for epileptic seizures to persist in the baboon *Papio papio*. *Exp Neurol*. 1985;89:250–258.
46. Heinemann U, Konnerth A, Pumain R, Wadman WJ. Extracellular calcium and potassium changes in chronic epileptic brain tissue. *Adv Neurol*. 1986;44:641–661.
47. Timofeev I, Steriade M. Neocortical seizures: initiation, development and cessation. *Neuroscience*. 2004;123:299–336.
48. Jiruska P, Csicsvari J, Powell AD, Fox JE, Chang WC, Vreugdenhil M, Li X, Palus M, Bujan AF, Dearden RW, Jefferys JGR. High-frequency network activity, global increase in neuronal activity and synchrony expansion precede epileptic seizures in vitro. *J Neurosci.* 2010;30:5690–5701.
49. Matyash V, Kettenmann H. Heterogeneity in astrocyte morphology and physiology. *Brain Res Brain Res Rev*. 2010;63:2–10.
50. Wallraff A, Kohling R, Heinemann U, Theis M, Willecke K, Steinhauser C. The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. *J Neurosci*. 2006;26:5438–5447.
51. Seifert G, Carmignoto G, Steinhauser C. Astrocyte dysfunction in epilepsy. *Brain Res Brain Res Rev.* 2010;63:212–221.
52. Fukuda T, Kosaka T. Gap junctions linking the dendritic network of GABAergic interneurons in the hippocampus. *J Neurosci*. 2000;20:1519–1528.
53. Tamas G, Buhl EH, Lorincz A, Somogyi P. Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. *Nat Neurosci*. 2000;3:366–371.
54. Galarreta M, Hestrin S. A network of fast-spiking cells in the neocortex connected by electrical synapses. *Nature*. 1999;402:72–75.
55. Mancilla JG, Lewis TJ, Pinto DJ, Rinzel J, Connors BW. Synchronization of electrically coupled pairs of inhibitory interneurons in neocortex. *J Neurosci*. 2007;27:2058–2073.
56. Beierlein M, Gibson JR, Connors BW. A network of electrically coupled interneurons drives synchronized inhibition in neocortex. *Nat Neurosci*. 2000;3:904–910.
57. Draguhn A, Traub RD, Schmitz D, Jefferys JGR. Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro. *Nature*. 1998;394:189–192.
58. Schmitz D, Schuchmann S, Fisahn A, Draguhn A, Buhl EH, Petrasch-Parwez E, Dermietzel R, Heinemann U, Traub RD. Axo-axonal coupling: a novel mechanism for ultrafast neuronal communication. *Neuron*. 2001;31:831–840.
59. Mercer A, Bannister AP, Thomson AM. Electrical coupling between pyramidal cells in adult cortical regions. *Brain Cell Biol*. 2006;35:13–27.
60. Wang Y, Barakat A, Zhou HW. Electrotonic coupling between pyramidal neurons in the neocortex. *Plos One* 2010;5.
61. Jefferys JGR. Influence of electric fields on the excitability of granule cells in guinea-pig hippocampal slices. *J Physiol*. 1981;319:143–152.
62. Francis JT, Gluckman BJ, Schiff SJ. Sensitivity of neurons to weak electric fields. *J Neurosci*. 2003;23:7255–7261.
63. Deans JK, Bikson M, Jefferys JGR. Sensitivity of coherent oscillations in rat hippocampus to AC electric fields. *J Physiol* 2007;583:555–565.
63a. Dudek FE, Snow RW, Taylor CP. Role of electrical interactions in synchronization of epileptiform bursts. *Adv Neurol*. 1986;44:593–617.
64. Frohlich F, McCormick DA. Endogenous electric fields may guide neocortical network activity. *Neuron*. 2010;67:129–143.
65. Holt GR, Koch C. Electrical interactions via the extracellular potential near cell bodies. *J Comput Neurosci*. 1999;6:169–184.
66. Vanhatalo S, Holmes MD, Tallgren P, Voipio J, Kaila K, Miller JW. Very slow EEG responses lateralize temporal lobe seizures: an evaluation of non-invasive DC-EEG. *Neurology*. 2003;60:1098–1104.
67. Barolet AW, Morris ME. Changes in extracellular K^+ evoked by Gaba, Thip and baclofen in the guinea-pig hippocampal slice. *Exp Brain Res*. 1991;84:591–598.
68. Ogren JA, Bragin A, Wilson CL, Hoftman GD, Lin JJ, Dutton RA, Fields TA, Toga AW, Thompson PM, Engel J, Staba RJ. Three-dimensional hippocampal atrophy maps distinguish two common temporal lobe seizure-onset patterns. *Epilepsia*. 2009;50:1361–1370.
69. Bragin A, Azizyan A, Almajano J, Wilson CL, Engel J. Analysis of chronic seizure onsets after intrahippocampal kainic acid injection in freely moving rats. *Epilepsia*. 2005;46:1592–1598.
70. Allen PJ, Fish DR, Smith SJM. Very high-frequency rhythmic activity during SEEG suppression in frontal lobe epilepsy. *Electroencephalogr Clin Neurophysiol*. 1992;82:155–159.
71. Fisher RS, Webber WRS, Lesser RP, Arroyo S, Uematsu S. High-frequency EEG activity at the start of seizures. *J Clin Neurophysiol.* 1992;9:441–448.
72. Gotman J, Levtova V, Olivier A. Frequency of the electroencephalographic discharge in seizures of focal and widespread onset in intracerebral recordings. *Epilepsia*. 1995;36:697–703.
73. Finnerty GT, Jefferys JGR. 9–16 Hz oscillation precedes secondary generalization of seizures in the rat tetanus toxin model of epilepsy. *J Neurophysiol.* 2000;83:2217–2226.
74. Derchansky M, Jahromi SS, Mamani M, Shin DS, Sik A, Carlen PL. Transition to seizures in the isolated

immature mouse hippocampus: a switch from dominant phasic inhibition to dominant phasic excitation. *J Physiol* (*Lond*). 2008;586:477–494.
75. Sackellares JC. Seizure prediction. *Epilepsy Curr*. 2008;8:55–59.
76. Bikson M, Fox JE, Jefferys JGR. Neuronal aggregate formation underlies spatiotemporal dynamics of nonsynaptic seizure initiation. *J Neurophysiol*. 2003;89:2330–2333.
77. Khosravani H, Pinnegar CR, Mitchell JR, Bardakjian BL, Federico P, Carlen PL. Increased high-frequency oscillations precede in vitro low-Mg^{2+} seizures. *Epilepsia*. 2005;46:1188–1197.
78. Csicsvari J, Hirase H, Czurkó A, Mamiya A, Buzsaki G. Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving Rat. *J Neurosci*. 1999;19:274–287.
79. Stead M, Bower M, Brinkmann BH, Lee K, Marsh WR, Meyer FB, Litt B, Gompel J, Worrell GA. Microseizures and the spatiotemporal scales of human partial epilepsy. *Brain*. 2010;133:2789–2797.
80. Worrell GA, Parish L, Cranstoun SD, Jonas R, Baltuch G, Litt B. High-frequency oscillations and seizure generation in neocortical epilepsy. *Brain*. 2004;127:1496–1506.
81. Jacobs J, Levan P, Chatillon CE, Olivier A, Dubeau F, Gotman J. High frequency oscillations in intracranial EEGs mark epileptogenicity rather than lesion type. *Brain*. 2009;132:1022–1037.
82. Fisher RS, Webber WRS, Litt B, Uematsu S, Lesser RP. High-frequency electroencephalogram activity at the start of seizures. Ann Neurol. 1991;30:291.
83. Carlen PL, Skinner F, Zhang L, Naus C, Kushnir M, Perez Velazquez JL. The role of gap junctions in seizures. *Brain Res Brain Res Rev*. 2000;32:235–241.
83a. Ziemann AE, Schnizler MK, Albert GW, Severson MA, Howard MA., Welsh MJ, Wemmie JA. Seizure termination by acidosis depends on ASIC1a. *Nat Neurosci*. 2008;11:816–822,.
84. Schindler K, Elger CE, Lehnertz K. Increasing synchronization may promote seizure termination: evidence from status epilepticus. *Clin Neurophysiol*. 2007;118:1955–1968.
85. Lothman EW. Seizure circuits in the hippocampus and associated structures. *Hippocampus*. 1994;4: 286–290.
86. Paré D, De Curtis M, Llinás R. Role of the hippocampal-entorhinal loop in temporal lobe epilepsy: extra- and intracellular study in the isolated guinea pig brain in vitro. *J Neurosci.* 1992;12:1867–1881.
87. Bragin A, Csisvari J, Penttonen M, Buzsáki G. Epileptic afterdischarge in the hippocampal-entorhinal system: current source density and unit studies. *Neuroscience*. 1997;76:1187–1203.
88. Finnerty GT, Jefferys JGR. Investigation of the neuronal aggregate generating seizures in the rat tetanus toxin model of epilepsy. *J Neurophysiol*. 2002;88: 2919–2927.
89. Khosravani H, Carlen PL, Velazquez JLP. The control of seizure-like activity in the rat hippocampal slice. *Biophys J*. 2003;84:687–695.
90. Arellano JI, Munoz A, Ballesteros-Yanez I, Sola RG, De Felipe J. Histopathology and reorganization of chandelier cells in the human epileptic sclerotic hippocampus. *Brain*. 2004;127:45–64.
91. Cossart R, Bernard C, Ben Ari Y. Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signalling in epilepsies. *Trends Neurosci*. 2005;28:108–115.
92. Foffani G, Uzcategui YG, Gal B, de la Prida LM. Reduced spike-timing reliability correlates with the emergence of fast ripples in the rat epileptic hippocampus. *Neuron.* 2007;55:930–941.
93. Jiruska P, Finnerty GT, Powell AD, Lofti N, Cmejla R, Jefferys JGR. High-frequency network activity in a model of non-lesional temporal lobe epilepsy. *Brain*. 2010;133:1380–1390.
94. Axmacher N, Mormann F, Fernandez G, Elger CE, Fell J. Memory formation by neuronal synchronization. *Brain Res Brain Res Rev*. 2006;52:170–182.
95. Bragin A, Engel JJ, Wilson CL, Fried I, Mathern GW. Hippocampal and entorhinal cortex high-frequency oscillations (100–500 Hz) in human epileptic brain and in kainic acid–treated rats with chronic seizures. *Epilepsia*. 1999;40:127–137.
96. Jiruska P, Jefferys JGR. High-frequency pre-seizure activity and seizure prediction. In: Schelter B, Timmer J, Schulze-Bonhage A, eds. *Seizure Prediction in Epilepsy: From Basic Mechanisms to Clinical Applications*. Weinheim: Wiley-VCH; 2008: 169–174.
97. Ylinen A, Bragin A, Nádasdy Z, Jandó G, Szabo I, Sik A, Buzsáki G. Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *J Neurosci*. 1995;15:30–46.
98. Bragin A, Engel JJ, Wilson CL, Vizentin E, Mathern GW. Electrophysiologic analysis of a chronic seizure model after unilateral hippocampal KA injection. *Epilepsia.* 1999;40:1210–1211.
99. Staba RJ, Frighetto L, Behnke EJ, Mathern G, Fields T, Bragin A, Ogren J, Fried I, Wilson CL, Engel J. Increased fast ripple to ripple ratios correlate with reduced hippocampal volumes and neuron loss in temporal lobe epilepsy patients. *Epilepsia*. 2007;48:2130–2138.
100. Bikson M, Hahn PJ, Fox JE, Jefferys JGR. Depolarization block of neurons during maintenance of electrographic seizures. *J Neurophysiol*. 2003;90: 2402–2408.
101. Bikson M, Lian J, Hahn PJ, Stacey WC, Sciortino C, Durand DM. Suppression of epileptiform activity by high frequency sinusoidal fields in rat hippocampal slices. *J Physiol.* 2001;531:181–191.
102. Kile KB, Tian N, Durand DM. Low frequency stimulation decreases seizure activity in a mutation model of epilepsy. *Epilepsia*. 2010;51:1745–1753.

Chapter 15

Imaging of Hippocampal Circuits in Epilepsy

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Temporal lobe epilepsy (TLE) is the most common variant of adult human epilepsy. Although many brain regions are affected by TLE, the hippocampus and associated parahippocampal cortices are critically implicated. Clinical evidence supporting this contention includes a characteristic TLE-associated hippocampal pathology, termed *mesial temporal sclerosis*, and the efficacy of amygdalahippocampectomy in the treatment of TLE, in which removal of the affected hippocampus and amygdala eliminates seizures in many patients with mesial TLE. Temporal lobe epilepsy is an acquired seizure disorder, usually developing with a prolonged latency following some form of brain injury in the patient's past, such as a central nervous function (CNS) infection, head trauma, or a prolonged seizure episode such as status epilepticus (SE) or complex febrile seizures. Advancing our knowledge of the mechanisms underlying epilepsy necessarily involves studying the dynamic properties of neurons in situ, as they function in their physiological circuit, with all of the emergent complexities involved in the generation of the integrated output of brain regions.

Several animal models of TLE exist. A majority involve experimental induction of a brain injury through administration of a chemoconvulsant or electrical stimulation, both of which induce an episode of SE. Following resolution of SE, a high proportion of animals go on to develop spontaneous seizures with hippocampal involvement within weeks to months. One such model, involving pilocarpine administration as the chemoconvulsant, is one of the major models used to study TLE. The development of epilepsy after pilocarpine treatment follows three distinct stages.[1–3] (1) After administration of pilocarpine, the hippocampus begins to exhibit high-frequency spiking, which develops into electroencephalographic (EEG) seizures, repeating every 3–5 min and eventually leading to continuous seizures that can last for several hours (SE) unless they are terminated by administration of drugs such as diazepam

alone, diazepam with pentobarbital, or phenytoin. (2) Following termination of SE, there is a subsequent latent period during which animals exhibit a relatively normal EEG and behavior; this period may last for days to weeks. (3) Signaling the end of the latent period is the emergence of spontaneous recurring seizures, which persist for the life of the animal. Seizure frequencies vary from several daily to sporadic seizures two or three times per week or occurring as clusters every 5 to 8 days.[3]

How might an epileptogenic injury (e.g., SE) trigger the subsequent delayed emergence of spontaneous seizures in TLE? The characteristic hippocampal pathology found in human TLE patients, including patterned segmental neuronal loss, sclerosis, and mossy fiber sprouting, can be reproduced in animal models of TLE.[4] Damage caused by SE includes CA3 and CA1 pyramidal cells, hilar mossy cells, and specific types of interneurons; dentate granule cells and CA2 pyramidal neurons tend to be preserved. Neuronal damage induced by SE is not limited to cell death. Altered cell morphology has also been described as a result of SE in neurons that survive this brain injury. This injury-induced anatomical plasticity includes the appearance of mossy fiber sprouting.[5] In addition, SE triggers other biological responses, including an increased rate of neurogenesis in the dentate gyrus,[6] increased activity of microglia, and induction of reactivity in astrocytes.[7]

In addition to the cell death and anatomical changes in neurons and glia described above, SE induces significant functional plasticity in surviving hippocampal neurons. This plasticity is triggered by alterations in transcriptional, translational, and posttranslational regulation of the function of transcription factors, ion channels, neurotransmitter receptors, ion and neurotransmitter transporters, and kinases in many different neuron types in the hippocampus. Notable examples of this type of functional plasticity include alterations in ion channel function, such as calcium currents,[8] hyperpolarization-activated mixed cationic channels[9,10] and potassium channels,[11] and changes in neurotransmitter receptors, including $GABA_A$ receptors[12,13] and glutamate receptors,[14] as well as in chloride transporters[15] and excitatory amino acid transporters.[16–18]

Although important, the role that these anatomical, morphological, pathological, and functional plasticity changes play in hippocampal circuit activity alterations in epileptic brains remains an open question. We simply do not know how the hippocampus becomes hyperexcitable. We also do not know how hyperexcitable cells integrate into a pathologically functioning circuit, contributing to induction of spontaneous recurrent seizures. These critical questions cannot be easily addressed by conventional EEG or single-cell recording techniques. Advancing our knowledge of the mechanisms underlying epilepsy necessarily involves studying the dynamic properties of neurons in situ, as they function in their physiological circuit, with all of the emergent complexities involved in generation of the integrated output of brain regions. In recent years, optical imaging technologies, collectively termed *functional imaging*, have developed rapidly, have provided us with the ability to conduct detailed neuronal circuit characterizations, and constitute perhaps the most powerful tools available to begin to address emergent function in both normal and pathological neuronal circuits. In this chapter, we will describe the basic tools and mechanisms of functional imaging and their application to in vitro hippocampal circuit characterization in naive and epileptic animals.

FUNCTIONAL IMAGING

Why Functional Imaging?

In order to understand network dysfunction in epileptic brain, it is necessary to characterize circuit dynamics comparing naive and epileptic brain. In conventional electrophysiological recording, cellular properties can be characterized in detail for a single cell and cellular interactions can be studied among at most two or three cells. Functional imaging, on the other hand, provides the ability to characterize dynamic properties of multiple cells (>100 cells) simultaneously. Activity can be monitored in individual hippocampal subregions and/or the entire hippocampal circuit. Using functional imaging, differences in signal propagation patterns can be distinguished in naive tissue and tissue from epileptic brain. Multicellular functional imaging also provides the ability to isolate unusual cell behavior in epileptic brain that may not be detected by single-cell recording techniques

simply because the population of such abnormal cells may be small and the chances of targeting the particular population of rare cells for electrophysiological recording may be correspondingly small. In another example, one can map the dynamic connectivity of multiple neurons by sequentially stimulating subsets of neurons optically using either photolysis of caged neurotransmitters or optogenetic approaches. One can then ask whether epileptic brains exhibit different patterns or prevalence of neuronal connectivity compared to naive animals.

Spatial and Temporal Resolution

The results obtained with various functional imaging approaches have the potential to be tremendously informative. However, dynamic imaging of neuronal circuit function is challenging, with achievement of the necessary spatial and temporal resolution required to resolve neuronal responses constituting the most difficult aspect of optical recording. For example, neurons and their processes are small, with a spatial extent measured in micrometers. In contrast, the circuits within which neurons function may extend millimeters to 1 cm or more. Studies examining circuit function must retain cellular resolution while probing areas spanning regions orders of magnitude larger in size (Fig. 15–1). The difficulty of achieving spatial resolution is compounded by the demanding temporal requirements of circuit recordings. The fundamental neuronal activities are action potential (AP) firing and synaptic transmission, with an AP lasting for 0.5–2 ms and a synaptic response lasting for 10–50 ms. To capture these events, any imaging method should have a temporal resolution of 1 kHz or better. To date, no single microscopic imaging approach can fulfill the requirements described above. However, integration of a combination of multiple imaging approaches can begin to accomplish this goal.

Various Types of Functional Imaging

VOLTAGE-SENSITIVE DYE IMAGING

Voltage-sensitive dye imaging (VSDI) monitors neuronal activity though the use of voltage-sensitive fluorescent or nonfluorescent dye molecules that are inserted into the cell membrane.[19,20] Changes in membrane potential are in essence changes in the electrical environment in which the voltage-sensitive dye (VSD) resides (the membrane), and elicit voltage-dependent shifts in the absorption spectra and/or shifts in the fluorescence emission spectra of a VSD. One can monitor AP firing and synaptic transmission using this technique. Optical acquisition systems in VSDI frequently have high temporal resolution exceeding 1 kHz frame rates, a significant strength of this form of imaging. The most commonly used VSD imaging method is wide-field microscopy combined with image acquisition using a fast charge-coupled device (CCD) camera (Table 15–1). Due to this high temporal resolution, the CCD camera must be sensitive enough to detect low light levels since the amount of light captured

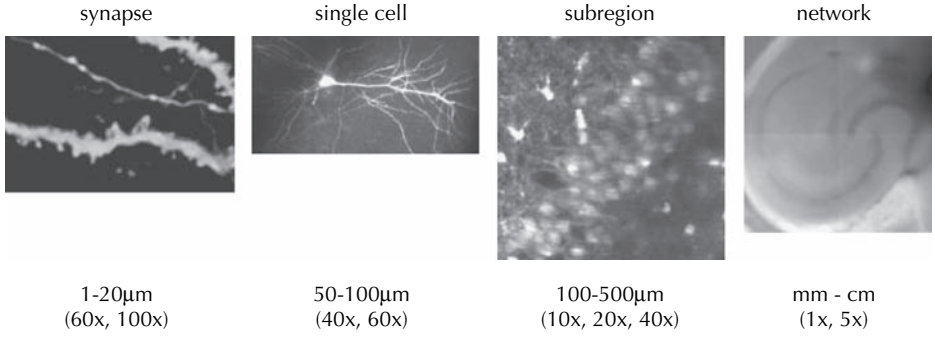

Figure 15–1. Spatial constraints of brain imaging studies. Image examples, typical image size, and microscopy objective lens to be used to study the dynamic function of these components. From left to right: (1) three-dimensional reconstruction of neuronal processes expressing the GFP molecule; (2) CA1 pyramidal cells in a slice filled with a dye; (3) CA3 pyramidal cells in a slice loaded with a calcium indicator dye; (4) hippocampus slice loaded with a VSD.

Table 15–1 **Various Forms of Functional Imaging**

	Experiment Scale	Probes, Loading Method	Microscopy
VSD imaging	Network	Bulk-loading VSD Genetic probe	Wide-field microscopy with fast CCD camera
	Single-cell	Internally injecting by patch pipette Genetic probe	
Calcium imaging	Multicellular	Bulk-loading AM-ester form of dyes Genetic probe	Confocal or two-photon microscopy Fast confocal microscopy with fast CCD camera
	Single-cell, synapse	Internally loading salt form of dyes Genetic probe	
Chloride imaging	Multicellular	Bulk-loading dye Genetic probe	Two-photon microscopy with point scan or line scan
	Single-cell	Internally injecting dyes Transgenic, viral infection	
Uncaging	Network multicellular single-cell synapse	Bath application Internally by patch pipette	Point-scanning device attached to wide-field microscopy, confocal microscopy, or two-photon microscopy UV or two-photon excitation
Channelrhodopsin	Network multicellular single-cell	Genetic	Blue light source (light-emitting diode, laser, display device) attached to a wide-field microscope, confocal microscope, or two-photon microscope

during a 1 ms or shorter epoch (one frame) is limited. The CCD camera also has to have a high readout speed, low dark noise, and low readout noise. In VSD experiments in our laboratory, we use a low-noise back-thinned CCD camera that has a smaller number of pixels (80x80 pixels), achieving an image acquisition rate of 2 kHz.[21] Conventional laser scanning confocal microscopy and multiphoton microscopy are not suitable for VSD imaging of neuronal circuit function due to their slow image acquisition rate (typically 1–10 Hz). Alternatively, multibeam scanning devices such as a spinning disk microscope, a swept-field microscope, and a multibeam multiphoton microscope are, in principle, sufficiently fast to allow VSD imaging.

Most VSDs are lipophilic, and so can be bulk-loaded by applying a dye solution to the tissue. The bulk-loading method is suitable for monitoring network activity with a low-magnification objective lens. However, in this form of recording, it is hard to identify single-cell morphology or dendrites of a particular cell in bulk-loaded slice tissue. Many VSDs also suffer from a low signal-to-noise ratio. Because changes in VSD signal responses to neuronal activity can be as small as 0.1%–1%, event averaging is often utilized to enhance the signal-to-noise ratio. Event averaging usually must be accompanied by triggered stimulation, such as an electrical pulse train of a certain frequency, although in some cases, spontaneous events can be averaged post hoc following an experiment based on event detection algorithms.

In addition, VSDs can be internally loaded into neurons through a patch pipette in a conventional electrophysiology setup. With this loading method, one can study dynamic properties of synaptic transmission within a single neuron. In some cases, neuronal connectivity can be studied using only optical approaches by combining VSD imaging with a photolytic uncaging system.[22] Recently, successful genetically encoded voltage-sensitive probes have been developed.[23] With this new technology,

Table 15–2 **Temporal Characteristics of Various Forms of Imaging**

	Image Size	Temporal Resolution
Wide-field microscopy with a fast CCD camera	80 × 80 pixels	2 kHz
Confocal microscopy and two-photon microscopy	512 × w512 pixels	1–10 Hz
	Line scan	200–500 Hz
Fast confocal microscopy with a fast CCD camera	512 × 512 pixels	25 Hz
Multibeam two-photon microscopy	128 × 128 pixels	500 Hz

one can characterize the activity of a specific neuron at a high magnification and the activity of an entire network at a low magnification within the same system.

CALCIUM IMAGING

Calcium imaging (Cal·I) of hippocampal tissue slices has been ongoing for decades, with the use of multiphoton microscopy in these studies reported since the 1990s.[24,25] Conventional Cal·I uses a calcium indicator dye loaded into cells. Calcium imaging is based on a change in fluorescence emission spectra (e.g., OGB-1 and fluo3) or a change in absorption spectra (e.g., fura-2) of calcium indicator dyes upon calcium ion binding. This process is reversible and follows the chemical equilibrium between cytosolic free Ca^{2+}, the ion free form, and the ion bound form of the calcium indicator dye. There are three major routes of calcium influx into the cytosol in neurons. One is through voltage-gated calcium channels, which, due to the high depolarization threshold of these channels, usually only occurs when neurons fire APs. Another route is through *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic transmission, in which activated receptors conduct large amounts of calcium. Lastly, activation of certain G-protein-coupled receptors may trigger the activation of phospholipase C and the release of inositol trisphosphate (IP3), followed by activation of IP3 receptors in endoplasmic reticulum, causing Ca^{2+} release from internal calcium stores. Due to the high affinity of the calcium indicator for calcium, once Ca^{2+} binds to a calcium indicator dye, even though Ca^{2+} is pumped out of cells and sequestered in intracellular stores continuously, it takes several tens to several hundreds of milliseconds to unbind from the dye and restore dye fluorescence to basal levels. Thus, due to the long-duration response of calcium indicators, Cal·I equipment normally does not require millisecond temporal resolution and conventional research CCD cameras, scanning confocal microscopy, and two-photon microscopy can be used in Cal·I experiments (Table 15–1).

Monitoring neuronal activity from multiple neurons (multicellular imaging) can be accomplished by bulk-loading the acetoxymethyl (AM)-ester form of calcium indicator dyes. Fura-2 AM and OGB-1 AM are widely used. With this loading method, due to the relatively low cytoplasmic dye concentrations achieved (10 μM or less), it is difficult to identify the dendrites and axon of a particular neuron, especially in pyramidal cells in CA regions or granule cells in the dentate gyrus of hippocampal slices. Instead, one can monitor signal changes from the soma of multiple neurons. While the temporal response of Cal·I is slower than that of VSDI, changes in signal corresponding to cellular activity are very high, often 5%–100% in fluorescence during a response. This facilitates monitoring of cellular activity without event averaging. Since fluorescence emission from bulk-loaded slices is weak, we typically use high numerical aperture water-immersion objective lenses with modest magnifications (16x, 20x, and 40x). With these magnifications, it is possible to monitor individual cellular behaviors of entire hippocampal subregions (CA1, CA3, DG, etc.). For reference, a 40x confocal image field could contain 100–300 cells, while a 16x image field could contain 500–1000 neurons, all of which are accessible for imaging responses (Fig. 15–1). Confocal microscopy or two-photon microscopy is preferable for imaging slices at moderate to high spatial resolution since wide-field epifluorescence microscopy with a CCD camera collects fluorescence from the entire slice depth, confounding interpretation of the resulting responses. However, conventional confocal microscopy or two-photon microscopy still suffers from slow image

acquisition speeds (poor temporal resolution) because excitation spots must be scanned pixel by pixel, reducing the typical imaging speed to 1 to 10 Hz frame rates (Table 15–2). In our laboratory, we use an advanced form of confocal microscopy, known as *swept-field microscopy*, which incorporates multibeam scanning to speed acquisition rates, coupled with an electron multiplying CCD (EM-CCD) camera capable of recording at frame rates exceeding 1 kHz. Using this combination of technologies, one can achieve a real experimental acquisition rate of 25 Hz for images with 512x512 pixels or 300 Hz at 128x128 pixels (Table 15–2).

Calcium indicators can also be internally loaded into neurons through patch pipettes using a conventional electrophysiology setup.[26] With this loading method, one can study dynamic properties of fine processes or even activity in individual synaptic spines (Table 15–1). In addition, there are many genetically encoded calcium indicator probes available that can be used to study single-cell activity, multicellular activity, and even network properties.

CHLORIDE ION IMAGING

$GABA_A$ receptor-mediated inhibition depends on maintenance of the intracellular chloride concentration at low levels, 2–10 mM.[27] This is accomplished through the activity of two chloride transporters expressed in neurons: a chloride extruder, potassium chloride cotransporter 2 (KCC2), and a chloride accumulator, sodium-potassium chloride cotransporter 1 (NKCC1). The activity of NKCC1 predominates in the developing central nervous system, and as a consequence, the intracellular chloride concentration is elevated to 10–40 mM[28] and GABA consequently is excitatory. As development progresses, KCC2 transporter function increases, intracellular chloride concentrations decrease, and GABA channel activation therefore elicits hyperpolarization. Chloride transporter expression and function are activity dependent and change during the development of epilepsy.[15] As a consequence, depolarizing GABAergic synaptic responses may be recapitulated in epilepsy. To characterize this phenomenon at the cellular and circuit levels, it is crucial to understand the variation in the intracellular chloride level of individual cells and how this may change in epileptic brains. Monitoring cytosolic chloride levels can be accomplished using chloride ion indicators, including quinoline dyes such as *N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE).[27] MQAE displays fluorescence quenching when dye molecules collide with chloride ions. Thus, at higher chloride concentrations, MQAE exhibits lower fluorescence emission and vice versa. The apparent dissociation constant for MQAE is 40 mM in neuronal cells. As in the case of Cal·I, circuit studies utilizing chloride imaging are most powerful when conducted using moderate magnification. Since the peak absorption wavelength of MQAE is in the ultraviolet (UV) range, imaging tissue continuously causes phototoxicity. Thus, two-photon excitation, with its reduced photodamage, is a preferable way of conducting imaging studies utilizing this dye. Genetic probes have also been developed for chloride imaging and provide a powerful way to characterize both static cytosolic chloride concentrations and variations in these levels during dynamic activity.[28,29]

Various Stimulation Methods in Imaging Studies

ELECTRICAL STIMULATION

The most simple and direct way of characterizing the input-output relationship of hippocampal circuits is to electrically stimulate a specific pathway and image the response from a specific subregion. One can control stimulus frequency and magnitude and conduct repeated trials to obtain averaged responses. For example, electrical stimulation in the direct entorhinal cortical afferent to area CA1, the temporoammonic pathway, induces neuronal activity in the dendrites of CA1 pyramidal neurons.[21]

SPONTANEOUS AND DRUG-INDUCED NETWORK ACTIVITY

Another way of characterizing network circuitry is to use a model that exhibits spontaneous network activity. For example, developing brains exhibit spontaneous network activity called *early network oscillations* (ENOs) and *giant depolarizing potentials* (GDPs). These network oscillations differ from an epileptic form of activity yet share many of its characteristics. For example, CA1 and CA3 neurons

exhibit synchronous network oscillations that are driven or governed by GABAergic neuronal activity. Drug-induced epileptiform activity (e.g., 4-AP and/or bicuculline-induced activity) can also be monitored using Cal·I approaches.[30]

PHOTOLYSIS OF CAGED COMPOUNDS

Synaptic input can be mimicked by using so-called caged neurotransmitters. Caged compounds usually contain a UV light-sensitive chemical group that, upon irradiation with UV light, breaks and releases a neurotransmitter (or other messenger molecules) as a product of the photo reaction. Caged compounds can be applied in the extracellular (imaging) solution during an experiment and then released with UV light being applied to small area, producing an uncaged neurotransmitter.[31] When this method is combined with two-photon excitation instead of UV irradiation, neurotransmitter is released in a three-dimensionally focused volume, exactly mimicking the spatial dynamics of synaptic neurotransmitter release at a spine (Table 15–1). If this technology is combined with high-resolution two-photon microscopy, the functional mapping of synaptic activity can be obtained. When it is combined with lower- to moderate-magnification objective lenses and Cal·I or VSD imaging, one can study cellular connectivity among multiple cells.

CHANNELRHODOPSIN/OPTOGENETIC APPROACHES

Channelrhodopsin (CR) is a light-sensitive cationic channel isolated from alga that can be introduced into neurons either by viral transfection or by construction of transgenic mice.[32] Because its expression is genetically encoded, CR can be expressed in restricted cell types when under the control of cell-specific promoters. Channelrhodopsin is activated by irradiating blue light, which, when sufficiently strong, can induce AP firing. Unlike extracellular electrode stimulation, CR stimulation is performed remotely by light and causes no physical damage to the tissue (Table 15–1). By appropriately designing the optical system, one could activate multiple targets within a tissue without significant delay.[33] Combining the CR stimulation method with functional imaging requires careful consideration of the absorption spectra and emission spectra of each element (CR, VSD, calcium indicator, etc.), as well as the use of appropriate optical filters.

IMAGING THE EPILEPTIC BRAIN: REPRESENTATIVE EXAMPLES

Dentate Gyrus Gating Function

In normal brain, the hippocampus receives input from the neocortex via the entorhinal cortex (EC; Fig. 15–2). In the main canonical trisynaptic excitatory hippocampal network, signals propagate from layer II of the EC to the dentate gyrus (DG) through the *perforant* pathway, then from the granule cells of the DG to CA3 via the output axons of these neurons, the mossy fibers. Area CA3 pyramidal neurons interconnect reciprocally via recurrent axon collaterals and, in addition to sending output to the contralateral hippocampus, project to

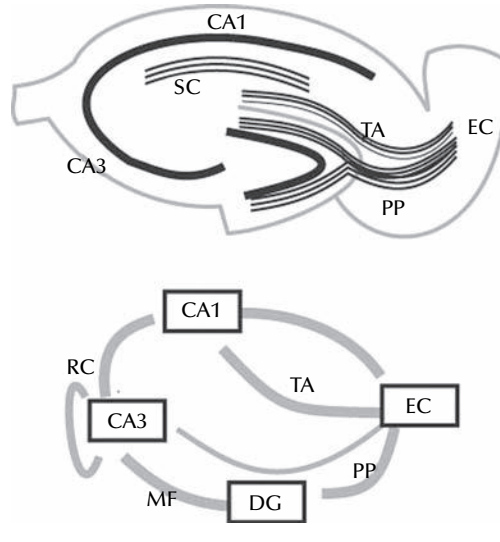

Figure 15–2. Schematic illustration of hippocampus structure in a horizontal slice and corresponding major excitatory pathways. In the trisynaptic pathway (TSP), the signal propagates from layer II of the entorhinal cortex (EC) to the dentate gyrus (DG) through the perforant path (PP), then from the DG to CA3 via mossy fibers (MF). CA3 neurons interconnect via a recurrent network (RC) and to CA1 through the Schaffer collaterals (SC). CA1 output to layer 5 of the EC occurs both directly and indirectly via the subiculum. The hippocampus contains another excitatory input, called the *temporoammonic* (TA) pathway, in which signal propagates from layer 3 of the EC to directly to CA1 distal dendrites.

pyramidal neurons in area CA1 through an axon collateral termed the *Schaffer* collateral pathway. CA1 outputs to layer 5 of the EC directly or indirectly via the subiculum. This signal transduction loop is referred to as the *trisynaptic pathway* (TSP; Fig. 15–2), although there are actually four to five synapses in a more comprehensive excitatory limbic loop. This type of excitatory interconnectivity is inherently unstable and prone to uncontrolled activation, so intrinsic regulators of unchecked activity are also present in the hippocampus. As an example of one such regulator, in the normal hippocampus, the DG → CA3 excitatory relay is limited (gated or filtered) by complex inhibitory mechanisms as well as by the low intrinsic excitability of dentate granule cells. These inhibitory mechanisms include feedforward GABA synaptic inhibition, feedback inhibition, and tonic GABAergic currents.[34]

How can the properties of this EC → DG → CA3 circuit be visualized with functional imaging? Previous work in our laboratory used fluorescence VSD (RH795 and/or JPW 3031) to characterize hippocampal circuit function in brain slices prepared from normal adult rats.[35,36] The VSD was bulk-loaded and imaged with a 4x objective lens using a fast CCD camera at a frame rate of 2 kHz. The VSD response to perforant path stimulation (100 Hz, four pulses) was imaged in the DG/CA3 area. Upon stimulation, the inner molecular layer of the DG showed a strong excitatory response. This excitatory response spread to the dentate granule neuron cell body layer and also to the proximal hilus, but only minimally activated the CA3 pyramidal cell layer (Fig. 15–3A). This indicates that dentate granule cells did not fire APs, and the response to perforant path activation was predominantly a subthreshold postsynaptic response. If granule cells did fire synchronous APs, the excitatory response to perforant path activation should have propagated through the mossy fibers and subsequently excited CA3 cells. The lack of dentate granule cell firing in response to perforant path activation was verified using Cal·I (Fig. 15–3B). Calcium transients in bulk-loaded neuronal somata only occur following AP firing, and are not seen with subthreshold depolarization such as an excitatory postsynaptic potential (EPSP). Under normal control conditions, only a small proportion of dentate granule cells exhibit calcium transients following perforant path activation (3%–5% on average). However, if inhibition is blocked with the $GABA_A$ antagonist picrotoxin, this proportional activation of dentate granule cells increases to 60%–65% following perforant path activation (Fig. 15–3B, middle panel). Despite the strong electrical stimulation, dentate granule cells are regulated by strong inhibitory mechanisms. These experiments demonstrate the inhibition-dependent "gating" aspect of DG function.

Breakdown of the dentate gate has been hypothesized to be a primary contributor to seizure generation in epilepsy. In animals with fully developed epilepsy, we have investigated whether the dentate gating function was disrupted. Surprisingly, the gating function was intact in these chronically affected animals.[35] This finding is consistent with several TLE studies[37,38] that indicated that inhibition within the dentate remains operative and suppresses seizure propagation from the entorhinal cortex. In contrast, recent studies from our laboratory, examining DG function in animals in the latent period preceding epilepsy onset, 1 week after pilocarpine-induced SE, showed markedly disrupted dentate gating functions[15] (Fig. 15–3B, bottom panel), with 60% of cells activating in response to perforant path stimulation, comparable to the results of control studies in the presence of a GABA antagonist (Fig. 15–3B, middle panel). This suggests that the gating function is corrupted by SE-induced processes and remains corrupted during the latent period (7 days after SE), only recovering at the point of development of chronic epilepsy (1 month or longer after SE).

Calcium Imaging of Epileptiform Activity

Network oscillations are associated with synchronous activity of large numbers of neurons and occur as a component of both normal and pathological circuit function. Investigation of individual cell behavior during network activity is critical in understanding the mechanisms regulating the generation of these types of responses. Voltage-sensitive dye imaging can capture network activity; however, it is not widely used in this context due to the necessity of event averaging and difficulty in identifying individual cell morphology, both constraints

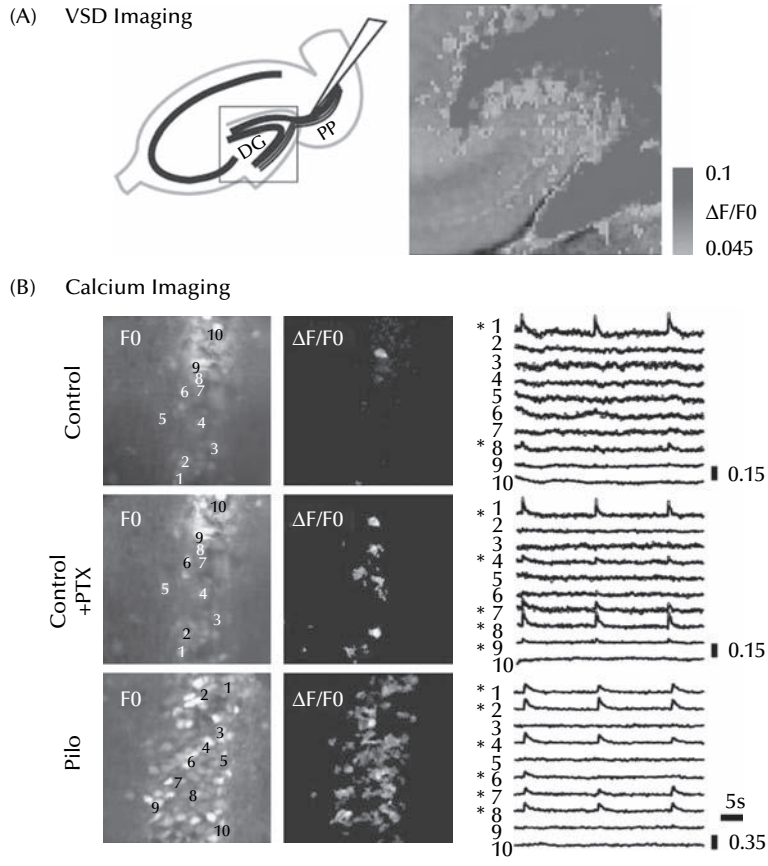

Figure 15–3. Dentate gyrus (DG) gate function. **A.** A schematic of the hippocampus illustrates the major afferent pathways to the hippocampus and the position of the stimulation electrode used to activate the perforant path (PP). A VSD image of a hippocampal slice from a control animal, containing most of each dentate blade and CA3. A snapshot at 25 ms after a burst stimulus applied to the PP generates a strong voltage response (dark color) in the DG that is minimally transmitted as excitation in CA3. **B.** Upper panels: Calcium imaging of the DG from a control animal and an experimental animal 7 days after injection with pilocarpine. The PP pathway was stimulated while imaging the response from calcium indicator–loaded tissues. Images on the left show baseline fluorescence, and second images show normalized fluorescence changes after electrical stimulation. Traces show normalized fluorescence changes on a randomly selected population of 10 cells from the images. Asterisks (*) indicate cells that responded to the electrical stimulation. Despite strong electrical stimulation, the majority of dentate granule cells did not have a response in control animal. Middle panels: In the presence of 100 μM picrotoxin in the imaging solution, the same electrical stimulation recruited more responsive cells, demonstrating disruption of inhibitory mechanisms. Lower panel: In a slice prepared from an animal 1 week after pilocarpine injection (during the latent period), there was a significant increase in dentate granule cell activation following PP stimulation. (Image courtesy of Dr. Cho.)

imposed by the low signal-to-noise ratios inherent in this mode of imaging. Calcium imaging, on the other hand, has proven to be useful in monitoring individual cell activity in cortical and hippocampal structures during synchronous circuit activity.[39–47] The hippocampus and cortex of young rodents (P3–5) generate recurrent spontaneous network oscillations, which are hypothesized to contribute to the consolidation of circuit connectivity. Some of these events are driven by GDPs[48] and other variants of network oscillation also occur, usually at a developmentally earlier stage.[41,42] Cell firing during these synchronous network bursts occurs at low frequencies (0.5 Hz or less). As was observed in previous work,[41,42] Cal·I can capture this network activity in simultaneous recordings of hundreds of individual cells. Figure 15–4 show an example of spontaneous synchronous network bursting captured

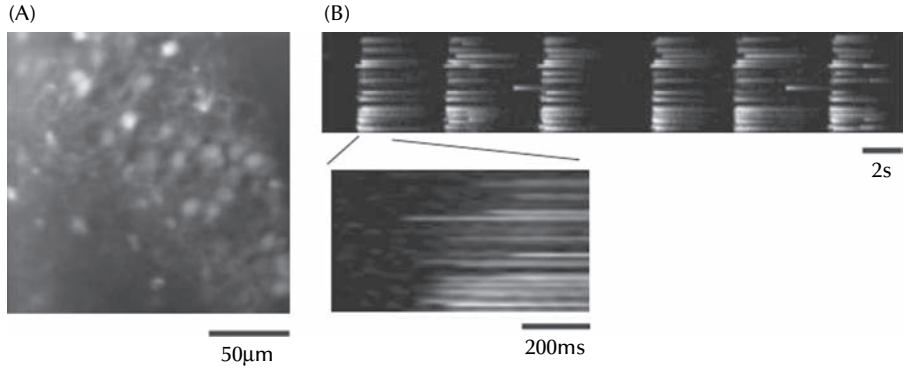

Figure 15–4. Calcium imaging of synchronous network activity captured in area CA3b from a slice prepared from a mouse aged P4. **A.** Snapshot of a calcium dye–loaded slice. **B.** The change in fluorescence was measured for multiple cells and shown as a raster plot. The insert shows a zoomed image of the onset of a synchronous network burst. The images were captured at 100 Hz by a fast confocal microscope equipped with an EM-CCD camera (128x128 pixels). Note the stereotypic activation order for individual cells in multiple bursts.

in the CA3b area from a slice prepared from P4 mouse (a raster plot depicts the responses of multiple cells). The images were captured at a 100 Hz frame rate using a fast confocal microscope equipped with an EM-CCD camera (128x128 pixel images). The spontaneous synchronous network events occurred repeatedly, that is, six times in 30 sec traces. These calcium transients arise from neurons firing APs, triggering the opening of voltage-gated calcium channels. The inset shows a raster plot zoomed on the rising phase of the signal change for the first network burst. The fast acquisition speed (100 Hz) was necessary to resolve differences in onset time of individual cell responses during synchronous network events. By analyzing the onset time of calcium transients for multiple cells for multiple network bursts, we determined that activation was not a random stochastic event. In contrast, bursts had a stereotypic, deterministic firing order, with individual neurons playing defined, repeated roles in burst generation. This can be visualized by sorting the raster plot by the activated order in the first network burst and determining how this trend was maintained in subsequent network bursts. This example illustrates that some cells are always activated earlier than others and some cells are always activated later than others. We hypothesize that intrinsic cellular properties and/or intrinsic connectivity determine these differing roles for individual neurons. In future studies, it will be interesting to determine if there is any association between onset time and type of cell, that is, between interneurons or principal cells. Given the resemblance of these early synchronous network bursts in the developing brain to interictal bursts characteristic of epilepsy, information gleaned from these studies may inform additional efforts to dissect the burst structure of epileptiform events in slices prepared from epileptic animals.

CONCLUSIONS

Development and application of integrated dynamic imaging approaches examining neuronal circuit function shows significant promise in furthering our understanding of the mechanisms underlying epileptogenesis, epilepsy, and seizure generation.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Scorza FA, Arida RM, Naffah-Mazzacoratti Mda G, Scerni DA, Calderazzo L, Cavalheiro EA. The pilocarpine model of epilepsy: what have we learned? *An Acad Bras Cienc*. 2009;81(3):345–365.

2. Curia G, Longo D, Biagini G, Jones RS, Avoli M. The pilocarpine model of temporal lobe epilepsy. *J Neurosci Methods*. 2008;172(2):143–157.
3. Goffin K, Nissinen J, Van Laere K, Pitkanen A. Cyclicity of spontaneous recurrent seizures in pilocarpine model of temporal lobe epilepsy in rat. *Exp Neurol*. 2007;205(2):501–505.
4. Sarkisian MR. Overview of the current animal models for human seizure and epileptic disorders. *Epilepsy Behav*. 2001;2(3):201–216.
5. Parent JM, Lowenstein DH. Mossy fiber reorganization in the epileptic hippocampus. *Curr Opin Neurol*. 1997;10(2):103–109.
6. Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J Neurosci*. 1997;17(10):3727–3738.
7. Wetherington J, Serrano G, Dingledine R. Astrocytes in the epileptic brain. *Neuron*. 2008;58(2):168–178.
8. Remy S, Beck H, Yaari Y. Plasticity of voltage-gated ion channels in pyramidal cell dendrites. *Curr Opin Neurobiol*. 2010;20(4):503–509.
9. Shah MM, Anderson AE, Leung V, Lin X, Johnston D. Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. *Neuron*. 2004;44(3):495–508.
10. Jung S, Bullis JB, Lau IH, Jones TD, Warner LN, Poolos NP. Downregulation of dendritic HCN channel gating in epilepsy is mediated by altered phosphorylation signaling. *J Neurosci*. 2010;30(19):6678–6688.
11. Bernard C, Anderson A, Becker A, Poolos NP, Beck H, Johnston D. Acquired dendritic channelopathy in temporal lobe epilepsy. *Science*. 2004;305(5683):532–535.
12. Pathak HR, Weissinger F, Terunuma M, Carlson GC, Hsu FC, Moss SJ, et al. Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. *J Neurosci*. 2007;27(51):14012–14022.
13. Kapur J. Acute molecular and fundtional charges in neurotransmission during early status epilepticus. *Epilepsia*. 2009;50:180–181.
14. Doherty J, Dingledine R. Reduced excitatory drive onto interneurons in the dentate gyrus after status epilepticus. *J Neurosci*. 2001;21(6):2048–2057.
15. Pathak HR, Weissinger F, Terunuma M, Carlson GC, Hsu FC, Moss SJ, et al. Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. J Neurosci. 2007;27(51):14012–14022.
16. Crino PB, Jin H, Shumate MD, Robinson MB, Coulter DA, Brooks-Kayal AR. Increased expression of the neuronal glutamate transporter (EAAT3/EAAC1) in hippocampal and neocortical epilepsy. *Epilepsia*. 2002;43(3):211–218.
17. Eid T, Ghosh A, Wang Y, Beckstrom H, Zaveri HP, Lee TSW, et al. Recurrent seizures and brain pathology after inhibition of glutamine synthetase in the hippocampus in rats. *Brain*. 2008;131:2061–2070.
18. Heilig C, Koliatsos V, Sepkuty J, Heilig K, Chen SL, Xiang MH, et al. Transgenic mouse model for the human GLUT1-deficiency syndrome. *Ann Neurol*. 2004;56:S12.
19. Loew LM, Cohen LB, Dix J, Fluhler EN, Montana V, Salama G, et al. A naphthyl analog of the aminostyryl pyridinium class of potentiometric membrane dyes shows consistent sensitivity in a variety of tissue, cell, and model membrane preparations. *J Membr Biol*. 1992;130(1):1–10.
20. Obaid AL, Loew LM, Wuskell JP, Salzberg BM. Novel naphthylstyryl-pyridium potentiometric dyes offer advantages for neural network analysis. *J Neurosci Methods*. 2004;134(2):179–190.
21. Carlson GC, Coulter DA. In vitro functional imaging in brain slices using fast voltage-sensitive dye imaging combined with whole-cell patch recording. *Nat Protocols*. 2008;3(2):249–255.
22. Xu X, Olivas ND, Levi R, Ikrar T, Nenadic Z. High precision and fast functional mapping of cortical circuitry through a combination of voltage sensitive dye imaging and laser scanning photostimulation. *J Neurophysiol*. 2010;103(4):2301–2312.
23. Akemann W, Mutoh H, Perron A, Rossier J, Knopfel T. Imaging brain electric signals with genetically targeted voltage-sensitive fluorescent proteins. *Nat Methods*. 2010;7(8):643–649.
24. Yuste R, Katz LC. Control of postsynaptic Ca^{2+} influx in developing neocortex by excitatory and inhibitory neurotransmitters. *Neuron*. 1991;6(3):333–344.
25. Smetters D, Majewska A, Yuste R. Detecting action potentials in neuronal populations with calcium imaging. *Methods*. 1999;18(2):215–221.
26. Yasuda R, Nimchinsky EA, Scheuss V, Pologruto TA, Oertner TG, Sabatini BL, et al. Imaging calcium concentration dynamics in small neuronal compartments. *Sci STKE*. 2004;2004(219):pl5.
27. Marandi N, Konnerth A, Garaschuk O. Two-photon chloride imaging in neurons of brain slices. *Pflugers Archiv (Eur J Physiol)*. 2002;445(3):357–365.
28. Berglund K, Schleich W, Krieger P, Loo LS, Wang D, Cant NB, et al. Imaging synaptic inhibition in transgenic mice expressing the chloride indicator, Clomeleon. *Brain Cell Biol*. 2006;35(4–6):207–228.
29. Kuner T, Augustine GJ. A genetically encoded ratiometric indicator for chloride: capturing chloride transients in cultured hippocampal neurons. *Neuron*. 2000;27(3):447–459.
30. Fellin T, Gomez-Gonzalo M, Gobbo S, Carmignoto G, Haydon PG. Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices. *J Neurosci*. 2006;26(36):9312–9322.
31. Haydon PG, Ellis-Davies GC. Ultra-high-speed photochemical stimulation of neurons. *Nat Methods*. 2005;2(11):811–812.
32. Zhang F, Wang LP, Boyden ES, Deisseroth K. Channelrhodopsin-2 and optical control of excitable cells. *Nat Methods*. 2006;3(10):785–792.
33. Shoham S, O'Connor DH, Sarkisov DV, Wang SS. Rapid neurotransmitter uncaging in spatially defined patterns. *Nat Methods*. 2005;2(11):837–843.
34. Coulter DA, Carlson GC. Functional regulation of the dentate gyrus by GABA-mediated inhibition. - *Prog Brain Res*. 2007;163:235–243.
35. Ang CW, Carlson GC, Coulter DA. Massive and specific dysregulation of direct cortical input to the hippocampus in temporal lobe epilepsy. *J Neurosci*. 2006;26(46):11850–11856.
36. Ang CW, Carlson GC, Coulter DA. Hippocampal CA1 circuitry dynamically gates direct cortical inputs preferentially at theta frequencies. *J Neurosci*. 2005;25(42):9567–9580.

37. Sloviter RS, Zappone CA, Harvey BD, Frotscher M. Kainic acid–induced recurrent mossy fiber innervation of dentate gyrus inhibitory interneurons: possible anatomical substrate of granule cell hyper-inhibition in chronically epileptic rats. *J Comp Neurol*. 2006;494(6):944–960.
38. Wu K, Leung LS. Enhanced but fragile inhibition in the dentate gyrus in vivo in the kainic acid model of temporal lobe epilepsy: a study using current source density analysis. *Neuroscience*. 2001;104(2): 379–396.
39. Garaschuk O, Linn J, Eilers J, Konnerth A. Large-scale oscillatory calcium waves in the immature cortex. *Nat Neurosci*. 2000;3(5):452–459.
40. Stosiek C, Garaschuk O, Holthoff K, Konnerth A. In vivo two-photon calcium imaging of neuronal networks. *Proc Natl Acad Sci USA*. 2003;100(12):7319–7324.
41. Allene C, Cattani A, Ackman JB, Bonifazi P, Aniksztejn L, Ben-Ari Y, et al. Sequential generation of two distinct synapse-driven network patterns in developing neocortex. *J Neurosci.* 2008;28(48):12851–12863.
42. Allene C, Cossart R. Early NMDA receptor-driven waves of activity in the developing neocortex: physiological or pathological network oscillations? *J Physiol* (*Lond*). 2010;588(1):83–91.
43. Crepel V, Aronov D, Jorquera I, Represa A, Ben-Ari Y, Cossart R. A parturition-associated nonsynaptic coherent activity pattern in the developing hippocampus. *Neuron*. 2007;54(1):105–120.
44. Sasaki T, Kimura R, Matsuki N, Ikegaya Y. Integrative spike dynamics of rat CA1 neurons: an in situ multineuronal imaging study. *J Physiol.* 2006;574(1):195–208.
45. Sasaki T, Matsuki N, Ikegaya Y. Metastability of active CA3 networks. *J Neurosci*. 2007;27(3):517–528.
46. Sasaki T, Takahashi N, Matsuki N, Ikegaya Y. Fast and accurate detection of action potentials from somatic calcium fluctuations. *J Neurophysiol*. 2008;100(3): 1668–1676.
47. Takahashi N, Sasaki T, Usami A, Matsuki N, Ikegaya Y. Watching neuronal circuit dynamics through functional multineuron calcium imaging (fMCI). *Neurosci Res*. 2007;58(3):219–225.
48. Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev*. 2007;87(4):1215–1284.

Chapter 16

Normal and Pathological High-Frequency Oscillations

Richard J. Staba

During natural waking and sleep behavior, the occurrence of spontaneous rhythmic field potentials, for example theta (3–8 Hz), gamma (30–80 Hz), and high-frequency oscillations (HFOs; 80–600 Hz), correspond with an increase in the rate and synchrony of neuronal firing. Compared to theta and gamma oscillations, networks supporting the generation of HFOs can be smaller in size and events shorter in duration, but the increase in neuronal firing and synchrony of discharges that occurs on a temporal scale of a few milliseconds or less can be much greater. Because HFOs can facilitate synaptic transmission through local networks, these events are implicated in information processing and consolidation of memory. Alterations to neuronal networks associated with epilepsy can also generate abnormal or pathological HFOs (pHFOs) that are believed to reflect fundamental neuronal disturbances associated with brain areas capable of generating spontaneous epileptic seizures. However, distinguishing normal HFOs from pHFOs is not always simple, particularly in studies involving intracranial brain recordings in presurgical patients with medically refractory epilepsy. This chapter will describe some of the spatial and temporal properties of HFOs observed in normal and epileptic mammalian brain and roles HFOs could play in normal brain function and epilepsy. Important to any description of HFOs are the putative mechanisms that support the generation of normal HFOs and pHFOs, and how pHFOs could be clinically useful in the treatment of epilepsy.

NORMAL HIGH-FREQUENCY OSCILLATORY NETWORK ACTIVITY

Spontaneous HFOs

In limbic structures of several mammalian species, including rodents, bats, nonhuman primates, and humans, spontaneous HFOs termed *ripples* contain spectral power between 80 and 200 Hz and have a duration of tens to hundreds of milliseconds.[1–4] The largest-amplitude ripples are found within the pyramidal cell layer of hippocampal subfield CA1 (Fig. 16–1A) and are also present in CA3, subiculum, entorhinal cortex, and amygdala.[5,6] In vivo studies in rodents have not observed normal ripples in the granule cell layer of dentate gyrus, although a study in cats observed ripple-frequency "minispindles" that occurred in CA1 but were also present in the hilus of the dentate gyrus.[7]

Hippocampal ripples occur more frequently during awake immobility and slow wave sleep (SWS) than in states characterized in animals by prominent theta rhythms such as exploratory behavior and rapid eye movement (REM) sleep. During episodes of SWS, hippocampal ripples can occur bilaterally and are often associated with large-amplitude slow waves called *sharp waves* (SPWs) generated in hippocampal CA3 (Fig. 16–1A).[8] Multielectrode studies show that there is strong coherence between ripples recorded in CA1 over distances of 4 or 5 mm suggesting that neuronal activity coordinated on a temporal scale of several milliseconds can occur across large areas of hippocampus.[5,9,10]

Neocortical HFOs

Spontaneous ripple-frequency HFOs are also present in neocortex. In cats, neocortical HFOs occur most frequently during episodes of SWS and states of ketamine anesthesia, and their appearance in isolated cortical tissue suggests that intracortical circuits can support the generation of HFOs.[11] Neocortical HFOs are often associated with the electroencephalogram (EEG) depth-negative component of the

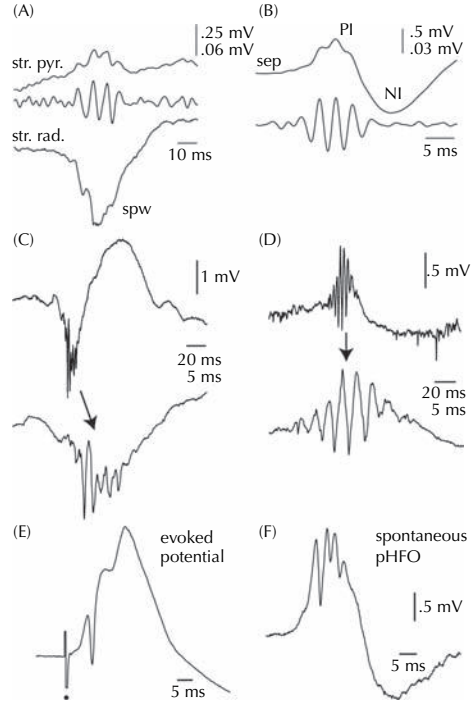

Figure 16–1. Examples of normal HFO and pHFOs. **A.** Spontaneous ripple recorded in CA1 of normal rat. The ripple is present in the wide bandwidth (0.1 Hz–1 kHz; top) and the corresponding bandpass filter (80–200 Hz; middle) traces recorded just above the pyramidal cell layer (str. pyr.). A sharp wave (spw) is shown in the wide-bandwidth trace recorded below the pyramidal cell layer within the stratum radiatum (str. rad.; bottom). **B.** Averaged somatosensory evoked potential (sep; 1 Hz–2 kHz; top trace) recorded in rat barrel cortex during contralateral whisker stimulation. The bandpass filter trace (200–600 Hz; bottom) more clearly shows the fast ripple-frequency HFO superimposed on the earliest component of the biphasic positive-negative (P1-N1) slow wave. **C.** Wide-bandwidth (1 Hz–3 kHz; top trace) EEG interictal spike associated with a pHFO recorded in entorhinal cortex ipsilateral to seizure onset in a patient with MTLE. The pHFO occurs on the descending limb of the depth-negative component of the interictal spike. The bottom trace more clearly shows the pHFO on a shorter time scale. **D.** Spontaneous pHFO in the absence of an EEG interictal spike. The pHFO was recorded in subiculum ipsilateral to seizure onset in a patient with bilateral MTLE. **E.** Averaged evoked potential recorded in human epileptic hippocampus during electrical stimulation of the ipsilateral entorhinal cortex. The evoked response consists of a short-latency population spike superimposed on a large-amplitude population postsynaptic potential. A stimulation artifact (denoted by the black circle) precedes the onset of the evoked potential by ~5 ms. **F.** Wide-bandwidth (1 Hz–3 kHz) spontaneous pHFO recorded from the same patient and microelectrode as in panel E. Superimposed on the EEG interictal spike is a pHFO that strongly resembles a burst of population spikes.

neocortical slow wave oscillation (0.5–1 Hz). Coherence among neocortical HFOs is strong over distances up to 10 mm within individual gyri, while the coherence weakens between HFOs in different gyri.[11]

In rat and human neocortex, somatosensory evoked potentials (SEPs) are associated with HFOs that contain spectral frequencies between 200 and 600 Hz (Fig. 16–1B). In rats, rapid mechanical stimulation of the vibrissae or electrical stimulation of the thalamic ventrobasal nuclei can evoke HFOs in somatosensory barrel cortex.[12–14] In humans, peripheral nerve stimulation elicits SEPs that contain HFOs with different components of the HFO arising from thalamic and cortical circuits.[15–20]

Sensory-evoked neocortical HFOs are typically superimposed on the earliest components of the biphasic positive-negative SEP (Fig. 16–1B) that can propagate in phase over several millimeters in rat barrel cortex.[13] Simultaneous stimulation of individual vibrissae evokes HFOs that propagate across barrel cortex and can constructively interact in a fashion that results in a supralinear summation of HFOs within sites of interaction.[21,22] These latter studies suggest that the locally facilitated HFO response could reflect a recruitment of additional neurons that discharge due to the in-phase interactions between propagating HFOs.

Neuronal Correlates of HFOs in Normal Mammalian Brain

Much of what is known regarding the neuronal activity associated with ripples derives from in vivo wide-bandwidth recordings in rat hippocampus. In CA1 of behaving rats, the SPW-ripple complex is associated with a significant increase in firing from pyramidal cells and several types of interneurons. Pyramidal cells discharge during the trough of the extracellular ripple oscillation but do not fire during each cycle of the ripple.[23] Interneurons such as basket cells that provide perisomatic inhibition to pyramidal cells can fire at ripple frequencies, and the timing of their discharges coincides closely with those from pyramidal cells.[24] Furthermore, recording from pyramidal cells reveals the presence of an intracellular oscillatory potential that corresponds with extracellular ripple oscillation and is likely mediated by chloride ions due to the activation of $GABA_A$ receptors from discharging basket cells.[9] These latter data suggest that hippocampal ripples reflect fast inhibitory postsynaptic potentials (IPSPs) that can powerfully regulate the firing and timing of pyramidal cell discharges.

Excitatory and inhibitory neurons are also involved in the generation of neocortical HFOs. Fast-spiking cells, presumably GABA-containing neurons, discharge bursts of action potentials at intraburst intervals that correspond with the frequency of the extracellular HFO.[11] Fast-spiking cell discharges can precede those from regular spiking cells, which occur during the trough of the extracellular HFO.[25] Similar to the coordinated pattern of firing between pyramidal cells and interneurons during hippocampal ripples, neocortical HFOs could reflect IPSPs from fast-spiking cells that regulate the timing and firing of regular spiking cells.

Mechanisms Generating Normal HFOs

The single-neuron studies discussed in the preceding section indicate that inhibitory networks could play an important role during hippocampal ripples and neocortical HFOs. Inhibitory interneurons can entrain pyramidal cells or other interneurons into a rhythmic pattern of firing,[26] and it is possible that phasic activation of hippocampal interneurons by external input could produce rhythmic IPSPs that regulate the timing of pyramidal cell discharges.[9] In contrast, some studies have shown that blocking $GABA_A$ receptor activation does not abolish spontaneous hippocampal ripples or evoked neocortical HFOs.[27,28] However, under conditions of reduced inhibition, ripples contain higher spectral frequencies and significantly longer durations, and can be associated with epileptiform discharges, all of which raises the question of whether ripples under these conditions in vitro are the same as those in vivo. Furthermore, blocking $GABA_A$-mediated inhibition in a hippocampal slice can generate ripple-frequency HFOs in the isolated dentate gyrus that reflect synchronously bursting granule cells.[29] While there is no direct evidence for ripples in the normal dentate gyrus, studies show that abnormal ripple-frequency HFOs do

occur in the epileptogenic dentate gyrus of epileptic rats.[30,31]

Electrotonic coupling mediated through gap junctions is another mechanism that could support the generation of HFOs. In vitro studies show that chemical agents that interfere with gap junction communication between hippocampal neurons suppress ripples, while manipulations intended to increase gap junction conductance are associated with an increase in ripples.[32] Furthermore, SPW-ripple complexes occur less frequently in hippocampal slices from mice deficient in a gene encoding a neuronal gap junction subunit than in tissue slices from littermate controls.[33] These in vitro data are consistent with the suppression of CA1 ripples in vivo in rats administered halothane anesthesia, which can reduce electronic coupling.[9] In addition, computer simulations predict the generation of ripples in hippocampal networks that contains axon-to-axon gap junctions between pyramidal cells.[34] These data suggest that electrical signaling through gap junctions may contribute to the synchronous neuronal discharges associated with ripples.

Nonsynaptic mechanisms such as ephaptic or field effects could also be involved with the generation of HFOs.[35,36] Ephaptic interactions arise when depolarizing currents associated with neuronal action potentials generate an electric field in the extracellular space that depolarizes adjacent neurons.[37] The probability that ephaptic interactions could trigger action potentials in nearby neurons increases if neurons are in close proximity to each other and neighboring neurons are already close to firing threshold. Field effects occur on a larger spatial scale and can arise when local populations of neurons generate large-amplitude field potentials, such as SPWs, that depolarize nearby inactive neurons.[38] Given the organized parallel arrangement of principal neurons in hippocampus and neocortex, the summation of extracellular currents across local principal cell networks could be sufficient to depolarize and coordinate the firing during hippocampal ripples and neocortical HFOs.

Physiological Role of HFOs

Spontaneous ripples have been implicated in information transfer between hippocampal and neocortical structures during sleep.[39] During SWS, the large-scale neuronal activation associated with SPWs and the increase in precisely timed discharges of pyramidal cells during ripples provide ideal conditions for synaptic transmission to extrahippocampal sites. In vitro evidence indicates that SPW-like stimulation is effective in inducing long-term potentiation in deep layers of entorhinal cortex,[40] which are cellular layers that receive hippocampal output and project to neocortical sites. In addition, there is evidence that ripples are temporally coupled with forebrain spindle oscillations that occur regularly during episodes of SWS.[41] Coordinated discharges along hippocampal and neocortical pathways could be related to processes associated with memory consolidation when short-term hippocampal memory traces are transferred to neocortical networks for long-term storage. A recent study in patients with medically refractory epilepsy found a significant correlation between ripples in hippocampus and rhinal cortex,[42] similar to the coherence of ripples between hippocampus and parahippocampal structures in rats.[5] In this patient study, rates of ripples in rhinal cortex measured after a brief daytime nap correlated with the number of successfully recalled items learned during a prior cognitive task. While these latter data are consistent with the possible role of normal ripples in memory consolidation, not all HFOs in the epileptic brain are normal.

ABNORMAL HFOS IN EPILEPSY

Spatiotemporal Properties of Pathological HFOs

In animal models of chronic limbic epilepsy, HFOs with spectral frequencies between 250 and 600 Hz, termed *fast ripples*, occur in dentate gyrus, subfields CA1 and CA3, subiculum, and entorhinal cortex (Figs. 16–1C, 16–1D). Fast ripples are found in rats that exhibit recurrent spontaneous seizures, but not in rats that have been subjected to an epileptogenic insult, such as status epilepticus, that do not exhibit spontaneous epileptic seizures.[43] These findings and the unique association between interictal fast ripples and sites of seizure onset indicate that fast ripples are pathological.[44]

In the intrahippocampal kainate model of mesial temporal lobe epilepsy (MTLE), in addition to fast ripples, interictal ripple-frequency HFOs can be found in the epileptogenic dentate gyrus, an area where normal ripples do not occur in normal rats (Fig. 16–2).[30] Similar to fast ripples, ripple-frequency HFOs appeared within days to weeks after kainate injection, but only in rats that later exhibit spontaneous seizures. In addition, shorter latencies to the first appearance of fast ripples or ripple-frequency HFOs in dentate gyrus correlated with shorter latencies to the first spontaneous seizure.[30] These data indicate that spectral frequency alone is not sufficient for distinguishing normal ripples from ripple-frequency pHFOs, but like fast ripples, ripple-frequency oscillations in epileptogenic dentate gyrus are also pathological. Importantly, these finding indicate that the occurrence and location of pHFOs can predict, although are not required for, the development of seizures after an epileptogenic insult and thereby provide a biomarker for epileptogenesis.

It has been proposed that pHFO-generating neuronal clusters represent a basic mechanism underlying limbic epilepsy.[44] Support for this hypothesis derives from studies that show that the size and location of pHFO-generating sites in dentate gyrus become stable over time,[45] but application of bicuculline, an antagonist to inhibitory $GABA_A$ receptors, causes these areas to increase in size.[46] In addition, the number of pHFO-generating clusters in dentate gyrus correlates with seizure frequency, and the spectral power of these oscillations increases during the transition to hippocampal hypersynchronous seizures.[45,47] These findings suggest that spontaneous seizures arise when reduction in tonic inhibitory influences results in the coalescence and synchronization of pHFO-generating sites.

Differences between Normal and Pathological HFOs

Studies in the rat hippocampus have identified several important differences between normal ripples and pHFOs.[48] Normal ripples arise from a relatively large area of tissue that involves the controlled firing of pyramidal cells and interneurons, and the extracellular ripple largely reflects summated IPSPs from precisely timed interneuron discharges. In contrast, pHFO-generating sites can be localized to small, discrete neuronal clusters often embedded in tissue that does not generate pHFOs,[46] although HFOs observed in scalp EEG recordings suggest that neocortical sites could be larger than those found in epileptogenic hippocampus.[49] Figure 16–2 shows an example of the local generation of spontaneous pHFOs in epileptic dentate gyrus. The pHFO recorded on electrode contact 2, positioned within the granule cell layer, does not appear on contact 1, located 200 μm from contact 1, and the voltage of the pHFO reverses in polarity in the depth beginning on contact 3. During pHFOs, there is no consistent firing pattern between interneurons and pHFOs, although principal cell discharges occur during the trough of the extracellular pHFO.[43,50] It is believed that pHFOs reflect bursts of population spikes arising from synchronized discharges of abnormally bursting principal cells and possibly interneurons (compared to Figs. 16–1E and 16–1F).[31]

It is not clear how abnormal synchrony is generated during pHFOs, but recording in CA3 of normal hippocampal slices bathed in high-potassium medium showed that blockade of ionotropic glutamatergic signaling reduces synchronous discharges between pyramidal cells and attenuates pHFO-like field events.[51] The same study demonstrated that increasing the fidelity of pyramidal cell discharges increases pHFO amplitude, whereas disrupting intrinsic action potential–generating mechanisms decreases pHFO amplitude. These data suggest that increased chemical transmission through recurrent excitatory synapses between pyramidal cells can generate pHFOs. However, another study recording in CA3 of hippocampal slices from epileptic rats offers an alternative explanation.[52] In this study, a reduction in neuronal spike timing was associated with a decrease in ripple power and an increase in fast ripple power, although the amplitude of fast ripples was much *lower,* not *higher*,[31] compared to that of ripples. Furthermore, increasing spike timing in epileptic CA3 restored ripples and decreased fast ripples, suggesting that in vitro, fast ripples emerge from an abnormal reduction in neuronal synchrony, not an *increase* in synchrony. It could be argued that ripples and

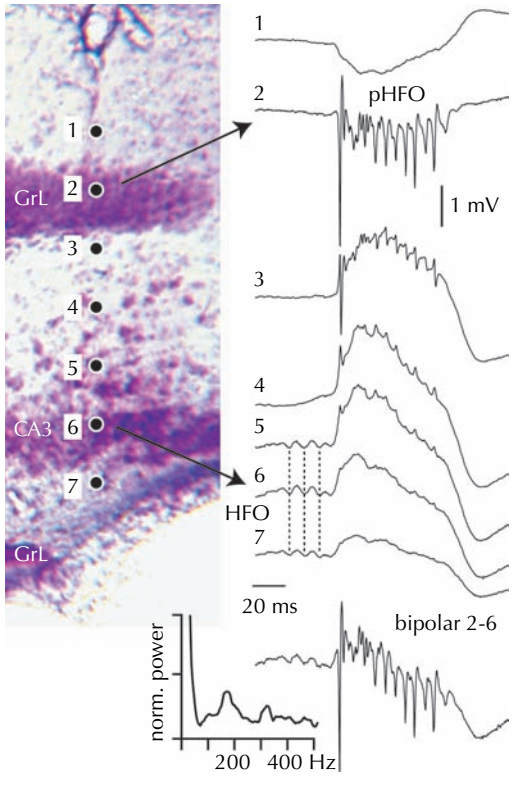

Figure 16–2. Multicontact electrode array recording spontaneous pHFOs in the dentate gyrus of an epileptic rat. A local fast ripple and a ripple-frequency HFO occur on electrode contacts numbered 2 and 6 in the granule cell layer (GrL) and area CA3, respectively. There is no evidence for a pHFO on contact 1, and the pHFO on contact 2 reverses in polarity in the depth starting on contact 3. Note that there is no reversal in potential associated with the ripple-frequency HFO recorded between contacts 5 and 7. A bipolar recording between contacts 2 and 6 (bottom trace) shows the occurrence of both HFOs, and the corresponding normalized power spectral histogram (left) shows the different spectral frequencies associated with each HFO. The bipolar recording does contain the spatial information to distinguish between the two local networks, each supporting the generation of pHFOs. Circles and numbers on the histological section (left) denote the approximate position of electrode contacts that was based on histological analysis and the voltage-depth profile of evoked potentials. Electrode contact spacing is 200 µm. Adapted from ref. 48.

fast ripples from the epileptogenic CA3 in this latter study were both pathological and that each HFO reflects bursts of population spikes with differing degrees of neuronal synchrony.[48] Clearly, there is a need for additional studies that will differentiate normal from pathological HFOs in vitro and in vivo.

HFOs in Patients with Epilepsy

The first human studies on interictal HFOs were carried out using microelectrodes positioned in the hippocampus and entorhinal cortex of patients with medically refractory MTLE.[2,43] Several of the properties of fast ripples recorded in patients resemble those of fast ripples in epileptic rats, including spectral frequency, duration, association with the seizure-onset zone,[43,53] and generation in cellular layers of entorhinal cortex that support evoked population spike discharge and abnormal synchrony of burst firing.[54] Microelectrode studies in patients also demonstrated that rates of fast ripples are highest during SWS and remain elevated during REM sleep.[55] With respect to pathological substrates and the hippocampal sclerosis often observed in patients with MTLE, higher rates of fast ripples in hippocampus correlate with severity of local atrophy,[56] and higher rates of fast ripples and lower rates of ripples correlate with lower neuron densities in Ammon's horn and dentate gyrus.[57] These data suggest that rates of fast ripple and ripple discharge reflect the severity of epileptogenicity and thus have clinical value in determining the treatment for patients with MTLE. In addition, morphological abnormalities associated with hippocampal sclerosis could promote the generation of fast ripples, although a recent study showed that fast ripples are present in a nonlesional animal model of temporal lobe epilepsy (TLE).[58]

It is not yet possible to distinguish normal from pathological ripple-frequency HFOs in human mesial temporal lobe limbic structures, but presumably normal hippocampal ripples share several important characteristics with normal ripples found in the nonprimate hippocampus. Human ripples have similar spectral frequencies; occur bilaterally, with the highest rate during SWS and the lowest during REM sleep[2,53,55]; and arise from broad areas of tissue supporting generation and synchrony of neuronal discharges.[54] Single-neuron studies in humans show that putative interneurons reach their highest rate of firing during the onset of the ripple, whereas pyramidal rates peak during the maximum amplitude of the ripple event[59] (Figs. 16–3A–C). During individual cycles of the ripple, human pyramidal cells fire during the trough of the ripple, while interneurons discharge between 0.0 and 1.0 ms

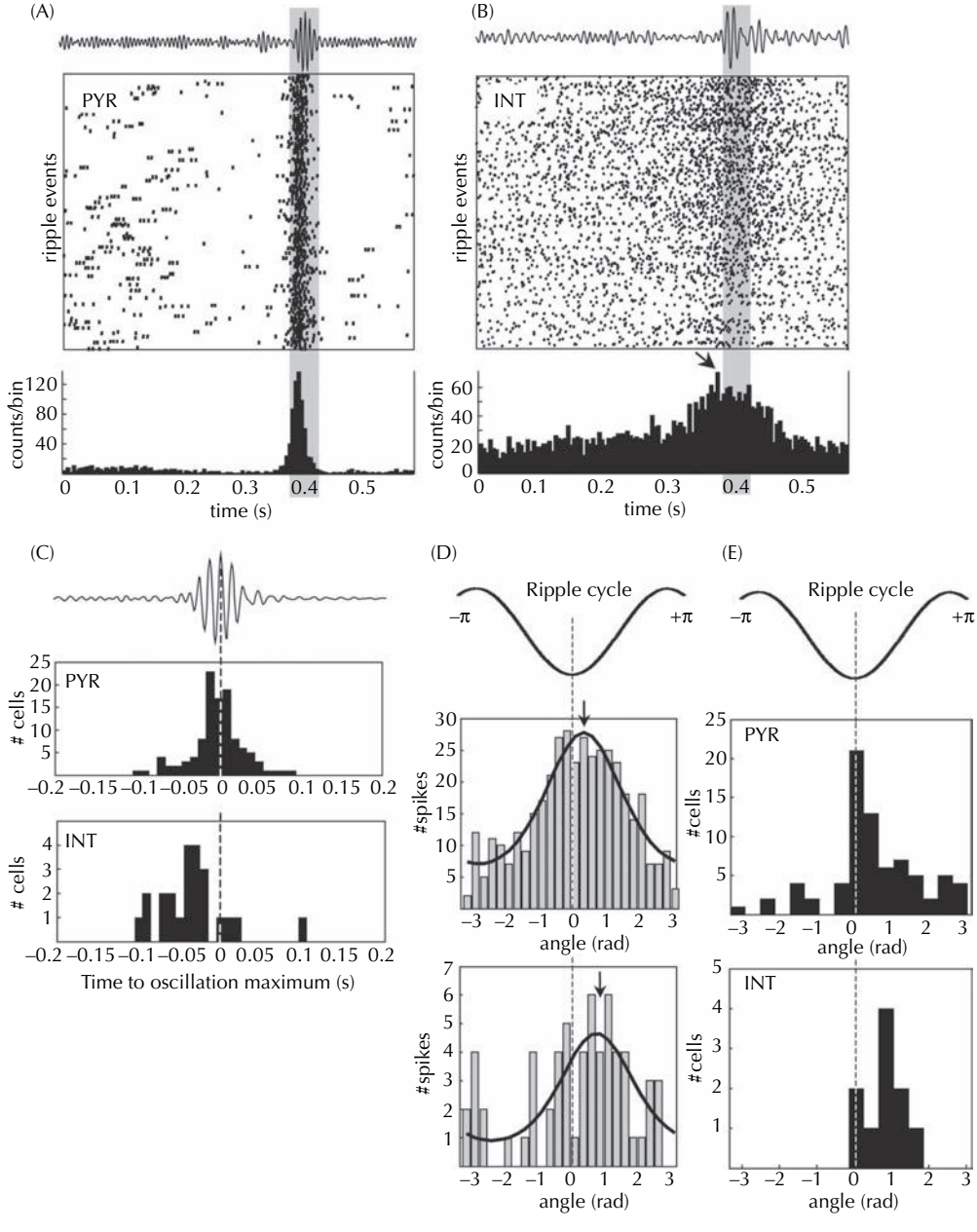

Figure 16–3. Single-neuron correlates of human hippocampal ripples. **A.** Raster plot of putative pyramidal cell (PYR) firing (horizontal axis) during individual ripple events (vertical axis). The trace at the top shows an example of a ripple oscillation. The cumulative histogram on the bottom shows that maximum PYR firing occurs during the ripple. **B.** Same as in panel A, but for a putative interneuron (INT). Maximum INT firing occurs before the peak amplitude of ripple oscillation (denoted by the arrow in the histogram at the bottom). **C.** Cumulative histograms show that maximum firing rates of most PYR cells occur near the peak amplitude of the ripple, indicated by the dashed line. In contrast, the highest firing rates of INT occurred ~50 ms before the ripple. **D.** Phase histogram of spike firing from a single PYR (top) and INT (bottom) in relation to the phase angle of the ripple shown atop the histograms. The trough of the ripple cycle is a zero phase angle (dashed line). A von Mises function fitted to each phase histogram (continuous solid line) shows that the mean firing phase for the PYR cell was 0.5 (± 0.9) radians and for the INT it was 0.8 (± 0.7) radians (denoted by arrows). The difference in firing phase between PYR and INT (~0.3) corresponds to an average 0.5 ms delay for a 100 Hz ripple oscillation. **E.** Cumulative histograms summarizing PYR and INT firing in relation to the phase of the ripple cycle. Most PYR cells and the INT discharged during the trough of the ripple (0 radians; denoted by the dashed line). Adapted from ref. 59.

of pyramidal cells, which is similar to the well-timed firing between pyramidal cells and some interneurons during rat hippocampal ripples (Figs. 16–3D and 16–3E).

Interest in pHFOs as markers of epileptogenic areas in presurgical patients has driven the development of many clinical electrophysiology systems used with standard clinical depth and subdural grid electrodes to support higher sampling rates and provide greater bandwidth. Studies using these systems confirm the strong association between pHFOs and epileptogenic regions, particularly fast ripples compared to ripple-frequency HFOs.[60–63] In addition, a patient study found pHFOs associated with epileptogenic lesions that included focal cortical dysplasia and nodular heterotopia, but rates of pHFOs were more strongly linked to areas of seizure onset than to anatomical lesion.[64] These latter data suggest that pHFOs are not limited to a specific type of epilepsy, but could be a fundamental property of epileptogenicity common to many types of epilepsy. Moreover, these results suggest that in some types of epilepsy, electrophysiological disturbances can be remote from anatomical abnormalities.

Interictal Spikes and HFOs

Recording of EEG interictal spikes can be useful for delineating the epileptogenic region, but the spatial extent of interictal spikes is usually more widespread than the seizure onset zone. Studies demonstrate a significant spatial and temporal association between interictal spikes and HFOs, although 40% to 50% of HFOs occur independently of interictal spikes.[61] Thus, abnormal HFOs may occur on the rising component, peak or the descending component of an interictal spike or may not be associated with a spike at all. [60,65–67] One study found that the occurrence of interictal spikes containing abnormal HFOs along with HFOs alone was more sensitive in predicting the seizure-onset area than the occurrence of interictal spikes alone, with no HFOs.[61] Furthermore, in a separate study that used surgical outcome to verify the boundaries of the epileptogenic region, patients with a good surgical outcome had a significantly larger proportion of abnormal HFO-generating sites removed than patients with a poor surgical outcome.[68] In contrast, there was no difference in the number of interictal spike (with no HFOs) or seizure onset sites removed between good and poor surgical outcome groups. The results from these latter studies need to be confirmed with studies using a greater number of patients. Nevertheless, the results suggest that pHFOs not only delineate the epileptogenic region better than interictal spikes with no pHFOs, but also better than EEG identification of the site of seizure onset.

Seizure Generation and pHFOs

In animals and human patients with epilepsy, the power of HFOs can increase several seconds to tens of minutes before seizure onset.[30,69–76] Focal cortical seizures that secondarily generalize are characterized by a relatively small area of pHFO generation at seizure onset that can move along the cortex and increase in size as the seizure progresses.[77] It is hypothesized that an increase in size and synchrony among pHFO-generating sites can trigger seizures,[30,44] but not all seizures are associated with an increase in HFO power and some seizures can be accompanied by a reduction in HFO power during onset.[78] It is possible that a preictal increase in HFO power that reflects bursts of population spikes could indicate enhanced synchronization of principal cell networks that reach a threshold supporting the spread of ictal discharges. In contrast, a preictal increase in HFOs that reflect IPSPs could be an indication of interneuronal network activation to suppress propagation of epileptiform discharges, whereas a reduction in these latter-type HFOs might fail to prevent the spread of ictal discharges. A more thorough understanding of the neuronal mechanisms during the ictal transition will help clarify the role of pHFOs during seizure genesis.

CONCLUSIONS

Basic research studies on HFOs indicate that these local oscillatory field potentials correspond with an increase in the rate and synchrony of neuronal discharges. There is compelling evidence supporting the view that normal hippocampal ripples and neocortical HFOs in the normal brain reflect IPSPs of interneurons that regulate the firing and timing of postsynaptic

principal cells. In contrast, substantial evidence suggests that pHFOs in the epileptic brain reflect bursts of population spikes that arise from local clusters of abnormal synchronously firing principal cells. Studies in animals and human patients with epilepsy demonstrate a strong association between interictal pHFOs and sites of seizure onset, and during some hippocampal and neocortical seizures, an increase in pHFOs preceding seizure onset. Furthermore, in animals, the early appearance of pHFOs after an epileptogenic insult correctly predicts the occurrence of spontaneous epileptic seizures. These results suggest that pHFOs can accurately identify the epileptogenic region, that is, brain areas necessary and sufficient for generating spontaneous seizures, and a unique biological marker of epileptogenicity and epileptogenesis. Future studies are needed to improve the criteria for differentiating normal HFOs from pHFOs in clinical recordings and develop noninvasive means for recording spontaneous HFOs. By successfully addressing these issues, it is anticipated that pHFOs could be used in clinical studies to optimize surgical treatment of medically refractory epilepsy, as well as in studies that seek to develop new therapies for treatment and possible prevention of epilepsy.

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REFERENCES

1. Buzsaki G, Horvath Z, Urioste R, Hetke J, Wise K. High-frequency network oscillation in the hippocampus. *Science.* 1992;256:1025–1027.
2. Bragin A, Engel J Jr, Wilson CL, Fried I, Buzsaki G. High-frequency oscillations in human brain. *Hippocampus*. 1999;9:137–142.
3. Ulanovsky N, Moss CF. Hippocampal cellular and network activity in freely moving echolocating bats. *Nat Neurosci*. 2007;10:224–233.
4. Skaggs WE, McNaughton BL, Permenter M, Archibeque M, Vogt J, Amaral D, Barnes CA. EEG sharp wave and sparse ensemble unit activity in the macaque hippocampus. *J Neurophysiol*. 2007;98:898–910.
5. Chrobak JJ, Buzsaki G. High-frequency oscillations in the output of the hippocampal-entorhinal axis of the freely behaving rat. *J Neurosci*. 1996;16:3056–3066.
6. Ponomarenko AA, Korotkova TM, Haas HL. High frequency (200 Hz) oscillations and firing patterns in the basolateral amygdala and dorsal endopiriform nucleus of the behaving rat. *Behav Brain Res*. 2003;141:123–129.
7. Kanamori N. Hippocampal minispindle wave in the cat: the different distribution of two types of waves. *Neurosci Res.* 1986;4:152–156.
8. Buzsaki G. Hippocampal sharp waves: their origin and significance. *Brain Res.* 1986;398:242–252.
9. Ylinen A, Bragin A, Nadasdy Z, Jando G, Szabo I, Sik A, Buzsaki G. Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *J Neurosci*. 1995;15:30–46.
10. Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsaki G. Fast network oscillations in the hippocampal CA1 region of the behaving rat. *J Neurosci*. 1999;19:1–4.
11. Grenier F, Timofeev I, Steriade M. Focal synchronization of ripples (80–200 Hz) in neocortex and their neuronal correlates. *J Neurophysiol*. 2001;86:1884–1898.
12. Kandel A, Buzsaki G. Cellular-synaptic generation of sleep spindles, spike-and-wave discharges, and evoked thalamocortical responses in the neocortex of the rat. *J Neurosci*. 1997;17:6783–6797.
13. Jones MS, Barth DS. Spatiotemporal organization of fast (>200 Hz) electrical oscillations in rat vibrissa/barrel cortex. *J Neurophysiol*. 1999;82:1599–1609.
14. Staba RJ, Brett-Green B, Paulsen M, Barth DS. Effects of ventrobasal lesion and cortical cooling on fast oscillations (>200 Hz) in rat somatosensory cortex. *J Neurophysiol*. 2003;89:2380–2388.
15. Cracco RQ, Cracco JB. Somatosensory evoked potential in man: far field potentials. *Electroencephalogr Clin Neurophysiol*. 1976;41:460–466.
16. Eisen A, Roberts K, Low M, Hoirch M, Lawrence P. Questions regarding the sequential neural generator theory of the somatosensory evoked potential raised by digital filtering. *Electroencephalogr Clin Neurophysiol/Evoked Potentials Sect*. 1984;59:388–395.
17. Curio G, Mackert BM, Burghoff M, Koetitz R, Abraham-Fuchs K, Harer W. Localization of evoked neuromagnetic 600 Hz activity in the cerebral somatosensory system. *Electroencephalogr Clin Neurophysiol*. 1994;91:483–487.
18. Nakano S, Hashimoto I. Comparison of somatosensory evoked high-frequency oscillations after posterior tibial and median nerve stimulation. *Clin Neurophysiol*. 1999;110:1948–1952.
19. Gobbele R, Waberski TD, Simon H, Peters E, Klostermann F, Curio G, Buchner H. Different origins of low- and high-frequency components (600 Hz) of human somatosensory evoked potentials. *Clin Neurophysiol*. 2004;115:927–937.

20. Ritter P, Freyer F, Curio G, Villringer A. High-frequency (600 Hz) population spikes in human EEG delineate thalamic and cortical fMRI activation sites. *Neuroimage.* 2008;42:483–490.
21. Barth DS. Submillisecond synchronization of fast electrical oscillations in neocortex. *J Neurosci.* 2003;23:2502–2510.
22. Staba RJ, Ard TD, Benison AM, Barth DS. Intracortical pathways mediate nonlinear fast oscillation (>200 Hz) interaction in rat barrel cortex. *J Neurophysiol.* 2004;93:2934–2939.
23. Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsaki G. Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving rat. *J Neurosci.* 1999;19:274–287.
24. Klausberger T, P.J.M, Marton LF, Roberts JDB, Cobden PM, Buzsaki G, Somogyi P. Brain-state and cell-type specific firing of hippocampal interneurons in vivo. *Nature.* 2003;42:844–848.
25. Jones MS, MacDonald KD, Choi B, Dudek FE, Barth DS. Intracellular correlates of fast (>200 Hz) electrical oscillations in rat somatosensory cortex. *J Neurophysiol.* 2000;84:1505–1518.
26. Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P. Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature.* 1995;378:75–78.
27. Behrens CJ, van den Boom LP, Heinemann U. Effects of the GABA(A) receptor antagonists bicuculline and gabazine on stimulus-induced sharp wave-ripple complexes in adult rat hippocampus in vitro. *Eur J Neurosci.* 2007;25:2170–2181.
28. Jones MS, Barth DS. Effects of bicuculline methiodide on fast (>200 Hz) electrical oscillations in rat somatosensory cortex. *J Neurophysiol.* 2002;88:1016–1025.
29. D'Antuono M, de Guzman P, Kano T, Avoli M. Ripple activity in the dentate gyrus of disinhibited hippocampus-entorhinal cortex slices. *J Neurosci Res.* 2005;80:92–103.
30. Bragin A, Wilson CL, Almajano J, Mody I, Engel J Jr. High-frequency oscillations after status epilepticus: epileptogenesis and seizure genesis. *Epilepsia.* 2004;45:1017–1023.
31. Bragin A, Wilson CL, Engel J Jr. Voltage depth profiles of high-frequency oscillations after kainic acid-induced status epilepticus. *Epilepsia.* 2007;48:35–40.
32. Draguhn A, Traub RD, Schmitz D, Jefferys JGR. Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro. *Nature.* 1998;394:189–192.
33. Maier N, Guldenagel M, Sohl G, Siegmund H, Willecke K, Draguhn A. Reduction of high-frequency network oscillations (ripples) and pathological network discharges in hippocampal slices from connexin 36-deficient mice. *J Physiol.* 2002;541:521–528.
34. Traub RD, Schmitz D, Jefferys JG, Draguhn A. High-frequency population oscillations are predicted to occur in hippocampal pyramidal neuronal networks interconnected by axoaxonal gap junctions. *Neuroscience.* 1999;92:407–426.
35. Jiruska P, Powell AD, Chang WC, Jefferys JG. Electrographic high-frequency activity and epilepsy. *Epilepsy Res.* 2010;89:60–65.
36. Weiss SA, Faber DS. Field effects in the CNS play functional roles. *Front Neural Circuits.* 2010;4:15.
37. Jefferys JG. Nonsynaptic modulation of neuronal activity in the brain: electric currents and extracellular ions. *Physiol Rev.* 1995;75:689–723.
38. Dudek FE, Snow RW, Taylor CP. Role of electrical interactions in synchronization of epileptiform bursts. *Adv Neurol.* 1986;44:593–617.
39. Buzsaki G. The hippocampo-neortical dialogue. *Cereb Cortex.* 1996;6:81–92.
40. Yun SH, Mook-Jung I, Jung MW. Variation in effective stimulus patterns for induction of long-term potentiation across different layers of rat entorhinal cortex. *J Neurosci.* 2002;22:RC214.
41. Siapas AG, Wilson MA. Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron.* 1998;21:1123–1128.
42. Axmacher N, Elger CE, Fell J. Ripples in the medial temporal lobe are relevant for human memory consolidation. *Brain.* 2008;131:1806–1817.
43. Bragin A, Engel J Jr, Wilson CL, Fried I, Mathern GW. Hippocampal and entorhinal cortex high-frequency oscillations (100–500 Hz) in human epileptic brain and in kainic acid–treated rats with chronic seizures. *Epilepsia.* 1999;40:127–137.
44. Bragin A, Wilson CL, Engel J Jr. Chronic epileptogenesis requires development of a network of pathologically interconnected neuron clusters: a hypothesis. *Epilepsia.* 2000;41:S144–S152.
45. Bragin A, Wilson CL, Engel J Jr. Spatial stability over time of brain areas generating fast ripples in the epileptic rat. *Epilepsia.* 2003;44:1233–1237.
46. Bragin A, Mody I, Wilson CL, Engel J Jr. Local generation of fast ripples in epileptic brain. *J Neurosci.* 2002;22:2012–2021.
47. Bragin A, Azizyan A, Almajano J, Wilson CL, Engel J Jr. Analysis of chronic seizure onsets after intrahippocampal kainic acid injection in freely moving rats. *Epilepsia.* 2005;46:1592–1598.
48. Engel J Jr, Bragin A, Staba RJ, Mody I. High-frequency oscillations: what is normal and what is not? *Epilepsia.* 2009;50:598–604.
49. Kobayashi K, Watanabe Y, Inoue T, Oka M, Yoshinaga H, Ohtsuka Y. Scalp-recorded high-frequency oscillations in childhood sleep-induced electrical status epilepticus. *Epilepsia.* 2010;51:2190–2194.
50. Grenier F, Timofeev I, Crochet S, Steriade M. Spontaneous field potentials influence the activity of neocortical neurons during paroxysmal activities in vivo. *Neuroscience.* 2003;119:277–291.
51. Dzhala VI, Staley KJ. Mechanisms of fast ripples in the hippocampus. *J Neurosci.* 2004;24:8896–8906.
52. Foffani G, Uzcategui YG, Gal B, Menendez de la Prida L. Reduced spike-timing reliability correlates with the emergence of fast ripples in the rat epileptic hippocampus. *Neuron.* 2007;55:930–941.
53. Staba RJ, Wilson CL, Bragin A, Fried I, Engel J Jr. Quantitative analysis of high frequency oscillations (80–500 Hz) recorded in human epileptic hippocampus and entorhinal cortex. *J Neurophysiol.* 2002;88:1743–1752.
54. Bragin A, Wilson CL, Staba RJ, Reddick M, Fried I, Engel J Jr. Interictal high frequency oscillations (80–500 Hz) in the human epileptic brain: entorhinal cortex. *Ann Neurol.* 2002;52:407–415.
55. Staba RJ, Wilson CL, Bragin A, Jhung D, Fried I, Engel J. High-frequency oscillations recorded in

human medial temporal lobe during sleep. *Ann Neurol.* 2004;56:108–115.
56. Ogren JA, Wilson CL, Bragin A, Lin JJ, Salamon N, Dutton RA, Luders E, Fields TA, Fried I, Toga AW, Thompson PM, Engel J Jr, Staba RJ. Three dimensional surface maps link local atrophy and fast ripples in human epileptic hippocampus. *Ann Neurol.* 2009;66:783–791.
57. Staba RJ, Frighetto L, Behnke EJ, Mathern GW, Fields TA, Bragin A, Ogren J, Fried I, Wilson CL, Engel J Jr. Increased fast ripple to ripple ratios correlate with reduced hippocampal volumes and neuron loss in temporal lobe epilepsy patients. *Epilepsia.* 2007;48:2130–2138.
58. Jiruska P, Finnerty GT, Powell AD, Lofti N, Cmejla R, Jefferys JG. Epileptic high-frequency network activity in a model of non-lesional temporal lobe epilepsy. *Brain.* 2010;133:1380–1390.
59. Le Van Quyen M, Bragin A, Staba R, Crepon B, Wilson CL, Engel J Jr. Cell type-specific firing during ripple oscillations in the hippocampal formation of humans. *J Neurosci.* 2008;28:6104–6110.
60. Urrestarazu E, Chander R, Dubeau F, Gotman J. Interictal high-frequency oscillations (100–500 Hz) in the intracerebral EEG of epileptic patients. *Brain.* 2007;130:2354–2366.
61. Jacobs J, LeVan P, Chander R, Hall J, Dubeau F, Gotman J. Interictal high-frequency oscillations (80–500 Hz) are an indicator of seizure onset areas independent of spikes in the human epileptic brain. *Epilepsia.* 2008;49:1893–1907.
62. Worrell GA, Gardner AB, Stead SM, Hu S, Goerss S, Cascino GJ, Meyer FB, Marsh R, Litt B. High-frequency oscillations in human temporal lobe: simultaneous microwire and clinical macroelectrode recordings. *Brain.* 2008;131:928–937.
63. Schevon CA, Trevelyan AJ, Schroeder CE, Goodman RR, McKhann G Jr, Emerson RG. Spatial characterization of interictal high frequency oscillations in epileptic neocortex. *Brain.* 2009;132:3047–3059.
64. Jacobs J, Levan P, Chatillon CE, Olivier A, Dubeau F, Gotman J. High frequency oscillations in intracranial EEGs mark epileptogenicity rather than lesion type. *Brain.* 2009;132:1022–1037.
65. Urrestarazu E, Jirsch JD, Le Van P, Hall J, Avoli M, Dubeau F, Gotman J. High-frequency intracerebral EEG activity (100–500 Hz) following interictal spikes. *Epilepsia.* 2006;47:1465–1476.
66. Bagshaw AP, Jacobs J, LeVan P, Dubeau F, Gotman J. Effect of sleep stage on interictal high-frequency oscillations recorded from depth macroelectrodes in patients with focal epilepsy. *Epilepsia.* 2008;50:617–628.
67. Kobayashi K, Jacobs J, Gotman J. Detection of changes of high-frequency activity by statistical time-frequency analysis in epileptic spikes. *Clin Neurophysiol.* 2009;120:1070–1077.
68. Jacobs J, Zijlmans M, Zelmann R, Chatillon CE, Hall J, Olivier A, Dubeau F, Gotman J. High-frequency electroencephalographic oscillations correlate with outcome of epilepsy surgery. *Ann Neurol.* 2010;67:209–220.
69. Traub RD, Whittington MA, Buhl EH, LeBeau FE, Bibbig A, Boyd S, Cross H, Baldeweg T. A possible role for gap junctions in generation of very fast EEG oscillations preceding the onset of, and perhaps initiating, seizures. *Epilepsia.* 2001;42:153–170.
70. Bragin A, Engel J Jr, Wilson CL, Vizentin E, Mathern GW. Electrophysiologic analysis of a chronic seizure model after unilateral hippocampal KA injection. *Epilepsia.* 1999;40:1210–1221.
71. Worrell GA, Parish L, Cranstoun SD, Jonas R, Baltuch G, Litt B. High-frequency oscillations and seizure generation in neocortical epilepsy. *Brain.* 2004;127:1496–1506.
72. Akiyama T, Otsubo H, Ochi A, Ishiguro T, Kadokura G, RamachandranNair R, Weiss SK, Rutka JT, Carter Snead C 3rd. Focal cortical high-frequency oscillations trigger epileptic spasms: confirmation by digital video subdural EEG. *Clin Neurophysiol.* 2005;116:2819–2825.
73. Jirsch JD, Urrestarazu E, LeVan P, Olivier A, Dubeau F, Gotman J. High-frequency oscillations during human focal seizures. *Brain.* 2006;129:1593–1608.
74. Ochi A, Otsubo H, Donner EJ, Elliott I, Iwata R, Funaki T, Akizuki Y, Akiyama T, Imai K, Rutka JT, Snead OC 3rd. Dynamic changes of ictal high-frequency oscillations in neocortical epilepsy: using multiple band frequency analysis. *Epilepsia.* 2007;48:286–296.
75. Khosravani H, Mehrotra N, Rigby M, Hader WJ, Pinnegar CR, Pillay N, Wiebe S, Federico P. Spatial localization and time-dependant changes of electrographic high frequency oscillations in human temporal lobe epilepsy. *Epilepsia.* 2008;50:605–616.
76. Ramachandran Nair R, Ochi A, Imai K, Benifla M, Akiyama T, Holowka S, Rutka JT, Snead OC 3rd, Otsubo H. Epileptic spasms in older pediatric patients: MEG and ictal high-frequency oscillations suggest focal-onset seizures in a subset of epileptic spasms. *Epilepsy Res.* 2008;78:216–224.
77. Akiyama T, Otsubo H, Ochi A, Galicia EZ, Weiss SK, Donner EJ, Rutka JT, Snead OCI. Topographic movie of ictal high-frequency oscillations on the brain surface using subdural EEG in neocortical epilepsy. *Epilepsia.* 2006;47:1953–1957.
78. Jacobs J, Zelmann R, Jirsch J, Chander R, Châtillon CE, Dubeau F, Gotman J. High frequency oscillations (80–500 Hz) in the preictal period in patients with focal seizures. *Epilepsia.* 2009;50:1780–1792.

Chapter 17

Interictal Epileptiform Discharges in Partial Epilepsy

Complex Neurobiological Mechanisms Based on Experimental and Clinical Evidence

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Seizures (also termed *ictal discharges*) represent the critical events and the primary clinical burden of an active epileptic condition. Between seizures, however, the brain of patients with epilepsy generates pathological patterns of activity, called *interictal epileptiform discharges* (IEDs), that are clearly distinguished from the activity observed during the seizure itself. The correlation between IEDs and ictal discharges in intractable partial epilepsies has been the subject of several studies (for review see refs. 1–4), yet no conclusion regarding the reciprocal relationship and interdependence of IEDs and ictal discharges has been reached to date. Indeed, the existing data findings have led to two opposite views that assign to IEDs either a preventive or a precipitating role in seizure occurrence.

Interest in the mechanisms underlying IEDs has been revived during the last decade by presurgical diagnostic studies that utilized prolonged video-electroencephalography (EEG) along with intracranial EEG monitoring over periods of several days. Analysis of IEDs recorded from the scalp and with intracranial electrodes in patients with partial epilepsy have focused on the relation between IED topographic distribution and seizure patterns, and on their occurrence during the preictal state.[5–10] The methods used to localize the electric source(s) within the brain volume that generate IEDs recorded using scalp EEG electrodes have been extensively reviewed and will not be considered in this chapter.[8,11–13] Intracranial recordings either with cortical surface grids/strips or with intracerebral electrodes have

been more useful to identify different IED patterns, since electrodes are positioned in closer proximity to the physiological IED generators. These studies, along with experimental evidence obtained from animal models of partial epilepsy, have demonstrated the existence of diverse IED electrical patterns. Focal IEDs show large pattern variability (including spikes, sharp waves, bursts of fast spikes, sequences of fast oscillation, etc.) even in the same patient/model (Fig. 17–1). Given this diversity, it is reasonable to assume that different types of IEDs may be mediated by distinct neurobiological mechanisms and play divergent functional roles with respect to ictogenesis. In this chapter, we will review the clinical and experimental evidence that demonstrates the multiplicity of IED patterns based on data obtained from humans and from experimental models of partial epilepsy and seizures. The neurobiological mechanisms responsible for the generation of different IEDs will also be considered.

DIFFERENT IED PATTERNS IN EPILEPTIC PATIENTS: SPIKES, SPIKE BURSTS, SHARP WAVES

As mentioned above, interictal patterns are diverse and variable in partial epilepsies. Fast events defined as interictal spikes are characterized by a large-amplitude, rapid component lasting 50–100 ms that is usually followed by a slow wave 200–500 ms in duration[14,15] (Fig. 17–1A). Highly reproducible interictal spikes are typical of cryptogenic and benign forms of epilepsy, such as epileptic disorders with Rolandic or occipital paroxysms.[16,17] In these clinical conditions, interictal spikes show a selective and specific regional distribution. In contrast, partial epilepsies secondary to brain lesions show more irregular interictal spikes, often associated with IED patterns that include sharp waves (characterized by a rapid component that lasts for 100–300 ms), bursts of spikes, fast oscillations, and repetitive, paroxysmal slow waves (Fig. 17–1B–E; but see ref. 18).

Interictal epileptiform discharges in partial pharmacoresistant epilepsies have been well characterized. In humans, simultaneous unit recordings and laminar field potential profiles obtained with intracortical multielectrodes during acute (surgical) corticography[19] or chronic presurgical monitoring[20,21] have revealed that interictal spikes are initiated by large potential deflections, consistent with paroxysmal depolarization shifts similar to those recorded experimentally.[22–25] Moreover, the cortical layers where these depolarizations occur differ according to whether the spike is locally generated or propagates from a distant area.[19,21] Finally, characterization of unit firing during

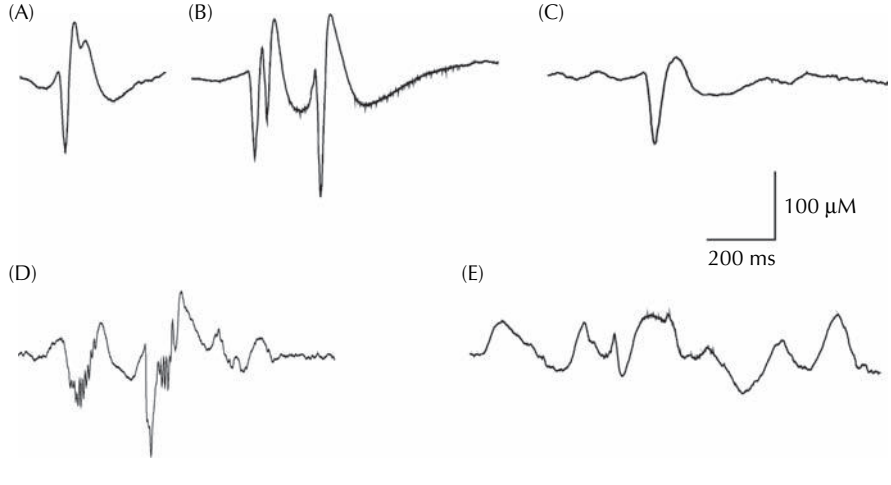

Figure 17–1. Interictal epileptic discharge patterns recorded in human partial epilepsies with intracranial electrodes. **A.** Interictal spike. **B.** Group of interictal spikes from neocortical dysplasia. **C. S**harp wave from a lesional partial epilepsy. **D.** Fast activity (brushes) riding on a spike recorded from a Taylor type II FCD. **E.** Paroxysmal slow activity superimposed on slow spikes recorded in a lesional partial epilepsy.

interictal spikes has demonstrated heterogeneity inside and outside the seizure onset zone, suggesting that IEDs are not a simple paroxysm of hypersynchronous excitatory activity, but rather represent the interplay of multiple distinct neuronal types within extended neuronal networks.[26]

Certain forms of human lesional partial epilepsies and cortical dysplasias show distinctive patterns that have specific diagnostic value.[27–29] One such condition is the Taylor-type II focal cortical dysplasia (FCD) that features IEDs characterized by high-frequency spikes and polyspikes, defined as *brushes*[30–32], that last for 100–200 ms, recur with a periodicity of 1–2 s and is enhanced during slow-wave sleep. Electrical stimulation has demonstrated that brushes in Taylor-type II focal dysplasias are followed by a desynchronization/depression of activity that lasts for 0.5–1 s and are associated with a higher threshold for the generation of further IEDs.[33] Interictal epileptiform discharges in temporal lobe epilepsy (TLE) with hippocampal sclerosis are less frequent than in focal dysplasias and consist of either spikes or sharp waves that are often undetectable on the scalp EEG.[34,35] In patients with severe hippocampal atrophy, large-amplitude spikes shorter than 100 ms with small-amplitude postspike slow activity have been reported. These IEDs increased in frequency and became rhythmic before and after ictal events.[36]

INTERICTAL SPIKES IN ACUTE AND CHRONIC ANIMAL MODELS IN VIVO

The temporal correlation between IEDs and ictal discharges has been analyzed in vivo in animal models mimicking both acute seizures and chronic epilepsy. Pioneering intracellular recordings obtained from neurons located in the *epileptic focus* induced by application of convulsants (e.g., penicillin) have demonstrated that interictal spikes correlate with paroxysmal depolarizing shifts of membrane potential leading to sustained action potential firing and at times followed by a robust hyperpolarization.[22,23,37] These studies have also shown that the transition to seizure is characterized by IED acceleration along with a decrease or disappearance of the postburst hyperpolarizing potential, a phenomenon that was proposed to result from the progressive accumulation of extracellular potassium.[38,39]

This transition pattern, however, has not been reproduced in chronic models of TLE. Thus, in both kindling and drug-induced status epilepticus (SE) models, the IED frequency either did not change or it decreased before the onset of an ictal event[40–43] (for review see ref. 2). Interictal spiking has also been analyzed in animals injected with kainic acid in one hippocampus; this represents a widely used chronic model that faithfully reproduces TLE. As in TLE patients, unilateral IEDs were reproducibly observed in this model in rats,[44,45] mice,[46] and guinea pigs (Carriero, Arcieri, and de Curtis, unpublished observations). More recently, video-EEG monitoring of the epileptic activity recorded during the latent and chronic periods in rats undergoing pilocarpine-induced SE has revealed that following the appearance of seizures, IEDs diminish in duration in the CA3 region and occur at higher rates in the amygdala.[47] Therefore, these findings suggest that IEDs undergo structure-specific changes following the appearance of spontaneous seizure activity. Little information about IEDs is available for other chronic models of partial epilepsy, such as posttraumatic models or models of cortical dysplasia studied in vivo.

IEDs IN ACUTE ANIMAL MODELS IN VITRO

Interictal epileptiform discharges can be studied in brain slices that are maintained in vitro following experimental procedures that favor epileptiform synchronization. Several reports have shown that IEDs are initiated by gradual enhancement and progressive recruitment of synaptic excitation that reaches the threshold for regenerative calcium currents.[48–50] This process further sustains recurrent excitation and promotes the synchronous firing of a large number of neurons that contribute to the buildup of a population event recognizable as a population spike/sharp wave. Excitatory postsynaptic potentials associated with these IEDs[25,51,52] are mediated by glutamate receptors of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and *N*-methyl-D-aspartate

(NMDA) subtypes.[53–57] Similar mechanisms of IED generation have also been identified in slices of the neocortex,[58–60] piriform cortex,[50,61] and entorhinal cortex (EC)[62] following a variety of pharmacological manipulations. Regenerative potentials sustained by high-voltage calcium spikes[61,63–66] and by a persistent fraction of the voltage-gated sodium current[67–69] also contribute to paroxysmal depolarizing shifts. Finally, interictal synchronization is further facilitated by nonsynaptic interactions mediated either by extracellular electric fields (ephaptic interactions) or by intercellular gap junctions[70,71] that exist between principal neurons or interneurons.[72–74]

Interictal epileptiform discharges and the associated glutamatergic paroxysmal shifts are typically observed during prolonged application of (1) drugs that interfere with GABAergic inhibition such as bicuculline, penicillin, and picrotoxin (Fig.17– 2A, B), (2) agonists of glutamatergic transmission such as kainic acid, or (3) solutions with an ionic composition that enhances neuronal excitability. However, epileptiform discharges can also be induced by drugs that boost both glutamatergic and GABAergic synaptic transmission, such as the potassium blocker 4-aminopiridine (4AP). Early studies have shown that 4AP induces two types of IEDs within the hippocampal formation. The first type is characterized by fast IEDs that are driven by the CA3 network and are abolished by AMPA receptor antagonists. The second type consists of slow IEDs that were spared by glutamatergic receptor blockers, but were abolished by GABAergic antagonists.[75] It was subsequently shown that these slow IEDs can be recorded from any limbic cortical area as well as from the neocortex in brain slices obtained from rats or mice (as well as in the human neocortex; see below). This evidence has recently been confirmed in several areas of the in vitro isolated guinea pig brain[76] (Fig. 17–2C).

Intracellular recordings in brain slices have demonstrated that the slow IEDs induced by 4AP are coupled to a complex intracellular potential consisting of hyperpolarizing and depolarizing components. Recently, similar GABAergic IEDs—which correlated in EC neurons with inhibitory postsynaptic potentials—have been reported in the isolated guinea pig brain maintained in vitro during glutamatergic receptor blockade[76,77]; under these pharmacological conditions, the slow IEDs continued to propagate within the hippocampal-entorhinal region and from one EC to the EC of the contralateral hemisphere (Fig. 17–2). These slow IEDs are abolished by $GABA_A$ receptor antagonists as well as by a mu-receptor agonist both in the brain slice and in the isolated guinea pig in vitro preparation, thus confirming that they reflect the synchronous activity of local GABAergic networks. It is unclear how the slow IEDs propagate during glutamatergic receptor blockade, but data obtained in brain slices suggest the involvement of nonsynaptic mechanisms such as transient increases in extracellular potassium and subsequent redistribution of this ion (see below). However, the involvement of long-range GABAergic pathways or syncytia-like connections among interneurons cannot be ruled out.[78,79]

Experiments performed in the in vitro isolated guinea pig brain, in which inhibition was reduced about 50% by short-lasting bicuculline systemic perfusions, have demonstrated the existence of IEDs sustained by bursting of inhibitory interneurons that precede by about 1 min the generation of a seizure-like discharge.[80] These IEDs were associated with inhibitory postsynaptic potentials in EC principal neurons of both superficial and deep layers. In addition, glutamatergic IEDs and GABAergic IEDs could be simultaneously induced by this short-lasting bicuculline application in the piriform cortex and in the EC, respectively. Hence, this evidence suggests that an epileptic brain can generate interictal activity sustained by both glutamatergic and GABAergic networks, and is in line with the occurrence of GABA-dependent IEDs, as reported in human cortical slices obtained from postsurgical specimens.[81–83] Interictal epileptiform discharges that correlate with synchronous inhibitory postsynaptic potentials in large groups of neurons are often associated with seizure onset.[80,84] It is therefore tempting to speculate that GABA-mediated interictal events may contribute to the enhancement of synchronization of local epileptic networks through a mechanisms of post-inhibition resetting of neuronal firing.[85,86]

Interictal epileptiform discharges have recently been analyzed by coupling electrophysiological recordings with imaging of activity-depended intracellular changes in calcium

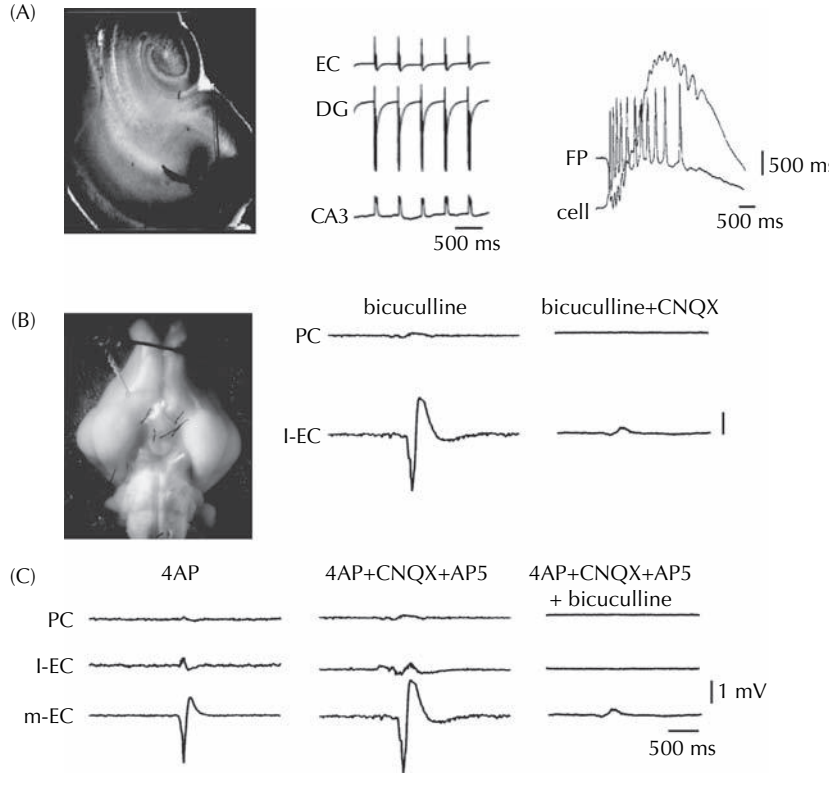

Figure 17–2. Interictal epileptic discharges analyzed in in vitro models of epileptiform synchronization. **A**. Glutamatergic interictal spikes induced in hippocampal slices (left panel) by application of 20 μM bicuculline. Field potentials recorded in the entorhinal cortex (EC), in the dentate gyrus (DG), and in the CA3 region are illustrated by the middle panel, while simultaneous intra- and extracellular recordings during an interictal spike in the DG are shown in the right panel. **B.** Glutamatergic interictal spikes induced in the in vitro isolated guinea pig brain (left panel) by arterial perfusion of bicuculline (50 μM). These IEDs are reduced by application of the glutamatergic receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). **C**. GABA-mediated interictal spikes. generated in the limbic cortices of the in vitro isolated guinea pig brain by arterial perfusion of 4AP (50 μM; left panel). Recordings were performed in the piriform cortex (PC), in the lateral and medial entorhinal cortices (l-EC and m-EC), and in the CA1 region of the hippocampus. These IEDs persisted after blockade of glutamatergic synaptic transmission with 10 μM CNQX and 100 μM AP5 (middle panel). Additional perfusion with the GABAa receptor antagonist bicuculline (50 μM) abolished the interictal spike (right panel).

concentration (for review see ref. 87). Increases in calcium signals have been identified during IEDs and ictal discharges in neurons, presumably as a correlate of neuronal firing.[88–90] In one of these studies, both IEDs and seizures appeared to be associated with calcium increases in astrocytes and these increases were independent of neuronal activation, thus suggesting that ictogenesis could be sustained exclusively by astrocyte activation and by the associated release of glutamate.[90] However, even though the contribution of glutamatergic glio-transmission to ictogenesis has been confirmed by other authors, the unique role of astrocyte in seizure initiation has not been replicated.[91] One recent report analyzed this issue in different experimental models of seizures and IEDs; these experiments showed that astrocyte calcium signaling contributes to sustained ictal activity, but is not involved in the generation of IEDs.[92]

HIGH-FREQUENCY OSCILLATIONS AS INTERICTAL EVENTS

High-frequency oscillations (HFOs) at >100 Hz have been recorded from cortical structures in humans and animal models, both under physiological conditions and in partial epilepsies (for review see ref. 93). Cortical HFOs at

100–200 Hz occur under physiological conditions, during the interictal state in patients presenting with partial epilepsy, and in animal models.[93–96] Intracranial EEG recordings obtained from pharmacoresistant patients suffering from mesial TLE have shown that HFOs are observed in coincidence with an interictal spike and in isolation. Further studies have confirmed these observations in TLE patients[97,98] and also in the epileptogenic region of patients with neocortical partial epilepsy.[99–101] Physiological HFOs (also termed *ripples*) are implicated in the process of memory consolidation[102] and represent population inhibitory postsynaptic potentials generated by principal neurons entrained by synchronously active interneuron networks.[103,104] Juxtacellular recordings obtained with microelectrodes in the human hippocampus during physiological ripples demonstrated that pyramidal cells fired preferentially at the highest amplitude of the ripple, while interneurons discharged earlier than pyramidal cells.[105] Pathological fast ripples that express very-high-rate oscillations (250–600 Hz) were recorded exclusively from epileptic tissue of epileptic patients and in animal models of TLE.[96,106,107] Fast ripples can be observed in the interictal state, while components in the beta-gamma frequency range are usually associated with ictal discharges.[108] Moreover, pathological fast ripples recorded in vivo, unlike ripples, are supported by synchronous burst firing of abnormally active principal neurons and are assumed to be independent on inhibitory neurotransmission.[93]

More recently, a different pattern of very-low-amplitude HFOs with high intrinsic rhythmicity (>250 Hz) has been identified in the seizure onset region in mesial TLE patients during the preictal period.[98,109,110] This activity—which is concealed in the intracranial recordings and can only be extracted by amplifying the appropriately filtered signal—occurs in coincidence with IEDs and sharp waves but also in the absence of any detectable IED.[98,111] In summary, HFOs may be interpreted as typical IEDs in partial epilepsies, and their association with other types of IEDs is not the rule.

The cellular and network mechanisms responsible for interictal HFOs have been analyzed in detail in in vitro brain slices exposed to pro-epileptic drugs. Physiological fast oscillations, either pharmacologically induced[85,112–114] or occurring spontaneously during up-down states,[115,116] were proposed to be supported by synchronization of inhibitory GABAergic networks via gap junctions with or without the contribution of glutamatergic networks. Faster oscillations, such as ripples, were also found to be supported by both excitatory and inhibitory transmission and gap junctions.[117,118] It is not clear whether the HFOs seen during interictal discharges in epileptic tissue are the same as physiological fast activities. Dzhala and Staley[106] and Behrens et al.[119] proposed that epileptic HFOs are initiated and synchronized by excitatory interactions between pyramidal cells in the hippocampus. More recent reports have proposed that HFOs generated at the onset of an ictal hippocampal discharge[120,121] correlate with transient GABAergic input,[122] indicating that GABAergic mechanisms are the source of epileptic HFOs at least in this case.

THE SLOW COMPONENT AFTER THE INTERICTAL DISCHARGES

Interictal epileptiform discharges recorded from the epileptogenic zone are assumed to be generated by synchronous neuronal firing. In line with experimental evidence,[23,24,123] presurgical intracranial studies in patients with partial epilepsy have demonstrated that the sharp component of a neocortical IED is followed by a depression of neuronal excitability. This phenomenon has been reported in mesial TLE patients analyzed with unit activity recordings[124–126] as well as by using the paired pulse stimulation paradigm. Thus, these studies provide evidence for the existence of inhibitory phenomena during the postspike slow wave.[127] Moreover, in Taylor type II focal dysplasia, a refractory period of 0.5–1 s has been identified after the interictal spike.[33] In this study, an enhanced threshold for spike generation was seen in brain regions that surround the epileptogenic zone during single-shock stimulations at 1 Hz that were performed to identify eloquent and symptomatogenic areas. Interestingly, no refractory period was observed after IEDs that were generated within the seizure-onset zone.

Early experimental studies[123] and more recent reports[128] have shown that glutamatergic IEDs are followed by a period of depression in excitability that may result from several mechanisms. Cortical surface IEDs

are usually larger then 1 mV, and they are associated with the simultaneous activation of large ensembles of neurons and possibly astrocytes. This synchronous activation generates massive neuronal firing that boosts recurrent synaptic activation and nonsynaptic field and direct interactions. Since GABAergic inhibitory interneurons are presumably preserved in the area of IED generation,[129–131] the activation of recurrent inhibitory networks during IED may be responsible for dampening neuronal excitability. Recurrent inhibition mediated by $GABA_A$ receptors lasts for 100 ms and could be reinforced and prolonged up to about 1 s by the activation of "slow" $GABA_B$ receptors. Since post-IED depression lasts longer than 1 s,[123] other mechanisms should be implicated in its generation. In line with this view, it has been shown in the piriform cortex that intra/extracellular pH changes associated with IEDs contribute to this depression by decoupling gap junctions.[132,133]

Intracranial studies in TLE patients have demonstrated that background activity and HFO are reduced in amplitude during the slow wave that follows an IED, suggesting post-IED depression.[100] Preliminary findings obtained at the Claudio Munari Epilepsy Surgery Center in Milan suggest that fast activity is reduced after an IED generated in the *irritative zone* surrounding the area of seizure onset,[134] whereas it is preserved and even enhanced after IEDs generated within the seizure-onset zone (Fig. 17–3). Thus, IEDs characterized by spikes or sharp waves generate a long-lasting period of inhibition/depression that dampens the fast,

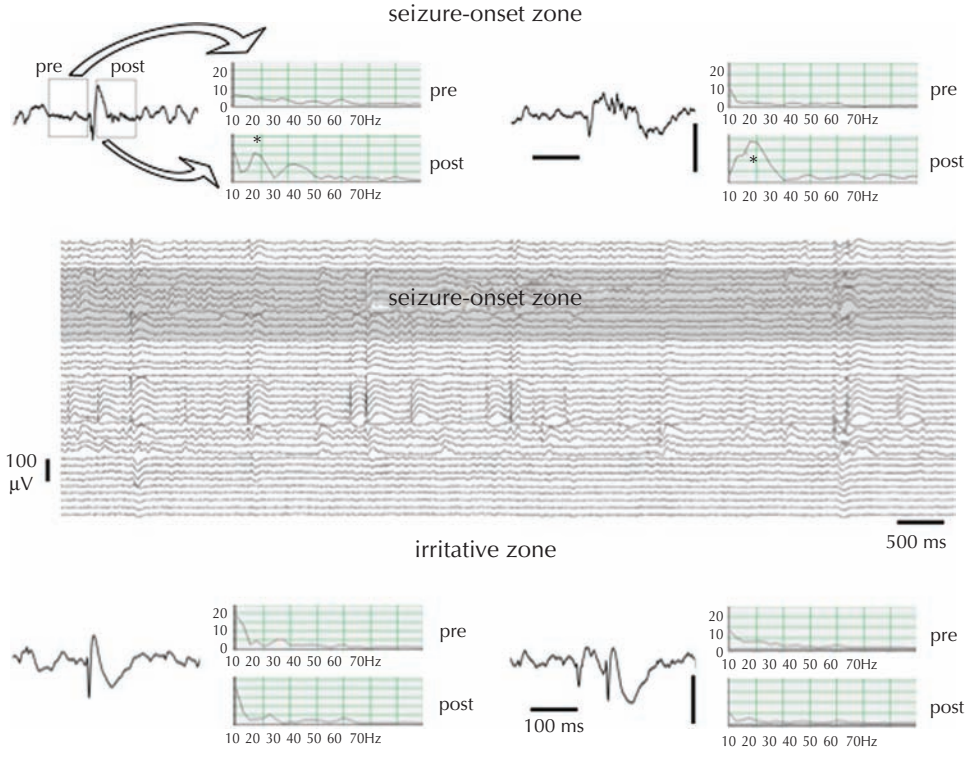

Figure 17–3. Intracranial recordings performed in a patient with cryptogenic partial epilepsy during presurgical evaluation of the epileptogenic region. The area of seizure onset is shaded in light gray in the central panel, which illustrates 10 s of continuous recording. Interictal spikes were observed in the seizure-onset zone (enlarged in the upper part of the figure) and in the surrounding irritative zone (lower part of the figure). Power spectra analysis was performed 500 ms before and after the spike component in the frequency range between 10 and 80 Hz. The frequency plots demonstrate the presence of fast activity at 20–40 Hz after the spikes recorded within the seizure-onset region (upper panels), whereas no fast activity was observed after spikes recorded in the irritative zone. Stereo-EEG recordings were kindly provided by Dr. S. Francione of the Caudio Munari Epilepsy Surgery Center.

transient increase in excitability that occurs during the spike/sharp wave. Postspike depression is typical of the tissue that borders the seizure-onset zone; hence, these findings may support the idea that some IEDs control brain hyperexcitability within the epileptic network and protect the region against seizure entrainment.

IN VITRO RECORDINGS OF IEDS FROM POSTSURGICAL BRAIN TISSUE

The fundamental mechanisms of IEDs have also been analyzed in postsurgical cortical slices of human brain tissue incubated in vitro for electrophysiological analysis. Human cortical slices in vitro do not generate spontaneous ictal discharges in standard saline bath solutions unless excitability is enhanced with various experimental procedures.[135] Yet, spontaneous IEDs can be recorded in postsurgical slices (that included the subiculum and the CA2 region) obtained from hippocampi of patients suffering from mesial TLE with Ammon horn sclerosis[82,136] as well as neocortical partial epilepsies.[81] As detailed in Chapter 14 in this book, interictal spikes in these studies were blocked by antagonists of either glutamate or $GABA_A$ synaptic transmission.[82,83] Hence, spontaneous IEDs in human cortical tissue in vitro are generated by both $GABA_A$ergic and glutamatergic synaptic conductances.

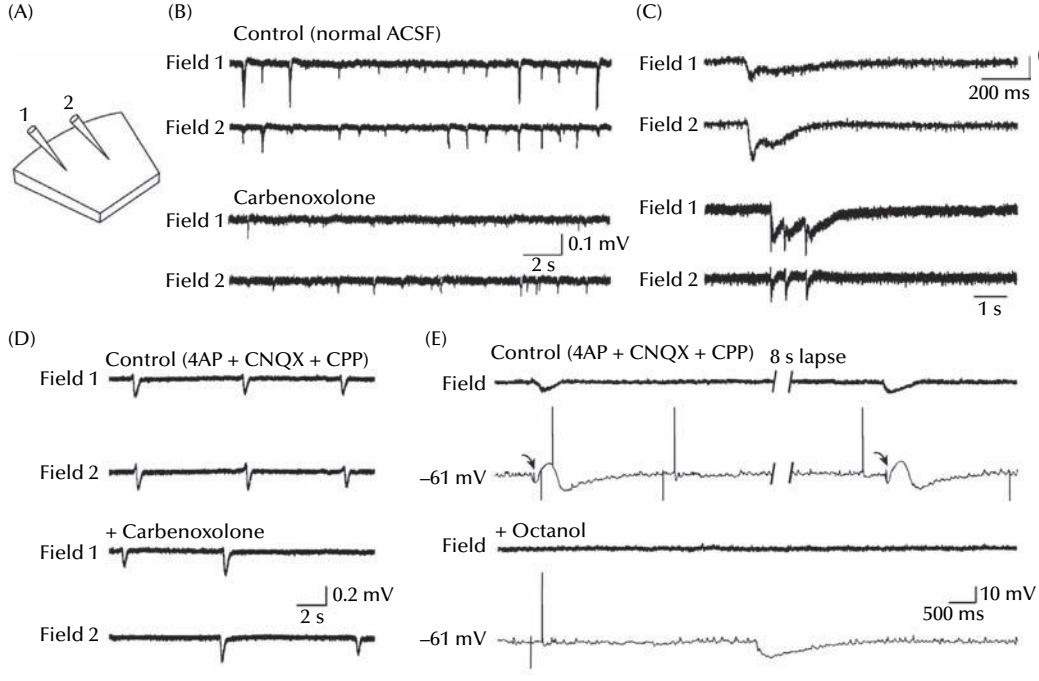

Figure 17–4. Effects of gap-junction decouplers on the synchronous activity generated by human neocortical slices in vitro. **A.** Schematic drawing of the location of the two recording extracellular microelectrodes used in the experiments shown in **B–D**; the interelectrode distance was approx. 2 mm. **B**. Carbenoxolone (0.3 mM) reduces the rate of occurrence and the amplitude of the spontaneous activity recorded in normal medium from an FCD slice. ACSF = artificial cerebro-spinal fluid; CPP = 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid. **C**. Expanded samples of this spontaneous activity; note that it consists of one (upper panel) to three (lower panel) fast transients riding on a slow negative shift. **D**. Carbenoxolone (0.3 mM) decreases the rate of occurrence and disrupts the synchronization of the spontaneous activity recorded during application of 4AP+glutamatergic receptor antagonists in a neocortical slice obtained from a TLE patient. **E**. Effects of octanol (1 mM) on the synchronous activity recorded with extracellular (Field) and sharp intracellular (K-acetate-filled; –61 mV) microelectrodes from a TLE neocortical slice treated with 4AP+glutamatergic receptor antagonists; note in the control sample that the field events were intracellularly mirrored by early hyperpolarization, followed by long-lasting depolarization (LLD) and terminated by prolonged hyperpolarization. Bath application of octanol abolishes the field events and the associated intracellular potentials, while long-lasting hyperpolarizing potentials continue to occur; note also the action potentials of small amplitude that occur during the early hyperpolarizing component (curved arrows) in the Control sample.

High-frequency oscillations characterized by very fast frequencies at 80–400 Hz have been reported to occur in postsurgical neocortical slices obtained from patients with drug-resistant TLE.[137] In this study, HFOs associated with interictal spikes did not require synaptic transmission, as they continued to occur in the presence of glutamatergic and GABAergic receptor antagonists; they were, however, abolished by application of drugs that are known to decouple gap junctions, such as carbanoxolone. The effects induced by gap junction decouplers have also been documented in human neocortical slices obtained from patients with FCD as well as from TLE patients.[138] This study showed that spontaneous IEDs recorded in the presence of normal medium from FCD tissue were reduced and, even more importantly, were no longer synchronized during carbenoxolone application (Fig. 17–4B); moreover, similar effects were seen when IEDs were elicited by 4AP in neocortical slices that had no obvious structural abnormality (Fig. 17–4D). These IEDs, when recorded in the presence of glutamatergic receptor antagonists, correspond intracellularly to a complex sequence of potentials that are dominated by a long-lasting depolarization (Fig. 17–4E) and are accompanied by transient increases in extracellular potassium.[139]

Electrophysiological analysis of slices of human FCD tissue has shown that during 4AP

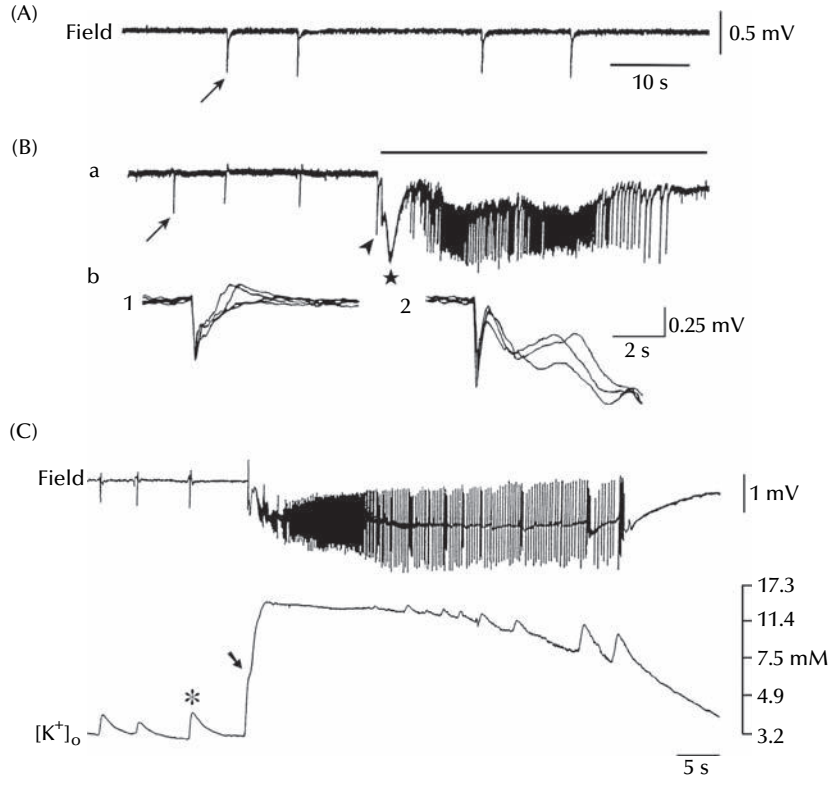

Figure 17–5. Synchronous activity induced by bath application of 4AP in neocortical slices obtained from TLE (i.e., presenting with no architectural anomaly) and FCD patients. **A.** Isolated field potentials (arrow) occur spontaneously in a TLE slice analyzed with field potential recording. **B.** Spontaneous field potential discharges recorded in an FCD slice; note that in this experiment, both isolated interictal field potentials (arrow) and an ictal discharge are shown in a. Note also that the onset of the ictal event is associated with the occurrence of a negative field potential (arrowhead) that is followed by a slow negative event (asterisk) leading to ictal discharge oscillations. In b, superimposed interictal discharges (1) and ictal discharge onsets (2) are illustrated. C. Field potential activity and concomitant changes in $[K^+]_o$ induced by 4AP in FCD tissue. Note that $[K^+]_o$ increases up to 4.5 mM during the isolated negative field events (asterisk), reaches values of approx. 6.4 mM during the negative-going field potential leading to the ictal discharge onset (arrow), and levels off to values of 12–14 mM during the ictal event.

application, IEDs that are mainly dependent on GABA receptor-mediated conductances may be instrumental in eliciting NMDA receptor-mediated ictal discharges.[140–142] As illustrated in Figure 17–5B, ictal discharges recorded from FCD slices were preceded by negative-going events resembling those seen in isolation during the interictal period; however, the field potentials leading to ictal discharge onset were always of larger amplitude and were followed by a secondary, slow negative field event from which ictal oscillations emerged. The ability of IEDs to initiate ictal synchronization in the human FCD tissue depends on the presence of a $GABA_A$ receptor–mediated mechanism that leads to sizable increases in extracellular potassium. As illustrated in Fig. 17–5C, the transient elevations in extracellular potassium associated with the IEDs that shortly preceded the ictal discharge onset were characterized by rises in extracellular potassium larger than those seen in association with similar field potentials occurring during the interictal period. A similar association of large elevations in extracellular potassium and ictal discharge onset has been observed in the deep layers of the EC[143] as well as in isolated hippocampal slices obtained from young rats.[144,145] Indeed, elevating extracellular potassium can disclose seizure activity both in vivo[146] and in vitro.[147,148] It should be emphasized that a similar 4AP treatment in human neocortical tissue without obvious structural abnormality induces only periodic, synchronous, interictal-like GABA receptor-mediated potentials[149] (Fig. 17–5A). Therefore, these in vitro data support the view that epileptogenicity is a functional feature of FCD tissue.

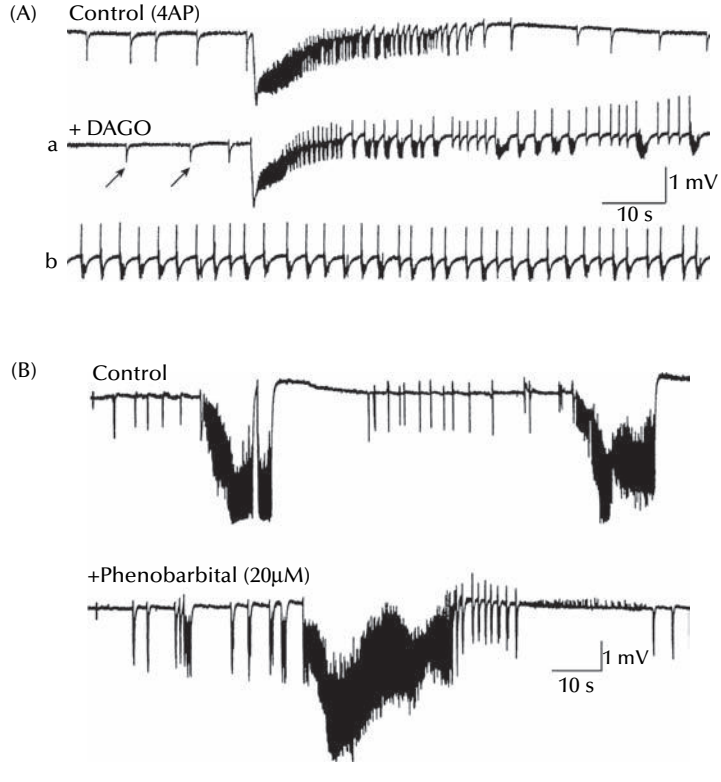

Figure 17–6. $GABA_A$ receptor function modulates ictal discharges in brain FCD slices.

A. Bath application of the μ-opioid receptor agonist [D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin (DAGO; 10 μM) reduces the amplitude of the isolated negative field events (arrows in a) and transforms ictal activity into regular, robust interictal discharges (b). a and b are a continuous recording that was started 2 min after the onset of DAGO application. **B.** Bath application of phenobarbital (20 μM) increases the duration of the interictal events and of the ictal discharges induced by 4AP in an FCD slice.

The role of GABA receptor–mediated synchronization in initiating ictal activity in FCD tissue is further supported by pharmacological manipulations aimed at decreasing or enhancing the function of $GABA_A$ receptors. $GABA_A$ receptor antagonists or activation of μ-opioid receptors (which blocks the release of GABA from interneuron terminals) made ictal discharges and GABA receptor-mediated interictal events disappear (Fig. 17–6A). Under both conditions, FCD slices generated recurrent epileptiform activity that lacked the features of an electrographic ictal event. Conversely, potentiating $GABA_A$ receptor function with minimal concentrations of phenobarbital[150,151] caused a prolongation of the ictal discharges along with potentiation of the slow interictal events (Fig. 17–6B).

CONCLUSIONS

Evidence reviewed in this chapter indicates that IEDs are heterogeneous in terms of both pattern and underlying mechanisms. It has been proposed that the core region in which focal seizures are generated is surrounded by an area that generates hypersynchronous activity (the irritative region) interposed between the seizure-onset area and the surrounding normal tissue.[134] Interictal epileptiform discharges are generated both in the epileptogenic zone and in the irritative region and can spread to (and thus be recorded from) adjacent healthy brain structures. Therefore, it is reasonable to conclude that interictal events are sustained by cellular and pharmacological mechanisms that vary according to the site of generation. Different IEDs sustained by GABAergic and glutamatergic networks could have opposite functions with respect to seizure generation, either precipitating it or preventing it, depending on the site where they originate within the epileptogenic tissue. According to this view, post-IED depression could be a selective feature of IEDs that occur in brain regions (such as the irritative region) in which neuronal homeostasis and synaptic networks are not drastically altered by the epileptogenic process. In seizure-onset regions, tissue damage could be more intense and post-IED depression may not be present or may be insufficient to dampen excitability, allowing IEDs to effectively trigger seizures.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Gotman J. Relationships between interictal spiking and seizures: human and experimental evidence. *Can J Neurol Sci.* 1991;18:573–576.
2. de Curtis M, Avanzini G. Interictal spikes in focal epileptogenesis. *Progr Neurobiol*. 2001;63:541–567.
3. Avoli M. Do interictal discharges promote or control seizures? Experimental evidence from an in vitro model of epileptiform discharge. *Epilepsia*. 2001;42(suppl 3):2–4.
4. Avoli M, Biagini G, de Curtis M. Do interictal spikes sustain seizures and epileptogenesis? *Epil Curr*. 2006;6:203–207.
5. Bast T, Oezkan O, Rona S, et al. EEG and MEG source analysis of single and averaged interictal spikes reveals intrinsic epileptogenicity in focal cortical dysplasia. *Epilepsia*. 2004;45:621–631.
6. Benar CG, Grova C, Kobayashi E, et al. EEG-fMRI of epileptic spikes: concordance with EEG source localization and intracranial EEG. *Neuroimage*. 2006;30:1161–1170.
7. Baumgartner C, Pataraia E. Revisiting the role of magnetoencephalography in epilepsy. *Curr Opin Neurol.* 2006;19:181–186.
8. Plummer C, Harvey AS, Cook M. EEG source localization in focal epilepsy: where are we now? *Epilepsia.* 2008;49:201–218.
9. Rose S, Ebersole JS. Advances in spike localization with EEG dipole modeling. *Clin EEG Neurosci.* 2009;40:281–287.
10. Vulliemoz S, Lemieux L, Daunizeau J, Michel CM, Duncan JS. The combination of EEG source imaging and EEG-correlated functional MRI to map epileptic networks. *Epilepsia*. 2010;51:491–505.
11. Alarcon G, Guy CN, Binnie CD, Walker SR, Elwes RD, Polkey CE. Intracerebral propagation of interictal activity in partial epilepsy: implications for source localisation. *J Neurol Neurosurg Psych*. 1994;57:435–449.
12. Bagshaw AP, Kobayashi E, Dubeau F, Pike GB, Gotman J. Correspondence between EEG-fMRI and EEG dipole localisation of interictal discharges in focal epilepsy. *Neuroimage*. 2006;30:417–425.
13. Rodin E, Constantino T, Rampp S, Wong PK. Spikes and epilepsy. *Clin EEG Neurosci*. 2009;40:288–299.
14. Kooi KA. Voltage-time characteristics of spikes and other rapid electroencephalographic transients: semantic and morphological considerations. *Neurology*. 1966;16:59–66.
15. Chatrian GE, Bergamini L, Dondey M, Klass DW, Lennox-Buchtal M. A glossary of terms most

commonly used by clinical electroencephalographers. *Electroencephalogr. Clin Neurol.* 1974;37:538–548.
16. Drury I, Beydoun A. Benign partial epilepsy of childhood with monomorphic sharp waves in centrotemporal and other locations. *Epilepsia*. 1991;32: 662–667.
17. Frost JD Jr, Hrachovy RA, Glaze DG. Spike morphology in childhood focal epilepsy: relationship to syndromic classification. *Epilepsia*. 1992;33:531–536.
18. Jiruska P, Finnerty GT, Powell AD, Lofti N, Cmejla R, Jefferys JG. Epileptic high-frequency network activity in a model of non-lesional temporal lobe epilepsy. *Brain.* 2010;133:1380–1390.
19. Ulbert I, Halgren E, Heit G, Karmos G. Multiple microelectrode-recording system for human intracortical applications. *J Neurosci Methods*. 2001;106:69–79.
20. Fabo D, Magloczky Z, Wittner L, et al. Properties of in vivo interictal spike generation in the human subiculum. *Brain*. 2008;131:485–499.
21. Ulbert I, Heit G, Madsen J, Karmos G, Halgren E. Laminar analysis of human neocortical interictal spike generation and propagation: current source density and multiunit analysis in vivo. *Epilepsia*. 2004;45 (suppl 4):48–56.
22. Matsumoto H, Marsan CA. Cortical cellular phenomena in experimental epilepsy: interictal manifeststions. *Exp Neurol*. 1964;80:286–304.
23. Prince D. Inhibition in "epileptic" neurons. *Exp Neurol*. 1968;21:307–321.
24. Prince DA. Cortical cellular activities during cyclically occurring inter-ictal epileptiform discharges. *Electroencephalogr Clin Neurophysiol*. 1971;31:469–484.
25. Johnston D, Brown TH. Interpretation of voltage-clamp measurements in hippocampal neurons. *J Neurophysiol*. 1983;50:464–486.
26. Keller CJ, Truccolo W, Gale JT, et al. Heterogeneous neuronal firing patterns during interictal epileptiform discharges in the human cortex. *Brain*. 2010;133:1668–1681.
27. Gambardella A, Gotman J, Cendes F, Andermann F. The relation of spike foci and of clinical seizure characteristics to different patterns of mesial temporal atrophy. *Arch Neurol*. 1995;52:287–293.
28. Francione S, Kahane P, Tassi L, et al. Stereo-EEG of interictal and ictal electrical activity of a histologically proved heterotopic gray matter associated with partial epilepsy. *Electroencephalogr Clin Neurophysiol*. 1994;90:284–290.
29. Tyvaert L, Hawco C, Kobayashi E, LeVan P, Dubeau F, Gotman J. Different structures involved during ictal and interictal epileptic activity in malformations of cortical development: an EEG-fMRI study. *Brain*. 2008;131:2042–2060.
30. Tassi L, Colombo N, Garbelli R, et al. Focal cortical dysplasia: neuropathological subtypes, EEG, neuroimaging and surgical outcome. *Brain*. 2002;125: 1719–1732.
31. Francione S, Nobili L, Cardinale F, Citterio A, Galli C, Tassi L. Intra-lesional stereo-EEG activity in Taylor's focal cortical dysplasia. *Epileptic Disord*. 2003; 5(suppl 2):S105–S114.
32. Spreafico R, Blumcke I. Focal cortical dysplasias: clinical implication of neuropathological classification systems. *Acta Neuropathol*. 2010;120:359–367.
33. de Curtis M, Tassi L, Lo Russo G, Mai R, Cossu M, Francione S. Increased discharge threshold after an interictal spike in human focal epilepsy. *Eur J Neurosci*. 2005;22:2971–2976.
34. Altafullah I, Halgren E, Stapleton JM, Crandall PH. Interictal spike-wave complexes in the human medial temporal lobe: typical topography and comparisons with cognitive potentials. *Electroencephalogr Clin Neurophysiol*. 1986;63:503–516.
35. Williamson PD, French JA, Thadani VM. Characteristics of medial temporal lobe epilepsy: II. Interictal and ictal scalp EEG, neuropsychological testing, neuroimaging, surgical results, and pathology. *Ann Neurol*. 1993;34:781–787.
36. Wilson C, Nix J, Szostak J. Functional requirements for specific ligand recognition by a biotin-binding RNA pseudoknot. *Biochemistry*. 1998;37: 14410–14419.
37. Dichter M, Spencer WA. Penicillin-induced interictal discharges from the cat hippocampus. I. Characteristics and topographical features. *J Neurophysiol*. 1969;32: 649–662.
38. Fertziger AP, Ranck JB Jr. Potassium accumulation in interstitial space during epileptiform seizures. *Exp Neurol*. 1970;26:571–585.
39. Dichter MA, Herman CJ, Selzer M. Silent cells during interictal discharges and seizures in hippocampal penicillin foci. Evidence for the role of extracellular K^+ in the transition from the interictal state to seizures. *Brain Res*. 1972;48:173–183.
40. Ralston BL. The mechanism of transition of interictal spiking foci into ictal seizure discharges. *Electroencephalogr Clin Neurophysiol Suppl*. 1958;10: 217–232.
41. Gotman J. Relationships between triggered seizures, spontaneous seizures, and interictal spiking in the kindling model of epilepsy. *Exp Neurol*. 1984;84:259–273.
42. Sherwin I. Ictal-interictal unit firing pattern differences in penicillin-induced primary and secondary epileptogenic foci. *Exp Neurol.* 1984;84:463–477.
43. Lange HH, Lieb JP, Engel J Jr, Crandall PH. Temporospatial patterns of pre-ictal spike activity in human temporal lobe epilepsy. *Electroencephalogr Clin Neurophysiol.* 1983;56:543–555.
44. Mathern GW, Cifuentes F, Leite JP, Pretorius JK, Babb TL. Hippocampal EEG excitability and chronic spontaneous seizures are associated with aberrant synaptic reorganization in the rat intrahippocampal kainate model. *Electroencephalogr Clin Neurophysiol.* 1993;87:326–339.
45. Bragin A, Engel J Jr, Wilson CL, Vizentin E, Mathern GW. Electrophysiologic analysis of a chronic seizure model after unilateral hippocampal KA injection. *Epilepsia.* 1999;40:1210–1221.
46. Le Duigou C, Bouilleret V, Miles R. Epileptiform activities in slices of hippocampus from mice after intra-hippocampal injection of kainic acid. J Physiol. 2008;586:4891–4904.
47. Bortel A, Levesque M, Biagini G, Gotman J, Avoli M. Convulsive status epilepticus duration as determinant for epileptogenesis and interictal discharge generation in the rat limbic system. *Neurobiol Dis*. 2010;40:478–489.
48. Ives AE, Jefferys JG. Synchronization of epileptiform bursts induced by 4-aminopyridine in the in vitro hippocampal slice preparation. *Neurosci Lett*. 1990;112:239–245.

49. Chamberlin NL, Traub RD, Dingledine R. Role of EPSPs in initiation of spontaneous synchronized burst firing in rat hippocampal neurons bathed in high potassium. *J Neurophysiol.* 1990;64:1000–1008.
50. Hoffman WH, Haberly LB. Bursting-induced epileptiform EPSPs in slices of piriform cortex are generated by deep cells. *J Neurosci*. 1991;11:2021–2031.
51. Ayala GF, Dichter M, Gumnit RJ. Genesis of epilectic interictal spikes. New knowledge of cortical feedback system suggests a neurophysiological explanation of brief paroxysms. *Brain Res*. 1973;52:1–17.
52. Traub RD, Wong RK. Cellular mechanism of neuronal synchronization in epilepsy. *Science*. 1982;216: 745–747.
53. Hablitz JJ. Picrotoxin-induced epileptiform activity in hippocampus: role of endogenous versus synaptic factors. *J Neurophysiol*. 1984;51:1011–1027.
54. Miles R, Traub RD, Wong RK. Spread of synchronous firing in longitudinal slices from the CA3 region of the hippocampus. *J Neurophysiol*. 1988;60: 1481–1496.
55. Traub RD, Miles R, Jefferys JG. Synaptic and intrinsic conductances shape picrotoxin-induced synchronized after-discharges in the guinea-pig hippocampal slice. *J Physiol.* 1993;461:525–547.
56. Stanton PK, Jones RS, Mody I, Heinemann U. Epileptiform activity induced by lowering extracellular. *Epilepsy Res.* 1987;1:53–62.
57. Bertram EH, Lothman EW. NMDA receptor antagonists and limbic status epilepticus: a comparison with standard anticonvulsant. *Epilepsy Res*. 1990;5: 177–184.
58. Lee WL, Hablitz JJ. Excitatory synaptic involvement in epileptiform bursting in the immature rat neocortex. *J Neurophysiol*. 1991;66:1894–1901.
59. Chagnac-Amitai Y, Connors BW. Synchronized excitation and inhibition driven by intrinsically bursting neurons in neocortex. *J Neurophysiol*. 1989;62: 1149–1162.
60. Hwa GGC, Avoli M. Hyperpolarizing inward rectification in rat neocortical neurons located in the superficial layers. *Neurosci Lett*. 1991;124:65–68.
61. Forti M, Biella G, Caccia S, de Curtis M. Persistent excitability changes in the piriform cortex of the isolated guinea-pig brain after transient exposure to bicuculline. *Eur J Neurosci.* 1997;9:435–451.
62. Jones RSG, Heinemann V. Synaptic and intrinsic responses of medial entorhinal cortical cells in normal and magnesium-free medium "in vitro." *J Neurophysiol*. 1988;59:1476–1496.
63. Schwartzkroin PA, Slawsky M. Probable calcium spikes in hippocampal neurons. *Brain Res*. 1977;135: 157–161.
64. Wong RKS, Prince DA, Basbaum AI. Intradendritic recordings from hippocampal neurons. *Proc Natl Acad Sci USA*. 1979;76:385–390.
65. Lopantsev V, Avoli M. Laminar organization of epileptiform discharges in the rat entorhinal cortex in vitro. *J Physiol.* 1998;509(pt 3):785–796.
66. de Curtis M, Radici C, Forti M. Cellular mechanisms underlying spontaneous interictal spikes in an acute model of focal cortical epileptogenesis. *Neuroscience*. 1999;88:107–117.
67. Auerbach JM, Segal M. A novel cholinergic induction of long-term potentiation in rat hippocampus. *J Neurophysiol*. 1994;2034–2040.
68. Franceschetti S, Guatteo E, Panzica F, Sancini G, Wanke E, Avanzini G. Ionic mechanisms underlying burst firing in pyramidal neurons: intracellular study in rat sensorimotor cortex. *Brain Res.* 1995;696: 127–139.
69. Azouz R, Jensen MS, Yaari Y. Ionic base of spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. *J Physiol*. 1996;492:211–223.
70. Traub RD, Dudek FE, Snow RW, Knowles WD. Computer simulations indicate that electrical field effects contribute to the shape of the epileptiform field potential. *Neuroscience*. 1985;15:947–958.
71. Jefferys JGR. Nonsynaptic modulation of neuronal activity in the brain: electric currents and extracellular ions. *Physiol Rev*. 1995;75:689–723.
72. Draguhn A, Traub RD, Schmitz D, Jefferys JG. Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro [see comments]. *Nature*. 1998;394:189–192.
73. Galarreta M, Hestrin S. A network of fast-spiking cells in the neocortex connected by electrical synapses. *Nature*. 1999;402:72–75.
74. Skinner FK, Zhang L, Velazquez JL, Carlen PL. Bursting in inhibitory interneuronal networks: a role for gap-junctional coupling. *J Neurophysiol*. 1999;81: 1274–1283.
75. Perreault P, Avoli M. 4-Aminopyridine-induced epileptiform activity and a GABA-mediated long-lasting depolarization in the rat hippocampus. *J Neurosci*. 1992;12:104–115.
76. Uva L, Avoli M, de Curtis M. Synchronous GABA-receptor-dependent potentials in limbic areas of the in-vitro isolated adult guinea pig brain. *Eur J Neurosci*. 2009;29:911–920.
77. Carriero G, Uva L, Gnatkovsky V, de Curtis M. Distribution of the olfactory fiber input into the olfactory tubercle of the in vitro isolated guinea pig brain. *J Neurophysiol*. 2009;101:1613–1619.
78. Aram JA, Michelson HB, Wong RKS. Synchronized GABAergic IPSPs recorded in the neocortex after blockade of synaptic transmission mediated by excitatory amino acids. *J Neurophysiol*. 1991;65:1034–1041.
79. Jinno S. Structural organization of long-range GABAergic projection system of the hippocampus. *Front Neuroanat*. 2009;3:13.
80. Gnatkovsky V, Librizzi L, Trombin F, de Curtis M. Fast activity at seizure onset is mediated by inhibitory circuits in the entorhinal cortex in vitro. *Ann Neurol*. 2008;64:674–686.
81. Kohling R, Lucke A, Straub H, et al. Spontaneous sharp waves in human neocortical slices excised from epileptic patients. Brain. 1998;121(pt 6):1073–1087.
82. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science*. 2002;298: 1418–1421.
83. Huberfeld G, Wittner L, Clemenceau S, et al. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. *J Neurosci*. 2007;27:9866–9873.
84. Lopantsev V, Avoli M. Participation of $GABA_A$-mediated inhibition in ictallike discharges in the rat entorhinal cortex. *J Neurophysiol*. 1998;79:352–360.
85. Whittington M, Traub RD, Jefferys JGR. Synchronized oscillations in interneuron networks driven by

metabotropic glutamate receptor activation. *Nature.* 1995;373:612–615.
86. Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P. Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature*. 1995;378:75–78.
87. Seifert G, Carmignoto G, Steinhauser C. Astrocyte dysfunction in epilepsy. *Brain Res Rev*. 2010;63:212–221.
88. Tashiro A, Goldberg J, Yuste R. Calcium oscillations in neocortical astrocytes under epileptiform conditions. *J Neurobiol*. 2002;50:45–55.
89. Kang N, Xu J, Xu Q, Nedergaard M, Kang J. Astrocytic glutamate release-induced transient depolarization and epileptiform discharges in hippocampal CA1 pyramidal neurons. *J Neurophysiol*. 2005;94:4121–4130.
90. Tian GF, Azmi H, Takano T, et al. An astrocytic basis of epilepsy. *Nat Med*. 2005;11:973–981.
91. Fellin T, Gomez-Gonzalo M, Gobbo S, Carmignoto G, Haydon PG. Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices. *J Neurosci.* 2006;26:9312–9322.
92. Gomez-Gonzalo M, Losi G, Chiavegato A, et al. An excitatory loop with astrocytes contributes to drive neurons to seizure threshold. *PLoS Biol*. 2010;8: e1000352.
93. Engel J Jr, Bragin A, Staba R, Mody I. High-frequency oscillations: what is normal and what is not? *Epilepsia*. 2009;50:598–604.
94. Allen PJ, Fish DR, Smith SJ. Very high-frequency rhythmic activity during SEEG suppression in frontal lobe epilepsy. *Electroencephalogr Clin Neurophysiol*. 1992;82:155–159.
95. Bragin A, Engel J Jr, Wilson CL, Fried I, Buzsaki G. High-frequency oscillations in human brain. *Hippocampus*. 1999;9:137–142.
96. Bragin A, Engel J Jr, Wilson CL, Fried I, Mathern GW. Hippocampal and entorhinal cortex high-frequency oscillations (100–500 Hz) in human epileptic brain and in kainic acid–treated rats with chronic seizures. *Epilepsia.* 1999;40:127–137.
97. Staba RJ, Wilson CL, Bragin A, Fried I, Engel J Jr. Quantitative analysis of high-frequency oscillations (80–500 Hz) recorded in human epileptic hippocampus and entorhinal cortex. *J Neurophysiol*. 2002;88:1743–1752.
98. Jacobs J, LeVan P, Chander R, Hall J, Dubeau F, Gotman J. Interictal high-frequency oscillations (80–500 Hz) are an indicator of seizure onset areas independent of spikes in the human epileptic brain. *Epilepsia*. 2008;49:1893–1907.
99. Worrell GA, Parish L, Cranstoun SD, Jonas R, Baltuch G, Litt B. High-frequency oscillations and seizure generation in neocortical epilepsy. *Brain.* 2004;127:1496–1506.
100. Urrestarazu E, Jirsch JD, LeVan P, Hall J. High-frequency intracerebral EEG activity (100–500 Hz) following interictal spikes. *Epilepsia*. 2006;47: 1465–1476.
101. Schevon CA, Trevelyan AJ, Schroeder CE, Goodman RR, McKhann G Jr, Emerson RG. Spatial characterization of interictal high frequency oscillations in epileptic neocortex. *Brain*. 2009;132:3047–3059.
102. Buzsáki G. The hippocampo-neocortical dialogue. *Cereb Cortex*. 1996;6:81–92.
103. Buzsáki G, Horváth Z, Urioste R, Hetke J, Wise K. High-frequency network oscillation in the hippocampus. *Science*. 1992;256:1025–1027.
104. Ylinen A, Bragin A, Nadasdy Z, et al. Sharp wave–associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *J Neurosci*. 1995;15:30–46.
105. Le Van Quyen M, Bragin A, Staba R, Crepon B, Wilson CL, Engel J Jr. Cell type–specific firing during ripple oscillations in the hippocampal formation of humans. *J Neurosci*. 2008;28:6104–6110.
106. Dzhala VI, Staley KJ. Mechanisms of fast ripples in the hippocampus. *J Neurosci.* 2004;24:8896–8906.
107. Jiruska P, Csicsvari J, Powell AD, et al. High-frequency network activity, global increase in neuronal activity, and synchrony expansion precede epileptic seizures in vitro. *J Neurosci.* 2010;30:5690–5701.
108. de Curtis M, Gnatkovsky V. Reevaluating the mechanisms of focal ictogenesis: the role of low-voltage fast activity. *Epilepsia.* 2009;50:2514–2525.
109. Jacobs J, Zelmann R, Jirsch J, Chander R, Dubeau CE, Gotman J. High frequency oscillations (80–500 Hz) in the preictal period in patients with focal seizures. *Epilepsia.* 2009;50:1780–1792.
110. Brazdil M, Halamek J, Jurak P, et al. Interictal high-frequency oscillations indicate seizure onset zone in patients with focal cortical dysplasia. *Epilepsy Res*. 2010;90:28–32.
111. Jacobs J, Kobayashi K, Gotman J. High-frequency changes during interictal spikes detected by time-frequency analysis. *Clin Neurophysiol*. 2010;122:32–42.
112. Whittington MA, Traub RD. Interneuron diversity series: inhibitory interneurons and network oscillations in vitro. *Trends Neurosci*. 2003;26:676–682.
113. Bartos M, Vida I, Jonas P. Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat Rev Neurosci*. 2007;8:45–56.
114. Mann EO, Paulsen O. Role of GABAergic inhibition in hippocampal network oscillations. *Trends Neurosci*. 2007;30:343–349.
115. Gnatkovsky V, Wendling F, de Curtis M. Cellular correlates of spontaneous periodic events in the medial entorhinal cortex of the in vitro isolated guinea pig brain. *Eur J Neurosci*. 2007;26:302–311.
116. Compte A, Reig R, Descalzo VF, Harvey MA, Puccini GD, Sanchez-Vives MV. Spontaneous high-frequency (10–80 Hz) oscillations during up states in the cerebral cortex in vitro. *J Neurosci*. 2008;28: 13828–13844.
117. Maier N, Nimmrich V, Draguhn A. Cellular and network mechanisms underlying spontaneous sharp wave-ripple complexes in mouse hippocampal slices. *J Physiol*. 2003;550:873–887.
118. Nimmrich V, Maier N, Schmitz D, Draguhn A. Induced sharp wave-ripple complexes in the absence of synaptic inhibition in mouse hippocampal slices. *J Physiol*. 2005;563:663–670.
119. Behrens CJ, van den Boom LP, Heinemann U. Effects of the GABA(A) receptor antagonists bicuculline and gabazine on stimulus-induced sharp wave-ripple complexes in adult rat hippocampus in vitro. *Eur J Neurosci*. 2007;25:2170–2181.
120. Khosravani H, Pinnegar CR, Mitchell JR, Bardakjian BL, Federico P, Carlen PL. Increased high-frequency oscillations precede in vitro low-Mg seizures. *Epilepsia*. 2005;46:1188–1197.

121. Lasztoczi B, Nyitrai G, Heja L, Kardos J. Synchronization of GABAergic inputs to CA3 pyramidal cells precedes seizure-like event onset in juvenile rat hippocampal slices. *J Neurophysiol*. 2009;102:2538–2553.
122. Lasztoczi B, Antal K, Nyikos L, Emri Z, Kardos J. High-frequency synaptic input contributes to seizure initiation in the low-[Mg^{2+}] model of epilepsy. *Eur J Neurosci*. 2004;19:1361–1372.
123. Lebovitz LB. Autorhythmicity of spontaneous interictal spike discharge at hippocampal penicillin focus. *Brain Res.* 1979;172:35–55.
124. Wyler AR, Ojemann GA, Ward AA Jr. Neurons in human epileptic cortex: correlation between unit and EEG activity. *Ann Neurol*. 1982;11:301–308.
125. Babb TL, Wilson CL, Isokawa-Akesson M. Firing patterns of human limbic neurons during stereoencephalography (SEEG) and clinical temporal lobe seizures. *Electroencephalogr Clin Neurophysiol.* 1987;66:467–482.
126. Isokawa-Akesson M, Wilson CL, Babb TL. Inhibition in synchronously firing human hippocampal neurons. *Epilepsy Res*. 1989;3:236–247.
127. Wilson CL, Khan SU, Engel J Jr, Isokawa M, Babb TL, Behnke EJ. Paired pulse suppression and facilitation in human epileptogenic hippocampal formation. *Epilepsy Res.* 1998;31:211–230.
128. de Curtis M, Librizzi L, Biella G. Discharge threshold is enhanced for several seconds after a spontaneous interictal spike in a model of focal epileptogenesis. *Eur J Neurosci*. 2001;14:1–6.
129. Davenport CJ, Brown WJ, Babb TL. GABAergic neurons are spared after intrahippocampal kainate in the rat. *Epilepsy Res*. 1990;5:28–42.
130. Esclapez M, Hirsch JC, Khazipov R, Ben-Ari, Bernard C. Operative GABAergic inhibition in hippocampal CA1 pyramidal neurons in experimental epilepsy. *Proc Natl Acad Sci USA*. 1997;94:12151–12156.
131. Prince DA, Jacobs K. Inhibitory function in two models of chronic epileptogenesis. *Epilepsy Res*. 1998;32:83–92.
132. de Curtis M, Manfridi A, Biella G. Activity-dependent pH shifts and periodic recurrence of spontaneous interictal spikes in a model of focal epileptogenesis. *J Neurosci.* 1998;18:7543–7551.
133. Spray D, Harris A, Bennet M. Gap junctional conductance is a simple and sensitive function of intracellular pH. *Science*. 1981;211:712–715.
134. Talairach J, Bancaud J. Lesion, "irritative" zone and epileptogenic focus. *Confin Neurol*. 1966;27: 91–94.
135. Avoli M, Louvel J, Pumain R, Kohling R. Cellular and molecular mechanisms of epilepsy in the human brain. *Prog Neurobiol*. 2005;77:166–200.
136. Wittner L, Huberfeld G, Clemenceau S, et al. The epileptic human hippocampal cornu ammonis 2 region generates spontaneous interictal-like activity in vitro. *Brain*. 2009;132:3032–3046.
137. Roopun AK, Simonotto JD, Pierce ML, et al. A nonsynaptic mechanism underlying interictal discharges in human epileptic neocortex. *Proc Natl Acad Sci USA*. 2010;107:338–343.
138. Gigout S, Louvel J, Kawasaki H, et al. Effects of gap junction blockers on human neocortical synchronization. *Neurobiol Dis.* 2006;22:496–508.
139. Louvel J, Papatheodoropoulos C, Siniscalchi A, et al. GABA-mediated synchronization in the human neocortex: elevations in extracellular potassium and presynaptic mechanisms. *Neuroscience*. 2001;105:803–813.
140. Mattia D, Olivier A, Avoli M. Seizure-like discharges recorded in human dysplastic neocortex maintained in vitro. *Neurology*. 1995;45:1391–1395.
141. Avoli M, Bernasconi A, Mattia D, Olivier A, Hwa GG. Epileptiform discharges in the human dysplastic neocortex: in vitro physiology and pharmacology. *Ann Neurol*. 1999;46:816–826.
142. D'Antuono M, Louvel J, Kohling R, et al. $GABA_A$ receptor–dependent synchronization leads to ictogenesis in the human dysplastic cortex. *Brain,* 2004;127:1626–1640.
143. Barbarosie M, Louvel J, D'Antuono M, Kurcewicz I, Avoli M. Masking synchronous GABA-mediated potentials controls limbic seizures. *Epilepsia.* 2002;43:1469–1479.
144. Avoli M, Louvel J, Kurcewicz I, Pumain R, Barbarosie M. Extracellular free potassium and calcium during synchronous activity induced by 4-aminopyridine in the juvenile rat hippocampus. *J Physiol*. 1996; 493(pt 3): 707–717.
145. Borck C, Jefferys JG. Seizure-like events in disinhibited ventral slices of adult rat hippocampus. *J Neurophysiol*. 1999;82:2130–2142.
146. Zuckermann EC, Glaser GH. Hippocampal epileptic activity induced by localized ventricular perfusion with high-potassium cerebrospinal fluid. *Exp Neurol*. 1968;20:87–110.
147. Traub RD, Dingledine R. Model of synchronized epileptiform bursts induced by high potassium in CA3 region of rat hippocampal slice. Role of spontaneous EPSPs in initiation. *J Neurophysiol*. 1990;64:1009–1018.
148. Traynelis SF, Dingledine R. Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. *J Neurophysiol*. 1988;59:259–276.
149. Avoli M, Mattia D, Siniscalchi A, Perreault P, Tomaiuolo F. Pharmacology and electrophysiology of a synchronous GABA-mediated potential in the human neocortex. *Neuroscience.* 1994;62:655–666.
150. Nicoll RA. Presynaptic action of barbiturates in the frog spinal cord. *Proc Natl Acad Sci USA*. 1975;72: 1460–1463.
151. Barker JL, McBurney RN. Phenobarbitone modulation of postsynaptic GABA receptor function on cultured mammalian neurons. *Proc R Soc Lond B Biol Sci*. 1979;206:319–327.

Chapter 18

$GABA_A$ Receptor Function in Typical Absence Seizures

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A typical absence is a nonconvulsive epileptic seizure that is characterized by a sudden and relatively brief impairment of consciousness, occurring concomitantly with a generalized, bilaterally synchronous *spike* (or *polyspike*) and *slow wave discharge* (SWD) at 2.5–4 Hz in the electroencephalogram (EEG).[1,2] Typical absence seizures are part of the multifaceted clinical and EEG presentation of many idiopathic generalized epilepsies (IGEs), but in childhood absence epilepsy (CAE) these seizures are the only neurological symptom and are not accompanied by either metabolic, neuropathological, or other neurological deficits.[1,2] The human studies reviewed in this chapter, therefore, will be those that relate to CAE, since this is the only IGE in which a putative causal link can be made between a typical absence seizure and the underlying genetic variants or pathophysiological mechanisms without the confounding effects of other epileptic and nonepileptic neurological phenotypes.

Typical absence seizures are genetically determined, and the classical 2.5–4 Hz SWD trait has been shown to be inherited in an autosomal dominant manner.[1,2] Indeed, there is a strong consensus in describing the occurrence of (typical) absence seizures as a familial disease with a complex multifactorial genetic background. There is also a general consensus, based on some old invasive studies and modern noninvasive imaging investigations, that typical absence seizures originate from abnormal electrical activities in reciprocally connected

thalamic and cortical territories—that is, in what is generally referred to as *thalamocortical networks*—with little or no involvement of other brain areas, including hippocampus, cerebellum, and limbic regions.[3–7] Key cellular elements of thalamocortical networks include pyramidal cells and interneurons of different cortical layers, the thalamocortical (TC) neurons of sensory thalamic nuclei and their main inhibitory input, that is, the GABAergic neurons of the nucleus reticularis thalami (NRT). Importantly, the notion that a typical absence seizure is "generalized" from the very start of the SWD has been recently challenged by the observation that the onset of an absence seizure in humans is associated with paroxysmal activation of discrete, often frontal and parietal cortical regions, spreading then to other cortical regions and to the thalamus.[3,5] The presence of a putative cortical *initiation site* had previously been shown in genetic rat models of typical absence seizures in which, differently from human absences, it is localized in the perioral region of the primary somatosensory cortex.[8,9] Indeed, direct application of the antiabsence drug ethosuximide at this putative initiation site, but not 1 mm away from it, readily abolishes absence seizures in freely moving rats.[10]

It is now well established that $GABA_A$ receptor ($GABA_AR$)–mediated inhibition consists of a phasic component (i.e., the classical inhibitory postsynaptic potentials [IPSPs]), which is generated by GABA interacting with synaptic $GABA_ARs$, and a tonic component (i.e., a persistent membrane hyperpolarization with increased conductance) that is due to GABA activation of perisynaptic or extrasynaptic $GABA_ARs$.[11] Abnormalities in both synaptic and extrasynaptic $GABA_ARs$ have undoubtedly been of primary significance among the various human molecular genetic alterations[12–15] that have in recent years provided support for the idea that IGEs, and in particular absence seizures, are channelopathies.[16] In this chapter, we first summarize recent evidence demonstrating that enhanced tonic $GABA_A$ inhibition is a common feature of both pharmacological and genetic models of absence epilepsy. In particular, enhanced tonic $GABA_A$ inhibition in TC neurons is both necessary and sufficient for the expression of typical absence seizures, and in genetic models it is not due to a neuronal abnormality but to a malfunction of the astrocytic GABA transporter GAT-1. The available data on phasic $GABA_A$ inhibition, on the other hand, suggest that the changes in this type of GABAergic function observed in animals and humans affected by typical absence seizures are both brain region and neuronal type specific. We will then discuss how all these findings concerning phasic and tonic $GABA_A$ inhibition fit within our current understanding of the pathophysiological mechanisms of typical absence seizures, and propose that the currently prevailing view of an overall GABAergic loss of function in typical absence seizures and CAE is no longer tenable.

TONIC $GABA_A$ INHIBITION

Enhanced Tonic $GABA_A$ Inhibition in Genetic Models of Typical Absence Seizures

The tonic $GABA_A$ current measured in vitro in TC neurons of the somatosensory ventrobasal thalamus in different genetic models of absence seizures is enhanced compared to the current in their respective control animals.[17] This is true for a polygenic rat model, that is, the Genetic Absence Epilepsy Rat from Strasbourg (GAERS)[18] (Fig. 18–1A1–2) and for various mouse models with known spontaneous monogenic mutations, including stargazer and lethargic mice[19] (Fig. 18–1A3). In particular, there is a clear developmental profile of this increased GABAergic function since in GAERS up to postnatal day 16, the current is similar to that in the nonepileptic control (NEC) strain but almost doubles in amplitude at postnatal day 17 (Fig. 18–1A2) and remains elevated well past the time of seizure onset[17] (around postnatal day 30 in this strain[18]). In contrast, no tonic $GABA_A$ current is detected in the GABAergic NRT neurons of GAERS and their respective nonepileptic control strain (unpublished observation), as it is indeed the case in normal Wistar rats.[20]

GAT-1 Malfunction Underlies Increased Tonic $GABA_A$ Inhibition

The enhanced tonic $GABA_A$ current of TC neurons in genetic absence models is not due

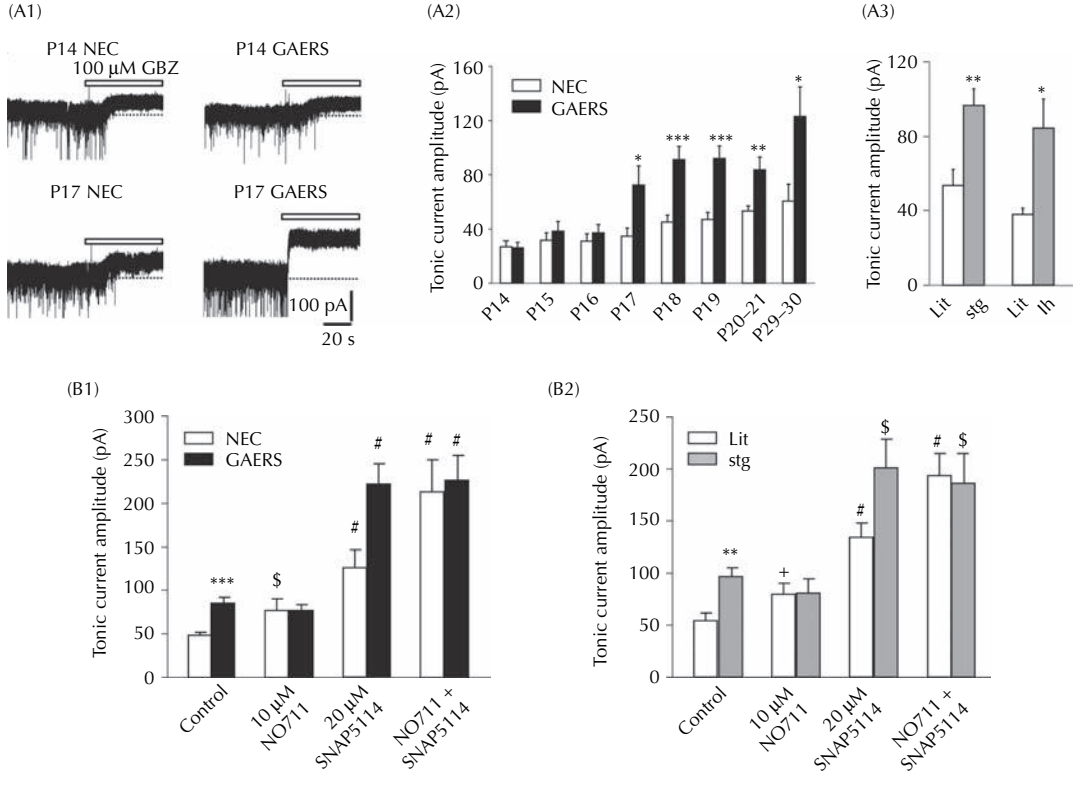

Figure 18–1. The enhanced tonic $GABA_A$ current in TC neurons of rat and mouse genetic models of typical absence seizures results from a malfunction in the GABA transporter GAT-1.

A1. Current traces from TC neurons of postnatal day (P) 14 and 17 nonepileptic control (NEC) and GAERS rats indicate the presence of a tonic $GABA_A$ current that is revealed as a shift in baseline current following the focal application of 100 µM gabazine (GBZ, white bars). The amplitude of the tonic current is about twofold larger in GAERS compared to NEC at P17. **A2**. Tonic current in NEC and GAERS at the indicated postnatal days. **A3**. Tonic $GABA_A$ current in stargazer (*stg*) (P19–21) and lethargic (*lh*) (P27–30) mice and respective control age-matched littermates (Lit). **B**. Comparison in P18–21 NEC and GAERS (**1**) and in P19–21 stargazer (*stg*) mice and littermates (Lit) (**2**) of the effects of blocking GAT-1 alone (by 10 µM NO711), GAT-3 alone (by 20 µM SNAP5114), and GAT-1 and GAT-3 together (NO + SNAP) on tonic $GABA_A$ current amplitude of TC neurons. **A2** and **A3**: $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, mutant versus nonmutant animals. **B1** and **B2**: $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, mutant versus nonmutant animals under control conditions; $+ p < 0.05$, $\$ p < 0.01$, and $\# p < 0.001$, drug versus nondrug for each strain. From ref. 17.

to increased vesicular GABA release, overexpression of extrasynaptic $GABA_A$Rs, or misexpression of synaptic $GABA_A$Rs, but results from a malfunction of the GABA transporter GAT-1,[17] despite its being far less abundant in the thalamus than GAT-3.[21] As shown in Fig. 18–1B1–2, in fact, (1) block of GAT-1 (by the selective blocker NO711) in GAERS and stargazer has no effect on the tonic current amplitude of TC neurons, (2) block of GAT-1 in the respective nonepileptic rats and mice increases tonic current amplitude to values similar to those seen in GAERS and stargazer mice, and (3) the compensatory increase in uptake by GAT-1 that is seen following block of GAT-3 (by the selective blocker SNAP5114) in nonepileptic animals is lost in both GAERS and stargazer mice.[17] A malfunction in GAT-1 also underlies the increased tonic $GABA_A$ current in TC neurons of lethargic mice.[17] These data support and enlarge previous observations that had shown reduced GABA uptake by GAT-1[22] and an increased level of extracellular GABA[23] in the ventrobasal thalamus of GAERS compared to NEC. In contrast to GAERS and stargazer mice, however, GABA transport by GAT-1 is reversed in lethargic mice, with this transporter being a source of ambient GABA.

Interestingly, NO711 increases tonic $GABA_A$ current by a similar amount in dentate gyrus granule cells of GAERS and NEC,[17] indicating that GAT-1 activity is not compromised in a brain area that does not participate in the generation of typical absence seizures[18] and where the distribution of this transporter is primarily neuronal.[24] Indeed, the tonic current of dentate gyrus granule cells is not different between GAERS and NEC.[17]

In summary, therefore, genetic models of typical absence seizures (i.e., in GAERS, stargazer, and lethargic mice) show a brain region–specific enhancement of tonic $GABA_A$ current, which in TC neurons is due to the increased extracellular GABA level resulting from a malfunction in GABA uptake by astrocytic GAT-1.

Enhanced Tonic $GABA_A$ Inhibition in the GHB Model: Role of $GABA_B$Rs

Systemic (or intrathalamic) injection of γ-hydroxybutyric acid (GHB) is undoubtedly the best-established pharmacological model of typical absence seizures,[25,26] and systemic administration of 4,5,6,7-tetrahydroisoxazolo[5,4-c] pyridin-3-ol) (THIP), a selective agonist at δ subunit-containing extrasynaptic $GABA_A$Rs,[27] has been shown to elicit SWDs in normal animals.[28] Therefore, whereas it is not surprising that THIP dose-dependently enhances the tonic $GABA_A$ current in TC neurons of normal Wistar rats,[17] one would not expect GHB, which does not bind to $GABA_A$Rs and is believed to elicit absence seizures by activation of $GABA_B$Rs,[26] to have an effect on the tonic $GABA_A$ current. However, GHB dose-dependently enhances the tonic $GABA_A$ current in TC neurons of normal Wistar rats[17] (Fig. 18–2A,B) at concentrations that are similar to the brain concentrations that elicit absence seizures in vivo.[29] This effect of GHB is not due to some aspecific action since it is abolished by the $GABA_B$R antagonist CGP55845 (Fig. 18–2B). Interestingly, application of CGP55845 alone significantly reduces the tonic $GABA_A$ current amplitude in TC neurons of Wistar rats to 74% of control values[17] (Fig. 18–2B), indicating that facilitation of extrasynaptic $GABA_A$Rs by $GABA_B$Rs contributes approximately one-quarter of the tonic $GABA_A$ current in normal rats. Importantly, CGP55845 also reduces the tonic current in GAERS, stargazer, and lethargic mice to about 55%, 65%, and 57%, respectively[17] (Fig. 18–2C), suggesting that facilitation of extrasynaptic $GABA_A$R function by $GABA_B$R activation contributes up to half of the tonic current in these genetic models.

In summary, therefore, a GAT-1 malfunction in thalamic astrocytes of mouse and rat genetic models leads to an increase in ambient GABA in the sensory thalamus, which in turn elicits an enhancement in tonic $GABA_A$ inhibition through both direct activation of extrasynaptic $GABA_A$Rs and indirect facilitation of extrasynaptic $GABA_A$Rs via activation of $GABA_B$Rs.

Enhanced Tonic $GABA_A$ Inhibition of TC Neurons Is Necessary and Sufficient for Typical Absence Seizure Generation

Assessing the impact of enhanced tonic $GABA_A$ current of TC neurons on the expression of absence seizures requires experiments in freely moving animals, in which both the behavioral and EEG components of the seizures can be studied without bias introduced by the concomitant use of anesthetics and/or analgesics. In fact, unrestrained and undrugged GAT-1 knockout (KO) mice, which have an enhanced tonic $GABA_A$ current in TC neurons, express ethosuximide-sensitive typical absence seizures[17] (Fig. 18–3A,F). Similarly, direct injection of the selective GAT-1 blocker NO711 into the ventrobasal thalamus by reverse microdialysis in normal Wistar rats triggers ethosuximide-sensitive typical absence seizures[17] (Fig. 18–3D,F). Moreover, in δ–$GABA_A$R KO mice, which exhibit markedly reduced $GABA_A$ inhibition in TC neurons, systemic administration of GHB fails to induce absence seizures[17] (Fig. 18–3F). Similarly, the intrathalamic injection of a δ subunit-specific antisense oligodeoxynucleotide in GAERS strongly decreases both the tonic $GABA_A$ current and spontaneous seizures 1–2 days after injection, whereas a missense oligodeoxynucleotide has no effect[17] (Fig. 18–3E). Finally, intrathalamic administration of the δ subunit-specific agonist THIP in normal Wistar rats elicits absence seizures in a concentration-dependent manner, an effect that is reversed by systemic administration of

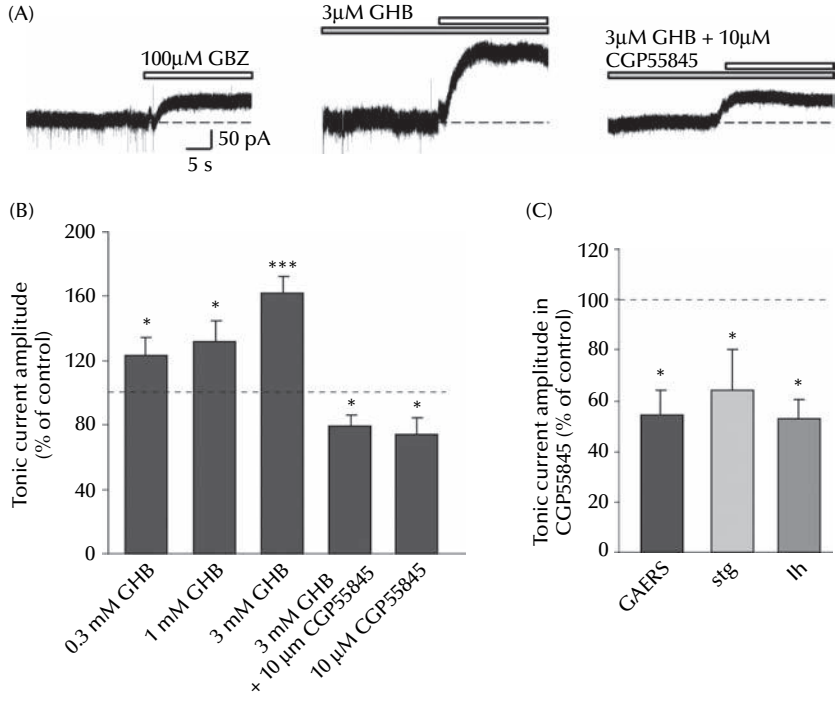

Figure 18–2. $GABA_B$Rs involvement in the tonic $GABA_A$ current of TC neurons in genetic and pharmacological models of typical absence seizures. **A**. Current traces from TC neurons of normal Wistar rats showing the GHB-elicited increase in tonic $GABA_A$ current and its block by the $GABA_B$ antagonist CGP55845 (10 μM). **B**. Comparison of the effects of different concentrations of GHB on tonic $GABA_A$ current amplitude in normal Wistar rats and its block by CGP55845 (10 μM). Note how CGP55845 alone decreases tonic current amplitude compared to control conditions. Values are normalized to the average tonic current in the absence of GHB or CGP55845. **C**. Effect of bath application of 10 μM CGP55845 on tonic $GABA_A$ current in GAERS, stargazer (*stg*) and lethargic (*lh*) mice. Values are normalized to the average tonic current amplitude in the absence of CGP55845. **B** and **C**: $^*p < 0.05$ and $^{***}p < 0.001$. From ref. 17.

ethosuximide[17] (Fig. 18–3C,F). Taken together, these data show that enhanced tonic $GABA_A$ inhibition in TC neurons is both necessary and sufficient for the generation of typical absence seizures. In addition, they provide a mechanistic explanation for the aggravation of absence seizures that is observed in humans and experimental models following systemic and intrathalamic administration of drugs that increase GABA levels, including tiagabine, a GABA uptake blocker, and vigabatrine, a GABA transaminase blocker.[18,30–32]

Significance of Tonic $GABA_A$ Inhibition in Typical Absence Epilepsy

The discovery of an increased tonic $GABA_A$ current in TC neurons represents the first abnormality that (1) is common to both well-established pharmacological and genetic models of typical absence seizures, irrespective of species and known upstream mutations, (2) is manifested both before and after the developmental onset of seizures, (3) contributes to the exquisite sensitivity of experimental absence seizures to selective $GABA_B$R agonists and antagonists, and (4) explains the aggravating effects of GABAergic drugs in both human and experimental absence seizures. Importantly, these results, together with the presence of powerful $GABA_A$ IPSPs in the majority of TC neurons during absence seizures in vivo[33,34] (Fig. 18–4A1–2; see below), lead to another significant conclusion, that is, that studies aiming at reproducing typical absence seizures by indiscriminately blocking $GABA_A$Rs of TC neurons[35–38] are inherently flawed.

The finding that the increased tonic $GABA_A$ inhibition in TC neurons of genetic models

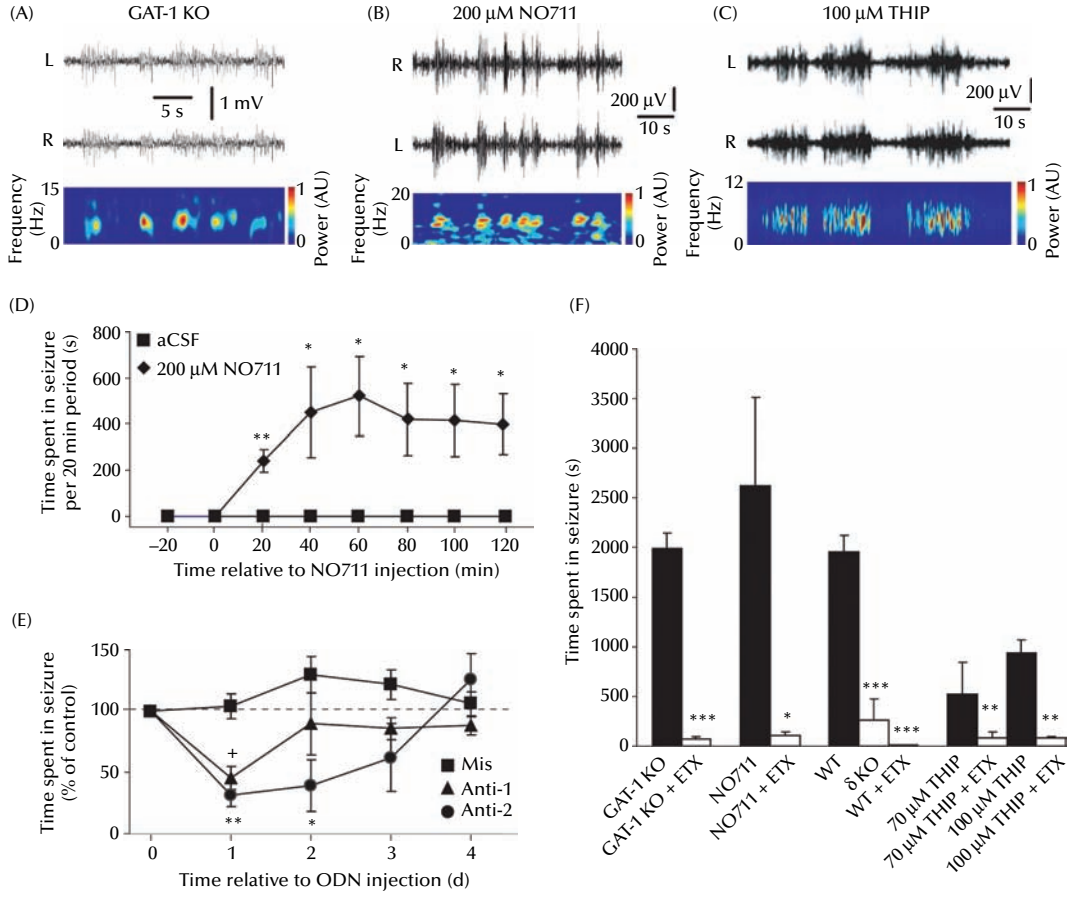

Figure 18–3. The tonic GABA$_A$ inhibition in TC neurons is both necessary and sufficient for typical absence seizure generation. **A**. Bilateral (L = left, R = right hemispheres) EEG traces from a freely moving GAT-1 knockout (KO) mouse showing spontaneous SWDs (spectrogram of the R trace is illustrated below). **B**. Bilateral EEG traces from a normal Wistar rat following intrathalamic administration by reverse microdialysis of 200 µM of the selective GAT-1 blocker NO711 (spectrogram of the L trace is illustrated below). **C**. Bilateral EEG traces from a normal Wistar rat following intrathalamic administration by reverse microdialysis of 100 µM THIP (spectrogram of the R trace is illustrated below). **D**. Time course of the induction of SWDs by intrathalamic administration of NO711. **E**. Spike-and-wave discharges are substantially reduced in GAERS 1 and 2 days following a single intrathalamic injection of a small and large dose of a δ-subunit antisense oligodeoxynucleotide (Anti-1 and Anti-2, respectively) but not of a missense oligo (Mis). **F**. Summary histograms showing the cumulative (over 1–2 h) time spent in seizures for different transgenic and pharmacological mice and rat models and the abolition of their SWDs by systemic administration of ethosuximide (100 or 200 mg/kg/ip). $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. From ref. 17.

is due to a malfunction of GAT-1 shifts the emphasis from a neuronal to an astrocytic etiology of absence epilepsy. The impaired GAT-1 activity in GAERS is not caused by decreased expression of GAT-1 mRNA or protein levels in either thalamus or cortex.[17] Also, GAT-1 cDNA from GAERS, stargazer, and lethargic mice presents no genetic variants, whereas the mutations responsible for absence seizures in stargazer and lethargic mice are not present in GAERS.[17] Potential abnormalities that may lead to reduced GAT-1 function, but have not yet been tested, include the inability of this protein to reach the outer astrocytic membrane, an alteration in its subcellular location, and/or abnormalities in its phosphorylation. Similarly, we are aware of no study that has investigated GAT-1 (or GAT-3) genetic variants in human CAE.

Experimental typical absence seizures can be elicited or aggravated by selective GABA$_B$R agonists and can be blocked by selective

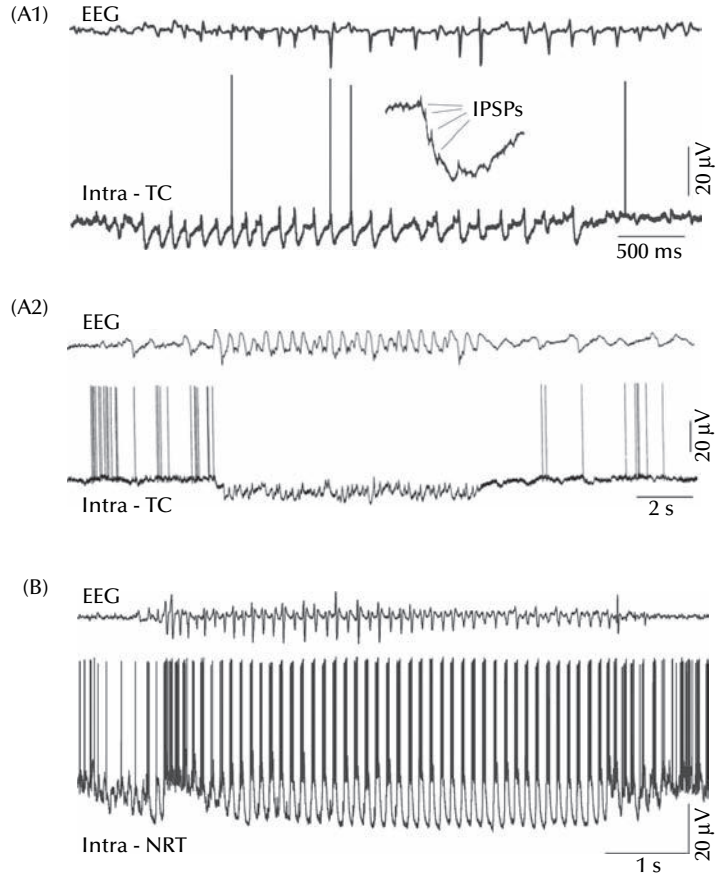

Figure 18–4. Intracellular correlates of TC and NRT neuron activity during spontaneous SWDs in vivo. **A1**. Intracellular recording (bottom trace) from a TC neurons in GAERS in vivo during a spontaneous SWD (top trace) shows the presence of bursts of IPSPs occurring in synchrony with each spike and wave complex. Note the individual IPSPs in the enlarged burst in the inset. **A2**. Intracellular recording (bottom trace) from cat TC neurons in vivo during an SWD (top trace) shows the presence of IPSPs superimposed on a long-lasting hyperpolarization that lasts for the entire duration of the SWD. **B**. Intracellular recording (bottom trace) from an NRT neurons in GAERS in vivo during a spontaneous SWD (top trace) shows the presence of a large low-threshold Ca^{2+} potential in synchrony with each spike-and-wave complex. A1 and B from ref. 1. A2 from ref. 33.

$GABA_B$R antagonists, applied either systemically or intrathalamically.[39–41] Because about 50% of the tonic $GABA_A$ current observed in TC neurons of GAERS, stargazer, and lethargic mice is abolished by a $GABA_B$R antagonist[17] (Fig. 18–2C), the behavioral and EEG effects of $GABA_B$R-selective drugs on typical absence seizures can no longer be explained simply by the ability of these drugs to affect $GABA_B$-mediated IPSPs and/or presynaptic $GABA_B$Rs, but should also take into account the positive modulation by $GABA_B$Rs of the tonic $GABA_A$ inhibition in TC neurons.

PHASIC $GABA_A$ INHIBITION

The picture concerning phasic $GABA_A$ inhibition in typical absence seizures is much more complex than that of tonic $GABA_A$ inhibition, since contradictory results have emerged from studies in various experimental models. For the sake of clarity, therefore, the analysis of available evidence on phasic $GABA_A$ inhibition will be presented separately for each main neuronal type of the thalamocortical network, that is, cortical neurons, TC neurons, and the GABAergic neurons of the NRT.

Phasic $GABA_A$ Inhibition in Cortex

Among the various mutations in $GABA_A$R subunits, those that have been identified in related or unrelated CAE patients are α1(S326fs328X), β3(P11S), β3(S15F), β3(G32R), γ2(R43Q), γ2(R139G), and γ2(IVS6+2T→G).[12–15,42] Functional analysis of these mutant proteins expressed in heterologous systems has shown that all of them bring about a marked decrease in GABA responses.[12] Importantly, humans carrying the γ2(R43Q) mutation have been shown to have a decreased short-interval intracortical inhibition assessed with transcranial magnetic stimulation, leading to cortical facilitation.[43] γ2(R43Q) mutant mice constructed by homologous recombination express spontaneous ethosuximide-sensitive absence seizures.[44] Moreover, despite the fact that the γ2 subunit has an almost ubiquitous expression and is apparently required for functional synaptic $GABA_A$Rs, these mice exhibit a brain region–specific alteration in phasic $GABA_A$ inhibition, since cortical layer 2/3 pyramidal cells but not TC or NRT neurons show a reduction in miniature IPSCs (mIPSCs) compared to age-matched wild-type littermates.[44] Whether the region specificity of this malfunction is also present in humans with the γ2(R43Q) mutation, or only occurs in mice because of some species-specific promoters or silencers, is not known at present. Nevertheless, together with showing the physiological effect of a human genetic variant in a "true" physiological system as opposed to heterologous expression systems, the key significance of this finding is that it is the first clear evidence that the downstream abnormalities of a human genetic variant in CAE can be brain region specific. Importantly, the cortex-specific abnormality in phasic $GABA_A$ inhibition that results from the human γ2(R43Q) mutation exquisitely complements previous experimental data showing that (1) in felines in vivo the localized block of $GABA_A$Rs by cortical application of bicuculline, which leaves intact phasic $GABA_A$ inhibition in the thalamus, does elicit SWDs,[33] and (2) in rat and mouse genetic models, phasic $GABA_A$ inhibition in TC neurons is either unaffected or increased compared to their respective non-epileptic controls[17,44–47] (see below).

On the basis of indirect evidence, decreased cortical GABAergic inhibition has also been suggested to occur in layer 2/3 regular spiking neurons[48] as well as in layer 5 pyramidal neurons[49] of adult Wistar Albino Glaxo/Rij (WAG) rats, another well-established polygenic model of typical absence seizures.[50] However, no change is observed in mIPSCs in pyramidal cell and interneurons of cortical layers 2–3 of young preseizure GAERS,[45] and cortical GABAergic inhibition is intact in the feline generalized penicillin epilepsy model.[51]

In summary, the evidence from a human mutation indicates that decreased phasic $GABA_A$ inhibition in cortex characterizes typical absence seizures, though further studies are needed to clarify the extent of this decrease in terms of different cortical regions, layers, and neuronal types: this additional work might shed light on the existing discrepancies concerning changes in phasic $GABA_A$ inhibition among experimental models.

Phasic $GABA_A$ Inhibition in TC Neurons

Reduced or absent phasic $GABA_A$R function in the thalamus also characterizes the current view of the pathophysiology of typical absence seizures. While this may be partly true for intra-NRT inhibition, it certainly does not hold for TC neurons. Thus, both in felines that show spontaneous or cortical bicuculline-induced SWDs[33] and in the well-established GAERS model,[34] the vast majority (60% and 94%, respectively) of TC neurons recorded in vivo during SWDs exhibit bursts of $GABA_A$ IPSPs, each tightly synchronized with the EEG spike and wave complex (Fig. 18–4A1–2). Indeed, the rise time, amplitude, frequency, and decay time constant of mIPSCs and spontaneous IPSCs (sIPSCs) measured in TC neurons in vitro do not differ between GAERS and NEC both prior to[45] and after spontaneous seizure manifestation[17] (i.e., postnatal days 12–25 and postnatal day 30, respectively), and paired pulse depression of evoked IPSCs is similar between the two strains[45] (Fig. 18–5B3). As mentioned earlier, no change in TC neuron IPSCs occurs in mice carrying the human γ2(R43Q) mutation,[44] and no change in IPSC properties has been detected in TC neurons of β3 KO mice[52] that show absence seizures as part of a much more complex neurological phenotype. Also, in lethargic, stargazer, and tottering mice,

sIPSCs in TC neurons are similar to those in their respective littermates,[17] and no difference has been found in evoked IPSCs in lethargic and tottering mice compared to control mice.[47] Finally, an increase in mIPSCs frequency, potentially leading to enhanced phasic inhibition, is observed in TC neurons of the absence seizure-prone DAB/2J mouse strain.[46]

In a manner similar to that of the genetic models, in the best-established pharmacological model of absence seizure, that is, the GHB model, a net increase in phasic $GABA_A$ inhibition is observed in TC neurons of the ventrobasal thalamus.[53] This is because whereas GHB reversibly and dose-dependently decreases the amplitude of all sensory[54] and corticothalamic EPSCs[53] (Fig. 18–5A1), the two main excitatory drives to TC neurons, at low concentrations (250 μM–1 mM) it only affects the $GABA_A$ IPSCs in some but not all TC neurons[53] (Fig. 18–5A2–3). In particular, 250 μM GHB produces an effect in <10% of tested TC neurons, whereas at 0.5–1 mM it decreases only half of the recorded IPSCs (Fig. 18–5A4–5). Thus, at brain concentrations (250 μM–1 mM) similar to those that elicit absence seizures in vivo,[29] GHB brings about not only the previously described $GABA_B$R- and K^+-dependent hyperpolarization[26,55] and an increase in tonic $GABA_A$ inhibition[17] (see above), but also an imbalance between the excitatory and inhibitory drives to TC neurons, leading to a net increase in their phasic $GABA_A$ inhibition. This, in turn, helps to impose the periodic phasic inhibition that sculptures the electrical behavior of TC neurons during SWDs, as observed in vivo in feline[56] and rat[57] models (Fig. 18–4A1–2).

On the basis of all this evidence from both genetic and pharmacological models, as well as from mice expressing the human γ2(R43Q) mutation, it is therefore surprising that the data in support of the presence of an enhanced or unchanged phasic GABAergic function in TC neurons have been selectively dismissed over the last 15 years, such that textbooks and topical reviews[58–62] still present the pathophysiological mechanisms of TC neuron activity during typical absence seizures as the one that is observed in vitro during application of a $GABA_A$R antagonist.[35,36,38] It is also surprising that the in vivo evidence that is often used to support this view is the ability of bicuculline (or other $GABA_A$R antagonists) to generate, in ketamine-xylazine anesthetized rats, strong field potentials at 3 Hz,[37] which are claimed to represent SWDs even if their sensitivity to ethosuximide or other antiabsence drugs was not tested. Indeed, it is highly unlikely that these field potentials represent SWDs, since the ketamine-xylazine combination, like other general anaesthetic, is known to abolish the SWDs of any well-established in vivo model of absence epilepsy.[18,51,63]

In summary, therefore, the presence of these serious issues of interpretation, together with the evidence showing an increased tonic and an unchanged/increased phasic $GABA_A$ inhibition in TC neurons of genetic and pharmacological models, strongly support the conclusion that the block of thalamic $GABA_A$Rs that continues to be used in many in vitro and some in vivo studies does at best elict some form of thalamic hyperexcitability of as yet unknown pathophysiological relevance, but it is definitely not the activity that is present during typical absence seizures.

Phasic $GABA_A$ Inhibition in NRT Neurons

The existance of contradictory data makes it difficult at present to draw a unifying picture of the changes in the phasic $GABA_A$ inhibition of NRT neurons that are associated with an absence seizure phenotype. Starting from inbred genetic models, mIPSCs recorded from NRT neurons of preseizure GAERS show a 67% higher frequency, a 25% larger amplitude, and a 40% faster decay than those in age-matched NECs.[45] Moreover, paired-pulse depression of evoked $GABA_A$ IPSCs is significantly smaller (46%) in NRT neurons of GAERS than in NECs[45] (Fig. 18–5B1–2). This smaller paired-pulse depression of IPSCs in GAERS would undoubtedly ensure a consistent hyperpolarizing drive to help promote the expression of the strong low-threshold Ca^{2+} potential-mediated bursts of action potentials that are the hallmark of NRT neuron firing at each spike and wave complex in vivo[56,57] (Fig. 18–4B). Moreover, an increase in mIPSC frequency, potentially leading to enhanced phasic $GABA_A$ inhibition, is observed in NRT neurons of the absence seizure-prone DAB/2J mouse strain.[46] Finally, an almost complete disappearance of the

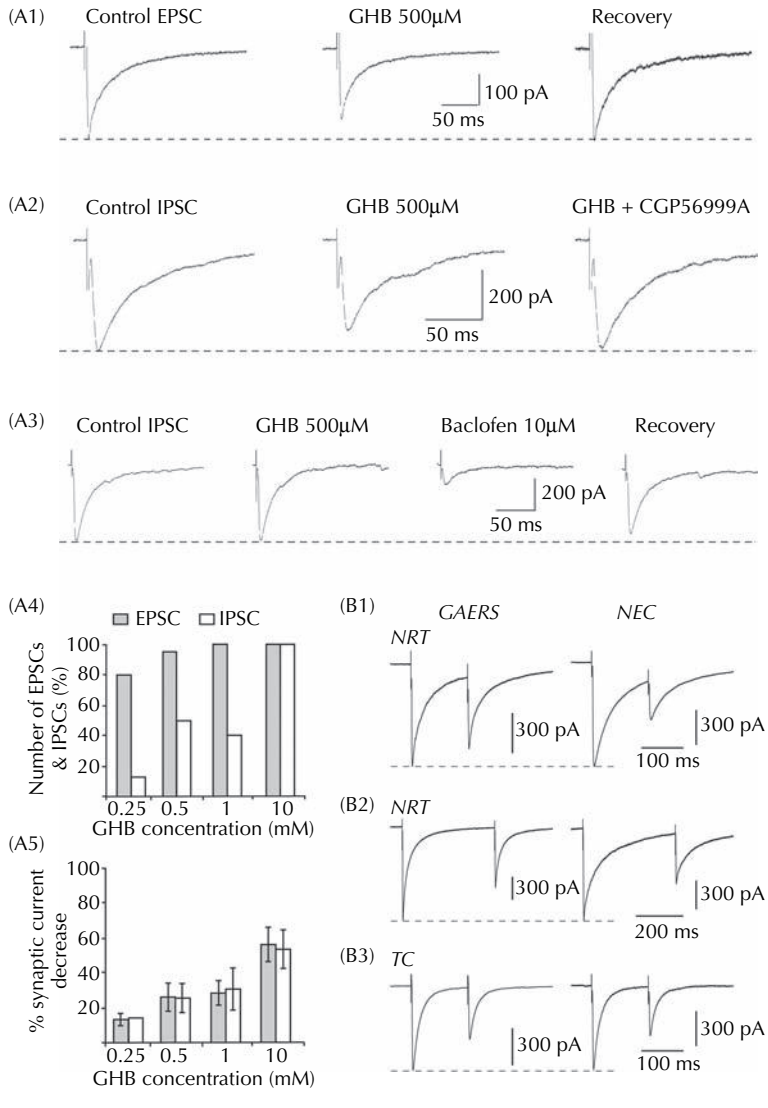

Figure 18–5. Phasic $GABA_A$ inhibition in TC and NRT neurons of genetic and pharmacological models of typical absence seizures. **A1**. γ-Hydroxybutyric acid reversibly decreases the amplitude of a cortical EPSC recorded in TC neurons. **A2**. Example of an NRT-derived IPSC (recorded in a TC neuron) that is decreased by GHB. The effect is blocked by the $GABA_B$ antagonist CGP56999A (100 nM). **A3**. Example of an NRT-derived IPSC (recorded in a TC neuron) that is unaffected by GHB (500 µM). Following washout of GHB, the IPSC is reversibly reduced by the selective $GABA_B$ agonist baclofen (10 µM). **A4**. All cortical EPSCs are decreased by GHB, while only a number of NRT-derived IPSCs are sensitive to low concentrations of this drug. **A5**. Plot showing the similar potency of GHB on cortical EPSCs and NRT-derived IPSCs. **B**. Paired-pulse depression of IPSCs in NRT neurons of GAERS is much smaller than in NEC than in GAERS for interstimulus interval similar (B**1**) but not longer (B**2**) than that of the SWDs. **B3**. Paired-pulse depression of IPSCs recorded in TC neurons is similar between GAERS and NEC. A from ref. 53. B from ref. 45.

α3 subunit, which in the thalamus is selectively expressed in NRT neurons,[64] has been reported in these GABAergic neurons from WAG rats,[65] suggesting a potential loss of function.

Surprisingly, however, α3 KO mice do not show spontaneous absence seizures and indeed exhibit a small reduction in GHB-elicited seizures compared to wild-type littermates, a result that has been interpreted as resulting from a powerful compensatory gain in phasic $GABA_A$ inhibition in NRT neurons.[66] On the other hand, since unknown mechanisms lead

to this unexpected compensatory increase in NRT $GABA_A$ inhibition following deletion of the α3 subunit, the lack of spontaneous absence seizures and the relative resistance to pharmacologically induced absence seizures may also be due to additional compensatory changes in phasic (or tonic?) $GABA_A$ inhibition in cortex, or tonic inhibition in thalamus, all of which were not investigated in that study.[66] Further evidence for a pro-absence role of a decreased phasic $GABA_AR$ function in NRT neurons has also come from the observation of an increase in high-frequency discharges at 3 Hz in β3 subunit KO mice, which show a massive reduction in sIPSP frequency and amplitude in NRT but no change in TC neurons.[52] Since these β3 KO mice exhibit a large variety of neurological deficits and are considered a model of Angelman's syndrome, it is difficult to unequivocally assign a causative role for this decreased intra-NRT inhibition in typical absence seizures.

In summary, whereas in inbred models and models with spontaneous mutations there is either an increase or no change in intra-NRT phasic $GABA_A$ inhibition, data from two transgenic mice suggest that a decrease in this NRT synaptic function has a pro-absence effect. Thus, it may be tempting to conclude that abnormalities in intra-NRT phasic $GABA_A$ inhibition are not a necessary condition for the expression of typical absence seizures, a view supported by the lack of changes in NRT IPSCs of mice carrying the human γ2(R43Q) mutation.[44] In this respect, it is worth noting that the antiabsence effect of clonazepam in humans and experimental models[67,68] may not be solely due to its ability to increase phasic $GABA_A$ inhibition in NRT,[69] since α3 subunit–containing $GABA_ARs$ that are the target of this benzodiazepine are also present in neocortical neurons.[70]

ROLE FOR PHASIC AND TONIC $GABA_A$ INHIBITION IN THE GENESIS OF ABSENCE SEIZURES

The evidence reviewed in this chaper suggests the following scenario concerning the cellular contribution of phasic and tonic $GABA_A$ inhibition to typical absence seizures. Decreased phasic $GABA_A$ inhibition in the neocortex leads or contributes to a paroxysmal development of normal 5–9 Hz oscillations in a discrete somatosensory cortical *initiation site.* This strong and highly synchronous cortical output powerfully excites the GABAergic neurons of the NRT, which respond by generating low-threshold Ca^{2+} potential-mediated bursts of action potentials at every spike-and-wave complex of the SWD. This rhythmic burst firing, in turn, results in bursts of IPSPs in TC neurons that override cortical excitation. Concomitantly, ambient GABA levels around TC neurons abnormally increase due to reduced GABA uptake by GAT-1, enhancing extrasynaptic $GABA_AR$ function directly, and indirectly by a $GABA_BR$-dependent facilitation. Enhanced tonic inhibition persistently hyperpolarizes the TC neurons and increases their membrane conductance, reducing their action potential output, with low-threshold Ca^{2+} potential-mediated bursts of action potentials rarely occurring. Furthermore, the responsiveness of TC neurons to excitatory sensory synaptic inputs is reduced, "gating" information flow through the thalamus, abolishing responsiveness to external stimuli, and causing behavioral arrest. Importantly, the rhythmic IPSP bursts entrain TC neuron output to each cycle of a SWD, providing sparse but synchronized input to the cortex and maintaining paroxysmal activity in thalamocortical networks.

CONCLUDING REMARKS

Despite the long-standing notion of an overall decrease in $GABA_AR$ function in typical absence seizures, a critical appraisal of the human and experimental evidence strongly supports the view of different, brain region-specific changes in both phasic and tonic $GABA_AR$-mediated inhibition in this nonconvulsive epileptic phenotype. In particular, decreased phasic $GABA_A$ inhibition characterizes cortical activity, though more studies are needed to disclose any neuronal type- and layer-specific abnormalities. Enhanced tonic inhibition, which results from a malfunction of the astrocytic GAT-1 transporters, together with unchanged or increased phasic inhibition, is present in TC neurons of both genetic and pharmacological models of typical absence seizures. Contradictory results in different species and transgenic animals and the reliance on unsuitable in vitro model systems

still do not provide a clear view of the changes in phasic $GABA_A$ inhibition that accompany the typical absence seizure in NRT neurons. Thus, thalamic and cortical model systems in which $GABA_A$Rs are indiscriminately blocked do not reproduce the complex pattern of alterations in these neurotransmitter receptors that is observed in human and experimental absence seizures, and are therefore of no value for understanding the cellular mechanisms operating in thalamocortical networks during typical absence seizures.

DISCLOSURE STATEMENT

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REFERENCES

1. Crunelli V, Leresche N. Childhood absence epilepsy: genes, channels, neurons and networks. *Nat Rev Neurosci*. 2002;3:371–382.
2. Blumenfeld H. Cellular and network mechanisms of spike-wave seizures. *Epilepsia*. 2005;46:21–33.
3. Holmes MD, Brown M, Tucker DM. Are "generalized" seizures truly generalized? Evidence of localized mesial frontal and frontopolar discharges in absence. *Epilepsia*. 2004;45:1568–1579.
4. Hamandi K, Salek-Haddadi A, Laufs H, Liston A, Friston K, Fish DR, Duncan JS, Lemieux L. EEG-fMRI of idiopathic and secondarily generalized epilepsies. *Neuroimage*. 2006;31:1700–1710.
5. Westmijse I, Ossenblok P, Gunning B, van Luijtelaar G. Onset and propagation of spike and slow wave discharges in human absence epilepsy: a MEG study. *Epilepsia*. 2009;50:2538–2548.
6. Bai X, Vestal M, Berman R, Negishi M, Spann M, Vega C, Desalvo M, Novotny EJ, Constable RT, Blumenfeld H. Dynamic time course of typical childhood absence seizures: EEG behaviour and functional magnetic resonance imaging. *J Neurosci*. 2010;30:5884–5893.
7. Szaflarski JP, Difrancesco M, Hirschauer T, Banks C, Privitera MD, Gotman J, Holland SK. Cortical and subcortical contributions to absence seizure onset examined with EEG/fMRI. *Epilepsy Behav*. 2010;18:404–413.
8. Meeren HKM, Pijn JPM, Van Liujtelaar ELJM, Coenen AML, da Silva FHL. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci*. 2002;22:1480–1495.
9. Polack PO, Guillemain I, Hu E, Deransart C, Depaulis A, Charpier S. Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *J Neurosci*. 2007;27:6590–6599.
10. Manning JP, Richards DA, Leresche N, Crunelli V, Bowery NG. Cortical-area specific block of genetically determined absence seizures by ethosuximide. *Neuroscience*. 2004;123:5–9.
11. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. *Nat Rev Neurosci*. 2005;6:215–229.
12. MacDonald RL, Kang J-Q, Gallagher MJ. Mutations in $GABA_A$ receptor subunits associated with genetic epilepsies. *J Physiol*. 2010;588:1861–1869.
13. Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, Berkovic SF. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet*. 2001;28:49–52.
14. Kananura C, Haug K, Sander T, Runge U, Gu W, Hallmann K, Rebstock J, Heils A, Steinlein OK. A splice-site mutation in *GABRG3* associated with childhood absence epilepsy and febrile convulsions. *Arch Neurol*. 2002;59:1137–1141.
15. Tanaka M, Olsen RW, Medina MT, Schwarz E, Alonso ME, Duron RM, Castro-Ortega R, Martinez-Juarez IE, Pascual-Castroviejo I, Machado-Salas J, Silva R, Bailey JN, Bai D, Ochoa A, Jara-Prado A, Pineda G, MacDonald RL, Delgado-Escueta AV. Hyperglycosylation and reduced GABA currents of mutated GABRB3 polypeptide in remitting childhood absence epilepsy. *Am J Hum Genet*. 2008;82:1249–1261.
16. Noebels JL. The biology of epilepsy genes. *Annu Rev Neurosci*. 2003;26:599–625.
17. Cope DW, Di Giovanni G, Fyson SJ, Orbán G, Errington AC, Lörincz ML, Gould TM, Carter DA, Crunelli V. Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. *Nat Med*. 2009;15:1392–1398.
18. Danober L, Deransart C, Depaulis A, Vergnes M, Marescaux C. Pathophysiological mechanisms of genetic absence epilepsy in the rat. *Prog Neurobiol*. 1998;55:27–57.
19. Fletcher CF, Frankel WN. Ataxic mouse mutants and molecular mechanisms of absence epilepsy. *Hum Mol Genet*. 1999;8:1907–1912.
20. Cope DW, Hughes SW, Crunelli V. $GABA_A$ receptor–mediated tonic inhibition in thalamic neurons. *J Neurosci*. 2005;25:11553–11563.
21. Pow DV, Sullivan RK, Williams SM, Scott HL, Dodd PR, Finkelstein D. Differential expression of the GABA transporters GAT-1 and GAT-3 in brains of rats, cats, monkeys and humans. *Cell Tissue Res*. 2005;320:379–392.
22. Sutch RJ, Davies CC, Bowery NG. GABA release and uptake measured in crude synaptosomes from Genetic Absence Epilepsy Rats from Strasbourg (GAERS). *Neurochem Int*. 1999;3:415–425.
23. Richards DA, Lemos T, Whitton PS, Bowery NG. Extracellular GABA in the ventrolateral thalamus of rats exhibiting spontaneous absence epilepsy: a microdialysis study. *J Neurochem*. 1995;65:1674–1680.
24. De Biasi S, Vitellaro-Zuccarello L, Brecha NC. Immunoreactivity for the GABA transporter-1 and GABA transporter-3 is restricted to astrocytes in the

rat thalamus. A light and electron-microscopic immunolocalization. *Neuroscience.* 1998;83:815–828.
25. Banerjee PK, Hirsch E, Snead OC III. γ-Hydroxybutyric acid induced spike and wave discharges in rats: relation to high-affinity [^{3}H]γ-hydroxybutyric acid binding sites in the thalamus and cortex. *Neuroscience*. 1993;56:11–21.
26. Crunelli V, Emri Z, Leresche N. Unravelling the brain targets of gamma-hydroxybutyric acid. *Curr Opin Pharmacol*. 2006;6:44–52.
27. Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA. Pharmacological characterization of a novel cell line expressing human α4β3δ $GABA_A$ receptors. *Br J Pharmacol*. 2002;136;965–974.
28. Fariello RG, Golden GT. The THIP-induced model of bilateral synchronous spike and wave in rodents. *Neuropharmacology*. 1987;26:161–165.
29. Snead OC. The gamma-hydroxybutyrate model of absence seizures: correlation of regional brain levels of gamma-hydroxybutyric acid and gamma-butyrolactone with spike wave discharges. *Neuropharmacology*. 1991;30:161–167.
30. Hosford DA, Wang Y. Utility of the lethargic (*lh/lh*) mouse model of absence seizures in predicting the effects of lamotrigine, vigabatrin, tiagabine, gabapentin, and topiramate against human absence seizures. *Epilepsia*. 1997;38:408–414.
31. Perucca E, Gram L, Avanzini G, Dulac O. Antiepileptic drugs as a cause of worsening seizures. *Epilepsia*. 1998;39:5–17.
32. Ettinger AB, Bernal OG, Andriola MR, Bagchi S, Flores P, Just C, Pitocco C, Rooney T, Tuominen J, Devinsky O. Two cases of nonconvulsive status epilepticus in association with tiagabine therapy. *Epilepsia*. 1999;40:1159–1162.
33. Steriade M, Contreras D. Relations between cortical and thalamic cellular events during transition from sleep patterns to paroxysmal activity. *J Neurosci.* 1995;15:623–642.
34. Pinault D, Leresche N, Charpier S, Deniau J-M, Marescaux C, Vergnes M, Crunelli V. Intracellular recordings in thalamic neurones during spontaneous spike and wave discharges in rats with absence epilepsy. *J Physiol*. 1998;509:449–456.
35. von Krosigk M, Bal T, McCormick DA. Cellular mechanisms of a synchronized oscillation in the thalamus. *Science*. 1993;261:361–364.
36. Bal T, von Krosigk M, McCormick DA. Synaptic and membrane mechanisms underlying synchronized oscillations in the ferret lateral geniculate nucleus in vitro. *J Physiol.* 1995;483:641–663.
37. Castro-Alamancos, M. A. Neocortical synchronized oscillations induced by thalamic disinhibition in vivo. *J Neurosci*. 1999;19:1–7.
38. Kleiman-Weiner M, Beenhakker MP, Segal WA, Huguenard JR. Synergistic roles of $GABA_A$ receptors and SK channels in regulating thalamocortical oscillations. *J Neurophysiol*. 2009;102:203–213.
39. Lui Z, Vergnes M, Depaulis A, Marescaux C. Involvement of intrathalamic $GABA_B$ neurotransmission in the control of absence seizures in the rat. *Neuroscience*. 1992;48:87–93.
40. Hosford DA, Lin FH, Kraemer DL, Cao Z, Wang Y, Wilson JT Jr. Neural network of structures in which $GABA_B$ receptors regulate absence seizures in the lethargic (*lh/lh*) mouse model. *J Neurosci*. 1995;15:7367–7376.
41. Snead OC 3rd. Antiabsence seizure activity of specific $GABA_B$ and gamma-hydroxybutyric acid receptor antagonist. *Pharmacol Biochem Behav*. 1996;53: 73–79.
42. Maljevic KK, Cobilanschi J, Tilgen N, Beyer S, Weber YG, Schlesinger F, Ursu D, Melzer W, Cossette P, Bufler J, Lerche H, Heils A. A mutation in the GABA(A) receptor alpha(1)-subunit is associated with absence epilepsy. *Ann Neurol*. 2006;59:983–987.
43. Fedi M, Berkovic SF, MacDonell RA, Curatolo JM, Marini C, Reutens DC. Intracortical hyperexcitability in humans with a $GABA_A$ receptor mutation. *Cereb Cortex*. 2008;18:664–669.
44. Tan HO, Reid CA, Single FN, Davies PJ, Chiu C, Murphy S, Clarke AL, Dibbens L, Krestel H, Mulley JC, Jones MV, Seeburg PH, Sakmann B, Berkovic SF, Sprengel R, Petrou S. Reduced cortical inhibition in a mouse model of familial childhood absence epilepsy. *Proc Natl Acad Sci USA*. 2007;104:17536–17541.
45. Bessaïh T, Bourgeais L, Badiu CI, Carter DA, Toth TI, Ruano D, Lambolez B, Crunelli V, Leresche N. Nucleus-specific abnormalities of GABAergic synaptic transmission in a genetic model of absence seizures. *J Neurophysiol*. 2006;96:3074–3081.
46. Tan HO, Reid CA, Chiu C, Jones MV, Petrou S. Increased thalamic inhibition in the absence seizure prone DBA/2J mouse. *Epilepsia*. 2008;49:921–925.
47. Caddick SJ, Wang C, Fletcher CF, Jenkins NA, Copeland NG, Hosford DA. Excitatory but not inhibitory synaptic transmission is reduced in lethargic (*Cacnb4*^{lh}) and tottering (*Cacna1a*^{tg}) mouse thalami. *J Neurophysiol*. 1999; 81:2066–2074.
48. Luhmann HJ, Mittmann T, van Luijtelaar G, Heinemann U. Impairment of introcortical GABAergic inhibition in a rat model of absence epilepsy. *Epilepsy Res*. 1995;22:43–51.
49. D'Antuono M, Inaba Y, Biagini G, D'Arcangelo G, Trancredi V, Avoli M. Synaptic hyperexcitability of deep layer neocortical cells in a genetic model of absence seizures. *Genes Brain Behav*. 2006;5:73–84.
50. Coenen AM, Drinkenburg WH, Inoue M, van Luijtelaar EL. Genetic models of absence epilepsy, with emphasis on the WAG/Rij strain of rats. *Epilepsy Res.* 1992;12:75–86.
51. Giaretta D, Kostopoulos G, Gloor P, Avoli M. Intracortical inhibitory mechanisms are preserved in feline generalized penicillin epilepsy. *Neurosci Lett*. 1985;59:203–208.
52. Huntsman MM, Porcello DM, Homanics GE, DeLorey TM, Huguenard JR. Reciprocal inhibitory connections and network synchrony in the mammalian thalamus. *Science*. 1999;283:541–543.
53. Gervasi N, Monnier Z, Vincent P, Paupardin-Tritsch D, Hughes SW, Crunelli V, Leresche N. Pathway-specific action of gamma-hydroxybutyric acid in sensory thalamus and its relevance to absence seizures. *J Neurosci*. 2003;23:11469–11478.
54. Emri Z, Antal K, Crunelli V. Gamma-hydroxybutyric acid decreases thalamic sensory excitatory postsynaptic potentials by an action on presynaptic $GABA_B$ receptors. *Neurosci Lett*. 1996;216:121–124.
55. Williams SR, Turner JP, Crunelli V. Gamma-hydroxybutyrate promotes oscillatory activity of rat

and cat thalamocortical neurons by a tonic $GABA_B$ receptor–mediated hyperpolarization. *Neuroscience.* 1995;66:133–144.
56. Steriade M, Contreras D. Spike-wave complexes and fast components of cortically generated seizures. I. Role of neocortex and thalamus. *J Neurophysiol*. 1998;80:1439–1455.
57. Slaght SJ, Leresche N, Deniau J-M, Crunelli V, Charpier S. Activity of thalamic reticular neurons during spontaneous genetically determined spike and wave discharges. *J Neurosci.* 2002;22:2323–2334.
58. McCormick DA, Contreras D. On the cellular and network bases of epileptic seizures. *Annu Rev Physiol*. 2001;63:815–846.
59. Destexhe A, Sejnowski TJ. Interactions between membrane conductances underlying thalamocortical slow-wave oscillations. *Physiol Rev*. 2003;83:1401–1453.
60. Budde T, Pape C-H, Kumar SS, Huguenard JR. Thalamic, thalamocortical and corticocortical models of epilepsy with an emphasis on absence seizures. In: Pitkanen A, Schwartzkroin PA, Moshe SL, eds. *Models of Seizures and Epilepsy*. Amsterdam: Elsevier; 2006:73–88.
61. Huguenard JR, McCormick DA. Thalamic synchrony and dynamic regulation of global forebrain oscillations. *Trends Neurosci*. 2007;30:350–357.
62. Beenhakker MP, Huguenard JR. Neurons that fire together also conspire together: is normal sleep circuitry hijacked to generate epilepsy? *Neuron.* 2009;62: 612–632.
63. Gloor P. Generalized cortico-reticular epilepsies. Some considerations on the pathophysiology of generalized bilaterally synchronous spike and wave discharges. *Epilepsia*. 1968;9:249–263.
64. Browne SH, Kang J, Akk G, Chiang LW, Schulman H, Huguenard JR, Prince DA. Kinetic and pharmacological properties of $GABA_A$ receptors in single thalamic neurons and $GABA_A$ receptor subunit expression. *J Neurophysiol*. 2001;86:2312–2322.
65. Liu XB, Coble J, van Luijtelaar G, Jones EG. Reticular nucleus–specific changes in alpha3 subunit protein at GABA synapses in genetically epilepsy-prone rats. *Proc Natl Acad Sci USA.* 2007;104:12512–12517.
66. Schofield CM, Kleiman-einer M, Rudolph U, Huguenard JR. A gain in $GABA_A$ receptor synaptic strength in thalamus reduces oscillatory activity and absence seizures. *Proc Natl Acad Sci USA*. 2009;106:7630–7635.
67. Mattson RH. General principles: selection of antiepileptic drug therapy. In: Levy R, ed. *Antiepileptic Drugs*. 4th ed. New York: Raven Press; 1995: 123–135.
68. Hosford DA, Wang Y, Cao Z. Differential effects mediated by $GABA_A$ receptors in thalamic nuclei in *lh/lh* model of absence seizures. *Epilepsy Res.* 1997;27:55–65.
69. Sohal VS, Keist R, Rudolph U, Huguenard JR. Dynamic $GABA_A$ receptor subtype-specific modulation of the synchrony and duration of thalamic oscillations. *J Neurosci.* 2003;23:3649–3657.
70. Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult. *Neuroscience.* 2000;101:815–850.

Chapter 19

$GABA_B$ Receptor and Absence Epilepsy

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BACKGROUND ON $GABA_B$ RECEPTORS

The type B receptor for gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, is a 7-transmembrane-domain G-protein coupled receptor. $GABA_B$ receptors ($GABA_BRs$), upon binding with their endogenous ligand GABA, mediate slow and sustained inhibitory responses via downstream Ca^{2+}/K^+ channels. Ligand activation of $GABA_BRs$ also initiates G-protein-dependent cell signaling pathways leading to long-term alterations in cellular functions within the central and peripheral nervous systems. Perturbations of the $GABA_B$ system during development may shift the excitatory/inhibitory balance, culminating in various

neurological conditions, including typical and atypical absence seizures. The acute administration of baclofen (a $GABA_BR$ agonist) to wild-type rodents is sufficient to induce an absence seizure phenotype. Exacerbation of absence seizures occurs when baclofen is administered in pharmacological and genetic rodent models of typical absence seizures, while $GABA_BR$ antagonists suppress the seizures. A similar GABAergic pharmacological response is observed in the AY-9944 model of chronic atypical absence seizures, characteristic of Lennox- Gastaut syndrome. AY-9944, a cholesterol synthesis inhibitor, is also known to increase the expression levels of $GABA_BRs$ within the rodent forebrain regions and alters $GABA_BR$ trafficking during development. The severity of atypical absence seizures in the $GABA_BR$-overexpressing transgenic mouse model is $GABA_BRs$ subunit specific. A more pronounced atypical absence seizure phenotype results from the overexpression of the R1a $GABA_BR$ subunit as opposed to R1b. The absence seizure phenotype is circuitry dependent, with exclusive thalamocortical involvement in typical absence seizures as opposed to thalamocortical-hippocampal recruitment in atypical absence seizures. The pathophysiology of typical absence seizures with normal cognitive development and atypical absence seizures associated with severe impairment in cognition involves $GABA_BR$-mediated neurotransmission and is circuitry dependent. The implications of these fundamental findings for the circuitry dependency and $GABA_BR$ subtype specificity for seizure severity are discussed in terms of clinical perspectives in the chapter, including the potential for new therapeutic interventions and future research directions.

A Novel GABA Receptor

GABA is the main inhibitory neurotransmitter in the brain and can activate both ligand-gated ion channels ($GABA_AR$ and $GABA_CR$) and metabotropic G-protein-coupled receptors ($GABA_BRs$). Activation of $GABA_AR$ and $GABA_CR$ results in the influx of chloride ions and the generation of a fast inhibitory postsynaptic potential (IPSP). For many years, $GABA_AR$ was thought to be the sole GABA-activated receptor in the brain. However, in 1979, Bowery et al. demonstrated in rat myocardial tissue the presence of a baclofen-activated GABA receptor, which was insensitive to the GABA agonist 3-aminopropane sulfonic acid (3-APS) and to the GABA antagonists bicuculline, picrotoxin, and penicillin.[1] This phenomenon was replicated in rat cerebellar, striatal, and cortical slices in which the K^+-evoked release of radiolabeled neurotransmitters could be reduced by GABA or baclofen but not by bicuculline or 3-APS, supporting the conclusion that bicuculline-insensitive receptors were present in the central nervous system.[2] These bicuculline-insensitive receptors were designated as $GABA_BRs$ to differentiate them from the bicuculline-sensitive $GABA_ARs$.[3]

Cloning and Characterization of the $GABA_BR$

The $GABA_BR$ was successfully cloned in 1997.[4] The first positive clone with a molecular weight of M_r of 106K was labeled as R1a $GABA_BR$, and the second protein with a molecular weight of M_r of 92K was identified as R1b $GABA_BR$. The R1b protein differed from the R1a protein in that the first 147 N-terminal residues are replaced by 18 other residues. Hence, the R1b protein probably had arisen from an alternative transcription start site.

Subsequently, the R2 $GABA_BR$ protein was cloned.[5–9] Since the cloning of the R1 and R2 isoforms of the $GABA_BR$, oligodimerization of the R1 and R2 subunits has been considered to be essential for the cell surface expression of the receptor and G-protein activation.[7,8] However, recent studies utilizing functional proteomics have demonstrated that the $GABA_BR$ actually is a heteromultimer of high-molecular-mass complex of R1, R2, and potassium channel tetramerization domain-containing (KCTD) proteins, in which different KCTD isoforms serve as auxiliary subunits to modulate the pharmacology and kinetics of the receptor response.[10]

Distribution of $GABA_BR$ in the Central Nervous System

The $GABA_BR$ is ubiquitously expressed throughout the mammalian central nervous system (CNS). R1a and R1b $GABA_BR$ subunits

are abundantly expressed in the cerebral cortex, especially in layer VIb, the pyramidal cell layer of the CA3–CA1 regions of the hippocampus, the granular layer of the dentate gyrus, medial habenula, basal ganglia, ventral tegmental area, olfactory bulb, thalamus, amygdala, and hypothalamus, as well as in cerebellar Purkinje and granule cells.[4,11,12] By contrast, $GABA_BR$ R2 mRNA is highly expressed in Purkinje cells of the cerebellum, and in the cerebral cortex, thalamus, and hippocampal pyramidal cells, while showing a low level of expression in the caudate/putamen and hypothalamus.[5,13] The R1a subunit is predominantly expressed in presynaptic terminals of glutamatergic neurons, in which it probably contributes to the inhibition of glutamate release, while the R1b subunit is thought to influence postsynaptic inhibition.[14–18] $GABA_BRs$ can also act as autoreceptors, inhibiting GABA release within presynaptic terminals.[19–21] As extrasynaptic/perisynaptic receptors, $GABA_BRs$ are thought to regulate GABA spillover from the synaptic cleft.[12,22] Although the $GABA_BR$ is primarily located on neuronal cell populations, it can also be found on astrocytes, implicated in reinforcing inhibitory or disinhibitory circuits through the release of glutamate or the uptake of GABA spillover.[23,24] The $GABA_BR$ has also been reported to be expressed on cultured activated microglia but not in myelin-containing oligodendrocytes[25]; in view of these findings, a role for $GABA_BR$ in modulating the release of interleukin-6[26] has been postulated, but the exact function of the microglial $GABA_BRs$ remains unclear.

Developmental Profile of $GABA_BRs$ in the CNS

The $GABA_BR$ subunits R1a and R1b protein levels decrease between postnatal day (P) 7 and adulthood in the rat cerebral cortex, the cerebellum, and, most drastically, the spinal cord.[27] Between P21 and P28 and during adulthood, no differences in R1a and R1b expression levels have been found in the brainstem, striatum, and hippocampus. However, in the cerebral cortex, R1a levels are higher than R1b levels between P2 and P14. The downregulation of R1a levels coincides with the upregulation of R1b levels as the animal matures. Finally, in the adult brain, there is twice as much R1b as R1a. Additionally, binding affinities for L-baclofen do not differ between the R1a and R1b isoforms. However, the R1 subunit of the $GABA_BR$ in adult animals have a higher (10-fold) binding affinity for L-baclofen than P1 animals. Levels of R2 in the cortical regions increase from P2 to P28, then gradually decreases as the animal approaches adulthood.[7] Interestingly, in the cerebellum and spinal cord, R2 levels peak during early postnatal stages and then decline with maturation, presenting with a sharper reduction in the spinal cord.[28]

PHYSIOLOGY OF THE $GABA_BR$

Intracellular Signal Transduction

The $GABA_BR$ regulates intracellular signaling via an adenylyl cyclase–cyclic adenosine monophosphate (cAMP)–dependent pathway.[29] Stimulation of the $GABA_BR$ with (–) baclofen can inhibit both basal and forskolin-stimulated adenylyl cyclase,[30] which in turn can be blocked by the addition of pertussis toxin, indicating the involvement of the $G_{1\alpha}$- and $G_{0\alpha}$- subunits of the G protein.[31,32] Interestingly, when both $GABA_B$ and other metabotropic neurotransmitter systems (noradrenergic, histaminergic, etc.) are stimulated simultaneously, adenylyl cyclase activity is significantly increased.[32–34] It is conceivable that this synergistic phenomenon is due to the interactions and cross-talk between various subtypes/subunits of the G-protein and the adenylyl cyclase complex from the many signaling pathways within the cell.[35–37] Therefore, this synergistic activation of the adenylyl cyclase pathway might affect neuronal activity on a more permanent basis.

Coupling of $GABA_BR$ to Ion Channels

The P/Q and N subtypes of Ca^{2+} channels[38–42] are inhibited through the binding of $\beta\gamma$ subunits released by G-proteins coupled to activated $GABA_BRs$.[43] In this way, $GABA_BRs$ function presynaptically as auto- or heteroreceptors,

respectively inhibiting the release of GABA or glutamate. At postsynaptic sites, activation of GABA$_B$Rs leads to release of the βγ complex from the G-protein, which then interacts with inwardly rectifying K^+ channels,[7,44] leading to a net increase in conductance and, thus, to an efflux of K^+ ions (Fig. 19–1). There is evidence that this process underlies the mechanism involved in the generation of slow inhibitory postsynaptic currents (IPSCs) and long-lasting hyperpolarization at the postsynaptic level.[45] Specifically, the Kir3 subtype of the K^+ channels appears to be involved in long-lasting hyperpolarization.[44,46]

TYPICAL VERSUS ATYPICAL ABSENCE SEIZURES

Two very different types of absence seizures are observed both clinically in children[47] and experimentally in rodents (Table 19–1).[48] Typical absence seizures (TASs) are generally considered to be benign because the seizures are characterized by ictal spike-wave discharges (SWDs) associated with only brief momentary behavioral and motor arrest. The seizures are easily controlled with antiepileptic drugs and are not associated with cognitive impairment[49,50]; lastly, they disappear during

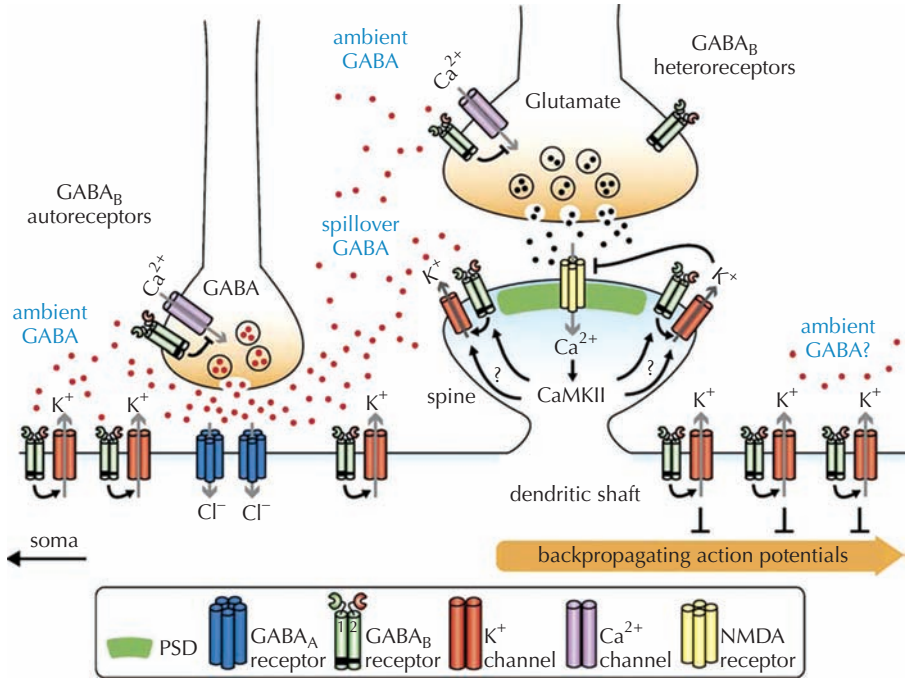

Figure 19–1. Localization and physiological roles of GABA$_B$ receptors. GABA$_B$ receptors are located presynaptically, postsynaptically, and on extrasynaptic membranes. Presynaptic GABA$_B$ receptors prevent neurotransmitter release by downregulating the activity of voltage-sensitive Ca^{2+} channels or by direct inhibition of the release machinery. GABA$_B$ autoreceptors inhibit the release of GABA, whereas GABA$_B$ heteroreceptors inhibit the release of glutamate and several other neurotransmitters. Some GABA$_B$ heteroreceptors are activated by ambient GABA, others probably by GABA spillover from inhibitory terminals. Postsynaptic GABA$_B$ receptors induce sIPSCs by activating Kir3-type K^+ channels, which hyperpolarizes the membrane, favors voltage-sensitive Mg^{2+} block of NMDA receptors, and shunts excitatory currents. GABA$_B$ receptors in spines and dendritic shafts are activated by spillover of GABA from adjacent terminals during population oscillations or during epileptiform activity, which may serve to regulate the excitability of the network and to counteract excess excitation. Dendritic GABA$_B$ receptors inhibit backpropagating action potentials through activation of K^+ channels, which may influence synaptic plasticity processes and action potential generation at the axon hillock. During high-frequency transmission, GABA depresses its own release by an action on GABA$_B$ autoreceptors, which permits sufficient NMDA receptor activation for the induction of LTP. In turn, activation of NMDA receptors and calcium/calmodulin-dependent protein kinase II (CaMKII) in dendritic spines enhances the sIPSC mediated by GABA$_B$ receptors and K^+ channels, which is proposed to influence the temporal resolution of synapses. From ref. 16.

Table 19–1 **Comparison of Features of TAS and AAS in Rodent Models and Humans**

	GAERS (TAS)	GABABR1bR2 Transgenic Hybrid Mouse (AAS)	Human (AAS)
EEG			
Bilaterally synchronous SWD	+	+	+
SWD frequency*	7–11 Hz	4–6 Hz	2 Hz
SWD from thalamus and cortex	+	+	+
SWD from hippocampus*	–	+	+
Ictal Behavior			
Staring, myoclonus	+	+	+
Movement during SSWD*	–	+	+
Precise EEG/behavioral correlation*	+	–	–
Pharmacology			
Blocked by ETO, VPA, TMD	+	+	+
Exacerbated by $GABA_AR$ and $GABA_BR$ agonists	+	+	+
Blocked by $GABA_BR$ antagonists	+	+	No data

*Characteristics that separate AASs from TASs. ETO ethosuximide; VPA valproic acid; TMD trimethadione; SWD spike-and-wave discharge; the $GABA_BR1bR2$ transgenic hybrid mouse is a mutant mouse overexpressing $GABA_B$ receptor subunits R1b and R2 in forebrain neurons; GAERS, Genetic Absence Epilepsy Rat from Strasbourg.

adolescence. In stark contrast, atypical absence seizures (AASs) are malignant[51,52] because they are progressive. The seizures are severe and characterized by ictal slow spike-wave discharges (SSWDs) associated with a prolonged ictal behavioral twilight state, during which semipurposeful movement is observed, and comorbid impairment in cognition is usually observed.[53–56] The impairment in cognition in AAS is thought to be caused by the involvement of limbic circuits,[54,57] which are spared in TAS.[58–60] The differences between typical and atypical absence seizures are summarized in Table 19–1.

ANIMAL MODELS OF TYPICAL ABSENCE SEIZURES

Genetic Absence Epilepsy Rat from Strasbourg (GAERS)

This rodent model is characterized by spontaneous SWDs at 7–11 Hz that start and end abruptly on a normal background electroencephalogram (EEG). The ictal events that are time locked with these discharges are staring and cessation of movement. GABAergic agonists and γ-hydroxybutyric acid (GHB) prolong the duration of SWDs in the GAERs, and the SWDs in this model are also exacerbated by pentylenetetrazole (PTZ), 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP), and penicillin.[61] The SWDs in the GAERS have been shown to originate from the facial somatosensory cortex, from which they spread to both hemispheres.[62] The seizures have a classic pharmacological profile, being enhanced by $GABA_AR$ and $GABA_BR$ agonists as well as by phenytoin and carbamazepine, and they are blocked by ethosuximide, benzodiazepines, and $GABA_BR$ antagonists. Enhancement of cognitive performance in GAERS was sustained in rats treated with $GABA_BR$ antagonists.[63]

WAG/Rij Rat

This is another commonly investigated genetic model of TAS, which presents the characteristics outlined in Table 19–1, as the GAERS do. Although the SWDs in the WAG/Rij have been shown to originate from the somatosensory cortex,[64] there is dissociation between the SWDs and sleep spindles in this model.[65] Within the thalamus, the bilateral 7–11 Hz SWD originates from the rostral pole of the nucleus reticularis (RTN) compared to the involvement of the entire RTN in sleep spindle

generation.[65] Levels of mRNA for most R1 GABA$_B$R subunits are diminished in the neocortex of WAG/Rij, whereas R2 mRNA levels are unchanged. R1 subunits fail to localize in the distal dendrites of WAG/Rij neocortical pyramidal cells, which may contribute to neocortical hyperexcitability.[66] The RTN and ventroposteromedial thalamic nucleus are coupled prior to the onset of SWDs in the WAG/Rij, suggesting that SWDs may derive from an intermixed delta-theta activity and that the seizure onset may require a reinforcement of intracortical and corticothalamic associations.[67] Higher doses of baclofen are required to depress pharmacologically isolated, stimulus-induced IPSPs generated by WAG/Rij neurons. Decreased function of presynaptic GABA$_B$Rs in the neocortex may contribute to neocortical hyperexcitability in this model.[68]

GHB-Treated Rodent

The bilateral 7–9 Hz SWD in this model originates from the thalamocortical circuitry, and G-protein-mediated mechanisms are involved in the pathogenesis of SWDs.[69] Intracortical perfusion of the GABA$_B$R antagonists, CGP 35348 and phaclofen, and the GHB receptor antagonist NCS 382, attenuated GHB-mediated changes in the basal and K^+-evoked release of GABA, which supports the idea that GHB induces a selective decrease in the basal and depolarization-induced release of GABA in cerebral cortex.[70] GHB most likely acts at the presynaptic GABA$_B$R.[60, 71] Similar to other animal models of typical absence, the hippocampus does not show SWDs in the GHB-treated rat (Fig. 19–2). There is some evidence that the GluR2 subunit may be involved in the initiation and maintenance of absence seizures induced by GHB.[72] Although neurogenesis appears to occur in convulsive seizures and in the kindling model, there is no evidence of this phenomenon in the GHB model.[73]

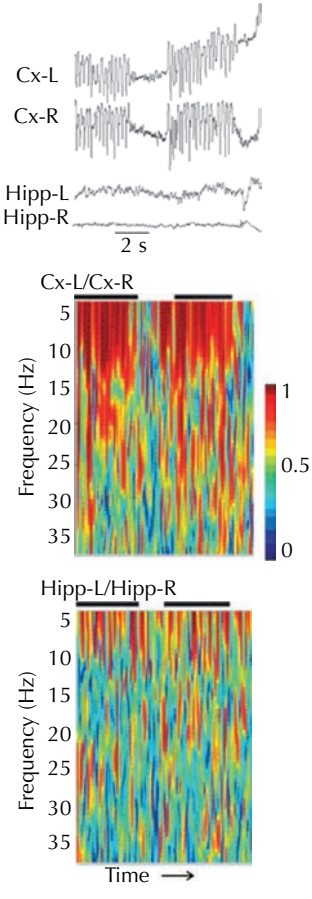

Figure 19–2. Temporal phase synchrony during absence seizures. Upper traces depict four simultaneous recordings in a rat injected with γ-butyrolactone (GBL), displaying two typical SWDs in the cortex (Cx-L and Cx-R, left and right cortical electrodes, respectively) and no apparent paroxysms in the hippocampi (Hipp-L and Hipp-R, left and right hippocampal electrodes, respectively). Lower color panels show the phase synchrony (color-coded, with red indicating the highest level of phase synchrony and blue the lowest) between the cortical recordings (upper panel) and between the two hippocampal recordings (lower panel). The black horizontal bars signal the two SWDs occurring in the cortex; the time axis is indicated (the synchrony plots correspond to the same length in time as the original traces above, ~12 s. Note the enhanced synchronization during the SWD between the cortical sites at almost all frequency ranges (from 5 to 35 Hz, *y*-axis), while the synchrony between the hippocampal electrodes shows many more fluctuations. From ref. 60.

lh/lh Mouse

In the lethargic (*lh/lh*) model, GABA$_B$R antagonists suppressed and GABA$_B$R agonists exacerbated absence seizures. GABA$_B$R activation may produce disinhibition, which is a possible role for GABA$_B$R in the expression of absence seizures in *lh/lh* mice.[74] Enhanced nuclear cyclic AMP–responsive element (CRE)- and activator protein 1 (AP-1) DNA-binding activities were found in the thalamocortical regions and have been implicated in the generation

and/or propagation of absence seizures in this model.[75] The repeated administration of the $GABA_BR$ antagonist CGP 46381 (60 mg/kg) attenuated seizure activity and the increased DNA-binding activities.[76] As well, $GABA_BR$ binding and synaptically evoked $GABA_BR$-mediated inhibition of *N*-methyl-D-aspartate (NMDA) responses were selectively increased, which suggested that enhanced $GABA_BR$-mediated synaptic responses may underlie absence seizures in *lh/lh* mice.[77]

ANIMAL MODELS OF ATYPICAL ABSENCE SEIZURES (AAS)

AY-9944-Treated Rodents

The administration of AY-9944, a cholesterol biosynthesis inhibitor at delta 7-reductase, to rodents produces a seizure phenotype that mimics AAS. The phenotype consists of slow SWD (SSWD), 4–5 Hz, during which the animal is able to move, and unlike TAS, there is no strict on-off relationship between the SSWD and the ictal event. In addition, also in contrast to the animal models of TAS, in the AY-9944 model the SSWD can be recorded from the hippocampus[78] (Fig. 19–3). A final difference is that in this model of AAS there is cognitive impairment, a characteristic not found in animal models of TAS.[79] As well, the AY-9944 model shows the characteristic pharmacology described above for the GAERS and detailed in Table 19–1. The AASs in the AY-9944 model are spontaneous, recurrent, and lifelong. AY-9944 is a cholesterol synthesis inhibitor that increases the expression levels and trafficking of the $GABA_BR$ in forebrain regions specifically during brain development, and lipid rafts participate in this process.[80] AY-9944 leads to prepubescent atypical absence onset[81] and to a maximum peak in adulthood.[82] There are no changes in histology[78,83] or neurogenesis[73] in this model.

Transgenic Mouse Models of Atypical Absence Seizures

Transgenic mice that overexpress the R1a $GABA_BR$ subtype show a phenotype characterized by atypical absence seizures associated with spontaneous, recurrent, bilaterally synchronous, 3–6 Hz SSWDs that are blocked by ethosuximide and exacerbated by baclofen. The discharges occur coincident with absence-like behaviors such as staring, facial myoclonus, and whisker twitching. However, similarly to the AY model described above, and in contrast to typical absence epilepsy models, these mice move during the ictal event, display SSWDs in both thalamocortical and limbic circuitry, exhibit impaired hippocampal synaptic plasticity, and display significantly impaired learning abilities (Fig. 19–4). A similar but less pronounced AAS phenotype results from the overexpression of the R1b subtype. The severity of AAS in the

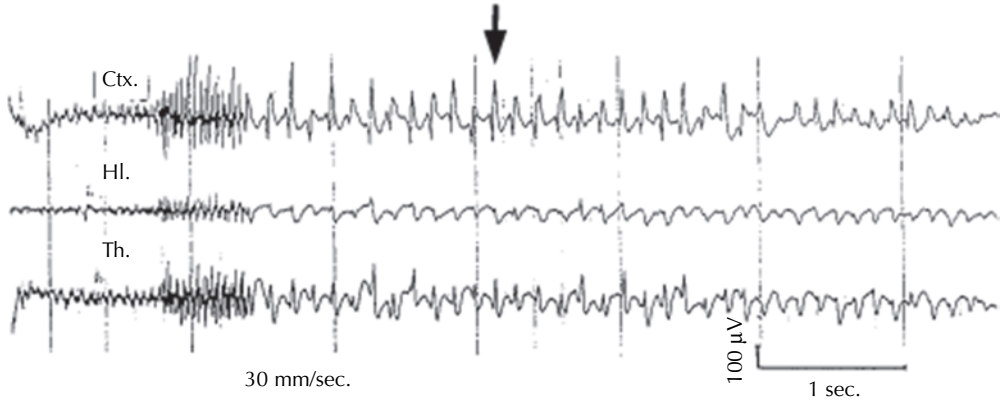

Figure 19–3. Baseline bipolar depth recording at P55 in the AY-9944 model. The figure illustrates the spontaneously recurrent 5 Hz spike-and-wave burst recorded from cortex (CTX.), hippocampus (Hi.), and thalamus (Th.) in a rat treated postnatally with AY-9944, better seen at 30 mm/s (arrow). The epileptiform activity was recorded from the hippocampus in all AY-9944-treated animals that had depth electrodes implanted ($n = 8$). From ref. 78.

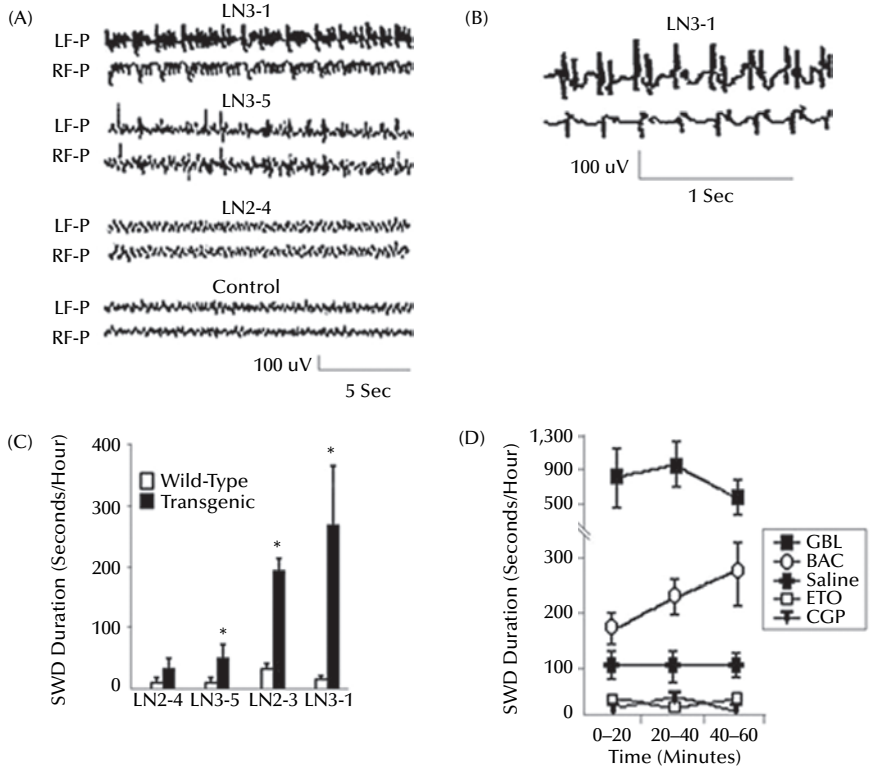

Figure 19–4. A. Baseline electrocorticography (ECoG) recording from the electrodes placed in cerebral cortex of a wild-type mouse and from the cortex of LN3–1, LN2–4, and LN3–5 transgenic mice. The traces denote differential signals between either the left frontal parietal electrodes (LF-P) or the right frontal parietal electrodes (RF-P). Note that the frequency of the seizure is 3–6 Hz. All subjects were 2 months of age or older at the time of the recordings. **B.** Higher-resolution tracing illustrating the spike-and-wave components of the ictal event from the cortex of an LN3–1 transgenic mouse. **C.** Comparison of the spontaneous SWD activity of the different transgenic mouse lines. Although the frequency of the seizure was consistent among the different lines, the amount of seizure activity per hour differed significantly among them. Note that the amount of seizure activity present in each line correlates with the amount of immunoreactive transgene expressed within each line (see Fig. 19–1). Data are presented as the mean and standard error of the seizure duration per hour of recording from $n = 10$ (LN2–4), $n = 11$ (LN3–5), $n = 10$ (LN2–3), and $n = 12$ (LN3–1) transgenic mice. **D.** The pharmacological responsiveness of the spontaneous SWD in the GABA$_B$R1a transgenic mice is consistent with absence epilepsy. The cumulative SWD duration in transgenic line LN3–1 was measured for 1 h either following saline injection or following the administration of the pro-absence drug γ-butyrolactone (GBL), the GABA$_B$R agonist (–)baclofen (BAC), the GABA$_B$R antagonist CGP 35348 (CGP), or the antiabsence drug ethosuximide (ETO). As shown in the figure, the duration of the SWD was significantly ($p < .01$, $n = 8$–10) reduced by pretreatment with either ETO or CGP and exacerbated by GBL or BAC. Note that the injection of the control saline was sufficient to reduce the SWD duration (compare panels C and D). Asterisks denote significant differences from their wild-type littermate ($p < .05$ Student's unpaired t-test). From ref. 84.

GABA$_B$R-overexpressing transgenic mouse model is GABA$_B$R subunit specific.[84,85]

GABA$_B$R-MEDIATED MECHANISMS IN TYPICAL AND ATYPICAL ABSENCE SEIZURES

The GABA$_B$R pharmacology in AAS and TAS is the same: GABA$_B$R agonists exacerbate and GABA$_B$R antagonists block both types of seizures.[48] GABA$_B$R agonists and antagonists act in this regard upon the thalamic circuitry involved in the bilateral synchronous SWDs in TAS[65,86] and SSWDs in AAS.[57] Intrathalamic neural networks serve as an intrinsic oscillatory unit within thalamocortical circuitry that drives the bilaterally synchronous SWDs, but their function depends upon reciprocal synaptic connectivity between excitatory thalamocortical relay neurons, excitatory corticothalamic

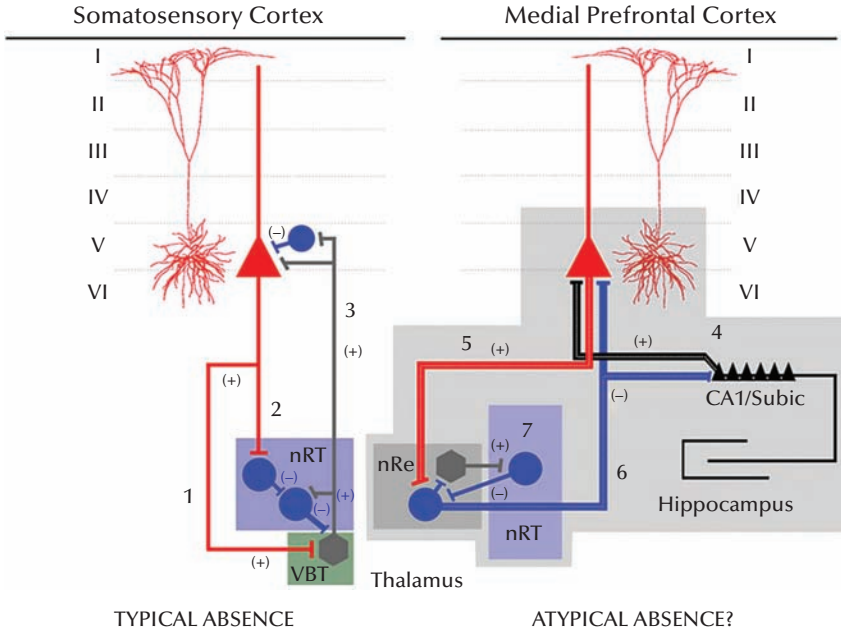

Figure 19–5. The well-established neuronal circuitry of TASs involves reciprocal connections between layer 5/6 of the perioral region of somatosensory cortex where the seizures are initiated, the ventrobasal thalamus (VBT; 1 and 3), and the caudal reticular nucleus of the thalamus (nRT; 2). The hypothesized circuitry of AASs (right), whereby the initiating epileptiform event for an AAS is postulated to occur in layer 5/6 of the medial prefrontal cortex (mPFC) and then project to the nucleus reunions of the thalamus (nRE; 5), which projects back to the mPFC and monosynaptically to the CA1 (6), which in turn projects to the mPFC (4). This reverberating circuit is modulated and driven by reciprocal intrathalamic connections between the nRE and the rostral nRT (7). Blue = GABAergic neurons; red = glutamatergic neurons. (–) indicates inhibition; (+) indicates excitation. Modified from ref. 57.

neurons, and inhibitory GABAergic neurons in the RTN and cortical interneurons.[87, 88] The $GABA_BR$ link[89] in this thalamocortical system that creates and promulgates the abnormal neuronal oscillatory activity within the circuitry, which manifests itself as either TAS or AAS, involves the low-voltage activated (LVA) Ca^{2+} channels. The LVA Ca^{2+} channel is found in both thalamus[90] and frontal cortex[91–93] and has the property of exquisite voltage sensitivity that allows regulation of oscillatory behavior within neuronal networks.[90,94] In the corticothalamic circuitry described in Fig. 19–5, powerful $GABA_BR$-mediated inhibition results in deinactivation of LVA Ca^{2+} channels with a resultant LVA Ca^{2+} current, rebound burst firing, and recurrent excitation of the RTN. In TAS this leads to recurrent excitation of the cerebral cortex, but in AAS, where the circuitry differs, midline thalamocortical neurons, most likely the nucleus reuniens (nRe) of the thalamus,[57] are activated and lead to oscillations within the cortical-thalamo-hippocampal circuitry. Thus, burst firing within either TAS or AAS circuitry depends upon LVA Ca^{2+} current, which is coupled to $GABA_BR$-mediated inhibition.

TYPICAL AND ATYPICAL ABSENCE SEIZURES ARE CIRCUITRY DEPENDENT

The absence seizure phenotype is circuitry dependent. Typical absence seizures (TAS) are generated from thalamocortical connections, and AASs are generated from cortical-thalamo-hippocampal circuitry[57,60] (Fig. 19–5). Recent data[57] suggest that the SSWDs in the R1a $GABA_BR$ transgenic model of AAS emanate from the cerebral cortex, midline thalamus, RTN, and the CA1 region of the hippocampus. A highly significant finding in those experiments was the noninvolvement of the ventrobasal nucleus of the thalamus (VB) in the SSWDs that characterize the AAS phenotype because VB is the major thalamocortical relay nucleus involved in TAS[58,64,95] (see Fig. 19–5).

Rather, the data showing the projection of the SSWDs to the CA1 in the R1a $GABA_B$R transgenic model of AAS[57] strongly suggest that the midline thalamic nucleus most likely involved in this genetic model of AAS is the nRe, since this is the midline thalamic nucleus that has direct projections to the CA1.[96–98] Because the nRe links CA1 and medial prefrontal cortex (mPFC),[97,98] the cortical component of the cortico-thalamo-hippocampal circuitry in AAS may well be the mPFC (Fig. 19–5).

The rationale for postulating a cortical focus for AAS is that TASs have been reported to arise from a cortical focus in humans[86] and animals.[64,95] Given the reciprocal projections of nRe to the mPFC[98,99] and RTN,[96] and given the pronounced direct monosynaptic projections from CA1/subiculum to the mPFC,[99] whether the initiating epileptiform event for an AAS within the proposed circuitry (Fig. 19–5) could take place in layer 5/6 of the mPFC requires further confirmation. Our postulation is conceivable because there are reciprocal projections from nRe to the mPFC[98,99] and RTN[96] and pronounced direct monosynaptic projections from CA1/subiculum to the mPFC.[99] However, there are no direct mPFC-to-CA1 projections, so it appears that the nRe serves as a critical relay from the mPFC to CA1—that is, as a reverberating loop that is modulated by reciprocal intrathalamic connections between the nRe and the RTN.[98,100,101] Taken together, all these data suggest that the EEG, behavioral, and cognitive phenotypes of the R1a $GABA_B$R transgenic model of AAS are dependent upon a reverberating neuronal circuitry that involves the mPFC, nRe, and the CA1 region of the hippocampus.

IMPAIRMENT OF LEARNING AND MEMORY IN ATYPICAL ABSENCE SEIZURES

$GABA_B$R Modulation of Learning and Memory

Several lines of evidence indicate that $GABA_B$R agonists impair learning and memory, whereas $GABA_B$R antagonists improve the cognitive processes. Administration of baclofen to patients resulted in memory loss reported in three different case studies.[102] In experimental models, posttraining intraperitoneal injection or intra-amygdala infusion of baclofen resulted in the impairment of retention on an inhibitory avoidance task.[103–105] Baclofen has also been shown to potentiate the amnesic effect of scopolamine on the performance of rats in the radial arm maze test, even though baclofen was unable to alter the performance when given separately.[106] However, intraseptal administration of baclofen reduced the correct choices and thus increased the errors in the radial maze test.[107] Other studies utilizing different learning paradigms demonstrated an impairing effect of baclofen on the performance of rats in the Y-maze[108] and the Morris water maze tests.[109] More recently, $GABA_B$R activation in the entorhinal cortex by direct infusion of baclofen inhibited spatial learning in the Morris water maze test.[110] This finding was dependent on a protein kinase A (PKA)-dependent pathway able to activate the two-pore, weakly inwardly rectifying K^+ (TWIK)-related potassium type 2 (TREK-2 K^+) channels in entorhinal neurons. Increased $GABA_B$R activity also has been shown to disrupt reconsolidation of a conditioned reward memory by facilitating the extinction of the normally resilient memory trace.[111] However, it has to be mentioned that one study has reported positive effects of baclofen on recognition memory in mice pretreated with methamphetamine.[112]

Conversely, $GABA_B$R antagonists have been demonstrated to improve cognitive performance and/or to reverse amnesia in animals,[50,104, 113–117] to enhance the cognitive performance in experimental TAS,[63,118] and to reverse the deficits in both cognition and long-term potentiation (LTP) in a pharmacological model of AAS.[79] There is recent clinical evidence that $GABA_B$R antagonists may improve cognition in human.[119,120] Although the majority of published data support the general thesis that $GABA_B$R agonists decrease and $GABA_B$R antagonists enhance the cognitive performance, mutant mice lacking R1a or R1b subunits appear to have impaired memory, which indicates that the R1a subunit is involved in familiar and novel object recognition and that, on the other hand, the R1b subunit is involved in acquisition and extinction of aversive memories.[121,122]

The mechanism of the cognition-enhancing effects of $GABA_B$R antagonists is thought to involve the inhibition of CREB2/CRE pathways by the reduction of CRE protein binding.[114] Alternatively, the behavioral effects

of $GABA_BR$ blockade also can be mediated by an increase in the levels of phosphorylated PKA[123] and synapsin Ia and IIa proteins,[124] and previously heightened release of somatostatin into the extracellular regions of the hippocampus was found in vitro.[125]

$GABA_BR$-Mediated Mechanisms in Impairment of Learning and Memory in Experimental Atypical Absence Seizures

As mentioned above, administration of AY-9944 to rats or mice early in development results in recurrent AAS similar to human atypical absence epilepsy in EEG, pharmacology, ictal behavior and developmental profile.[54,79] In addition, this model of AAS exhibits cognitive deficits that mirror those observed clinically in AAS, an invariable component of the Lennox-Gastaut syndrome. In vitro tests of synaptic plasticity in the AY-9944 model of AAS demonstrated impairments in CA1- LTP, paired-pulse facilitation (PPF), and presynaptic depression (PD). Furthermore, AY-9944 animals in the radial arm maze test showed fewer perfect entries, a greater number of errors, and a requirement for more training days to learn the task than saline-treated controls. The antiabsence drug ethosuximide significantly attenuated AAS in the AY model but failed to rescue the spatial learning abilities and working memory in AY animals.[79] However, a specific high-affinity $GABA_BR$ antagonist, CGP 35348, not only blocked the AAS, but also restored the LTP to baseline and reversed the cognitive deficit in the AY-9944 model. Importantly, dose-response studies showed that doses of CGP 35348 that failed to influence AAS activity in AY-9944-treated rats completely reversed the spatial working memory deficit in these animals. Thus, $GABA_BR$ antagonists are antiepileptic, reverse the learning deficit, and rescue the impaired CA1-LTP in experimental atypical absence epilepsy.[79] These data suggest that the cognitive dysfunction in the AY-9944 model of AAS is $GABA_BR$ dependent and that it is not influenced by AAS activity. In line with this hypothesis, transgenic mice overexpressing the R1a or R1b $GABA_BR$ subtype in forebrain[84,85] present with characteristic AAS, reduced hippocampal LTP and significantly impaired hippocampal learning functions.

CONCLUSION

$GABA_BR$-mediated mechanisms have been known for many years to be involved in the genesis and propagation of both TASs[126–129] and AASs,[78,84] as well as in the associated cognitive impairments.[54,79] Moreover, the data reviewed in this chapter indicate that TAS and AAS share the same pharmacological profile, because they share common thalamic circuitry perturbations, notably nRT involvement. However, TAS and AAS phenotypes differ in seizure severity, SWD characteristics, and impairment in learning abilities, mainly because they differ in the other parts of the circuitry involved. In TAS, cognition is not affected because the SWD is constrained within thalamocortical circuits and does not involve limbic circuits. In AAS, on the other hand, cognition is impaired because of the SWD involvement of limbic circuitry that is recruited through cortico-thalamo-hippocampal pathways,[130] hence the difference in seizure semiology and cognitive involvement.

Because of the demonstrated efficacy of $GABA_BR$ antagonists in preventing SWDs, the potential of $GABA_BR$ antagonists as powerful antiabsence drugs has been long proposed,[61] yet clinical trials of $GABA_BR$ are lacking both in TAS and AAS. Frequent refractory seizures and severe impairment in cognition represent the most disabling aspects of pediatric epilepsy and are the major contributors to the burden of illness in children affected by epilepsy.[131] Because of data suggesting that atypical absence seizures are independent of their comorbid cognitive deficits, yet both are $GABA_BR$ dependent,[79] there is a clinical imperative to initiate clinical trials of a $GABA_BR$ antagonist in the AASs observed in Lennox-Gastaut syndrome.[119,120]

DISCLOSURE STATEMENT

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REFERENCES

1. Bowery NG, Doble A, Hill DR, Hudson AL, Shaw JS, Turnbull MJ. Baclofen: a selective agonist for a novel type of GABA receptor proceedings. *Br J Pharmacol*. 1979;67:444P–445P.

2. Bowery NG, Hill DR, Hudson AL, et al. (–)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature*. 1980;283:92–94.
3. Hill DR, Bowery NG. 3H-Baclofen and 3H-GABA bind to bicuculline-insensitive GABA B sites in rat brain. *Nature*. 1981;290:149–152.
4. Kaupmann K, Huggel K, Heid J, et al. Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature*. 1997;386:239–246.
5. Jones KA, Borowsky B, Tamm JA, et al. GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature*. 1998;396:674–679.
6. White JH, Wise A, Main MJ, et al. Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature*. 1998;396:679–682.
7. Kaupmann K, Malitschek B, Schuler V, et al. GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature*. 1998;396:683–687.
8. Kuner R, Köhr G, Grünewald S, Eisenhardt G, Bach A, Kornau HC. Role of heteromer formation in $GABA_B$ receptor function. *Science*. 1999;283:74–77.
9. Martin SC, Russek SJ, Farb DH. Molecular identification of the human $GABA_B$R2: cell surface expression and coupling to adenylyl cyclase in the absence of $GABA_B$R1. *Mol Cell Neurosci*. 1999;13:180–191.
10. Schwenk J, Metz M, Zolles G, et al. Native GABA(B) receptors are heteromultimers with a family of auxiliary subunits. *Nature.* 2010;465:231–235.
11. Bischoff S, Leonhard S, Reymann N, et al. Spatial distribution of GABA(B)R1 receptor mRNA and binding sites in the rat brain. *J Comp Neurol*. 1999;412:1–16.
12. Fritschy JM, Meskenaite V, Weinmann O, Honer M, Benke D, Mohler H. GABAB-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. *Eur J Neurosci*. 1999;11:761–768.
13. Berthele A, Platzer S, Weis S, Conrad B, Tölle TR. Expression of GABA(B1) and GABA(B2) mRNA in the human brain. *Neuroreport.* 2001;12:3269–3275.
14. Billinton A, Upton N, Bowery NG. GABA(B) receptor isoforms GBR1a and GBR1b appear to be associated with pre- and post-synaptic elements respectively in rat and human cerebellum. *Br J Pharmacol*. 1999;126: 1387–1392.
15. Vigot R, Barbieri S, Bräuner-Osborne H, et al. Differential compartmentalization and distinct functions of $GABA_B$ receptor variants. *Neuron*. 2006;50: 589–60.
16. Bettler B, Tiao J Y-H. Molecular diversity, trafficking and subcellular localization of $GABA_B$ receptors. *Pharmcol Ther*. 2006;110:533–543.
17. Ulrich D, Besseyrias V, Bettler B. Functional mapping of $GABA_B$-receptor subtypes in the thalamus. *J Neurophysiol*. 2007;98:3791–3795.
18. Biermann B, Ivankova-Susankova K, Bradaia A, et al. The Sushi domains of $GABA_B$ receptors function as axonal targeting signals. *J Neurosci*. 2010;30: 1385–1394.
19. Pérez-Garci E, Gassmann M, Bettler B, Larkum ME. The $GABA_B$1b isoform mediates long-lasting inhibition of dendritic Ca^{2+} spikes in layer 5 somatosensory pyramidal neurons. *Neuron.* 2006;50:603–616.
20. Waldmeier PC, Kaupmann K, Urwyler S. Roles of $GABA_B$ receptor subtypes in presynaptic auto- and heteroreceptor function regulating GABA and glutamate release. *J Neural Transm*. 2008;115:1401–1411.
21. Raiteri M. Presynaptic metabotropic glutamate and $GABA_B$ receptors [Review]. *Handb Exp Pharmacol*. 2008;184: 373–407.
22. Kratskin I, Kenigfest N, Rio JP, Djediat C, Repérant J. Immunocytochemical localization of the $GABA_B$2 receptor subunit in the glomeruli of the mouse main olfactory bulb. *Neurosci Lett.* 2006;402:121–125.
23. Barbaresi P. Cellular and subcellular localization of the GABA(B) receptor 1a/b subunit in the rat periaqueductal gray matter. *J Comp Neurol*. 2007;505:478–492.
24. Andersson M, Blomstrand F, Hanse E. Astrocytes play a critical role in transient heterosynaptic depression in the rat hippocampal CA1 region. *J Physiol*. 2007;585: 843–852.
25. Charles KJ, Deuchars J, Davies CH, Pangalos MN. GABA B receptor subunit expression in glia. *Mol Cell Neurosci*. 2003;24:214–22.
26. Song DK, Suh HW, Huh SO, et al. Central $GABA_A$ and $GABA_B$ receptor modulation of basal and stress-induced plasma interleukin-6 levels in mice. *J Pharmacol Exp Ther*. 1998;287(1):144–9.
27. Malitschek B, Rüegg D, Heid J, et al. Developmental changes of agonist affinity at $GABA_B$R1 receptor variants in rat brain. *Mol Cell Neurosci.* 1998;12:56–64.
28. Fritschy JM, Sidler C, Parpan F, et al. Independent maturation of the GABA(B) receptor subunits GABA(B1) and GABA(B2) during postnatal development in rodent brain. *J Comp Neurol*. 2004;477:235–252.
29. Hill DR, Bowery NG, Hudson AL. Inhibition of $GABA_B$ receptor binding by guanyl nucleotides. *J Neurochem*. 1984;42:652–657.
30. Xu J, Wojcik WJ. Gamma aminobutyric acid B receptor-mediated inhibition of adenylate cyclase in cultured cerebellar granule cells: blockade by islet-activating protein. *J Pharmacol Exp Ther.* 1986;239:568–573.
31. Nishikawa M, Kuriyama K. Functional coupling of cerebral Gamma-aminobutyric acid (GABA)(B) receptor with adenylate cyclase system: effect of phaclofen. *Neurochem Int*. 1989;14:85–90.
32. Karbon EW, Enna SJ. Characterization of the relationship between gamma-aminobutyric acid B agonists and transmitter-coupled cyclic nucleotide-generating systems in rat brain. *Mol Pharmacol*. 1985;27:53–59.
33. Gerber U, Gähwiler BH. $GABA_B$ and adenosine receptors mediate enhancement of the K^+ current, IAHP, by reducing adenylyl cyclase activity in rat CA3 hippocampal neurons. *J Neurophysiol.* 1994;72:2360–2367.
34. Knight AR, Bowery NG. The pharmacology of adenylyl cyclase modulation by $GABA_B$ receptors in rat brain slices. *Neuropharmacology.* 1996;35:703–712.
35. Bayewitch ML, Avidor-Reiss T, Levy R, et al. Differential modulation of adenylyl cyclases I and II by various G beta subunits. *J Biol Chem*. 1998;273: 2273–2276.
36. Hou M, Duan L, Slaughter MM. Synaptic inhibition by glycine acting at a metabotropic receptor in tiger salamander retina. *J Physiol*. 2008;586:2913–2926.
37. Robichon A, Tinette S, Courtial C, Pelletier F. Simultaneous stimulation of GABA and beta adrenergic receptors stabilizes isotypes of activated adenylyl cyclase heterocomplex. *BMC Cell Biol*. 2004;5(25): 1–14.
38. Mintz IM, Bean BP. $GABA_B$ receptor inhibition of P-type Ca^{2+} channels in central neurons. *Neuron.* 1993;10:889–898.

39. Cardozo DL, Bean BP. Voltage-dependent calcium channels in rat midbrain dopamine neurons: modulation by dopamine and $GABA_B$ receptors. *J Neurophysiol*. 1995;74:1137–1148.
40. Huston E, Cullen GP, Burley JR, Dolphin AC. The involvement of multiple calcium channel sub-types in glutamate release from cerebellar granule cells and its modulation by $GABA_B$ receptor activation. *Neuroscience*. 1995;68:465–478.
41. Poncer JC, McKinney RA, Gähwiler BH, Thompson SM. Either N- or P-type calcium channels mediate GABA release at distinct hippocampal inhibitory synapses. *Neuron*. 1997;18:463–472.
42. Chen G, van den Pol AN. Presynaptic $GABA_B$ autoreceptor modulation of P/Q-type calcium channels and GABA release in rat suprachiasmatic nucleus neurons. *J Neurosci*. 1998;18:1913–1922.
43. Ikeda SR, Dunlap K. Voltage-dependent modulation of N-type calcium channels: role of G protein subunits [Review]. *Adv Second Messenger Phosphoprotein Res*. 1999;33:131–151.
44. Lüscher C, Jan LY, Stoffel M, Malenka RC, Nicoll RA. G protein-coupled inwardly rectifying K_+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron*. 1997;19:687–695. Erratum in: *Neuron*. 1997;19(4): following 945.
45. Pitler TA, Alger BE. Depolarization-induced suppression of GABAergic inhibition in rat hippocampal pyramidal cells: G protein involvement in a presynaptic mechanism. *Neuron*. 1994;13:1447–1455.
46. Slesinger PA, Stoffel M, Jan YN, Jan LY. Defective gamma-aminobutyric acid type B receptor-activated inwardly rectifying K^+ currents in cerebellar granule cells isolated from weaver and Girk2 null mutant mice. *Proc Natl Acad Sci USA*. 1997;94:12210–12217.
47. Stefan H, Olofsson OE, Snead OC 3rd. Typical and atypical absence seizures, myoclonic absences, and eyelid myoclonias. In: Engel P, Pedley T, eds. *Epilepsy: A Comprehensive Textbook*. New York: Lippincott Williams & Wilkins; 2008:573–584.
48. Cortez MA, Snead OC 3rd. Pharmacological models of generalized absence seizures in rodents. In: Pitkanen M, Schwartzkroin P, Moshe S, eds. *Animal Models of Seizures and Epilepsy*. New York: Elsevier; 2006:111–126.
49. Farwell JR, Dodrill CB, Batzel LW. Neuropsychological abilities of children with epilepsy. *Epilepsia*. 1985;26:395–400.
50. Getova D, Bowery NG. The modulatory effects of high affinity GABA(B) receptor antagonists in an active avoidance learning paradigm in rats. *Psychopharmacology*. 1998;137:369–373.
51. Boniver C, Dravet C, Bureau M, Roger J. Idiopathic Lennox-Gastaut syndrome. In: Wolf P, Dam M, Janz D, Dreifuss F, eds. *Advances in Epileptology*. Vol. 16. New York: Raven Press; 1987:195–200.
52. Kaminsa A, Ickowicz A, Plouin P, Bru MF, Dellatolas G, Dulac O. Delineation of cryptogenic Lennox-Gastaut syndrome and myoclonic astatic epilepsy using multiple correspondence analysis. *Epilepsy Res*. 1999;36:15–29.
53. Carmant L, Kramer U, Holmes GL, Mikati MA, Riviello JJ, Helmers Sl. Differential diagnosis of staring spells in children: a video-EEG study. *Pediatr Neurol*. 1996;14:199–202.
54. Chan KF, Murphy PA, Burnham MW, Cortez MA, Snead OC 3rd. Learning and memory impairment in rats with chronic atypical absence seizures. *Exp Neurol*. 2004;190:328–336.
55. Gastaut H, Zifkin BG. Secondary bilateral synchrony and Lennox-Gastaut syndrome. In: Niedermeyer E, Degen R, eds. *The Lennox-Gastaut Syndrome*. New York: Alan R. Liss; 1988:221–242.
56. Nolan M, Bergazar M, Chu B, Cortez MA, Snead OC 3rd. Clinical and neurophysiologic spectrum associated with atypical absence seizures in children with intractable epilepsy. *J Child Neurol*. 2005;20:404–410.
57. Wang X, Stewart L, Cortex MA, et al. The circuitry of atypical absence seizures in $GABA_B$R1a transgenic mice. *Pharmacol Biochem Behav*. 2009;94:124–130.
58. Banerjee PK, Hirsch E, Snead OC 3rd. Gamma-hydroxybutyric acid induced spike and wave discharges in rats: relation to high affinity [^{3}H]gamma-hydroxybutyric acid binding sites in the thalamus and cortex. *Neuroscience*. 1993;56:11–21.
59. Vergnes M, Marescaux C, Depaulis A, Micheletti G, Warter JM. Spontaneous spike and wave discharges in thalamus and cortex in a rat model of genetic petit mal-like seizures. *Exp Neurol*. 1987;96:127–136.
60. Perez-Velazquez J-L, Huo JZ, Dominguez LG, Leschenko Y, Snead OC 3rd. Typical versus atypical absence seizures: network mechanisms of the spread of paroxysms. *Epilepsia*. 2007;48:1585–1593.
61. Marescaux C, Vergnes M, Depaulis A. Genetic absence epilepsy in rats from Strasbourg [Review]. *J Neural Transm Suppl*. 1992;35:37–69.
62. Polack P-O, Guillemain I, Hu E, Deransart C, Depaulis A, Charpier S. Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *J Neurosci*. 2007;27:6590–6599.
63. Getova D, Bowery NG, Spassov V. Effects of $GABA_B$ receptor antagonists on learning and memory retention in a rat model of absence epilepsy. *Eur J Pharmacol*. 1997;320:9–13.
64. Meeren HK, Pijn JP, van Luijtelaar EL, Coenen AM, Lopes da Silva FH. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizure in rats. *J Neurosci*. 2002;22:1480–1495.
65. Meeren HK, Veening JG, Möderscheim TA, Coenen AM, van Luijtelaar G. Thalamic lesions in a genetic rat model of absence epilepsy: dissociation between spike-wave discharges and sleep spindles. *Exp Neurol*. 2009;217:25–37.
66. Merlo D, Mollinari C, Inaba Y, et al. Reduced $GABA_B$ receptor subunit expression and paired-pulse depression in a genetic model of absence seizures. *Neurobiol Dis*. 2007;25:631–641.
67. Sitnikova E, van Luijtelaar G. Electroencephalographic precursors of spike-wave discharges in a genetic rat model of absence epilepsy: power spectrum and coherence EEG analyses. *Epilepsy Res*. 2009;84:159–171.
68. Inaba Y, D'Antuono M, Bertazzoni G, Biagini G, Avoli M. Diminished presynaptic GABA(B) receptor function in the neocortex of a genetic model of absence epilepsy. *Neurosignals*. 2009;17:121–131.
69. Snead OC 3rd. Evidence for G protein modulation of experimental-generalized absence seizures in rat. *Neurosci Lett*. 1992;148:15–18.
70. Hu RQ, Banerjee PK, Snead OC 3rd. Regulation of gamma-aminobutyric acid (GABA) release in cerebral

cortex in the gamma-hydroxybutyric acid (GHB) model of absence seizures in rat. *Neuropharmacology.* 2000;39:427–439.
71. Snead OC 3rd. Relation of the [3H] gamma-hydroxybutyric acid (GHB) binding site to the gamma-aminobutyric acidB (GABAB) receptor in rat brain. *Biochem Pharmacol*. 1996;52:1235–1243.
72. Hu RQ, Cortez MA, Man HY, et al. Gamma-hydroxybutyric acid-induced absence seizures in GluR2 null mutant mice. *Brain Res*. 2001;897:27–35.
73. Scott BW, Chan KF, Wong G, et al. Cytogenesis in the adult rat dentate gyrus is increased following kindled seizures but is unaltered in pharmacological models of absence seizures. *Epilepsy Behav*. 2010;18:179–185.
74. Lin FH, Cao Z, Hosford DA. Increased number of $GABA_B$ receptors in the lethargic (*lh/lh*) mouse model of absence epilepsy. *Brain Res.* 1993;608:101–106.
75. Ishige K, Ito Y, Fukuda H. Characterization of absence seizure-dependent cyclic AMP responsive element-and activator protein 1 DNA-binding activities in lethargic (*lh/lh*) mice. *Neurosci Lett*. 1999;262:53–56.
76. Ishige K, Endo H, Saito H, Ito Y. Repeated administration of CGP 46381, a gamma-aminobutyric acid B antagonist, and ethosuximide suppresses seizure-associated cyclic adenosine 3' 5' monophosphate response element- and activator protein-1 DNA-binding activities in lethargic (*lh/lh*) mice. *Neurosci Lett*. 2001;297:207–210.
77. Hosford DA, Lin FH, Wang Y, et al. Studies of the lethargic (*lh/lh*) mouse model of absence seizures: regulatory mechanisms and identification of the *lh* gene. *Adv Neurol*. 1999;79:239–252.
78. Cortez MA, McKerlie C, Snead OC 3rd. A model of atypical absence seizures: EEG, pharmacology, and developmental characterization. *Neurology*. 2001;56: 341–349.
79. Chan KFY, Burnham WM, Jia ZP, Cortez MA, Snead OC 3rd. GABAB receptor antagonism abolishes the learning impairments in rats with chronic atypical absence seizures. *Eur J Pharmacol.* 2006;541:64–72.
80. Huo JZ, Cortez MA, Snead OC 3rd. GABA receptor proteins within lipid rafts in the AY-9944 model of atypical absence seizures. *Epilepsia.* 2009;50:776–788.
81. Persad V, Cortez MA, Snead OC 3rd. A chronic model of atypical absence seizures: studies of developmental and gender sensitivity. *Epilepsy Res*. 2002;48: 111–119.
82. Cortez MA, Cunnane SC, Snead OC 3rd. Brain sterols in the AY-9944 rat model of atypical absence seizures. *Epilepsia*. 2002;43:3–8.
83. Smith KA, Bierkamper GG. Paradoxical role of GABA in a chronic model of petit mal (absence)-like epilepsy in the rat. *Eur J Pharmacol*. 1990;176:45–55.
84. Wu Y, Wong CGT, Cortez MA, et al. Evidence for a critical role of forebrain GABA-B receptors in atypical absence seizures. *Neurobiol Dis*. 2007;26:439–451.
85. Stewart LS, Ying Wu, Eubanks J, et al. Mice over-expressing $GABA_B$R1b receptor subtype show evidence of an atypical absence seizure phenotype. *Epilepsy Behav*. 2009;14:577–581.
86. Holmes MD. Dense array EEG: methodology and new hypothesis on epilepsy syndromes. *Epilepsia*. 2008;49(suppl 3):3–14.
87. Steriade M. Sleep, epilepsy, and thalamic reticular inhibitory neurons. *Trends Neurosci.* 2005;28: 317–324.
88. Beenhakker MP, Huguenard JR. Neurons that fire together also conspire together: is normal sleep circuitry hijacked to generate epilepsy? *Neuron*. 2009;62:612–632.
89. Crunelli V, Leresche N. Childhood absence epilepsy: genes, channels, neurons, and networks. *Nat Rev Neurosci*. 2002;3:371–382.
90. Khosravani H, Zamponi GW. Voltage-gated calcium channels and idiopathic generalized epilepsies. *Physiol Rev*. 2006;86:941–966.
91. de la Peña E, Geijo-Barrientos E. Laminar localization, morphology, and physiological properties of pyramidal neurons that have the low-threshold calcium current in the guinea-pig medial frontal cortex. *J Neurosci*. 1996;16:5301–5311.
92. de la Peña E, Geijo-Barrientos E. Participation of low-threshold calcium spikes in excitatory synaptic transmission in guinea pig medial frontal cortex. *Eur J Neurosci.* 2000;12:1679–1686.
93. Karameh FN, Massaquoi SG. Intracortical augmenting responses in networks of reduced compartmental models of tufted layer 5 cells. *J Neurophysiol*. 2009;101:207–233.
94. Iftinca MC, Zamponi GW. Regulation of neuronal T-type calcium channels. *Trends Pharmacol Sci*. 2008;30:32–40.
95. Polack P-O, Guillemain I, Hu E, Deransart C, Depaulis A, Charpier S. Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *J Neurosci*. 2007;27:6590–6599.
96. Çavdar S, Onate FY, Çakmak YO, Ynanli H, Gülçebi M, Aker R. The pathways connecting the hippocampal formation, the thalamic reuniens nucleus and the thalamic reticular nucleus in the rat. *J Anat*. 2008;212:249–56.
97. Vertes RP. Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. *Neuroscience*. 2006;142:1–20.
98. Vertes RP, Hoover WB, Szigeti-Buck K, Leranth C. Nucleus reuniens of the midline thalamus: link between the medial prefrontal cortex and the hippocampus. *Brain Res Bull*. 2007;71:601–609.
99. Hoover WB, Vertes RP. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Struct Funct*. 2007;211:149–179.
100. Aker RG, Özyurt HB, Yanali HR, et al. $GABA_A$ receptor mediated transmission in the thalamic reticular nucleus of rats with genetic absence epilepsy shows regional differences: functional implications. *Brain Res*. 2006;1111:213–221.
101. Fuentealba P, Steriade M. The reticular nucleus revisited: intrinsic and network properties of a thalamic pacemaker. *Prog Neurobiol*. 2005;75: 125–141.
102. Sandyk R, Gillman MA. Baclofen-induced memory impairment. *Clin Neuropharmacol*. 1985;8:294–295.
103. Swartzwelder HS, Tilson HA, McLamb RL, Wilson WA. Baclofen disrupts passive avoidance retention in rats. *Psychopharmacology* (*Berl*). 1987;92:398–401.
104. Castellano C, Brioni JD, Nagahara AH, McGaugh JL. Post-training systemic and intra-amygdala administration of the GABA-B agonist baclofen impairs retention. *Behav Neural Biol.* 1989;52:170–179.

105. Castellano C, McGaugh JL. Oxotremorine attenuates retrograde amnesia induced by post-training administration of the GABAergic agonists muscimol and baclofen. *Behav Neural Biol*. 1991;56:25–31.
106. Sidel ES, Tilson HA, McLamb RL, Wilson WA, Swartzwelder HS. Potential interactions between GABAb and cholinergic systems: baclofen augments scopolamine-induced performance deficits in the eight-arm radial maze. *Psychopharmacology* (*Berl*). 1988;96:116–120.
107. Stackman RW, Walsh TJ. Baclofen produces dose-related working memory impairments after intraseptal injection. *Behav Neural Biol*. 1994;61:181–185.
108. DeSousa NJ, Beninger RJ, Jhamandas K, Boegman RJ. Stimulation of $GABA_B$ receptors in the basal forebrain selectively impairs working memory of rats in the double Y-maze. *Brain Res*. 1994;641:29–38.
109. McNamara RK, Skelton RW. Baclofen, a selective $GABA_B$ receptor agonist, dose-dependently impairs spatial learning in rats. *Pharmacol Biochem Behav*. 1996;53:303–308.
110. Deng PY, Xiao Z, Yang C, et al. GABA(B) receptor activation inhibits neuronal excitability and spatial learning in the entorhinal cortex by activating TREK-2 K^+ channels. *Neuron*. 2009;63:230–243.
111. Heinrichs SC, Leite-Morris KA, Carey RJ, Kaplan GB. Baclofen enhances extinction of opiate conditioned place preference. *Behav Brain Res*. 2010;207:353–359.
112. Arai S, Takuma K, Mizoguchi H, et al. $GABA_B$ receptor agonist baclofen improves methamphetamine-induced cognitive deficit in mice. *Eur J Pharmacol*. 2009;602:101–104.
113. Bianchi M, Panerai AE. Reversal of scopolamine-induced amnesia by the $GABA_B$ receptor antagonist CGP 35348 in the mouse. *Cogn Brain Res*. 1993;1:135–136.
114. Castellano C, Cestaari V, Cabib S, Puglisis-Allegra S. Strain-dependent effects of post-training GABA receptor agonists and antagonists on memory storage in mice. *Psychopharmacology*. 1993;111:134–138.
115. Helm KA, Haberman RP, Dean SL, et al. $GABA_B$ receptor antagonist SGS742 improves spatial memory and reduces protein binding to the cAMP response element (CRE) in the hippocampus. *Neuropharmacology*. 2005;48:956–964.
116. Nakagawa Y, Takashima T. The $GABA_B$ receptor antagonist CGP36742 attenuates the baclofen- and scopolamine-induced deficit in Morris water maze task in rats. *Brain Res*. 1997;766:101–106.
117. Pitsikas N, Rigamonti AE, Cella SG, Muller EE. The $GABA_B$ receptor and recognition memory: possible modulation of its behavioral effects by the nitrergic system. *Neuroscience*. 2003;118:1121–1127.
118. Getova DP, Bowery NG. Effects of high-affinity $GABA_B$ receptor antagonists on active and passive avoidance responding in rodents with gamma-hydroxybutyrolactone-induced absence syndrome. *Psychopharmacology*. 2001;157:89–95.
119. Froestl W, Gallagher M, Jenkins H, et al. SGS742: the first $GABA_B$ receptor antagonist in clinical trials. *Biochem Pharmacol*. 2004;68:1479–1487.
120. Davies S, Castañer R. SGS-742. *Drugs Future* 2005;30:248–253.
121. Jacobson LH, Kelly PH, Bettler B, Kaupmann K, Cryan JF. GABA B(1) receptor isoforms differentially mediate the acquisition and extinction of aversive taste memories. *J Neurosci*. 2006;26:8800–8803.
122. Jacobson LH, Kelly P, Bettler B, Kaupmann K, Cryan JF. Specific roles of GABA-B(1) receptor isoforms in cognition. *Behav Brain Res*. 2007;181:158–162.
123. Sunyer B, Shim KS, An G, Höger H, Lubec G. Hippocampal levels of phosphorylated protein kinase A (phosphor-S96) are linked to spatial memory enhancement by SGS742. *Hippocampus*. 2009;19:90–98.
124. John JP, Sunyer B, Höger H, Pollak A, Lubec G. Hippocampal synapsin isoform levels are linked to spatial memory enhancement by SGS742. *Hippocampus*. 2009;19:731–738.
125. Nyitrai G, Kékesi KA, Emri Z, Szárics E, Juhász G, Kardos J. GABA(B) receptor antagonist CGP-36742 enhances somatostatin release in the rat hippocampus in vivo and in vitro. *Eur J Pharmacol*. 2003;478:111–119.
126. Crunelli V, Leresche N. A role for $GABA_B$ receptors in excitation and inhibition of thalamocortical cells. *Trends Neurosci*. 1991;14:16–21.
127. Snead OC 3rd. Evidence for $GABA_B$-mediated mechanisms in experimental generalized absence seizures. *Eur J Pharmacol*. 1992;213:343–349.
128. Hosford DA, Clark S, Cao Z, et al. The role of $GABA_B$ receptor activation in absence seizures of lethargic (*lh/lh*) mice. *Science*. 1992;257:398–401.
129. Liu Z, Vergnes M, Depaulis A, Marescaux C. Involvement of intrathalamic $GABA_B$ neurotransmission in the control of absence seizures in the rat. *Neuroscience*. 1992;48:87–93.
130. Wang X, Stewart L, Cortex MA, et al. The circuitry of atypical absence seizures in $GABA_B$R1a transgenic mice. *Pharmacol Biochem Behav*. 2009;94:124–130.
131. Wiebe S, Bellhouse DR, Fallahay C, Eliasziw M. Burden of epilepsy: the Ontario Health Survey. *Can J Neurol Sci*. 1999;26:263–270.

Chapter 20

Brainstem Networks

Reticulocortical Synchronization in Generalized Convulsive Seizures

Carl L. Faingold

Penfield and Jasper[1] proposed that generalized seizures involve the brainstem reticular formation (BRF) and other brain sites that are extensively connected to most other brain regions as a centrencephalic system. Considerable subsequent research has confirmed a major role for the BRF in the neuronal networks that subserve generalized convulsive seizure

generation. The BRF has been defined to include the core of the brainstem in the medulla, pons, and midbrain. The projections between brainstem nuclei, as well as those to and from the BRF, are massive, including all levels of the central nervous system (CNS) from spinal cord to cerebral cortex. Recent research on the anatomical and neurochemical diversity of nuclei within the BRF has been extensive, leading to a more complex view of the functions of the brainstem. However, these observations have not invalidated the original concept that the BRF has the potential to act as an extremely large network (reticulum) capable of "mass action" under certain circumstances, such as arousal or generalized convulsive seizure.[2–4] Depression of BRF neurons was originally and is still implicated as a major mechanism of action for depressant and anesthetic drugs.[2,5]

Direct brainstem or spinal cord stimulation can induce tonic muscular activity resembling the tonic convulsive component of generalized tonic-clonic seizures (GTCS).[6] In addition, the stereotypical and coordinated four limb generalized tonic and clonic motor movements seen during GTCS are proposed to be due to excessive activation of the normal locomotor network[7] that lies primarily in brainstem and spinal cord.[8] Thus, it has been proposed that generalized convulsive movements involve the recruitment of the normal locomotion network into the epilepsy network, which mediates GTCS. The concept of network interaction in epilepsy is an important advance in understanding how convulsions and other major alterations of brain organization occur, as discussed below.

The mechanisms that mediate convulsive seizures have been widely studied in a variety of animal models, involving chemical or electrical stimulation in normal animals, and these studies have yielded important insights into convulsive mechanisms. It has become clear that there is an important genetic component to human epilepsy susceptibility.[9] Thus, many specific genetic abnormalities occur in epileptic patients and their families, and genetically based animal models of epilepsy have also been discovered. The *two-hit* hypothesis (nature plus nurture) is proposed to be the most common cause of human epileptic syndromes.[9] Thus, a genetic predisposition is the first hit, and an inducing event such as a brain injury or infection is the second hit that triggers the onset of the epileptic syndrome.

AUDIOGENIC SEIZURES AS GENERALIZED CONVULSIVE EPILEPSY MODELS

The severe seizure strain of genetically epilepsy-prone rats (GEPR-9s) is a well-studied genetic model of generalized convulsive epilepsy.[10,11] These rats exhibit greater than normal susceptibility to seizures induced by convulsant drugs, hyperthermia, hyperbaric conditions, and electrical kindling, and GEPR-9s are also susceptible to sound-induced or audiogenic seizures (AGS). The AGS in GEPR-9s begin with wild running and proceed to tonic convulsions, ending in tonic hind limb extension, which is followed by postictal depression of consciousness. Susceptibility to AGS occurs in many other rodent strains, including the moderate-severity seizure strain of GEPR-3s, and AGS mechanisms have been studied in different rodent models worldwide.[11–15] In DBA mice, AGS have recently been shown to be an important model of human sudden, unexpected death in epilepsy, as discussed below.[16,17] Models of AGS have also been widely used to test anticonvulsant drugs, and drugs that are effective in human epilepsy are effective against AGS in the various models. Spontaneous seizures are seen rarely in AGS models, and their susceptibility to seizures is also associated with the two-hit hypothesis of genetic predisposition plus an environmental insult to the brain, as proposed for human epilepsy.[9] In GEPR-9s the first hit is a genetic defect in GABA-mediated inhibition,[11] and the second hit is an intense acoustic stimulus, which initiates the AGS.

NEURONAL NETWORK FOR AGS

The neuronal network required for AGS in GEPR-9s is confined exclusively to the brainstem,[6,10] including the lower brainstem primary auditory nuclei (Fig. 20–1A), but forebrain structures are not required for AGS.[10,18]

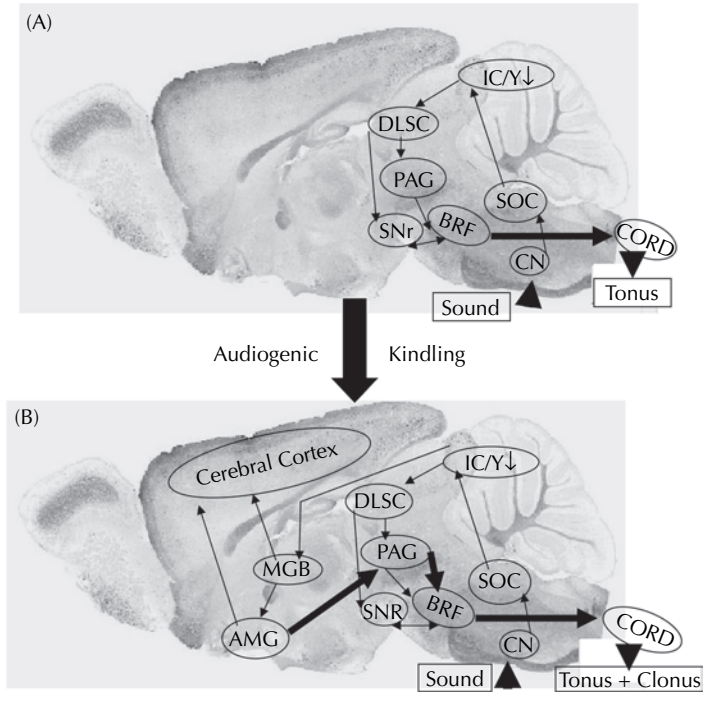

Figure 20–1. These diagrams show the neuronal network for audiogenic seizures (AGS) in genetically epilepsy-prone rats (GEPR-9s) and changes after AGS kindling. **A.** The AGS network (prior to AGS kindling) involves progression of the seizure via the brainstem primary auditory nuclei, including the cochlear nucleus (CN) and superior olivary complex (SOC) up to the inferior colliculus (IC). A deficit of GABA-mediated inhibition ($\gamma\downarrow$) in IC results in excessive auditory-evoked IC neuronal firing that initiates AGS. This involves projections to the normal locomotor pathway (deep layers of superior colliculus (DLSC) to periaqueductal gray (PAG), substantia nigra reticulata (SNr), and (pontine) brainstem reticular formation (BRF), which project to the spinal cord, mediating the convulsion. No forebrain structures are required. **B.** After periodic frequent repetitive AGS (audiogenic kindling) in GEPR-9s, the seizure network expands to include the medial geniculate body (MGB), amygdala (AMG), and cortex, which now exhibits epileptiform cortical EEG activity. The AMG is the most critical structure for generating the additional convulsive post-tonic clonus that is induced by AGS kindling.

Inferior Colliculus as the AGS Initiation Site—GABA Mechanisms

The inferior colliculus (IC) is the consensus initiation site for all AGS models, including GEPR-9s, GEPR-3s, and acoustically primed, thyroid-deficient and ethanol-withdrawn rats based on blockade, stimulation, and neuronal recording studies.[11] GABA is the major inhibitory neurotransmitter in the IC, mediating several different normal forms of acoustically evoked inhibition in the IC, including firing reductions normally induced by high-intensity acoustic stimuli.[11] Several forms of GABA-mediated inhibition are significantly reduced in GEPR-9s, leading to greatly increased IC neuronal firing (Fig. 20–2A,B), which acts as the triggering mechanism for AGS.[11] Direct stimulation of IC produces AGS susceptibility in normal rats.[19,20] Increased excitability, due to deficits of GABA-mediated inhibition, in IC neurons is also seen in the IC slice, and in other parts of the brain where it is associated with an 80% reduction of $\gamma 2$ subunit levels of $GABA_A$ receptors.[21–23]

Superior Colliculus in the AGS Network

Neurons in the deep layers of the superior colliculus (DLSC) are a major nonprimary auditory recipient of IC output and are strongly implicated in the AGS network, since blockade of DLSC blocks AGS.[19,24,25] The DLSC neurons are strongly implicated in triggering

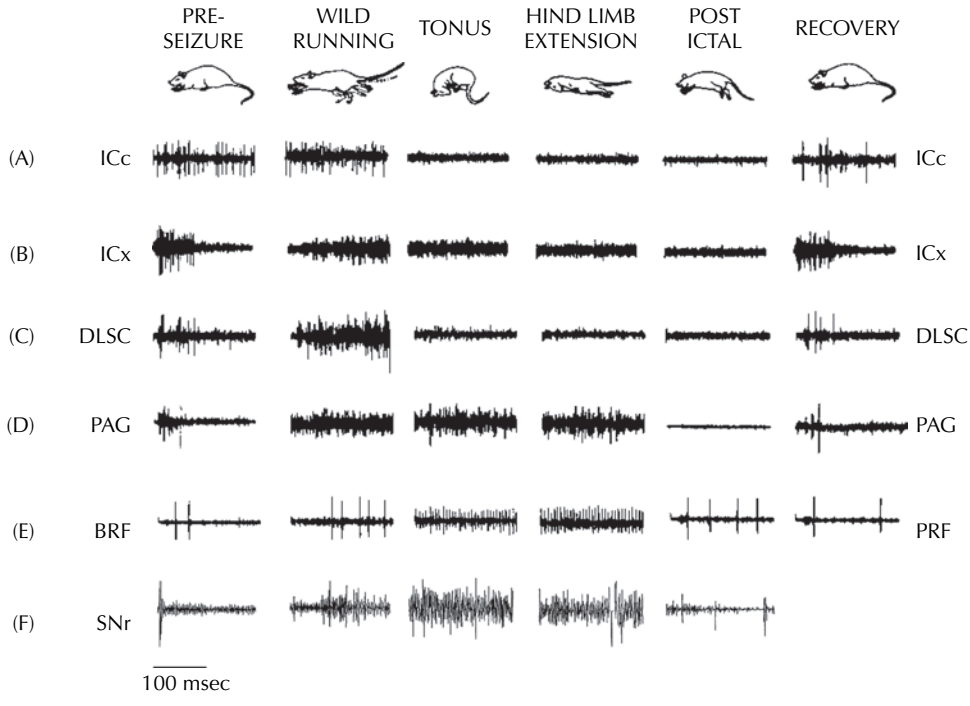

Figure 20–2. Composite behavioral activity in GEPR-9s and concurrent neuronal firing in the major network nuclei for AGS seen during each seizure behavior in the awake animal. The structures include (**A**) the inferior colliculus central (ICc) and (**B**) inferior colliculus external (ICx) nuclei; (**C**) the deep layers of superior colliculus (DLSC); (**D**) periaqueductal gray (PAG); (**E**) pontine brainstem reticular formation (BRF); and (**F**) substantia nigra reticulata (SNr). Thus, ICc and ICx firing is greatest during AGS initiation, while the greatest increase in DLSC neuronal firing occurs at the onset of wild running. The greatest increases in PAG, SNr, and BRF neuronal firing occur during the tonic and clonic phase(s) of AGS. The BRF and SNr are the only areas active during postictal depression of consciousness. Note: Recovery of SNr firing occurred but was not recorded in the example in F. (Acoustic stimulus parameters: 12 kHz tone burst, 100 ms duration, 5 ms rise-fall, 100 dB SPL, 2–4 Hz repetition rate. Each row is from the same rat.) Drawings according to ref. 45.

the wild running phase of AGS.[26,27] An abrupt onset of acoustically evoked firing at relatively high acoustic intensities was observed in DLSC neurons of GEPR-9s, which was significantly above the normal threshold. The DLSC neurons began to exhibit rapid tonic burst firing 1–2 s prior to the onset of the wild running behavior at the beginning of AGS (Fig. 20–2C).[26] The DLSC project to the spinal cord directly and via the pontine BRF and periaqueductal gray (PAG), and DLSC also project to the substantia nigra reticulata (SNr).[27–29]

PAG in the AGS Network

The PAG is a brainstem site that is extremely important in the neuronal network for AGS, since blockade of PAG blocks AGS, and PAG neurons are implicated in initiation of the tonic and clonic phases of AGS in GEPR-9s.[28,30] The PAG receives input from the DLSC and projects directly and indirectly to the spinal cord.[31–33] Acoustically evoked PAG neuronal responses in GEPR-9s are elevated compared to those in normal rats. The PAG neurons displayed tonic neuronal firing patterns during the wild running and tonic hind-limb extension phases of AGS (Fig. 20–2D).[28] In vitro studies indicate that the properties of PAG neurons exhibit abnormal membrane and synaptic properties in animals that are susceptible to AGS.[34]

Substantia Nigra in the AGS Network

The SNr is also implicated in control of seizures, including AGS in GEPR-9s, since blockade of SNr blocks AGS.[29] Acoustically evoked

SNr neuronal firing in awake normal rats was not detectable except at very high intensities, but the threshold for acoustically evoked neuronal firing in GEPR-9s was considerably lower (Fig. 20–3). The responsiveness of SNr neurons in GEPR-9s was also significantly greater than that in normal rats. During AGS in GEPR-9s, SNr neurons showed enhanced rapid firing (Fig. 20–2F). The SNr neurons showed occasional action potentials during postictal depression.

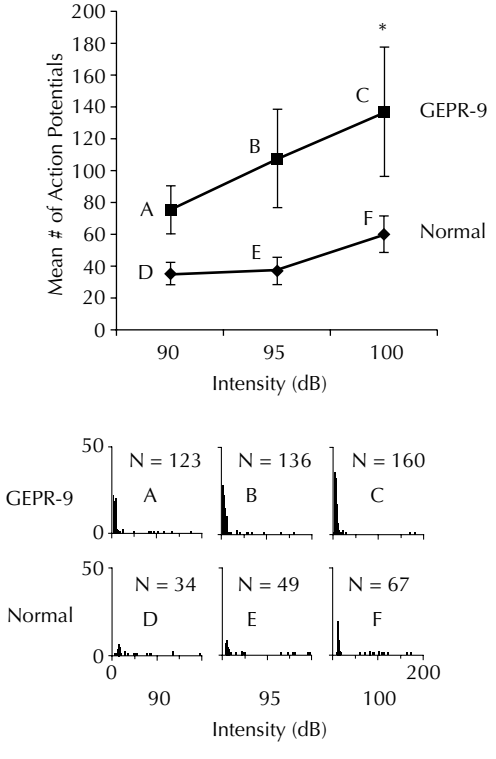

Figure 20–3. Comparison of acoustically-induced firing changes in the substantia nigra reticulata (SNr) in GEPR-9s compared to normal Sprague-Dawley rats. Acoustically evoked SNr neuronal firing in awake normal rats was not detectable except at very high intensities (≥90 dB SPL re; 0.0002 dyn/cm^2), but the threshold for acoustically-evoked neuronal firing in GEPR-9s was considerably lower. The upper graph shows the mean (n = 5 rats/group) number of acoustically-evoked action potentials/poststimulus-time histogram (PSTH) in GEPR-9s (**A–C**) compared to normal rats (**D–F**). There was a significantly greater response at C ($p < 0.05$, ANOVA) compared to control. The PSTHs below show typical examples from the graph above, presenting the number of action potentials (N) for each example. Each row is from the same rat. N = number of action potentials in the PSTH. (Acoustic stimulus parameters: 12 kHz tone burst, 100 ms duration, 5 ms rise-fall, 100 dB SPL, 0.5 Hz rate, 50 presentations.)

BRF in the AGS Network

Blockade within the pontine region of BRF blocks AGS susceptibility.[26] Neurons in the pontine BRF are implicated in triggering the tonic phase of AGS in GEPR-9s and GEPR-3s.[35,36] The BRF neurons in GEPR-9s exhibit precipitous intensity-evoked increases at a significantly lower intensity than normal rats. At the onset of AGS (wild running), the firing rate of BRF neurons increased and the rate of BRF firing increased dramatically as the tonic phase of the seizure began. During postictal depression BRF neuronal firing slowed, gradually returning to normal, but the BRF is one of two network nuclei active during this behavior[26,35] (Fig. 20–2E).

Changes in Nuclear Dominance during AGS

The neuronal recording data mentioned above indicate that changes occur in the dominance (greatest firing change) in each specific site within the requisite nuclei in the neuronal network for AGS during each sequential phase in a hierarchical manner during the behavioral phases of the convulsion[26] (Fig. 20–2). Thus, IC firing is greatest during AGS initiation, while the greatest increase in DLSC neuronal firing occurs just prior to the appearance of wild running. The greatest increases in PAG, SNr, and BRF neuronal firing occur during the tonic and clonic phase(s) of AGS (Fig. 20–2). The BRF is the major area active during postictal depression of consciousness, and may be indicative of the critical role of the BRF in maintaining "vegetative" cardiac and respiratory functions, which maintain the life of the animal when much of the rest of the brain is depressed.

RETICULAR FORMATION IN CONVULSANT-INDUCED GENERALIZED SEIZURE INITIATION

Brainstem reticular formation neuronal response changes are also implicated in other epilepsy models, including seizures induced by convulsant drugs.[37,38] Thus, the elevated

BRF neuronal response to sensory stimuli are induced by subconvulsant doses of convulsant drugs and is proposed to be a major mechanism involved in sensory initiation of seizures in this reflex epilepsy model. This model represents another example of the two-hit hypothesis of epilepsy. In this case, the first hit is the subconvulsant dose of bicuculline, pentylenetetrazol, or other convulsant drug. When the second hit—sensory stimulation—is presented, this triggers a generalized convulsive seizure in which BRF neurons appear to play a major role.[37] Normally, BRF neuronal responsiveness is very restrained, leading to minimal conduction of repetitive stimuli along the extensive ascending and descending BRF pathways. The BRF neuronal responses to sensory and other stimuli exhibit pervasive repetition-induced response habituation characterized by major declines in responsiveness with succeeding presentations of the stimulus and/or increases in the stimulus presentation rate.[37,38] When animals are given a subconvulsant dose of the $GABA_A$ receptor antagonist, bicuculline, or a number of other convulsant drugs and then subjected to sensory or electrical stimuli in one of several modalities, the sensory second hit can trigger convulsive seizures.[37] However, before seizure is induced, BRF neurons undergo a major increase in responsiveness to these various stimuli.[37,38] Extensive increases in BRF neuronal responsiveness seen in this epilepsy model would greatly enhance the output along the numerous pathways from the BRF. This intense output would tend to recruit and synchronize the firing of the large numbers of neurons in the multitude of CNS regions that receive BRF input, including the spinal cord, where the central program generators for the convulsive behaviors are localized.[39] Such a simultaneous and massive synchronization of neuronal firing is likely to play a key role in the establishment of the neuronal network subserving these generalized convulsive seizures. When the number and distribution of affected neurons reach a critical mass, the neuronal network for seizure is assembled, and the emergent properties of this network are expressed in triggering a generalized seizure by the sensory stimulus. This process could explain how the enhanced seizure susceptibility brought about genetically or by convulsant drug treatment would allow a sensory stimulus that was a modality-specific communicative signal to become the initiating factor in the pervasive cascading activation of the neuronal networks that ultimately produces the emergence of a generalized convulsive seizure. Similar mechanisms may also be operative in other forms of epilepsy, since the ability of sensory stimuli to trigger convulsions (reflex epilepsy) is also seen in many experimental epilepsy models and in human epilepsy.[11,13,40–42]

RETICULAR FORMATION PLASTICITY—CONDITIONAL MULTIRECEPTIVE NEURONS

The BRF is a nonprimary (sensory or motor) brain region, and this region contains a high proportion of cells that have been termed *conditional multireceptive* (CMR) neurons; other sites with high percentages of CMR neurons include the amygdala and certain regions of the cerebral cortex.[38] Unlike most primary neurons, CMR neurons exhibit extremely inconsistent responses to stimuli in the behaving animal, depending on the conditions affecting the organism, including vigilance states, drug treatment, and learning paradigms. The degree of CMR neuronal responsiveness is highly unpredictable, since these neurons are capable of exhibiting a major degree of response enhancement or diminution, depending on the external and internal milieu that the behaving organism is experiencing. This response plasticity can be either short-term or long-term in duration. Conditional multireceptive brain regions also have extensive connections to numerous brain areas, and the participation of CMR regions in any neuronal network can be highly variable. The mechanisms that allow BRF and other CMR neurons to undergo such a great increase in responsiveness are related to the physiological functions of these structures. Thus, most BRF neurons normally respond poorly to repetitive stimuli, and these declines in BRF neuronal responsiveness are associated with progressive reductions in excitatory postsynaptic potential (EPSP) amplitude, which can result in subthreshold EPSPs as stimuli are repeated.[37,38] These subthreshold EPSPs can be brought to threshold by blocking inhibition or enhancing excitation, which can be induced by pharmacological treatments. Thus, systemic or iontophoretic treatment with antagonists (bicuculline

or strychnine) of inhibitory neurotransmitter receptors can cause CMR neurons to become consistently responsive to external inputs (e.g., peripheral nerve, sensory, or electrical stimuli in the brain) to which these neurons did not previously appear to respond.[37,38] Conversely, agents that enhance GABA-mediated inhibition (e.g., barbiturates or benzodiazepines) or antagonize glutamate-mediated excitation (e.g., ketamine) can cause CMR neurons to become temporarily unresponsive to inputs that they responded to previously.[37,43]

SEIZURE REPETITION INDUCES NEURONAL NETWORK CHANGES

Audiogenic Kindling in GEPR-9s as a Model of Generalized Tonic-Clonic Seizures

The AGS models of epilepsy do not show epileptiform electroencephalographic (EEG) activity in the cortex during seizure, which is often considered the hallmark of human epilepsy (R. Naquet, personal communication). However, periodic AGS repetition (AGS kindling) in several strains of AGS-susceptible rats does result in the appearance of an epileptiform cortical EEG.[44–46] After AGS kindling in GEPR-9s, the tonic hind limb extension is followed by another convulsive behavior, post-tonic generalized clonus (PTC), and this sequence of convulsive behaviors closely mimics human GTCS, including the cortical epileptiform EEG. After AGS kindling in GEPR-9s, the seizure network undergoes permanent expansion from the brainstem and recruits forebrain structures, including the amygdala and cerebral cortex, as discussed below. This has led to the idea that unkindled AGS of GEPR-9s are a "larval" form of GTCS similar to human infantile spasms. After AGS kindling, the larval form develops into full-blown GTCS.[6]

IC Changes in Audiogenic Kindling

During AGS kindling (typically, 14 seizures over 7–14 days) in GEPR-9s,[45] IC neuronal firing in GEPR-9s showed significant increases in the number of acoustically-evoked action potentials by the second AGS repetition, which peaked at the 4th seizure, remaining elevated to about the same degree through the 14th seizure.[47] The increased IC neuronal firing was observed prior to the appearance of PTC. Although IC neuronal firing increases may subserve, at least, the initial increase in AGS severity, changes in neuronal firing in nuclei of the neuronal network for AGS efferent to the IC are likely to be responsible for the increased AGS severity that occurs after the fourth AGS repetition.

Reticular Formation Neuronal Firing Changes in Audiogenic Kindling

Although the BRF neuronal firing changes induced by AGS kindling in GEPR-9s have not been evaluated, the changes in BRF firing induced by AGS kindling in GEPR-3s have been studied.[36,48] The pontine BRF also plays a critical part in the neuronal network for AGS in GEPR-3s.[36] AGS in unkindled GEPR-3s culminate in generalized clonus, but AGS kindling in GEPR-3s results in a different additional seizure behavior, facial and forelimb (F & F) clonus, not seen prior to kindling, and which is also seen after AGS kindling in other AGS models.[44–46] After AGS kindling in GEPR-3s, BRF neuronal firing was greatly increased. Tonic BRF neuronal firing occurred during generalized clonus, which changed to burst firing after AGS kindling. Burst firing in BRF neurons also occurred during F & F clonus.[48] Increased neuronal firing and the change from tonic to burst firing suggest that AGS kindling involves increased BRF excitability. These data support an important role for BRF neurons in the generation of both the increase in generalized clonus and the F & F clonus induced by AGS kindling in GEPR-3s. Similar changes may also be induced in BRF neurons in GEPR-9s by AGS kindling.

Medial Geniculate Body Changes in Audiogenic Kindling

The medial geniculate body (MGB), which is the next major primary auditory nucleus efferent to the AGS-initiating site in IC, is not required for AGS induction prior to AGS

kindling. However, the MGB is strongly implicated in the kindled AGS network in GEPR-9s.[49] Blockade of the MGB blocks the PTC component of kindled AGS, causing the seizure to revert, reversibly, to the unkindled behavior pattern.[49] Audiogenic seizure kindling in GEPR-9s also results in changes in neuronal firing patterns in the MGB.[49] Acoustically-evoked MGB neuronal responses after AGS kindling in GEPR-9s were elevated, with an increased incidence of sustained firing associated with a loss of habituation. The MGB neurons exhibited rapid tonic firing during tonic seizures in GEPR-9s, suggesting that the MGB may be involved in the propagation of seizure activity. But MGB neuronal firing was silent during PTC, suggesting that MGB does not play a direct role in this behavior.

Amygdala Changes in Audiogenic Kindling

The amygdala appears to be the most critical site in seizure network expansion in AGS kindling in GEPR-9s, since focal blockade of this structure can block PTC, causing the AGS behaviors to revert temporarily to the unkindled pattern.[50] In addition, chemical stimulation of the amygdala in unkindled GEPR-9s results in susceptibility to AGS that closely mimics the AGS behavior pattern seen in kindled GEPR-9s.[51] Extensive increases in lateral amygdala neuronal firing are induced by AGS kindling in GEPR-9s.[43] These changes include significant increases in onset-only patterns and increases in acoustic responsiveness to over five times that of normal neurons. Amygdala neurons also fired tonically during tonic convulsions and exhibited burst firing during PTC. Greater acoustically-induced synchronization of lateral amygdala firing, as indicated by elevated responsiveness and increased concentration of firing near the stimulus onset, may be critical for mediating the behavioral and EEG changes induced by AGS kindling in GEPR-9s.

As noted above, GEPR-3s also undergo AGS kindling, and changes in amygdala firing also occur in this model.[52] Audiogenic seizure kindling in GEPR-3s results in F & F clonus, and this behavior is blocked by bilateral blockade of amygdala, causing the seizure behavior to revert to the unkindled generalized clonus pattern temporarily. Lateral amygdala neuronal responses to high-intensity acoustic stimuli are significantly increased after AGS kindling compared to unkindled GEPR-3s. Rapid firing occurred during wild running and generalized clonic behaviors before and after AGS kindling in GEPR-3s. Burst firing occurred during F & F clonus in GEPR-3s after AGS kindling, which was not seen in unkindled GEPR-3s. These findings indicate that amygdala neurons play a critical role in AGS kindling in GEPR-3s as well.[52]

Changes in the MGB-to-Amygdala Pathway in Audiogenic Kindling

Both the MGB and amygdala are implicated in the network expansion induced by AGS kindling, as described above. Lateral amygdala neuronal responses to MGB electrical stimulation in GEPR-9s are greatly increased by AGS kindling, compared to unkindled GEPR-9s, and a threshold reduction in amygdala neuronal responsiveness to MGB stimuli was also seen in AGS kindled GEPR-9s.[53] These findings indicate that AGS kindling increases the efficacy of the MGB-amygdala pathway in GEPR-9s, suggesting that synaptic plasticity in this pathway within the expanded seizure network is an important pathophysiological mechanism subserving AGS kindling.

Molecular Mechanisms of Audiogenic Kindling in Amygdala

Increased amygdala neuronal firing induced by AGS kindling may initiate permanent pathophysiological alterations in the amygdala, in part, by enhanced glutamate receptor-mediated excitation, since focal microinjection of *N*-methyl-D-aspartate (NMDA) into amygdala of unkindled GEPR-9s or GEPR-3s induces susceptibility to PTC in GEPR-9s or F & F clonus in GEPR-3s, which mimic AGS kindling in both AGS models temporarily.[51] When a cyclic adenosine monophosphate (cAMP) activator was microinjected into amygdala of unkindled GEPR-9s, seizures that mimic AGS kindling were also evocable, but this effect lasted indefinitely.[54] Conversely, when an NMDA antagonist or inhibitor of cAMP synthesis was

microinjected into the amygdala, the effects of AGS kindling were reversed, and the seizure pattern temporarily reverted to that seen prior to AGS kindling.[51,55,56] These data suggest that AGS kindling-induced repetitive NMDA receptor activation in the amygdala triggers a molecular cascade that critically involves activation of cAMP, which leads to the permanent expansion of the seizure neuronal network that is induced by AGS kindling.

PAG Changes in Audiogenic Kindling

Audiogenic seizure kindling in GEPR-9s also induces changes in PAG neuronal firing.[57] As noted above, the PAG is implicated in generalized clonus initiation in unkindled GEPR-9s. A pathway from the amygdala to PAG is well known.[58] The PAG neurons in kindled GEPR-9s showed significantly elevated responses to acoustic stimuli compared to unkindled GEPR-9s. During the wild running and tonic hind-limb extension, PAG neurons display tonic firing. Burst firing was seen in PAG during PTC that coincided temporally with expression of the PTC behavior in kindled GEPR-9s, which was never seen in unkindled GEPR-9s.[57] The burst pattern of neuronal activity is known to be associated with neuroplasticity, which suggests that neuroplastic changes are occurring in the PAG of GEPR-9s that may contribute importantly to AGS kindling-associated epileptic network expansion and emergence of PTC.

Plasticity in the Amygdala-to-PAG Pathway in Audiogenic Kindling

As noted above, pathways from the amygdala to PAG are well documented, and the PTC behavior induced by AGS kindling may involve a reentry of AGS activity from the amygdala to PAG, as shown by a recent study inGEPR-9s.[59] Electrical stimulation in amygdala evokes neuronal responses in PAG in unkindled GEPR-9s, but PAG responsiveness to these stimuli is significantly increased in AGS-kindled GEPR-9s compared to unkindled GEPR-9s. These findings suggest that AGS kindling involves plastic changes in the amygdala-to-PAG pathway, which may be an important epileptogenic mechanism, mediating the emergence of the PTC behavior that is induced by AGS kindling.

Cerebral Cortical Neuronal Firing in Audiogenic Kindling

Cortical epileptiform activity is induced during AGS kindling in GEPR-9s and in GEPR-3s, which is absent prior to AGS kindling.[45] Neuronal changes in the cortex induced by AGS kindling have not been evaluated in GEPR-9s, but perirhinal cortex neuronal firing changes induced by AGS kindling in GEPR-3s were examined.[60] As noted above, AGS kindling in GEPR-3s induces an additional behavior, F & F clonus, immediately following generalized clonus.[45] Focal bilateral blockade of the perirhinal cortex resulted in complete and reversible blockade of only the F & F clonic seizure behavior in AGS-kindled GEPR-3s but did not affect generalized clonus before or after AGS kindling.[60] Significant increases in perirhinal cortex neuronal responses to acoustic stimuli were also induced by AGS kindling in GEPR-3s.[60] During F & F clonus in GEPR-3s, burst firing appeared in these cortical neurons. These findings support a critical role for the perirhinal cortex in the neuronal network for AGS kindling in GEPR-3s associated with the emergence of epileptiform cortical EEG activity and suggest that the epileptiform EEG activity seen in the cortex in GEPR-9s may involve neuronal firing changes similar to those seen in GEPR-3s.

Cerebral Cortex Neuronal Firing in Convulsant Drug-Induced Seizures

Convulsant drugs, such as bicuculline, induce major response increases in the CMR neurons in BRF, as noted above, and cerebral cortex neuronal responses are also increased in this model.[61] Neuronal responses to sensory stimuli in BRF and pericruciate cortex were recorded simultaneously in awake animals, and the neuronal responses to sensory stimuli in both structures were greatly enhanced following administration of subconvulsant doses of bicuculline (Fig. 20–4). The degree of enhancement in BRF neurons is somewhat greater in

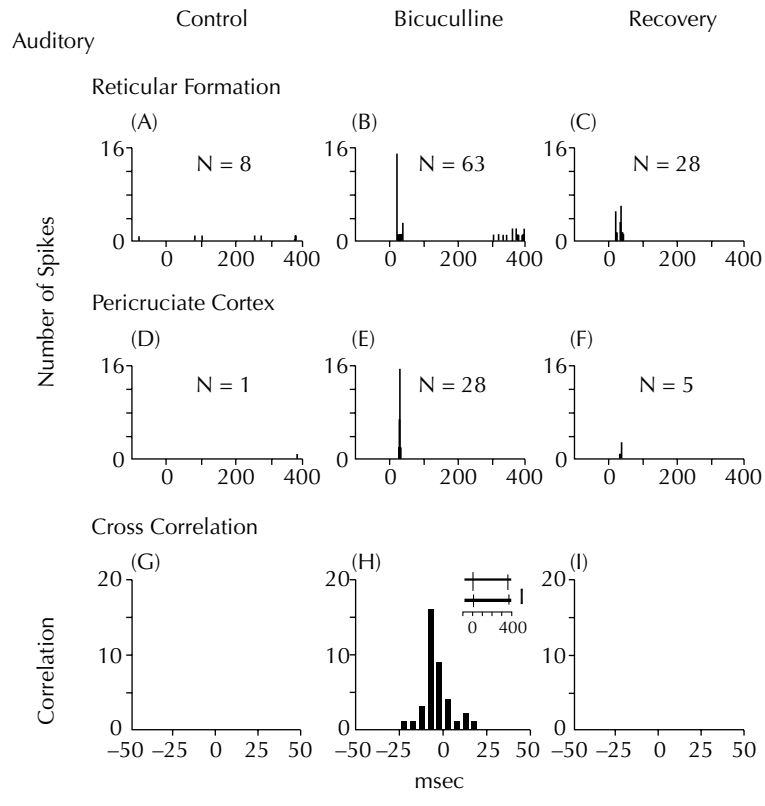

Figure 20–4. Typical example of the effect of a subconvulsant dose of bicuculline (0.08 mg/kg, i.v.) on neuronal firing in a midbrain BRF neuron (**A–C**) and simultaneously in a pericruciate cortex neuron (**D–F**), and the cross-correlogram (8 ms bin width) between these activities (**G–I**) in the awake cat. Few responses to the auditory stimulus were observed prior to bicuculline administration (**A,D**), with no detectable activity in the correlogram (**G**). Within 3 min of the drug's administration, both neurons became responsive to the stimulus (**B,E**), as illustrated by the peak in the peristimulus time histograms (PrSTHs). There was a major peak in the correlogram, with the midbrain BRF neurons firing consistently 15 ms prior to the cortical neuron, as shown by the negative values in the correlogram (**H**). (By convention, a negative value indicates that the BRF neurons fired first.) The neuronal responses gradually reverted to being minimally responsive 26 min after drug administration (**C,F**). No peak in the correlogram was present at this time (**I**). The inset in part H shows the three superimposed oscilloscope tracings for the raw data for both neurons (BRF in top trace) (calibration, 1 mV. *N* = number of action potentials in the PrSTHs. (Acoustic stimulus parameters: 1 ms duration click, 75 dB SPL, 0.5 Hz rate, 25 presentations, 400 ms duration, 1 ms bin width.) From ref. 61.

magnitude and occurs in a greater percentage of BRF neurons.[37,38] The latency of the responses to the stimuli is shorter in the BRF than in the cortex in 70% of cases. A large percentage of simultaneously recorded BRF and cortical neurons, which became responsive to the same stimulus, exhibited consistent convulsant-induced cross-correlations of firing, which were not present before drug treatment.[61] The latency and correlation data are consistent with the possibility that the BRF mediates the cortical enhancement. The bicuculline-induced correlation of firing of BRF and pericruciate neurons may be involved in the mechanism of initiation of convulsant-mediated seizure generalization induced by sensory stimuli. These findings may illuminate an important mechanism of reticulocortical synchronization.

INTERACTIONS BETWEEN NEURONAL NETWORKS

The CNS neuronal networks that mediate specific single physiological functions, such as hearing, can interact with other networks to produce many normal brain functions (e.g., acoustically-evoked startle), and such network interactions can also result in CNS disorders

(e.g., epilepsy). Such network interactions may also be important to the therapy of these disorders.[38] Network interactions often involve brain regions with high proportions of CMR neurons because of their extensive neuroplasticity, as noted above. Such network interactions are proposed to be a major mechanism for the induction of functional brain changes in positive directions, as in learning, or in negative directions, as in certain brain diseases. Network-mediated emergent properties of neurons can also be prominent targets for the action of CNS drugs in the intact brain.[7] The interactions between different networks can be competitive or cooperative as well as acute or chronic. Interactions between networks may involve sequential and/or simultaneous network activation. A critical nexus of network interaction in the production of generalized seizures is postulated to involve CMR brain regions, particularly the BRF. An example of sequential network interaction is the interaction of the normal auditory network and the normal locomotion network that occurs in AGS discussed above and illustrated in Fig. 20–1.

Epilepsy Network–Respiratory Network Interaction

Network interactions can also result in negative effects that have been observed in certain types of epileptic seizures, including that seen in sudden unexpected death in epilepsy (SUDEP). Human SUDEP is associated with respiratory difficulties in many human cases.[62] Many inbred and gene knockout mouse strains exhibit susceptibility to AGS, and in many of these mouse strains the seizure can be fatal.[11,16,17] DBA/1 and DBA/2 inbred strains of mice exhibit generalized convulsive seizures leading to respiratory arrest. These mice often die after AGS due to respiratory failure, and the incidence of death can be reduced or prevented by respiratory support, enhanced oxygen, or drugs that enhance respiration.[63] The DBA/2 and DBA/1 mouse animal models of SUDEP have been proposed to involve a negative sequential network interaction. This interaction is thought to involve activation of the sound-induced generalized convulsive seizure network that causes disruption of the normal respiratory network, with possible effects on cardiovascular networks. This network interaction leads directly to respiratory arrest immediately following the seizure (Fig. 20–5), which results in sudden death associated with subsequent cardiac failure, mechanisms proposed to be a major cause for human SUDEP.[16,17,62,63]

Pain Network–Epilepsy Network Interaction

Competition between networks can also exert positive effects. The neuronal network subserving analgesia is proposed to activate structures of the descending antinociceptive pathway that include the PAG,[64] which is implicated in epilepsy, as noted above. Periaqueductal gray neurons, unlike BRF neurons, are quiescent during postictal depression in GEPR-9s.[28] However, the PAG plays an important role during a significant period following postictal depression, during which the rats are less responsive to nociceptive stimuli. Thus, a reduction of pain responsiveness is noted in GEPR-9s, lasting for nearly 2 h after AGS.[65] Focal blockade of the PAG will prevent this form of analgesia.[65]

RETICULOCORTICAL SYNCHRONIZATION MECHANISMS

The CMR nature of BRF neurons may be a key mechanism in reticulocortical synchronization, since normally inactive BRF neurons can become highly active participants in neuronal networks that mediate seizures. Thus, in GEPR-9s, response habituation of BRF neurons to acoustic stimuli is significantly diminished[35] and may be a key element in the seizure network that mediates reticulocortical synchronization after AGS kindling.[11,26] Reticulospinal projections also become critical in epilepsy networks induced by convulsant drug interaction with stimulus-induced seizures, as discussed above.[37,38] The functional basis for the involvement of reticulospinal projections in motor convulsions may lie in the role that these projections are known to play in the normal neuronal network for locomotion.[8] The immense interconnectivity of BRF neurons provides extensive potential opportunities for

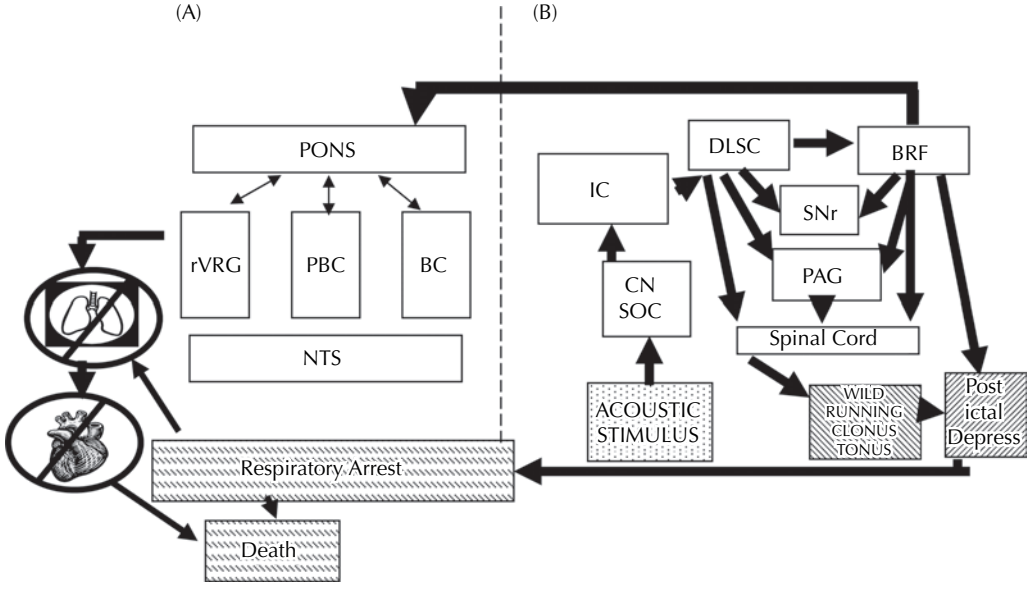

Figure 20–5. Diagram of network interactions involved in AGS that are postulated to occur in the DBA mouse models of human SUDEP, which exhibit AGS, leading to death due to respiratory depression. The diagram of elements of the normal network for respiration is shown in panel **A**. The diagram of the AGS network is shown in panel **B**. High-intensity acoustic stimuli activate the AGS network, which is postulated to involve a network interaction (based on that of GEPR-9s) between the normal brainstem primary auditory network nuclei cochlear nucleus (CN) and superior olivary complex (SOC) up to the level of the inferior colliculus (IC) and elements of the normal locomotion network, beginning in the deep layers of the superior colliculus (DLSC) and proceeding to the periaqueductal gray (PAG), substantia nigra reticulata (SNr), and brainstem (pontine) reticular formation (BRF), which project to the spinal cord. DBA mice that exhibit severe AGS (tonic hind limb extension) have postictal (seizure) depression of consciousness. The depression of consciousness is postulated to lead to BRF suppression, which produces a negative electrophysiological and neurochemical network interaction between the seizure network and the respiratory network. This leads to respiratory arrest and SUDEP, associated with subsequent cardiac failure. The latter events are believed to model a major cause of human SUDEP. Abbreviations: rVRG = rostral ventral respiratory group, PBC = pre-Bötzinger complex, BC = Bötzinger complex, NTS = nucleus tractus solitarius.

participation in various types of reticulocortical seizure networks, as originally suggested by Penfield and Jasper.[1]

The BRF neurons are known to be a key element in the neuronal network for the acoustic startle response.[66] High-intensity acoustic stimuli in an appropriate behavioral situation will evoke a major motor reaction (startle response) in humans and animals, but similar stimuli of lower intensities or in different behavioral situations do not evoke this motor response. In AGS-susceptible animals, the excessive output from IC neurons evoked by the high-intensity acoustic stimulus is projected to the hyperresponsive BRF neurons, and this results in intense output of BRF neurons along the reticulospinal pathway, which is critical in triggering the motor convulsions seen in AGS.[35] Thus, BRF neuronal hyperresponsiveness to sensory stimuli may lead to generalized seizures due to pathological intensification of the normal brain network that mediates the acoustic startle response. Since startle reactions can also be triggered by visual or somatosensory stimuli, the hyperresponsiveness of BRF neurons to these stimuli, which is induced by convulsant drugs, such as bicuculline, may also contribute to the generalized convulsions that are inducible by these stimuli in animals treated with otherwise subconvulsant doses of these convulsants.[37,38]

CONCLUSIONS

Penfield and Jasper[1] proposed that generalized seizures involve the BRF. Considerable research has confirmed a major role of the BRF in GTCS. Direct stimulation of the brainstem or spinal cord alone induces tonic muscular activity resembling the tonic convulsions of GTCS. Genetically epilepsy-prone rats

(GEPR-9s) are an epilepsy model that exhibits a genetically-based abnormally high sensitivity to seizure induction by a number of modalities, including high-intensity acoustic stimuli, which lead to convulsive AGS. The AGS in GEPR-9s end in tonic convulsions, and the neuronal network for these seizures requires only brainstem nuclei, including the BRF.[6] After periodic AGS repetition (AGS kindling) in GEPR-9s, the tonic seizures are followed by an additional behavior, PTC, and the resulting convulsive pattern closely mimics human GTCS, including cortical epileptiform EEG not seen prior to AGS kindling. After AGS kindling, the seizure network permanently expands from the brainstem to include the forebrain, leading to the idea that unkindled seizures of GEPR-9s are a "larval" form of GTCS similar to infantile spasms. After AGS kindling, the larval form develops into full-blown GTCS.[6]

The amygdala is a critical site in the seizure network expansion in AGS kindling, and extensive increases in amygdala neuronal firing are induced by AGS kindling. Focal blockade of amygdala blocks PTC, causing the seizures in kindled GEPR-9s to revert temporarily to the prekindled convulsive behavior. Microinjection of NMDA into amygdala of unkindled GEPR-9s induces PTC susceptibility, mimicking AGS kindling temporarily. However, when a cAMP activator (forskolin derivative) is microinjected into the amygdala of unkindled GEPR-9s, seizures, behaviorally identical to AGS kindling, are evocable indefinitely. These data suggest that AGS kindling-induced repetitive NMDA receptor activation in the amygdala triggers a molecular cascade, leading to the permanence of AGS kindling, in which activation of cAMP is critical, since pharmacological enhancement of cAMP levels in the amygdala leads to instant AGS kindling behavior after 2 AGS, which normally requires 14 AGS. This instant AGS kindling effect is also very long-lasting, similar to AGS kindling. Blockade of cAMP formation in amygdala of AGS-kindled GEPR-9s also reverses the effects of AGS kindling. Major amygdala projections to the brainstem, PAG, appear to be involved in PTC mechanisms, since extensive neuronal firing changes in PAG are also induced by AGS kindling. These findings suggest pathways and molecular mechanisms that may be important to epileptogenesis in human generalized tonic-clonic seizures.

DISCLOSURE STATEMENT

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REFERENCES

1. Penfield W, Jasper HH. *Epilepsy and the Functional Anatomy of the Human Brain*. Boston: Little, Brown; 1954.
2. Magoun HW. *The Waking Brain*. 2nd ed. Springfield, IL: Charles C. Thomas; 1963.
3. Kinomura S, Larsson J, Gulyas B, Roland PE. Activation by attention of the human reticular formation and thalamic intralaminar nuclei. *Science*. 1996;271(5248):512–515.
4. Norden AD, Blumenfeld H. The role of subcortical structures in human epilepsy. *Epilepsy Behav*. 2002;3(3):219–231.
5. Abulafia R, Zalkind V, Devor M. Cerebral activity during the anesthesia-like state induced by mesopontine microinjection of pentobarbital. *J Neurosci*. 2009;29(21):7053–7064.
6. Faingold, CL, Evans M.S. Pathophysiology of generalized tonic clonic seizures. In: Panayiotopoulos T, ed. *Atlas of Epilepsies*. Springer, Heidelberg: Springer; 2010;32:243–246.
7. Faingold CL. Emergent properties of CNS neuronal networks as targets for pharmacology: application to anticonvulsant drug action. *Prog Neurobiol*. 2004;72:55–85.
8. Grillner S, Jessell TM. Measured motion: searching for simplicity in spinal locomotor networks. *Curr Opin Neurobiol*. 2009;19(6):572–586.
9. Berkovic S, Mulley JC, Scheffer IE, Petrou S. Human epilepsies: interaction of genetic and acquired factors. *Trends Neurosci*. 2006;29(7):391–397.
10. Jobe P, Browning R. From brainstem to forebrain in generalized animal models of seizures and epilepsies. *Prog Epileptic Disord*. 2006;2:33–52.
11. Faingold CL. Role of GABA abnormalities in the inferior colliculus pathophysiology—audiogenic seizures. *Hear Res*. 2002;168(1–2):223–237.
12. Fuentes-Santamaria V, Alvarado JC, Herranz AS, Garcia-Atares N, Lopez DE. Morphologic and neurochemical alterations in the superior colliculus of the genetically epilepsy-prone hamster (GPG/Vall). *Epilepsy Res*. 2007;75(2–3):206–219.
13. Garcia-Cairasco N. A critical review on the participation of inferior colliculus in acoustic-motor and acoustic-limbic networks involved in the expression of acute and kindled audiogenic seizures. *Hear Res*. 2002;168(1–2):208–222.
14. Vinogradova LV, van Rijn CM. Anticonvulsive and antiepileptogenic effects of levetiracetam in the audiogenic kindling model. *Epilepsia*. 2008;49(7):1160–1168.
15. Li Sy, Xu DS, Jia HT. AGS-induced expression of Narp is concomitant with expression of AMPA receptor subunits GluR1 and GluR2 in hippocampus but not inferior colliculus of P77PMC rats. *Neurobiol Dis*. 2003;14(3)328–335.

16. Tupal S, Faingold CL. Evidence supporting a role of serotonin in modulation of sudden death induced by seizures in DBA/2 mice. *Epilepsia*. 2006;47(1): 21–26.
17. Faingold CL, Randall M, Tupal S. DBA/1mice exhibit susceptibility to audiogenic seizures followed by sudden death associated with respiratory arrest. *Epilepsy Behav*. 2010;17:436–440.
18. Ribak CE, Khurana V, Lien NT. The effect of midbrain collicular knife cuts on audiogenic seizure severity in the genetically epilepsy-prone rat. *J Hirnforsch*. 1994:35(2):303–311.
19. Millan MH, Meldrum BS, Faingold CL. Induction of audiogenic seizure susceptibility by focal infusion of excitant amino acid or bicuculline into the inferior colliculus of normal rats. *Exp Neurol*. 1986;91(3):634–639.
20. Sakamoto T, Mishina M, Niki H. Mutation of NMDA receptor subunit epsilon 1: effects on audiogenic-like seizures induced by electrical stimulation of the inferior colliculus in mice. *Brain Res Mol Brain Res*. 2002;102(1–2):113–117.
21. Li Y, Evans MS, Faingold CL. Inferior colliculus neuronal membrane and synaptic properties in genetically epilepsy-prone rats. *Brain Res*. 1994;660(2):232–240.
22. Evans MS, Cady CJ, Disney KE, Yang L, Laguardia JJ. Three brief epileptic seizures reduce inhibitory synaptic currents, GABA(A) currents, and GABA(A)-receptor subunits. *Epilepsia.* 2006;47(10):1655–1664.
23. Molnar LR, Fleming WW, Taylor DA. Alterations in neuronal gamma-aminobutyric acid(A) receptor responsiveness in genetic models of seizure susceptibility with different expression patterns. *J Pharmacol Exp Ther*. 2000;295 (3):1258–1266.
24. Ribak CE, Manio AL, Navetta MS, Gall CM. In situ hybridization for c-*fos* mRNA reveals the involvement of the superior colliculus in the propagation of seizure activity in genetically epilepsy-prone rats. *Epilepsy Res*. 1997;26(3):397–406.
25. Merrill MA, Clough RW, Jobe PC, Browning RA. Role of the superior colliculus and the intercollicular nucleus in the brainstem seizure circuitry of the genetically epilepsy-prone rat. *Epilepsia.* 2003;44(3):305–314.
26. Faingold CL. Neuronal networks in the genetically epilepsy-prone rat. *Adv Neurol.* 1999;79:311–321.
27. Faingold CL, Randall ME. Neurons in the deep layers of superior colliculus play a critical role in the neuronal network for audiogenic seizures: mechanisms for production of wild running behavior. *Brain Res*. 1999;815(2):250–258.
28. N'Gouemo P, Faingold CL. Periaqueductal gray neurons exhibit increased responsiveness associated with audiogenic seizures in the genetically epilepsy-prone rat. *Neuroscience.* 1998;84:619–625.
29. Millan MH, Meldrum BS, Boersma CA, Faingold CL. Excitant amino acids and audiogenic seizures in the genetically epilepsy-prone rat. II. Efferent seizure propagating pathway. *Exp Neurol*. 1988;99:687–698.
30. Yang L, Long C, Randall ME, Faingold CL. Neurons in the periaqueductal gray are critically involved in the neuronal network for audiogenic seizures during ethanol withdrawal. *Neuropharmacology*. 2003;44(2): 275–281.
31. Yang K, Ma WL, Feng YP, Dong YX, Li YQ. Origins of GABA(B) receptor-like immunoreactive terminals in the rat spinal dorsal horn. *Brain Res Bull*. 2002;58(5):499–507.
32. Bajic D, Proudfit HK. Projections of neurons in the periaqueductal gray to pontine and medullary catecholamine cell groups involved in the modulation of nociception. *J Comp Neurol*. 1999;405(3):359–379.
33. Mouton LJ, Holstege G. The periaqueductal gray in the cat projects to lamina VIII and the medial part of lamina VII throughout the length of the spinal cord. *Exp Brain Res*. 1994;101(2):253–264.
34. Yang L, Long C, Evans MS, Faingold C. Ethanol withdrawal results in aberrant membrane properties and synaptic responses in periaqueductal gray neurons associated with seizure susceptibility. *Brain Res*. 2002;957(1):99–108.
35. Faingold CL, Randall ME. Pontine reticular formation neurons exhibit a premature and precipitous increase in acoustic responses prior to audiogenic seizures in genetically epilepsy-prone rats. *Brain Res.* 1995;704:218–226.
36. Raisinghani M, Faingold CL. Pontine reticular formation neurons are implicated in the neuronal network for generalized clonic seizures which is intensified by audiogenic kindling. *Brain Res.* 2005b;1064:90–97.
37. Faingold CL, Riaz A. Neuronal networks in convulsant drug-induced seizures In: Faingold CL, Fromm GH, eds. *Drugs for Control of Epilepsy: Actions on Neuronal Networks Involved in Seizure Disorders*. Boca Raton, FL: CRC Press; 1992:213–251.
38. Faingold CL. Electrical stimulation therapies for CNS disorders and pain are mediated by competition between different neuronal networks in the brain. *Med Hypotheses*. 2008;71(5):668–681.
39. Jobe PC. Spinal seizures induced by electrical stimulation. In: Fromm GH, Faingold CL, Browning RA, Burnham WM, eds. *Epilepsy and the Reticular Formation: The Role of the Reticular Core in Convulsive Seizures*. New York: Alan R. Liss; 1987:81–91.
40. Szabo CA, Narayana S, Kochunov PV, Franklin CK, Knape K, Davis MD, Fox PT, Leland MM, Williams JT. PET imaging in the photosensitive baboon: case-controlled study. *Epilepsia*. 2007;48(2):245–253.
41. Trenite G. Photosensitivity, visually sensitive seizures and epilepsies. *Epilepsy Res*. 2006;1(Suppl 70): S269–S279.
42. Xue LY, Ritaccio AL. Reflex seizures and reflex epilepsy. *Am J Electroneurodiagnostic Technol*. 2006;46(1):39–48.
43. Feng HJ, Faingold CL. Repeated generalized audiogenic seizures induce plastic changes on acoustically evoked neuronal firing in the amygdala. *Brain Res*. 2002;932(1–2):61–69.
44. Marescaux C, Vergnes M, Kiesmann M, Depaulis A, Micheletti G, Warter JM. Kindling of audiogenic seizures in Wistar rats: an EEG study. *Exp Neurol*. 1987;97(1):160–168.
45. Naritoku DK, Mecozzi LB, Aiello MT, Faingold CL. Repetition of audiogenic seizures in genetically epilepsy-prone rats induces cortical epileptiform activity and additional seizure behaviors. *Exp Neurol*. 1992;115:317–324.
46. Dutra Moraes MF, Galvis-Alonso OY, Garcia-Cairasco N. Audiogenic kindling in the Wistar rat: a potential model for recruitment of limbic structures. *Epilepsy Res*. 2000;39(3):251–259.
47. N'Gouemo P, Faingold CL. Repetitive audiogenic seizures cause an increased acoustic response in inferior colliculus neurons and additional convulsive behaviors in the genetically-epilepsy prone rat. *Brain Res*. 1996;710(1–2):92–96.

48. Raisinghani M, Faingold CL. Identification of the requisite brain sites in the neuronal network subserving generalized clonic audiogenic seizures. Brain Res 2003;967(1–2): 113–122.
49. N'Gouemo P, Faingold CL. Audiogenic kindling increases neuronal responses to acoustic stimuli in neurons of the medial geniculate body of the genetically epilepsy-prone rat. *Brain Res*. 1997;761:217–224.
50. Feng HJ, Naritoku DK, Randall ME, Faingold CL. Modulation of audiogenically kindled seizures by gamma-aminobutyric acid-related mechanisms in the amygdala. *Exp Neurol*. 2001;172:477–481.
51. Raisinghani M, Feng HJ, Faingold CL. Glutamatergic activation of the amygdala differentially mimics the effects of audiogenic seizure kindling in two substrains of genetically epilepsy-prone rats. *Exp Neurol.* 2003;183(2):516–522.
52. Raisinghani M, Faingold CL. Neurons in the amygdala play an important role in the neuronal network mediating a clonic form of audiogenic seizures both before and after audiogenic kindling. *Brain Res.* 2005;1032(1–2):131–140.
53. Feng HJ, Faingold CL. Synaptic plasticity in the pathway from the medial geniculate body to the lateral amygdala is induced by seizure repetition. *Brain Res*. 2002;946:198–205.
54. Tupal S, Faingold CL. Precipitous induction of audiogenic kindling by activation of adenylyl cyclase in the amygdala. *Epilepsia*. 2010;51 (3):354–361.
55. Naritoku D, Randall ME, Faingold CL. Microinfusion of GABA agonists and 2-APH into the amygdala reduces seizure duration and clonus in repeated audiogenic seizures of the genetically epilepsy-prone rat. *Epilepsia*. 1989;30(5):698.
56. Tupal S, Faingold CL. Inhibition of adenylyl cyclase in amygdala blocks the effect of audiogenic seizure kindling in genetically epilepsy-prone rats. *Neuropharmacology.* 2010;59(1–2):107–111.
57. Tupal S, Faingold CL. Audiogenic kindling induces neuroplastic changes in the periaqueductal gray. *Brain Res.* 2010b2011;1377:60–66].
58. da Costa Gomez TM, Behbehani NM. An electrophysiological characterization of the projection from the central nucleus of the amygdala to the periaqueductal gray of the rat: the role of opioid receptors. *Brain Res*. 1995;689:21–31.
59. Faingold CL, Tupal S. Plasticity in the athway from amygdala to periaqueductal gray (PAG) is an important mechanism of epileptogenesis in audiogenic kindling (AK) *Am Epilepsy Soc Abs.* 2010;3.017.
60. Raisinghani M, Faingold CL. Evidence for the perirhinal cortex as a requisite component in the seizure network following seizure repetition in an inherited form of generalized clonic seizures. *Brain Res*. 2005:1048(1–2):193–201.
61. Faingold CL, Hoffmann WE, Caspary DM. Bicuculline-induced enhancement of sensory responses and cross-correlations between reticular formation and pericruciate cortex neurons. *Electroencephalogr Clin Neurophysiol*. 1983;55:301–313.
62. Bateman LM, Spitz M, Seyal M. Ictal hypoventilation contributes to cardiac arrhythmia and SUDEP: report on two deaths in video-EEG-monitored patients. *Epilepsia.* 2010;51 (5):916–920.
63. Faingold CL, Tupal S, Mhaskar Y, Uteshev VV. DBA mice as models of sudden unexpected death in epilepsy. In: Lathers C, Schraeder P, Bungo M, Leestma J, eds. *Sudden Death in Epilepsy: Forensic and Clinical Issues*. Boca Raton: Taylor and Francis; 2010:657–674.
64. Arvidsson UM, Riedl S, Chakrabarti JH, Lee AH, Nakano RJ, Dado HH, Loh PY, Law M, Wessendorf W, Elde R. Distribution and targeting of a mu-opioid receptor (MOR1) in brain and spinal cord. *J Neurosci*. 1995;15:3328–3341.
65. Samineni VJ, Premkumar LP, Faingold CL. Post-ictal analgesia in genetically epilepsy-prone rats involves cannabinoid receptors in the periaqueductal gray. *Brain Res.* 2011;1389:177–182.
66. Yeomans JS, Lee J, Yeomans MH, Steidl S, Li L. Midbrain pathways for prepulse inhibition and startle activation in rat. *Neuroscience.* 2006;142(4):921–929.

Chapter 21

On the Basic Mechanisms of Infantile Spasms

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The epileptic encephalopathies of early childhood are a challenge for both clinicians and basic scientists. For the clinician, these disorders are difficult to manage and most often respond inadequately to available therapies—particularly commonly used anticonvulsants. Indeed, disorders such as infantile spasms, Lennox-Gastaut syndrome, early infantile epileptic encephalopathy (EIEE or Otahara's syndrome), and severe myoclonic epilepsy of infancy (Dravet's syndrome) commonly lead to persistent drug-resistant seizures and very poor neurodevelopmental outcomes including intellectual disabilities. Thus, there has long been a need for research scientists to address the underlying cause of these disorders so that they can be understood and rational treatments can be developed based on an understanding of the basic mechanisms. Indeed, in 2007, the National Institute of Neurological Diseases and Stroke (NINDS) at the National Institutes of Health (NIH-USA) included the creation of animal models of these disorders in their Epilepsy Research Benchmarks to encourage study of their basic mechanisms (http://www.ninds.nih.gov/research/epilepsyweb/2007_benchmarks.htm).

INFANTILE SPASMS: THE CHALLENGES

From the perspective of basic scientists, numerous barriers have hampered the study of these catastrophic epilepsies of childhood. Of the early epileptic encephalopathies, infantile spasms is the most common and occurs in approximately 1 in every 3225 live births. Numerous reviews have described the clinical

features of this syndrome.[1–6] However, these clinical descriptions provide few direct clues as to how the seizures are produced. The spasms do not arise immediately at birth but usually begin between 3 and 12 months of age—with a peak incidence at 6 months. The spasms themselves almost always disappear with time but are replaced by other types of refractory epilepsy—such as focal seizures or Lennox-Gastaut syndrome. This observation has led to the notion that the spasms may be an age-dependent manifestation of an underlying abnormality and that, as the brain matures, the neuronal substrate for the seizures changes and the behavioral and electrographic manifestations of the abnormality change as well. As the name *infantile spasms* implies, the seizures that characterize this disorder are unusual and typically consist of very brief (a few seconds) bilaterally symmetric contractions of muscles of the extremities, neck, and/or trunk, which result in brief flexion and/or extension jerking movements—leading originally to the use of the term *spasm*. The spasms can vary in their intensity from jerking movements that are very dramatic to very subtle ones that at times are hardly noticeable. Within a child, the intensity of the jerks can also wax and wan in their intensity. While the spasms can occur as single isolated events, they are observed more commonly in clusters, which can number more than 100 over a few minutes. In addition, these seizures commonly occur during periods of sleep-wake transitions.

The electroencephalographic (EEG) correlates of the behavioral spasms are equally unique and distinguish this syndrome from all other forms of epilepsy. Similar to the accompanying behaviors, the ictal complex of spasms is only a few seconds in duration. Figure 21–1 shows an example of an ictal complex. Typically, it begins as a generalized slow wave that is followed by an electrodecremental response (temporary flattening of the EEG) and a subsequent run of fast spiking activity.[7] However, there are often variations on this theme in which each of the components of an ictal event can be absent. Such variations can occur between patients and can occur from time to time in the same patient.[7] Electroencephalographic recordings during the interictal period show equally unusual electrographic events. Indeed, a defining and diagnostic feature of infantile spasms is a neurophysiological event called *hypsarrhythmia*.[8] Figure 21–2 shows an example of this highly abnormal EEG activity. This electrographic pattern is most often recorded during non–rapid eye movement (non-REM) sleep and consists of a seemingly chaotic mixture of asynchronous very-high-voltage slow waves intermixed with frequent large multifocal spikes. Classically, hypsarrhythmia has been described as random high-voltage slow waves and spikes that occur asynchronously in all cortical areas and change from one instant to the next. However as with the ictal complexes, variations in this pattern have been described.[9]

To study the basic mechanisms of infantile spasms, a very significant challenge is reproducing the distinct clinical features of this syndrome—that is, brief behavioral flexor/extensor spasms—and the signature ictal complexes and hypsarrhythmia in experimental systems. However, since the features of the syndrome are unique, if researchers were able to reproduce the features of infantile spasms in the laboratory, this would provide significant opportunities for advances in the understanding of its pathophysiology.

Another challenge in approaching infantile spasms is its highly variable etiology. Unlike temporal lobe epilepsy, in which status epilepticus is thought to be a primary cause, and which can be modeled in animals both in vivo and in experimentally simpler in vitro models, infantile spasms are associated with a very large number (over 200) of clinically distinct conditions.[3,10,11] Approximately 80% of patients are classified as having symptomatic infantile spasms, since they have a clearly identified underlying cause or are developmentally delayed. The remaining 20% have no identifiable cause and are referred to as *cryptogenic.* However, with advances in genetic, imaging, and other diagnostic testing, this percentage is likely to decrease in the coming years. Some of the broad categories associated with symptomatic infantile spasms are (1) central nervous system (CNS) infections such as meningitis, (2) developmental brain abnormalities or malformations such as cortical dysplasia, (3) hypoxic-ischemic encephalopathy, and (4) genetic syndromes such as Down syndrome and tuberous sclerosis complex. In terms of genetic causes, numerous single-gene mutations, deletions, or duplications have been linked to infantile spasms. These genes include *Arx*, *CDKL5*, and *MAG12*.[12–14] Interestingly, these have quite different functions in the

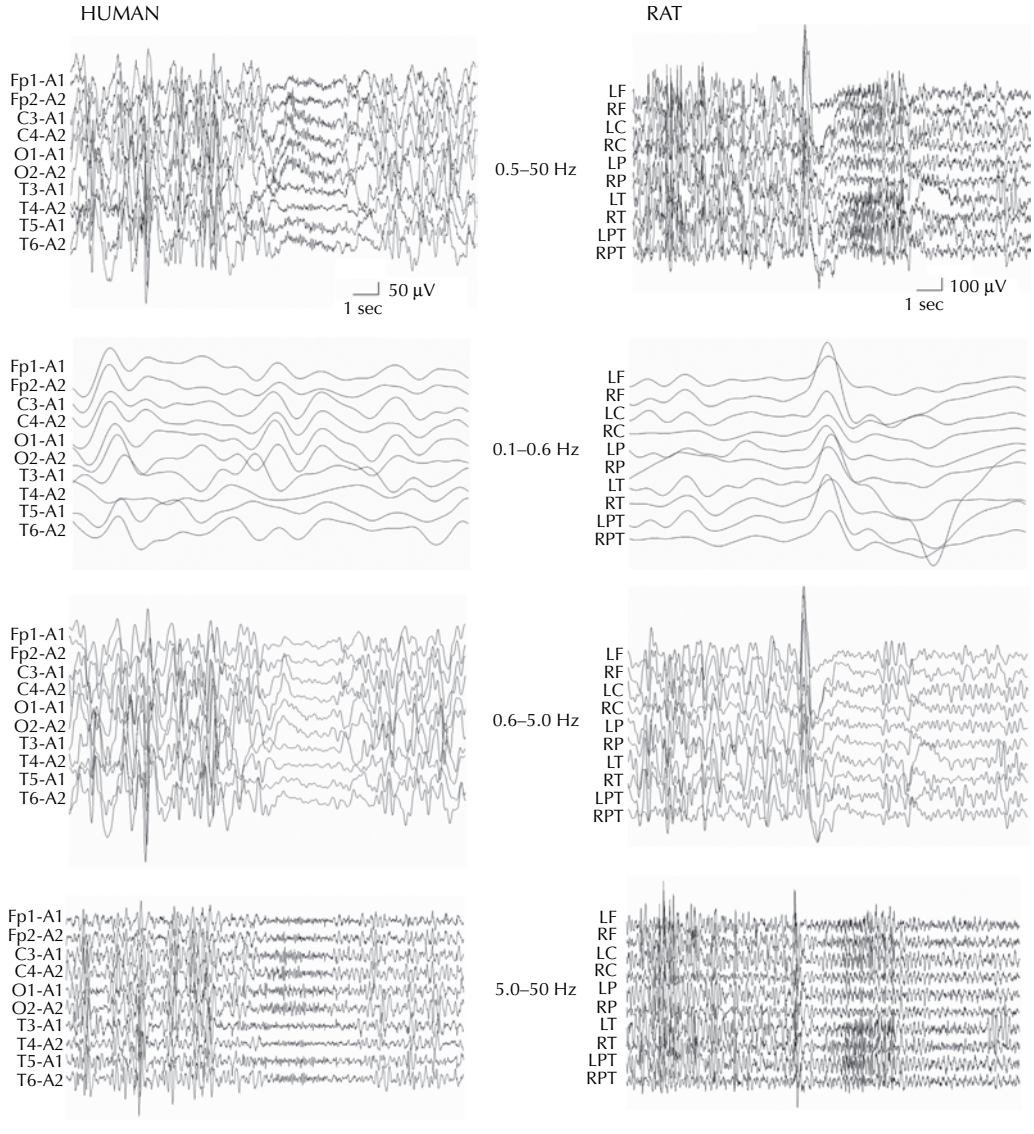

Figure 21–1. Ictal EEG discharges recorded in a human infant with infantile spasms and a rat that was infused in neocortex with tetrodotoxin (TTX). Infusion was initiated in infancy and persisted for 28 days. The top two panels show the original recordings. In the lower panels, selective bandpass filters (0.1–0.6 Hz, 0.6–5 Hz, and 5–50 Hz) were applied. In the recordings from the human, the ictal event consisted primarily of an electrodecremental response. For the rat, the ictal event consisted of an initial slow wave followed by an electrodecrement with superimposed fast activity. The electrodecremental response is clearly evident at frequencies of 0.6–5.0 Hz in both the human and the rat. The high-frequency fast activity predominates at frequencies of 5–50 Hz. The montage for the rat had electrodes positioned in: LF, RF—left and right frontal cortex; LC, RC—left and right central cortex; LP, RP—left and right posterior cortex; LT, RT—left and right temporal cortex; LPT, RPT—left and right posterior temporal cortex. The montage for the human corresponds to the International System of electrode placement. From ref. 60.

brain, which further amplifies the highly variable origins of infantile spasms.

The variable etiology of infantile spasms has led to speculation on the existence of a common mechanism(s) shared by all patients even though the associated and presumed causative conditions are so different.[3,15–18] Undoubtedly, it is quite remarkable that conditions as different as Down syndrome and meningitis can result in a seizure disorder with the same unique

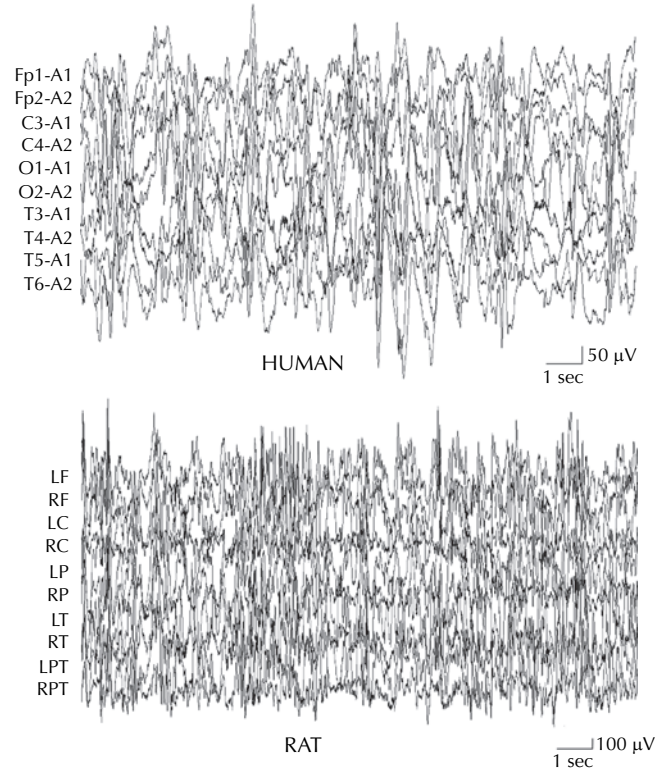

Figure 21–2. Comparison of interictal hypsarrhythmic patterns recorded in a human with infantile spasms and a rat whose neocortex was infused with TTX. The montages for the rat and human are the same as in Fig. 21–1. From ref. 60.

stereotyped clinical and electrographic features. How could a hypoxic-ischemic episode at birth, a mutation of a single gene such as *TSC1*, or a tumor all produce hypsarrhythmia and the ictal events of infantile spasms? Given that the product of more than 200 highly varied clinical conditions is the same (i.e., spasms), there must be a common final path that all these conditions converge on that produces this seizure disorder. There have been several suggestions as to what the shared mechanism might be, and these will be discussed later in this chapter in concert with potential new animal models of infantile spasms.

Another challenge in understanding the origins of infantile spasms is identifying where in the brain these seizures are generated. The EEG abnormalities are recognized as being generalized events. However, similar to the generalized spike-and-wave discharges of absence seizures, in which thalamic nuclei are thought to play key synchronizing roles, if researchers could identify the site(s) of spasm generation, this would greatly accelerate the discovery of the underlying mechanisms. Based on clinical observations, a general consensus has emerged in recent years that spasms likely result from abnormal interactions between cortical and brainstem neural networks.[5,10,16,19] Observations favoring a cortical contribution are that some children with infantile spasms have cortical malformations or injuries and that seizures in children can be eliminated by removing cortical abnormalities during epilepsy surgery. Moreover, positron emission tomography (PET) imaging studies have reported regional hypometabolism in the cortex of a majority of children with this disorder.[20–22] Hypsarrhythmia, since it is generalized across cortex, also suggests significant abnormalities there. On the other hand, altered brainstem function is suggested by the occurrence of abnormal sleep patterns and the common occurrence of spasms during sleep-wake transitions.[23] However, it is obvious even from this brief summary that there is a paucity

of data directly implicating specific neural networks of the brainstem or cortex in seizure generation.

Finally, potential clues to the mechanisms underlying infantile spasms come from its unique profile of pharmacological responsiveness. As mentioned earlier, spasms in infants are usually unresponsive to conventional anticonvulsants. However, in 1958, spasms were reported to respond to treatment with adrenocorticotropic hormone (ACTH).[24] The rationale behind the initiation of this therapeutic strategy is unclear. However, since that time, a number of clinical trials have been completed and it has been shown that, while not all children respond to ACTH, between 42% and 87% (depending on the study) of patients do respond by achieving complete cessation of clinical spasms (both behavioral and EEG) and elimination of hypsarrhythmia on prolonged EEG recordings.[25–28] The effects of ACTH are considered to be all-or-none and are in this respect unique in epilepsy therapeutics. Many have considered the complete elimination of hypsarrhythmia to be critical in order to prevent the continued progression of the disorder to other forms of epilepsy and impairments in cognition. Thus, an all-or-none effect has been considered an important endpoint in clinical trails—at least in the United States.

The site and the mechanism of action of ACTH remain an enigma. In fact, the blood-brain barrier is thought to be quite impermeable to ACTH, so its action in the CNS has been questioned. However, high doses of ACTH are often used therapeutically, and some think that enough ACTH gets into the CNS to be effective.[27] Alternatively, ACTH could be acting on the adrenal to stimulate the release of steroids, which in turn could act on the brain to suppress spasms. There is evidence that steroids can be effective in suppressing spasms, but in a head-to- head trial, prednisone was found to be less effective than high-dose ACTH.[27] This has led some to conclude that ACTH may be acting centrally to suppress seizures. Yet, questions linger since prednisone or prednisolone is effective in some patients, as the United Kingdom Infantile Spasms Study (UKISS) has emphasized.[28,29] Currently in the United States, ACTH therapy is considered "probably effective" in the treatment of infantile spasms by a joint commission of the American Academy of Neurology and the Child Neurology Society (AAN/CNS).[30] Nonetheless, it is clear that current therapies are not ideal. ACTH, the recommended treatment, can have significant side effects in some patients, especially when high doses are used.

It also remains a possibility that other treatments will be effective for subtypes of spasms, depending on their etiology. For instance, vigabatrin appears to be quite effective in suppressing spasms in children with tuberous sclerosis complex (TSC); it has been used in Europe for well over a decade and was approved in the United States in 2009 for this purpose.[31] However, it appears to be less effective in patients with spasms of other etiologies. In this regard, results of a UKISS study suggest that vigabatrin may be less effective than steroids.[28] Much interest has been engendered by a recent report suggesting that treatment of TSC patients with vigabatrin before the onset of seizures can be preventive.[32]

In this regard, some reports have emphasized the importance of treating children not necessarily before but as soon as possible after the onset of spasms, since results suggest that early treatment can lead to better long-term outcomes, including a reduction in the proportion of patients with epilepsy and impaired cognition.[33,34] It is thought that later treatment may still suppress spasms, but it may not be as effective in ameliorating their long-term sequelae. While these ideas remain controversial, they are in keeping with the notion that infantile spasms is a progressive disorder—akin to some other forms of epilepsy—and that the spasms themselves have consequences for normal brain development. However, it is important to note that even if the spasms are controlled by currently used therapies, many infants will still develop epilepsy and/or cognitive deficits, including autistic features, regardless of the etiology of the condition (with the caveat that cryptogenic cases, i.e., those without a clear pathology, usually have better outcomes). Whether this is because the child was treated too late in the course of the syndrome or because the therapies were simply ineffective in blunting the progression of this disorder is unclear. However, until very recently, the lack of animal models of infantile spasms has prevented a detailed examination of whether the syndrome can be progressive and the possible mechanisms underlying epileptogenesis.

INFANTILE SPASMS: THE QUESTIONS

Based on this review of some of the challenges associated with treating and understanding the pathophysiology of infantile spasms, there are many questions that need to be answered if we are to begin to understand the cellular and molecular mechanisms responsible for this seizure disorder and develop new and hopefully rational therapies for these children. The following list summarizes some of the questions.

1. How can one interpret the natural course of this seizure disorder in terms of its underlying mechanisms? Why do spasms occur in clusters during infancy? Is the disorder progressive? And if so, what are the mechanisms underlying the dramatic changes that occur over time? Are there underlying mechanisms of epileptogenesis? Or is the precipitating mechanism(s) unchanging and the alterations (e.g., in seizure phenotype, from infantile spasms to Lennox-Gastaut syndrome or subsequent focal seizures) that occur with time a reflection of a relatively static mechanism(s) interacting with the ongoing development of the brain, its neurons, and maturing networks? Or is it a combination of interactions between a progressive epileptogenesis and brain development?
2. If there is a shared final common path between the 200 conditions associated with the disorder and the products—the behavioral spasms, associated EEG abnormalities, and impaired cognition—what is it? If there are unique treatments for subtypes of infantile spasms (e.g., based on different etiologies: i.e., vigabatrin for TSC), does this mean that there are parallel paths for generating infantile spasms? Or, in this instance, is vigabatrin acting upstream before convergence on the final pathway?
3. Why do some but not all infants with the same underlying condition (e.g., perinatal asphyxia or TSC) develop spasms? Are there additional contributing factors? Is there a genetic predisposition?
4. Where in the brain do the spasms originate? Do they arise from cortex and/or brainstem, as some suggest, or somewhere else? And if so, where in cortex and which brainstem nuclei are responsible? And importantly, what neuronal subtypes in each area not only participate in spasm generation but also initiate the seizures? For instance, are principal neurons the key participants and initiators, and do different subpopulations of interneurons (some possibly dysfunctional) contribute in unique ways to spasms or hypsarrhythmia?
5. What is the basic mechanism underlying hypsarrhythmia? Where is it generated, and which neuronal subtypes participate in its generation? Do hypsarrhythmia and the ictal complexes of spasms share common mechanisms or are they independent electrophysiological phenomenon? What is more detrimental for long-term outcomes, hypsarrhythmia or the spasms themselves?
6. Why do spasms wax and wane in their intensity? What does this say about the underlying mechanisms? How well does behavioral spasm intensity correlate with alterations in ictal complexes recorded simultaneously? If they correlate or not, can this provide clues to underlying mechanisms?
7. Why do children with infantile spasms become cognitively impaired—many severely so? Do the spasms themselves contribute in any way to cognitive decline? What is the role of hypsarrhythmia in these developmental disabilities?
8. What is the mechanism of action of ACTH therapy? And what can this tell us about the mechanisms responsible for spasm generation?
9. How will new therapies be developed not only to control the spasms but also to prevent the development of other epileptic phenotypes and improve the cognitive outcome? Will one drug type be sufficient or is a cocktail of drugs required to target different processes?
10. As many as 50% of children with infantile spasms are currently refractory to all available therapies—including ACTH. Does this suggest that there are different mechanisms for spasm generation in these children, and will they require different types of treatment?

INFANTILE SPASMS: THE OPPORTUNITIES

It has long been thought that an understanding of the basic mechanisms of infantile spasms would be accelerated by the creation of animal models that would permit a detailed examination of the underlying cellular and molecular causes. However, until recently, this has proved to be a difficult undertaking for several reasons. The rational approach for the creation of animal models would be to reproduce one of the clinical conditions most commonly associated with the disorder in immature laboratory animals with the hope that these animals would develop a disorder mimicking what is seen in humans. For example, there are numerous animal models of neonatal hypoxic-ischemic encephalopathy, which have been studied for many years, but there are no reports of these animals having a condition like infantile spasms—although some can develop seizures later in life.[35] Perhaps this result, although disappointing, may not be unanticipated since not every child who suffers a hypoxic-ischemic episode will develop infantile spasms. Perhaps a more considered approach would be to choose a disorder in which the frequency of spasms is very high. The genetic disorder TSC is a good example since the frequency of infantile spasms in these children can be as high as 40%–50%.[36] Considering the advances made in human and mouse molecular genetics over the past decade, creating mutant mouse models that reproduce genetic disorders in children has become commonplace. And indeed, several mouse models of TSC have been created. Yet, while some of these mice develop severe epilepsy, a condition similar to infantile spasms has never been reported despite extensive experimental investigations of these mice.[37,38] Such findings have led some investigators to speculate that perhaps the rodent brain is simply not sufficiently advanced in evolutionary terms to reproduce the highly stereotyped neurological abnormalities that characterize the more complex human infant brain. However, this does not appear to be the case because in the last few years, seven different laboratories have reported models of infantile spasms either in mice or in rats. Although these are very early days in the characterization and study of all of these animal models, there is every reason to hope that one or more of them will provide unparalleled opportunities to study the basic mechanisms of this disorder.

Before reviewing the features of the animal models, it is important to discuss the criteria a model needs to meet in order to be considered a valid model of this syndrome. Unfortunately, at this early stage in model characterization, there appears to be little consensus on what features of the human condition an animal model must possess for it to be consider a valid model. In 2006 a report from a NIH/NINDS workshop on animal models of pediatric epilepsy was published. Based on the discussions at the workshop by leaders in the field, a list of criteria for an "ideal" model of infantile spasms was developed.[39] These included:

1. Unprovoked spasms early in postnatal development that occur in clusters
2. EEG correlates of the ictal complex (including the electrodecremental response)
3. Abnormal interictal EEG—hypsarrhythmia
4. Response to clinically relevant treatments (e.g., ACTH and/or vigabatrine)
5. Behavioral/cognitive sequelae

In this regard, the use of the term "*ideal*" needs to be emphasized. In numerous reviews and discussions of models, it is often noted that it is unlikely that any one animal model will meet all of these criteria but that this does not prevent it from advancing our understanding of the mechanisms of infantile spasms.[40] Perhaps a more useful list needs to be agreed upon by the scientific community that includes the *minimal* criteria required to model this disorder. And if these criteria are not met, it would not be considered a model of infantile spasms. Of course, this would not mean that the model is not important in the study of the basic mechanisms of epilepsy—if the animals had other types of seizures—or in the study of the consequences of gene mutations in a genetically engineered mouse that mimics gene mutations associated with human spasms. It would just mean that the model would not be useful in answering questions like those listed earlier concerning this syndrome. Recently, one review mentioned minimum criteria consisting of (1) an abnormal EEG, (2) spontaneous seizures that begin in the postnatal period but not necessarily spasms, and (3) cognitive deficits.[41] Another review considered (1) seizures in infancy and (2) ACTH

responsiveness as minimal criteria.[42] Clearly, significant debate and discussion will be necessary to resolve such differences of opinion. However, ultimately, the utility of any animal model will depend on its ability to answer basic questions concerning the pathophysiological mechanisms of this disorder. It is hard to envision how an animal model would be useful in studying infantile spasms if it does not at least include behavioral spasms and the EEG correlate of ictal complexes observed clinically. It would also be preferable if the spasms were initiated in infancy. It may also be necessary to construct models of infantile spasms refractory to the current clinically used treatments in order to identify new approaches. In the reviews that follow, we will attempt to highlight the features of the proposed animal models and their advantages in terms of addressing pressing questions concerning the pathophysiology of this disorder.

INFANTILE SPASMS: PROPOSED ANIMAL MODELS

The Conditional Deletion of the Aristaless-related-homeobox Gene (*Arx*) Mouse Model

The X-linked *Arx* gene encodes a transcription factor that is likely involved in early cortical development.[43] Mutations of this gene can be associated with a variety of clinical phenotypes including mental retardation, epilepsy, and the infantile spasm syndrome, X-linked lissencephaly with abnormal genitalia (XLAG) [41]. *Arx* appears to be critical in neurodevelopmental processes such as progenitor cell proliferation and neuronal migration. Based on observations that patients with XLAG and mice with similar mutations of *Arc* have deficits in interneuron migration from the basal ganglion,[43,44] Kato and Dobyns hypothesized that infantile spasms—at least in XLAG—could be due to deficits in cortical interneuron function and thus coined the term *interneuronopathy* as the basis for infantile spasms in these patients.[18] Indeed, the loss of interneurons from cortex could be a mechanism that many conditions converge on to produce infantile spasm. Thus, interneuronopathies could be critical component of a final common path to the production of spasms.

To test the interneuronopathy hypothesis, Marsh and colleagues selectively eliminated *Arx* from interneurons arising from the basal ganglion by crossing mice carrying a floxed *Arx* allele with Dlx5/6^{CIG} mice.[45] Neuroanatomical studies have confirmed deficits in subpopulations of interneurons in the cortex. Importantly, EEG recordings have shown that male mice carrying this mutation have epilepsy. Hypsarrhythmia was not observed, However, spasm-like behaviors were observed in adulthood, and these were reported to be correlated with a spike and slow-wave complex followed by an electrodecrement on EEG recordings. Surprisingly, video-EEG recordings from younger mice did not show similar spasms but instead limbic seizures. There is no easy explanation for the late onset of spasm-like events in this animal model. Electroencephalographic recordings from infant mice can be challenging, and the authors were only able to record for 24 h in three mutant male mice. Perhaps more prolonged recordings would reveal the presence of earlier spasms in these mice. Nonetheless, the direct link between human genetic abnormalities and the creation of relevant mutant mice is a powerful approach. Discoveries made with this model and the development of experimental tools to study interneuron deficits could lead to testing of the interneuronopathy hypothesis in other animal models of infantile spasms. The development of new technologies that allow routine video-EEG recordings over extended periods from infant mice would accelerate progress in characterizing such mouse models.

The Triple Repeat Expansion Model of *Arx*

This mouse model is based on the discovered link between mutations in the *Arx* gene and infantile spasms reviewed above. Since knockout of *Arx* in mice results in perinatal lethality,[43,44] Price and colleagues used a knockin strategy to engineer a targeted expansion of the first polyalanine tract in *Arx*.[46] This is the mutation very commonly associated with infantile spasms in humans.[47] Multiple neurobehavioral phenotypes were discovered in the resultant mice. As anticipated, based on *Arx* expression in the precursors of neocortical interneurons, the loss of subpopulations of interneurons was

observed. Behavioral spasms were observed in both control and mutant mice between postnatal days 7 and 11. Two types were described: low- and high-amplitude spasm-like movements. There were no differences between the frequency of the low-amplitude myoclonic events in mutant mice compared to controls. However, there were twice as many high-amplitude movements in the mutant mice. While EEG recordings on postnatal days 7 –11 were not possible, it was reported that EEG recordings on postnatal days 16–20 showed a high-amplitude slow wave followed by attenuation of the background EEG activity concurrent with brief myoclonic jerks. In older mice (24 to 70 days of age), spasms were not observed; instead, limbic motor seizures as well as 6s spike wave bursts—accompanied by behavioral arrest—were seen. Hypsarrhythmia was not reported in this mouse model. However, cognitive impairment and abnormal social interactions suggestive of an autism phenotype were observed.

Thus, this animal model has several desirable features for a model of infantile spasms. Again, the lack of quality EEG recordings from infant mice are a significant barrier to a full description of the spasm phenotype in infant mice. It would be interesting to know what the EEG correlates are of the high-amplitude spasm-like movements in control mice. Study of this mouse model in concert with the *Arx* conditional knockout described above should lead to a better understanding of the role interneuron loss may play in the pathophysiology of seizures and, in particular, infantile spasms. Study of the origins of the neurobehavioral abnormalities in these mice could address the roles of interneuron loss and/or seizures in these cognitive and autistic phenotypes that are often seen in patients with a history of infantile spasms.

The NMDA Model

Intraperitoneal injections of *N*-methyl-D-aspartate (NMDA), a glutamate receptor agonist, in rats on postnatal days 12 to 18 produce hyperflexion and tonic spasms of the body.[48,49] These have been reported to be concurrent with a slow wave and a subsequent electrodecrement on EEG recordings from cortex, although other neurophysiological discharges can occur.[50] Recently, this model has been modified by prenatal treatment with betamethasone on gestational day 15.[50] Although the rationale underlying this experimental strategy has not been fully articulated, it appears that it is an attempt to mimic prenatal stress. This treatment sensitized rats to NMDA since they responded at lower doses of the drug. In addition, rats became responsive to ACTH in that the latency to onset of NMDA-induced spasms was increased. Since the seizures were not blocked, this is not fully in keeping with the all-or-none effects of ACTH seen clinically. Additionally, ACTH usually takes days to become effective in children and commonly does not act immediately, as reported for this model. The drawback of this model is that the spasms are evoked by NMDA and do not occur spontaneously. However, as with other models described below, it could be useful in rapidly screening drugs for efficacy against spasms—assuming that the scientific community as a whole concludes that the spasms evoked by NMDA are relevant to those that occur clinically.

The Down Syndrome–Ts65Dn Mouse Model

The incidence of epilepsy in Down syndrome is significant; approximately 8% of patients have epilepsy and a third of these have infantile spasms. The Ts65Dn mouse, which is segmentally trisomic for the distal end of chromosome 16, is an accepted model of Down syndrome.[51] While spontaneous seizures have never been reported in these mice (with the caveat that audiogenic seizures can be triggered in these animals by intense noise), these mice have increased gamma-aminobutyric acid B ($GABA_B$) receptor-mediated currents, which likely result from overexpression of the GIRK2 channel due to the extra copy of this gene on chromosome 16. Since $GABA_B$ receptor agonists have convulsant properties in rodents, Cortez and colleagues thought that it is possible that treatment with these drugs might induce seizures and possibly even spasms in this mouse model.[52] Results showed that such treatments produced absence seizures in control mice but clusters of extensor spasms in the Ts65Dn mouse. Each spasm in a cluster appeared to be associated with burst discharges on EEG recordings that were separated by a marked attenuation in cortical

activity. This type of activity was observed from 1 week to 2 months postnatally. Mice were also used to screen the effectiveness of a number of anticonvulsants. Valproic acid, ACTH, ethosuximide, and vigabatrin were all reported to be effective in suppressing spasms. Like the clinically reported effectiveness of vigabatrin in TSC patients, results from this mouse model suggest that infantile spasms in Down syndrome patients may have a unique pharmacological responsiveness—which could include more conventional anticonvulsants. However, it would be important to know if the enhanced $GABA_B$ receptor function also occurs in Down syndrome patients. The advantage of this mouse model is its ease of use in drug screening. A drawback is that seizures are not spontaneous and are evoked by drug treatment. The consequences of the spasms in terms of epilepsy or neurobehavioral deficits have not yet been reported.

The CRH Hypothesis

Since ACTH is effective in suppressing infantile spasms, Baram and colleagues have pursued the molecular mechanisms by which it acts.[53] It is known from neuroendocrinology studies that systemic ACTH treatment can act through classic negative feedback systems to downregulate the expression of corticotropin releasing hormone (CRH) in the CNS.[54] This can be mediated in part by steroids that are released from the adrenal or by ACTH itself. Importantly, CRH has been shown to be a potent convulsant drug and is particularly effective in infant rats.[55] Thus, a reasonable hypothesis has been proposed that ACTH acts in infantile spasms to suppress CRH expression and thereby decrease neuronal excitability. This hypothesis has been extended to suggest that prenatal or perinatal stress may be a common factor in the etiology of infantile spasms and may be an important part of a final common path that leads to the generation of spasms.[17] It is likely that many infants with spasms have undergone some form of stress earlier in their lives. Stress is thought to increase CRH levels in the brain, and ACTH treatment may act to normalize CRH levels. While investigators continue to actively pursue these ideas, there are several shortcomings that limit enthusiasm for them at this time. It is well known that CRH produces severe limbic motor seizures in infant rats and has never been reported to produce spasm-like behaviors. Moreover, EEG recordings during CRH-induced seizures show rhythmic sharp activity consistent with seizures arising from the limbic system.[56] Neither ictal complexes nor hypsarrhythmia have been reported in recordings from CRH-treated mice. Thus, despite the strength of the rationale underlying the CRH/stress hypothesis, the link between infantile spasms and CRH remains strictly hypothetical at this time. However, it is possible that treatment of the brain with exogenous CRH is unable to mimic the effects of its release locally from neurons. And if a more physiological condition could be produced in which the effects of endogenous CRH were evoked, spasm-type seizures and EEG correlates might be seen.

The Multiple-Hit Model

Based on the notion reviewed earlier that infantile spasms results from abnormal interactions between cortical and subcortical networks—particularly those in the brainstem—Scantlebury and colleagues developed a multiple-hit model of infantile spasms.[57] In this model, investigators have simultaneously produced pathological abnormalities in cortical and subcortical brain areas of infant rats. To accomplish this on postnatal day 3, doxorubicin is injected intraventricularly and lipopolysaccharide is injected into the right cerebral cortex. Doxorubicin is an anthracycline chemotherapeutic agent that injures and kills neurons through oxidative stress, while lipopolysaccharide is a toxin released from gram-negative bacteria that is able to damage white matter and activate inflammatory cells. Two days after these treatments, rats are injected intraperiotoneally with *p*-chlorophenylalanine to deplete serotonin in the brain by blocking the synthesizing enzyme, tryptophan hydroxylase. This is used in an attempt to increase neuronal excitability. Rats display clusters of flexor or extensor spasms from postnatal day 4 to postnatal day 13, and other seizure types begin to emerge after postnatal day 9 that resemble limbic motor seizures. The EEG correlates of spasms are complex. Similar to humans, spasms have electrographic correlates in only 49% of events. Electrodecremental responses (i.e., attenuation of background activity) were

observed in 27% of spasms that had electrographic correlates. The remaining 73% of spasms with electrographic correlates coincided with spike and sharp-wave discharges and/or fast rhythmic activity. The interictal EEG was also abnormal and consisted of high-amplitude spikes and slow waves. Treatment with ACTH was found to be ineffective in this model, and vigabatrine suppressed seizures but only transiently on postnatal day 5. The rats also displayed numerous neurobehavioral deficits including learning and memory deficits, stereotypes, and impaired socialization suggesting a possible autism-like phenotype. As would be expected from the treatments used, neuroanatomical examination of the brains of these rats showed marked neuropathologies including a reduction in the thickness of the right cortex and diffuse damage to the corpus callosum, striatum, hippocampus, and thalamus, among other structures. The advantage of this model is that it may reproduce the drug-resistant symptomatic refractory spasms that are encountered clinically and therefore could be useful in screening for new treatments for this patient population.

The TTX Model of Focal Neocortical Inactivation

In contrast to many of the models of infantile spasms, the rationale behind using local infusion of tetrodotoxin (TTX) in the brain to induce infantile spasms in rat pups is not immediately apparent. Indeed, this experimental strategy was initially employed to examine the role of neuronal activity in postnatal development of the hippocampus, not to develop a model of childhood epilepsy.[58,59] Unexpectedly, however, rats treated in this way developed very brief seizures, and later it was discovered that these seizures closely resembled infantile spasms.[60] In this model, TTX (10 μM) is chronically (for 28 days) infused by an osmotic minipump into either the hippocampus or the neocortex of rats beginning on postnatal days 10–12. Identical infusions a few days later do not produce spasms or seizures. 2-Deoxyglucose studies showed that the treatment produces a region of neuronal inactivation approximately 2.5 cm in diameter centered on the tip of the infusion cannula. Thus, this treatment may mimic the regional neocortical hypometabolism reported from PET studies of patients with infantile spasms.[20–22] Initial behavioral observations showed that seizures begin sometime before postnatal day 21 and consist of brief (1–2 s) flexions or extensions of the trunk and/or forelimbs. Multichannel video-EEG recordings show that behavioral spasms are concurrent with a generalized ictal complex that is virtually identical to that seen in humans. Figure 21–1 compares an ictal complex from a rat to that recorded from a patient. The complex begins with a large slow wave followed by an electrodecremental response on which runs of spikes are subsequently superimposed. The spasms frequently occur in clusters of as many as 50 spasms, and most often occur during sleep-wake transitions. Interictally, patterns of hypsarrhythmia are recorded in the majority of animals having spasms, and these have a frequency spectrum very similar to that recorded in human infants. Figure 21–2 shows an example of hypsarrhythmia from a rat in comparison to that from a human. The spasms usually persist for 2 months, but eventually rats develop severe limbic motor seizures by 6 months of age. The pharmacological responsiveness to ACTH and vigabatrin has not been evaluated, nor have learning and memory deficits been fully characterized in these animals. The advantage of this model is the robust nature of the EEG recordings and how closely they mimic those of humans. Thus, the model could be exploited to study the cellular neurophysiology of infantile spasms. Indeed, a recent study using high digital sampling rates followed by compressed spectral array analysis and bandpass filtering showed that high-frequency EEG activity occurs throughout ictal events in these animals and prominently during the electrodecrement that is recorded using conventional EEG filter settings.[61] Studies are needed to determine the age of onset of spasms in the animals and to characterize their evolution to the condition seen on postnatal day 21—a process possibly akin to epileptogenesis.

Recently, Frost and Hrachovy[3] proposed the developmental desynchronization model for infantile spasms. Like the interneuropathy and CRH/stress models discussed above, this model is an attempt to describe a common pathophysiological mechanism that could result from the myriad etiologies associated with this disorder. Put simply, this model suggests that infantile spasms result from a

temporal desynchronization of two or more ongoing neurodevelopmental processes. The desynchronization could be caused by injury to the brain or a mutation in a gene important for neuronal development, among the many other etiologies for this disorder. At the systems, cellular, and molecular levels, normal brain development is thought to be dependent on reinforcing interactions between networks of cells and networks of molecules. It is hypothesized that when one developing system is delayed or accelerated relative to its maturational partners, a developmental desychronization results that produces infantile spasms. Features of the TTX model are consistent with the concept of developmental desynchronization since it is well known that neuronal activity plays an important role in the normal course of neuron and network maturation.[62] By suppressing neuronal activity with TTX in a portion of the neocortex, one would expect that the maturation of neurons in that region would be altered and possibly delayed compared to their synaptic partners at distant sites. Thus, the development of TTX-exposed cells should be out of sync with that of unexposed cells that continue developing at a normal pace. At this time, it is unclear how such maturational desynchronization could lead to infantile spasms, but different types of desynchonization resulting from different etiologies would have to converge on a *critical system* that would initiate the pathophysiological mechanisms in a shared or common pathway leading to spasms in children. Validated animal models could be important tools used to support or refute such theoretical models of pathogenesis and should help identify hypothesized critical systems and common pathways—if they exist.

CONCLUSIONS

We are at a very early stage in studying the basic mechanisms of infantile spasms. However, features of several promising animal models have been described, and more may be forthcoming in the near future. Technical challenges are substantial in studying seizures in infant rodents, but they are not insurmountable. The basic science and clinical communities need to evaluate each of the proposed animal models and develop minimal criteria for what they consider to be a valid animal model of this disorder. Once validated, models should provide an unprecedented opportunity to study the mechanisms responsible for this catastrophic epilepsy and hopefully provide new avenues for effective therapies.

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REFERENCES

1. Riikonen R. Epidemiological data of West syndrome in Finland. *Brain Dev*. 2001;23:539–541.
2. Hrachovy RA. West's syndrome (infantile spasms). Clinical description and diagnosis. *Adv Exp Med Biol*. 2002;497:33–50.
3. Frost JD Jr, Hrachovy RA. Pathogenesis of infantile spasms: a model based on developmental desynchronization. *J Clin Neurophysiol*. 2005;22:25–36.
4. Shields WD. Infantile spasms: little seizures, BIG consequences. *Epilepsy Curr*. 2006;6:63–69.
5. Dulac O, Soufflet C, Chiron C. et al. What is West syndrome? *Int Rev Neurobiol*. 2002;49:1–22.
6. Lacy JR, Penry JK. *Infantile Spasms*. New York: Raven Press; 1976.
7. Kellaway P, Hrachovy RA, Frost JD Jr, et al. Precise characterization and quantification of infantile spasms. *Ann Neurol*. 1979;6:214–218.
8. Gibbs EL, Fleming MM, Gibbs FA. Diagnosis and prognosis of hypsarrhythmia and infantile spasms. *Pediatrics*. 1954;13:66–73.
9. Hrachovy RA, Frost JD Jr, Kellaway P. Hypsarrhythmia: variations on the theme. *Epilepsia*. 1984;25:317–325.
10. Frost JD Jr, Hrachovy RA. *Infantile Spasms*. Boston: Kluber Academic Publishers; 2003.
11. Baram TZ. Pathophysiology of massive infantile spasms: perspective on the putative role of the brain adrenal axis. *Ann Neurol*. 1993;33:231–236.
12. Kitamura K, Yanazawa M, Sugiyama N, et al. Mutation of *ARX* causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with

abnormal genitalia in humans. *Nat Genet*. 2002;32: 359–369.
13. Weaving LS, Christodoulou J, Williamson SL, et al. Mutations of *CDKL5* cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. *Am J Hum Genet*. 2004;75:1079–109
14. Marshall CR, Young EJ, Pani AM, et al. Infantile spasms is associated with deletion of the *MAGI2* gene on chromosome 7q11.23-q21.11. *Am J Hum Genet.* 2008;83:106–111.
15. Dulac O, Chiron C, Robain O, et al. Infantile spasms: a pathophysiological hypothesis. *Semin Pediatr Neurol*. 1994;1:83–89.
16. Lado FA, Moshe SL. Role of subcortical structures in the pathogenesis of infantile spasms: what are possible subcortical mediators? *Int Rev Neurobiol*. 2002;49:115–140.
17. Baram TZ. Models for infantile spasms: an arduous journey to the Holy Grail. *Ann Neurol*. 2007;61:89–91.
18. Kato M, Dobyns WB. X-linked lissencephaly with abnormal genitalia as a tangential migration disorder causing intractable epilepsy: proposal for a new term, "interneuronopathy." *J Child Neurol*. 2005;20: 392–397
19. Chugani HT, Shewmon DA, Sankar R, et al. Infantile spasms: II. Lenticular nuclei and brain stem activation on positron emission tomography. *Ann Neurol*. 1992;31:212–219.
20. Chugani HT, Shields WD, Shewmon DA, et al. Infantile spasms: I. PET identifies focal cortical dysgenesis in cryptogenic cases for surgical treatment. *Ann Neurol*. 1990;27:406–413.
21. Metsahonkala L, Gaily E, Rantala H. et al. Focal and global cortical hypometabolism in patients with newly diagnosed infantile spasms. *Neurology*. 2002;58: 1646–1651.
22. Itomi K, Okumura A, Negoro T, et al. Prognostic value of positron emission tomography in cryptogenic West syndrome. *Dev Med Child Neurol*. 2002;44:107–111.
23. Hrachovy RA, Frost JD Jr, Kellaway P. Sleep characteristics in infantile spasms. *Neurology*. 1981;31: 688–693.
24. Sorel, L, Dusaucy-Bauloye, A. Findings in 21 cases of Gibbs' hypsarrhythmia; spectacular effectiveness of ACTH. *Acta Neurol Psychiatr Belg*. 1958;58:130–141.
25. Hrachovy RA, Frost JD Jr, Kellaway P, et al. Double-blind study of ACTH vs. prednisone therapy in infantile spasms. *J Pediatr*. 1983;103:641–645.
26. Hrachovy RA, Frost JD Jr, Glaze DG. High-dose, long-duration versus low-dose, short-duration corticotropin therapy for infantile spasms. *J Pediatr*. 1994;124: 803–806.
27. Baram TZ, Mitchell WG, Tournay A et al. High-dose corticotropin (ACTH) versus prednisone for infantile spasms: a prospective, randomized, blinded study. *Pediatrics*. 1996;97:375–379.
28. Lux AL, Edwards SW, Hancock E, et al. The United Kingdom Infantile Spasms Study comparing vigabatrin with prednisolone or tetracosactide at 14 days: a multicentre, randomised controlled trial. *Lancet.* 2004;364: 1773–1778.
29. Kossoff EH, Hartman AL, Rubenstein JE, et al. High-dose oral prednisolone for infantile spasms: an effective and less expensive alternative to ACTH. *Epilepsy Behav*. 2009;14:674–676.
30. Mackay MT, Weiss SK, Adams-Webber T, et al. Practice parameter: medical treatment of infantile spasms: report of the American Academy of Neurology and the Child Neurology Society. *Neurology*. 2004;62: 1668–1681.
31. Willmore LJ, Abelson MB, Ben-Menachem E, et al. Vigabatrin: 2008 update. *Epilepsia.* 2009;50:163–173.
32. Jozwiak S, Kotulska K, Domanska-Pakiela D, et al. Antiepileptic treatment before the onset of seizures reduces epilepsy severity and risk of mental retardation in infants with tuberous sclerosis complex. *Eur J Paediatr Neurol*. 2011. [Epub ahead of print.]
33. Lombroso CT. A prospective study of infantile spasms: clinical and therapeutic correlations. *Epilepsia*. 1983;24:135–158.
34. Kivity S, Lerman P, Ariel R, et al. Long-term cognitive outcomes of a cohort of children with cryptogenic infantile spasms treated with high-dose adrenocorticotropic hormone. *Epilepsia*. 2004;45:255–262.
35. Kadam SD, White AM, Staley KJ, et al. Continuous electroencephalographic monitoring with radiotelemetry in a rat model of perinatal hypoxia-ischemia reveals progressive post-stroke epilepsy. *J Neurosci*. 2010;30:404–415.
36. Chu-Shore CJ, Major P, Camposano S, et al. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia*. 2010;51:1236–1241.
37. Zeng LH, Xu L, Gutmann DH, et al. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol*. 2008;63:444–453.
38. Meikle L, Pollizzi K, Egnor A, et al. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci*. 2008;28:5422–5432.
39. Stafstrom CE, Moshe SL, Swann JW, et al. Models of pediatric epilepsies: strategies and opportunities. *Epilepsia*. 2006;47:1407–1414.
40. Stafstrom CE. Infantile spasms: a critical review of emerging animal models. *Epilepsy Curr*. 2009;9: 75–81.
41. Marsh ED, Golden JA. Developing an animal model for infantile spasms: pathogenesis, problems and progress. *Dis Model Mech*. 2009;2:329–335.
42. Stafstrom CE, Holmes GL. Infantile spasms: criteria for an animal model. *Int Rev Neurobiol*. 2002;49: 391–411.
43. Kitamura K, Yanazawa M, Sugiyama N, et al. Mutation of *ARX* causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet*. 2002;32: 359–369.
44. Colombo E, Collombat P, Colasante G, et al. Inactivation of *Arx*, the murine ortholog of the X-linked lissencephaly with ambiguous genitalia gene, leads to severe disorganization of the ventral telencephalon with impaired neuronal migration and differentiation. *J Neurosci*. 2007;27:4786–479
45. Marsh E, Fulp C, Gomez E, et al. Targeted loss of *Arx* results in a developmental epilepsy mouse model and recapitulates the human phenotype in heterozygous females. *Brain*. 2009;132:1563–1576.
46. Price MG, Yoo JW, Burgess DL, et al. A triplet repeat expansion genetic mouse model of infantile spasms syndrome, *Arx*(GCG)10+7, with interneuronopathy,

spasms in infancy, persistent seizures, and adult cognitive and behavioral impairment. *J Neurosci*. 2009;29:8752–8763.
47. Poirier K, Eisermann M, Caubel I, et al. Combination of infantile spasms, non-epileptic seizures and complex movement disorder: a new case of *ARX*-related epilepsy. *Epilepsy Res.* 2008;80:224–228.
48. Mares P, Velisek L. *N*-methyl-D-aspartate (NMDA)-induced seizures in developing rats. *Brain Res Dev Brain Res*. 1992;65:185–189.
49. Kabova R, Liptakova S, Slamberova R, et al. Age-specific *N*-methyl-D-aspartate-induced seizures: perspectives for the West syndrome model. *Epilepsia*. 1999;40:1357–1369.
50. Velisek L, Jehle K, Asche S, et al. Model of infantile spasms induced by *N*-methyl-D-aspartic acid in prenatally impaired brain. *Ann Neurol*. 2007;61:109–119.
51. Salehi A, Faizi M, Belichenko PV, et al. Using mouse models to explore genotype-phenotype relationship in Down syndrome. *Ment Retard Dev Disabil Res Rev.* 2007;13:207–214.
52. Cortez MA, Shen L, Wu Y, et al. Infantile spasms and Down syndrome: a new animal model. *Pediatr Res.* 2009;65:499–503.
53. Brunson KL, vishai-Eliner S, Baram TZ. ACTH treatment of infantile spasms: mechanisms of its effects in modulation of neuronal excitability. *Int Rev Neurobiol*. 2002;49:185–197.
54. Brunson KL, Khan N, Eghbal-Ahmadi M, et al. Corticotropin (ACTH) acts directly on amygdala neurons to down-regulate corticotropin-releasing hormone gene expression. *Ann Neurol*. 2001;49:304–312.
55. Baram TZ, Hirsch E, Snead OC III, et al. Corticotropin-releasing hormone-induced seizures in infant rats originate in the amygdala. *Ann Neurol*. 1992;31:488–494.
56. Baram TZ, Schultz L. Corticotropin-releasing hormone is a rapid and potent convulsant in the infant rat. *Brain Res Dev Brain Res*. 1991;61:97–101.
57. Scantlebury MH, Galanopoulou AS, Chudomelova L, et al. A model of symptomatic infantile spasms syndrome. *Neurobiol Dis*. 2010;37:604–612.
58. Galvan CD, Hrachovy RA, Smith KL, et al. Blockade of neuronal activity during hippocampal development produces a chronic focal epilepsy in the rat. *J Neurosci*. 2000;20:2904–2916.
59. Galvan CD, Wenzel JH, Dineley KT, et al. Postsynaptic contributions to hippocampal network hyperexcitability induced by chronic activity blockade in vivo. *Eur J Neurosci*. 2003;18:1861–1872.
60. Lee CL, Frost JD Jr, Swann JW, et al. A new animal model of infantile spasms with unprovoked persistent seizures. *Epilepsia.* 2008;49:298–307.
61. Frost JD Jr, Lee CL, Hrachovy RA, et al. High frequency EEG activity associated with ictal events in an animal model of infantile spasms. *Epilepsia*. 2011;52:53–62.
62. Katz LC, Shatz CJ. Synaptic activity and the construction of cortical circuits. *Science*. 1996;274:1133–1138.

Chapter 22

Fast Oscillations and Synchronization Examined with In Vitro Models of Epileptogenesis

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For many years, interictal spikes, recorded in a patient's electroencephalogram (EEG), were considered to be a primary indicator that the patient might indeed have epilepsy and that the patient's seizures (when they occurred) were likely to begin in the vicinity of the interictal spike generator. That interictal spikes could often be recorded at the scalp, without the necessity of invasive recording procedures, contributed to their clinical usefulness. A question of continuing importance to basic epilepsy research was naturally this: what factors determine whether "epileptogenic tissue" will produce interictal spikes,[1] unequivocal seizure activity, or something intermediate?

It now appears, however, that very fast oscillations (VFO, >70–80 Hz), in electrocorticographic (ECoG) or depth electrode recordings, may have more localizing significance than do interictal spikes.[2–5] Furthermore, recent in vitro studies of human epileptogenic tissue indicate that VFO is the primary event during an interictal field transient—meaning that such field transients always contain VFO, possibly

riding upon slower fields produced by synaptic currents. Conversely, however, VFO can also occur alone, without the synaptic components; in contrast, the slower synaptic components do not occur without VFO[6] (see the later discussion). Numerous reports, as well, have documented that localized VFO (typically lasting for seconds) precedes electrographic seizures.[7–12] It remains to be determined whether such VFO causes the seizures or is rather the first manifestation of a pathological tissue state (awaiting more precise characterization) that is prone to generate both VFO and also seizure activity.

Unfortunately, VFO detection requires, in general, invasive recording techniques and is therefore not available for the clinical evaluation of most epilepsy patients. Nevertheless, understanding the pathogenesis of epilepsy clearly requires learning more about VFO mechanisms. In this chapter, we shall review relevant ultrastructural, in vitro slice, and computer modeling data. We shall also review evidence that proposed in vitro VFO mechanisms are applicable to the in situ brains of actual epilepsy patients. The gist of the chapter is that VFO requires the presence of sufficient gap junctional coupling between principal neurons and that VFO does not require chemical synapses—and indeed may even be facilitated by the blockade of chemical synapses.

DATA INDICATING THE EXISTENCE OF GAP JUNCTIONS BETWEEN PRINCIPAL CORTICAL NEURONS

Before considering very fast network oscillations, let us first discuss their proposed structural underpinnings: electrical coupling between the axons of principal neurons. This discussion is essential, especially given that the subject remains controversial. Our treatment here is compressed; the subject is analyzed in more depth in our recent monograph.[13] The issues are controversial because more data are required, because gap junction pharmacology is difficult, and perhaps because the ideas are novel.

That principal cortical neurons are electrically coupled was shown first, with dual intracellular recordings, by MacVicar and Dudek[14] and was recently confirmed by Mercer et al.[15] An essential issue, however, is to determine *what parts* of the neurons are coupled together: axons, somata, dendrites, or some combination. This issue is important, because the functional properties of gap junctional coupling—in particular, whether action potentials pass from one cell to another—strongly depend on the electrophysiological behavior of the membranes at, and close to, the site of coupling. There is no evidence (of which we are aware) for somatic coupling in mature tissue. There *is* evidence for axonal coupling of two basic types: structural and functional. The structural evidence can, in turn, be broken down into (1) dye coupling and (2) ultrastructure. It has long been known that pyramidal neurons can be dye-coupled and that alkaline pH increases the extent of coupling.[16,17] (The conductance of at least some sorts of gap junctions is pH-sensitive.) In addition, however, Schmitz et al.[18] demonstrated with confocal imaging of CA1 pyramidal neurons that it was "kissing" *axons* that allowed for the passage of fluorescent dye from one neuron to another (by light microscopic criteria). So far as ultrastructure goes, putative gap junctions between hippocampal mossy fibers have been shown by transmission electron microscopy, and a connexin36-containing gap junction has been visualized with freeze-fracture immunogold replica labeling (FRIL) on a mossy fiber axon (Fig. 22–1 and ref. 19).

So far as *functional* evidence goes, Schmitz et al.[18] demonstrated the existence of spikelets in CA1 pyramidal neurons, in media blocking synaptic transmission, and following stimulation of stratum oriens (which contains CA1 pyramidal cell axon collaterals). Such spikelets had a number of relevant properties: (1) they followed stimulation at hundreds of Hertz without failures; (2) they were voltage-dependent; (3) they were suppressed by intracellular acidification and by the gap junction blocker carbenoxolone (which, in control experiments, was shown not to alter intrinsic axonal membrane properties); and (4) they actually propagated along axons. The most parsimonious explanation of these findings, especially in view of the dye coupling, was that the spikelets represented decrementally conducted axonal action potentials induced in one neuron by electrical coupling with one or more axons of other neuron(s).

An alternative means of electrical coupling—dendrite to dendrite—is potentially available to

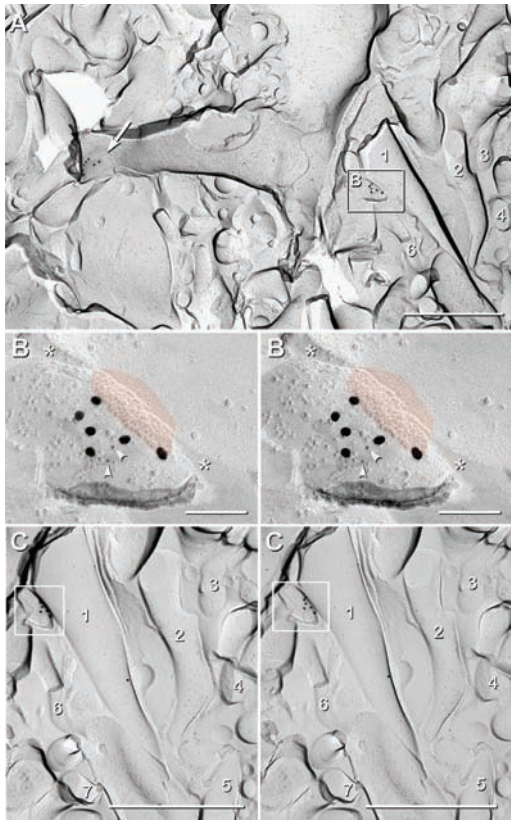

Figure 22–1. Freeze-fracture immunogold replica labeling image of a gap junction on a hippocampal mossy fiber axon (CA3c stratum lucidum, 150 g rat). **A.** Freeze-fracture replica showing two gap junctions labeled with two different gold bead-complexed antibodies identifying connexin36; the beads are 18 nm and 6 nm. The left gap junction (arrow) lies on a dendrite. The right one (box B) lies on mossy fiber axon 1 (other axons in the image are also numbered). Scale bar, 1 μm. **B.** Dual stereoscopic images of the gap junction in box B above. Arrowheads mark the 6 nm gold beads. The gap junction plaque, containing about 100 connexons, has been shaded in red. Asterisks mark the extracellular space, which is ~3 nm at the gap junction site. Scale bar, 100 nm. **C.** Stereoscopic view with the replica tilted. Scale bar, 1 μm. From ref. 19.

CA3 pyramidal neurons. It turns out that in the rat (but not necessarily in the mouse), at least some mossy fiber synapses are mixed, that is, they contain both chemical and gap junctional components.[20] Thus, it is possible that one pyramidal cell dendrite could be coupled indirectly to another dendrite through a common mossy fiber. It is not known if this mechanism is available to other types of principal neurons.

The dye coupling data of Church and Baimbridge[16] provide a clue as to *how many* other pyramidal cells any one pyramidal cell might be coupled to: even under optimal pH conditions (i.e., during alkalinization), any one cell is likely to couple to only a few (fewer than four or five, averaging about three) other cells. Additionally, the dye-coupling data in Schmitz et al.[18] indicate that, at least in CA1, any two pyramidal neurons that are coupled are likely to have somata within about 300 μm of each other.

IN VITRO MODELS OF VFO SUGGEST THAT CHEMICAL SYNAPSES ARE NOT REQUIRED FOR THEIR GENERATION

While in vitro hippocampal VFO can occur in conditions of normal neurotransmission,[21,22] it is important to note that VFO also occurs in vitro with low-calcium media (blocking synaptic transmission entirely), in the presence of cocktails of synaptic blockers, and with either gamma-aminobutyric acid A ($GABA_A$) or alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)/kainate receptors selectively blocked[8,21,23,24] (see the later discussion). An example is shown in Fig. 22–2. These data imply that interneurons cannot be critical for VFO generation, at least in vitro, even if phasic inhibitory postsynaptic potentials (IPSPs) occur in vivo during ripples[25]: the latter IPSPs are plausibly explained as an epiphenomenon of a primary VFO generator involving the plexus of pyramidal cell axons.[26,27] The data also provide support for the notion that gap junctional coupling is the primary between-neuron interaction necessary for population VFO: chemical synapses are not necessary, and no other type of known between-neuron interaction can plausibly explain the phenomenon.

BRIEF VFO, GENERATED BY NONSYNAPTIC MECHANISMS, OCCURS DURING INTERICTAL BURSTS IN HUMAN EPILEPTOGENIC TISSUE IN VITRO

During the physiologically normal in vivo hippocampal events called *sharp-wave/ripples*

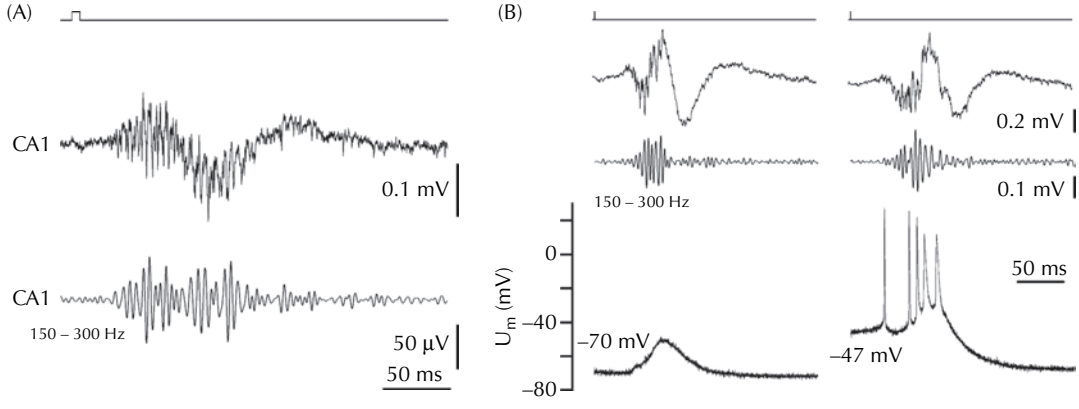

Figure 22–2. Sharp wave ripple complexes in vitro (mouse hippocampal CA1 minislice) do not require $GABA_A$ receptors. When $GABA_A$ receptors are blocked, the complexes can be evoked by pressure ejection of potassium chloride (KCl; 1 M) into stratum radiatum (upper traces), although they do not occur spontaneously. **A.** Stratum radiatum field potential of a KCl-evoked sharp wave ripple in 10 µM gabazine to block $GABA_A$ receptors. A 150–300 Hz filtered trace is shown below, highlighting the ripple. **B.** Combined extracellular (above) and intracellular (putative pyramidal cell, below) recordings during a KCl-induced sharp wave ripple with blocked $GABA_A$ receptors. The cell was held at –70 mV (left) to show the compound glutamatergic excitatory postsynaptic potential (EPSP) during the sharp wave without phasic IPSPs. (Phasic IPSPs do occur in baseline conditions in vivo[25] and in vitro.[22]) When the cell was held depolarized at –47 mV (right), it generated a burst during the sharp wave ripple, but again without evidence of phasic IPSPs. From ref. 24.

(or *physiological sharp waves*), VFO occurs superimposed on a field transient lasting for tens of milliseconds.[25,28] Analogously to this, and actually discovered first, VFO occurs superimposed on epileptiform field transients in *disinhibited* hippocampal slices,[29,30] providing additional evidence that VFO does not require phasic synaptic inhibition. A number of different instances have since been published, demonstrating VFO occurring before, during, and after interictal discharges: in vitro, in brain slices from experimental animals[31]; in vivo, in experimental animals that also have spontaneous seizures[32]; and in situ in the brains of patients with epilepsy[7,11,33] (see Fig. 22–4C for an example in a subdural grid recording from an epileptic infant who had a subcortical dysplasia).

Figure 22–3 provides an example of VFO in association with an interictal burst in human brain studied in vitro (data from ref. 6). (The patient had epilepsy secondary to mesial temporal sclerosis.) For comparison, the figure shows paroxysmal events, having quite similar morphology, in a foramen ovale recording from the same patient prior to surgery and in a network model of a cortical column. The network model contained synaptic excitation and inhibition, and paroxysmal events were simulated by a transient increase in the conductance of gap junctions between pyramidal cell axons. Both in the human tissue in vitro and in the network model, there was a poor correlation between particular synaptic conductances and the VFO; indeed, VFO persisted after blockade of chemical synapses, both in the tissue and in the model. On the other hand, blockade of gap junctions with carbenoxolone eliminated both the VFO and the paroxysmal interictal discharge; correspondingly, in the model, if gap junction conductances were too small, neither VFO nor paroxysmal discharges occurred. The effects of carbenoxolone in the experiment are unlikely to reflect nonspecific effects of the compound on chemical synapses, as the use of specific drugs known to block chemical synapses did not abolish the VFO. Similarly, carbenoxolone has been shown not to influence the time course or amplitude of axonal action potentials in hippocampal pyramidal cell axons and in dentate granule cell mossy fiber terminals.[18]

The data of Roopun et al.[6] therefore suggest that VFO is not just an epiphenomenon of epileptic discharges, but rather may have a causal relationship to such discharges—although the exact nature of this putative relationship remains to be worked out. In our opinion, the most economical hypothesis at present is this: that VFO arises from the electrically coupled

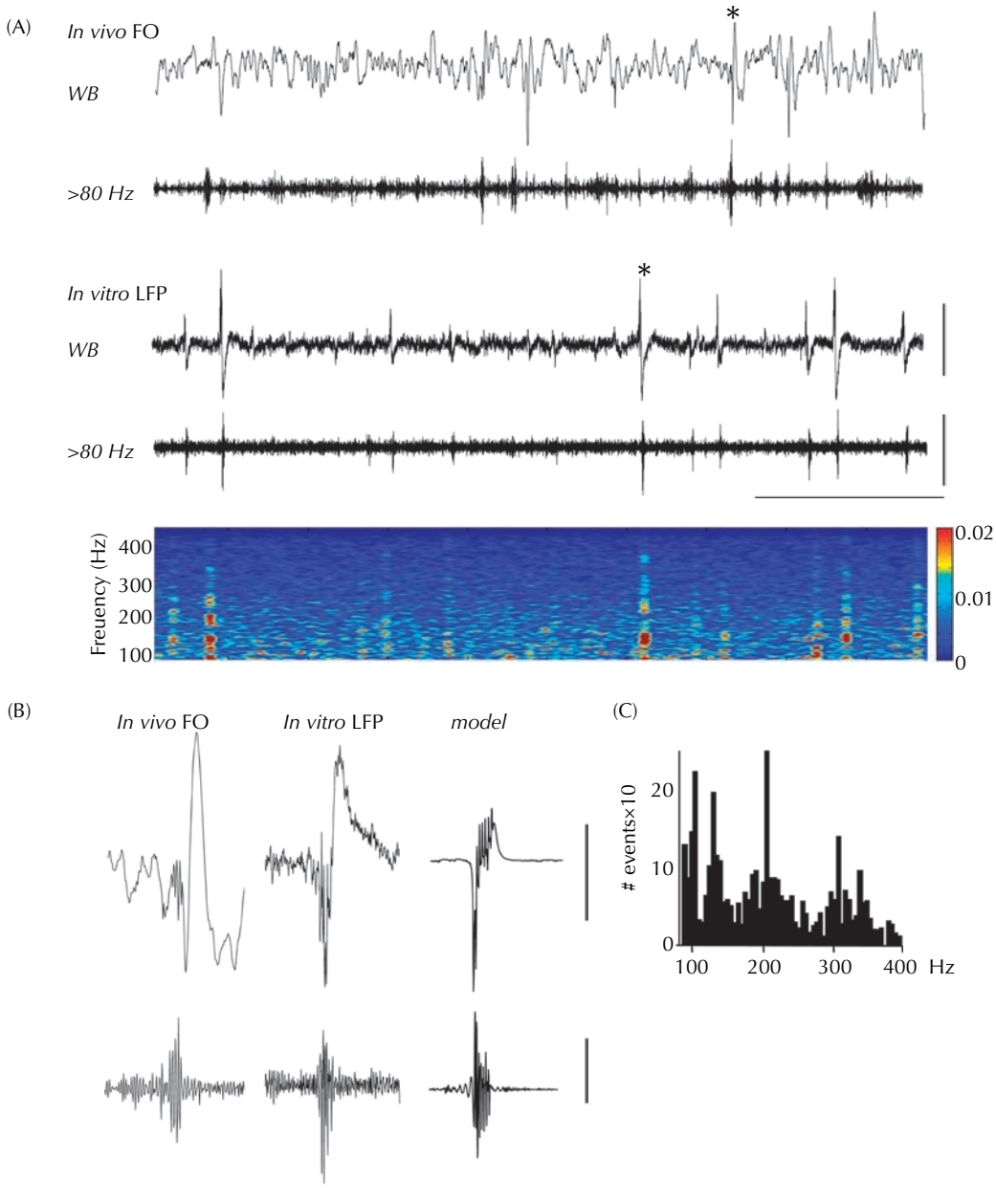

Figure 22–3. Very fast oscillations (VFOs) are spontaneously generated in human temporal neocortical slices in vitro. **A.** Foramen ovale (FO) electrodes, implanted in a patient with right medial temporal lobe sclerosis, demonstrate spike-and-wave interictal events recorded in wide bandpass (WB) mode. The bandpass filtered trace (20 s duration) illustrates that oscillatory activity above 80 Hz is observed coincidently with the sharp-wave complex. In vitro local field potential (LFP) recordings in slices of superior mediotemporal cortex (20 s duration), resected from the same patient, demonstrate spontaneous sharp-wave discharges. The bandpass filtered trace reveals VFO behavior associated with the sharp wave discharges, as can be seen in the color-coded spectrogram of the activity illustrated. **B.** Comparison of two selected (denoted by the asterisk in **A**) events from in vivo FO and in vitro LFP—inverted for reference to the far-field potential FO data—reveal a similar location of the VFO activity at the initial stage of the sharp-wave event. A cortical column model, containing synaptic (excitatory and inhibitory) and gap junctional connections, reproduced the VFO and the initial low-frequency envelope of the interictal discharge. Scale bars **(A)** 500 μV (upper), 200 μV (lower) and 5 s, **(B)** 200 μV (upper), 100 μV (lower). Scale bars for the model "field" are arbitrary. **C.** Histogram displays the total number of detected VFO events from 80 to 400 Hz ($n = 20$, epochs of data = 60 s). Bin width of histogram was 5 Hz. From ref. 6.

plexus of pyramidal cell axons, as described in Traub et al.,[27,34] and that the coherently and rapidly oscillating plexus of axons, in turn, orthodromically drives nearby downstream neurons, thereby inducing the occurrence of approximately synchronized synaptic currents.

EXAMPLE OF MORE SUSTAINED VFO PRIOR TO AN ELECTROGRAPHIC SEIZURE IN A PATIENT'S BRAIN IN SITU

Not only does VFO occur transiently during sharp wave/ripples and in association with interictal epileptiform events, but VFO can also occur stably, on a time scale of seconds, prior to an electrographic seizure.[12] Figures 22–4A and 22–4B show details of such prolonged VFO, as recorded with a subdural grid implanted over the frontal lobe of a child with intractable seizures attributed to a focal dysplasia. (See also Fig. 22–5A for the demonstration of similar phenomenology in a single ECoG electrode.) Typically in such cases, the preseizure VFO is both more localized in space and of much smaller amplitude than the succeeding electrographic seizure proper. Both the conditions permitting this type of stable VFO and the mechanisms of the VFO-seizure transition require explanation. Unfortunately, the necessary intracellular recordings and pharmacological manipulations are difficult or impossible to perform in vivo, particularly in the brains of patients. A rational plan of research will include, therefore, studies of VFO mechanisms in vitro (with human or animal tissue) and with network models, and will also include testing of model predictions with those in situ measurements that can feasibly be performed. In the next two figures, we will illustrate how this can be done.

VFO CAN BE ELICITED IN NEOCORTICAL SLICES IN NONSYNAPTIC CONDITIONS ASSOCIATED WITH SPIKELETS IN DEEP PYRAMIDAL NEURONS

Figure 22–5A illustrates approximately 0.5 s of about a 150 Hz VFO, in a subdural grid recording from an epileptic patient, that occurred prior to an electrographic seizure. Figure 22–5B shows, for comparison, field oscillations at a similar frequency (without, however, the succeeding electrographic seizure) in a rat neocortical slice. The recording conditions in Fig. 22–5B are striking. First, the tissue was "activated" by nanomolar kainate in the bath, which is likely to contribute to a sustained cortical up state.[35] Second, phasic chemical synaptic receptors were pharmacologically blocked. Finally, alkaline artificial cerebrospinal fluid (ACSF) was pressure-ejected onto the slice just prior to the VFO event. Thus, we may conclude that the VFO in Fig. 22–5B does not depend on chemical synapses and is almost certainly generated by gap junctions. In addition, it was possible to obtain an intracellular recording (from a layer 5 intrinsic bursting, "IB," presumed pyramidal neuron) simultaneously with the field recording. This neuron generated both full action potentials and spikelets (fast prepotentials)—indicative of action potential generation at a distance from the soma, and presumably in the axon—with both types of event (action potentials and spikelets) phase-locked, on average, to the population spikes in the field. Again, the occurrence of spikelets is reminiscent of VFO recorded in the hippocampus in nonsynaptic conditions,[21] wherein the spikelets are highly likely to derive from axons.[18]

An expanded view of the field/intracellular recordings is shown in the lower left part of Fig. 22–5. For comparison, the lower right part shows how these patterns are replicated in a network model of 15,000 multicompartment pyramidal neurons, interconnected only by axonal gap junctions, without chemical synapses. In this network model, it is possible to demonstrate directly that somatic spikelets arise from antidromically, and decrementally, conducted axonal action potentials. The in vitro data then provide a plausible scenario concerning how VFO could be generated in local regions of cortex in situ; stated another way, the in vitro data provide a plausible explanation for temporal aspects of VFO, including the network frequency and the tendency of individual pyramidal neurons to generate spikelets. But what about the spatial aspects of VFO? In Fig. 22–4A, for example, VFO occurs in several subdural grid electrodes and hence involves several square centimeters of cortex.

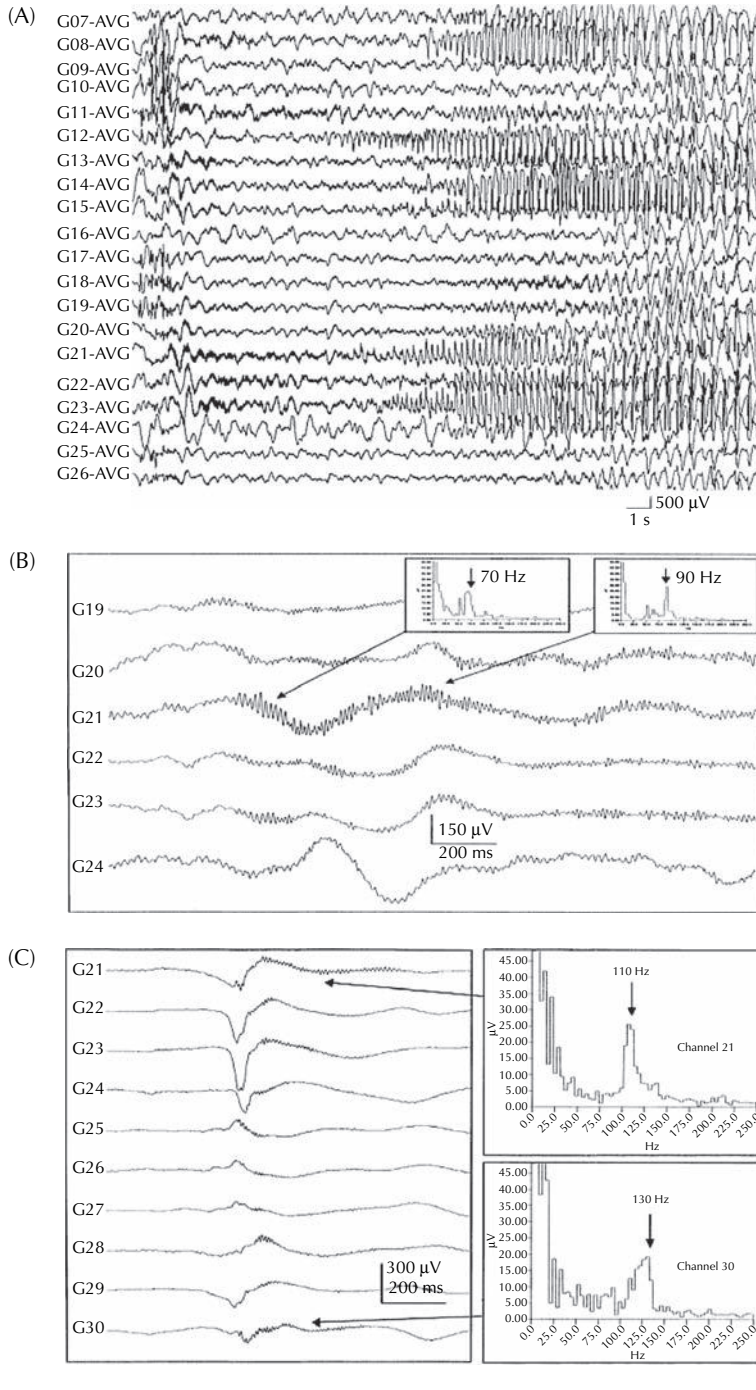

Figure 22–4. Brief, relatively localized, VFO leading to a more extensive electrographic seizure in a child with a subcortical dysplasia. A focal seizure in a child is preceded by localized very fast oscillatory activity. The patient, age 13 months, had a right frontal subcortical dysplasia, subsequently removed surgically. The EEG activity was recorded with a subdural grid of electrodes. **A.** Electrographic seizure preceded by low-amplitude very fast activity restricted to a few recording sites (including G11, G13, and G21–G23). **B.** Using a different recording technique, to give a better signal:noise ratio, shows that the very fast activity (preceding a seizure) contains frequency components of 70–90 Hz. **C.** An interictal burst recorded from the same patient contains superimposed VFO at 110–130 Hz; compare it with the in vitro and in vivo interictal spikes in Fig. 22–3. Signals recorded and analyzed by T. Baldeweg, H. Cross, and S. Boyd. From Ref. 7.

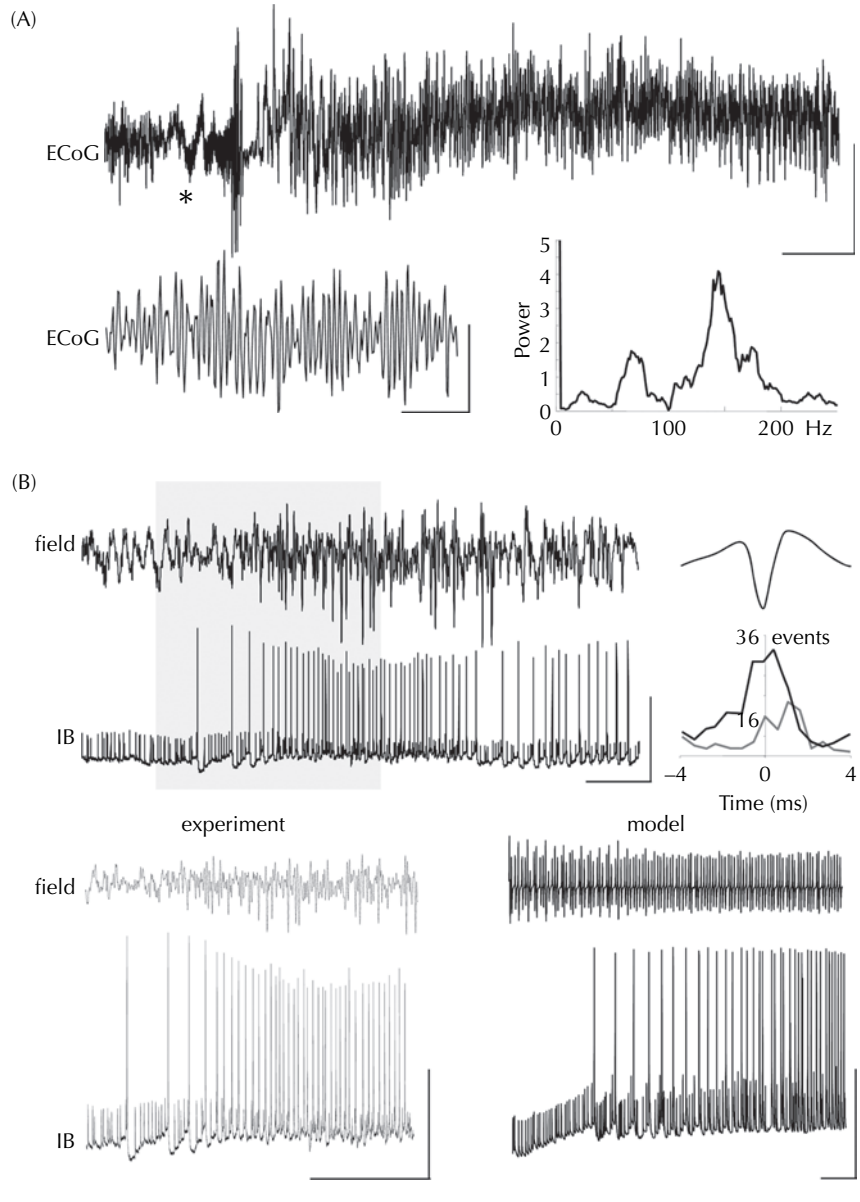

Figure 22–5. Very fast oscillations (VFO) occur at the surface of human epileptic brain; in layer 5 of rat neocortex, in vitro, with chemical synapses blocked; and in a detailed network model of neurons coupled by gap junctions without chemical synapses. **A.** An ECoG recording from epileptic frontal neocortex (patient B of ref. 38). The portion marked by * is expanded below. The power spectrum is from 1 s of data. Scale bars: 100 μV, 11 s; 10 μV, 100 ms. **B.** Upper traces are field and intracellular recordings (IB, or intrinsic bursting cell) showing VFO in layer 5 of rat temporal neocortex in nonsynaptic conditions: AMPA, NMDA, and $GABA_A$ receptors were blocked with SYM2206, AP5, and gabazine, respectively. The bath contained kainate, and alkaline artificial cerebrospinal fluid (ACSF) was pressure ejected onto the slice just before the trace begins. Scale bars; 0.1 mV (field), 50 mV (cell), 500 ms. The graph (right) shows a pooled incidence plot (bin width 0.5 ms) for 500 field VFO periods with full spike data plotted as the gray line and spikelet data as the black line.

The data in the gray box were expanded at the lower left: scale bars 0.2 mV, 40 mV, and 400 ms. Simulation data are shown at the lower right: field potential of very fast network oscillation (above, spectral peak at 112 Hz) and simultaneous "intracellular recording" (below). Scale bars 50 mV (cell), 100 ms. Note the mixture of full action potentials and spikelets, as in the experiment. The model consisted of 15,000 multicompartment IB pyramidal cells, with gap junctions between axons. A ramping bias current (–0.5 to 0.5 nA) was applied to the illustrated cell to show that spikelets are more common at hyperpolarized somatic membrane potentials and full spikes at depolarized membrane potentials. From ref. 8.

Could there be a spatial structure to this VFO, which might provide clues concerning the cellular mechanisms by which VFO is generated in situ? We consider this question next.

SPATIAL PATTERNS OF VFO IN HUMAN EPILEPTIC BRAIN ARE REPLICATED WITH A SIMPLE MODEL BASED ON LOCALIZED ELECTRICAL COUPLING BETWEEN PYRAMIDAL NEURONS

Neocortical slices contain thousands of neurons, as do our "detailed" multicompartment network models. On the other hand, cortical surfaces of many square centimeters contain millions of neurons. In vitro slices on this scale are difficult to prepare, and it is difficult to perform computer simulations of highly detailed network models on this scale as well. One approach for modeling, however, is to abstract the key physical principles for VFO that have been reasonably established from in vitro slices and detailed models, as in Fig. 22–5. Thus, the in vitro recordings and the simulation in Fig. 22–5 are similar enough that we may ask: what is it that really makes the model "tick"? In the model, VFO is generated by "percolation" of spikes from axon to axon under conditions in which (1) there is a low rate of spontaneous axonal firing and (2) each axon is coupled to a small number of other axons (on average)—but to more than *one* other axon (on average). (If each axon connects to less than one other axon, on average, then collective network behavior is impossible.) These physical ideas can be captured in an extremely reduced type of model called a *cellular automaton* with locally random connectivity.[8] In such a model, each element represents an axon that is either at rest, is firing, or is refractory. The model behavior is determined by a small number of parameters, each of which has physical meaning: (1) the time scale, determined by how long it takes for an action potential to cross from one axon to another (estimated to be about 0.25 ms); (2) a spatial scale, corresponding to the maximum separation of two neuronal somata that is compatible with a gap junction forming between them (estimated to be hundreds of microns, extrapolating from the data of Schmitz et al.[18]); (3) the mean number of axons that any one axon couples to, which must be more than one and is probably less than about three (from dye coupling data); and (4) the mean rate of spontaneous activity, which is, at present, difficult to determine experimentally.

Such a model predicts that VFO will not be spatially uniform, provided that neurons are only allowed to couple to other neurons not too far away. In these latter conditions, VFO waves start as small "blobs" that then propagate and destructively interfere with other propagating

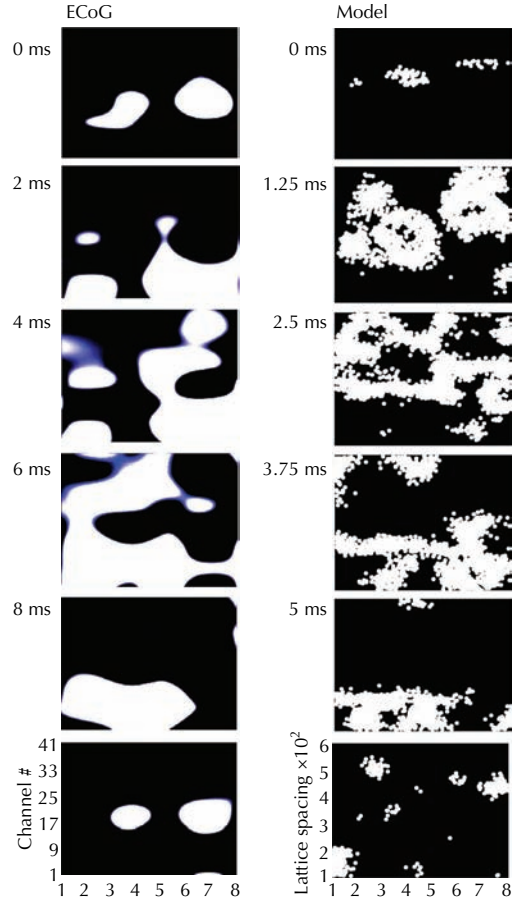

Figure 22–6. Comparison of spatiotemporal patterns in ECoG data and in cellular automaton model data. The left side shows successive frames of activity, every 2 ms, from an 8 × 6 subdural array of electrodes (with 1 cm spacing) in patient B of ref. 38. The right side shows frames of activity every 1.25 ms from a cellular automaton model with 480,000 "cells" in an 800 × 600 array with sparse, localized gap junctional connectivity (mean index <i> = 1.33, connectivity footprint = 25 lattice spacings) and rare spontaneous action potentials. From ref. 8.

blobs. The result is a statistical structure to the spatial activity that, qualitatively, resembles the spatial structure in human ECoG data (in a small number of patients so far; Fig. 22–6). Videos of the comparison between model prediction and ECoG data are to be found in the supplementary data of ref. 8.

In summary, then, the spatial patterns of VFO, predicted by a model based on gap junctional coupling and not using chemical synapses, are qualitatively similar to VFO patterns observed in situ in the human brain. This provides indirect support for the notion that VFO in situ, at least of the sort that precedes epileptic events, is indeed generated by electrical coupling between principal neurons. In the Discussion, we shall consider the clinical implications of our postulated mechanism for epilepsy-related VFO.

DISCUSSION: CLINICAL IMPLICATIONS

The evidence that in vitro VFO is generated by nonsynaptic mechanisms is, we believe, compelling[6,8,21,34]:

1. Very fast oscillation occurs in slices bathed in a low-calcium medium or in a medium in which one or multiple types of phasic synaptic transmission are blocked with specific receptor blockers.
2. In vitro VFO is affected by pH and gap junction blockers in a manner consistent with a requirement for gap junctions.
3. Network models (and a more abstract cellular automaton theory), based on the postulate of gap junctional coupling between principal neurons, account for the frequency of VFO and also for the firing patterns of individual pyramidal neurons, including frequent spikelets. The models also account for the increase in frequency with alkalinization (which presumably increases gap junction conductances).

The clinically relevant question here, however, concerns the extent to which one can reasonably extrapolate the in vitro data to the in situ epileptic brain. Bathing in situ human brain in a low- calcium medium, or with cocktails that block synaptic transmission, is, of course, not possible; nor are we aware of pH measurements in human epileptic tissue in situ (although such measurements might be feasible and would be especially useful if combined with simultaneous ECoG recording). Spikelets have been recorded intracellularly in vivo from the hippocampus of cats[36] and mice,[37] but gap junctional dependence has not been proven, and the technology does not yet exist for safe intracellular recording in human tissue in situ.

The direct resemblances between in vitro and in vivo (epileptic) VFO consist of two main elements: a common frequency range and—in a small number of patients—spatial patterns that are predicted by a gap junction model. In addition, the same human tissue that is epileptogenic in situ is capable of producing nonsynaptic VFO when studied in vitro.[6] The remainder of the evidence that in vivo epileptic VFO is gap junction-dependent is negative and consists of the following: so far as we are aware, there is no cortical preparation that has been shown to generate VFO by synaptic mechanisms. The gap junction dependence of in vivo epileptic VFO is therefore, in our opinion, the most reasonable available hypothesis.

If it is indeed true that VFO in epilepsy is generated by gap junctional coupling between principal neurons, it would matter in a number of ways, both in terms of basic epilepsy mechanisms and in terms of treatment approaches:

1. First, it would provide clues concerning epileptogenesis, the process by which previously normal brain tissue becomes capable of initiating seizures. Thus, it may be that gap junctions appear in abnormal places, in excessively great numbers, or with abnormal physiological properties. In order to pursue these important questions, it is first necessary to identify what gap junction proteins are responsible for electrical coupling between principal neurons.
2. Second, it would provide clues to the events responsible for initiating individual seizures. Specifically, VFO is favored to occur (in vitro, at least) by alkalinization—presumably opening gap junctions—and by relative blockade of synaptic transmission. Such effects could arise in tissue as a result of glial dysfunction or of abnormalities in one or more transporter systems.

3. Given the association between VFO and the initiation of seizures, it makes sense to attempt to target VFO as a possible strategy for seizure prevention. Such attempts might be directed against gap junctions per se, although with a possibility of undesirable side effects, given the normal widespread distribution of gap junctions in the brain (e.g., inferior olive, nucleus reticularis thalami, cortical and cerebellar interneurons), in the retina, and, of course, elsewhere in the body. Alternatively, other tissue factors—yet to be identified experimentally—that predispose to VFO might be targeted.

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REFERENCES

1. Traub RD, Wong RKS. Cellular mechanism of neuronal synchronization in epilepsy. *Science*. 1982;216:745–747.
2. Jacobs J, LeVan P, Chander R, Hall J, Dubeau F, Gotman J. Interictal high-frequency oscillations (80–500 Hz) are an indicator of seizure onset areas independent of spikes in the human epileptic brain. *Epilepsia.* 2008;49:1893–1907.
3. Jacobs J, LeVan P, Chatillon CE, Olivier A, Dubeau F, Gotman J. High frequency oscillations in intracranial EEGs mark epileptogenicity rather than lesion type. *Brain.* 2009;132: 1022–1037.
4. Khosravani H, Mehrotra N, Rigby M, Hader WJ, Pinnegar CR, Pillay N, Wiebe S, Federico P. Spatial localization and time-dependent changes of electrographic high frequency oscillations in human temporal lobe epilepsy. *Epilepsia*. 2008;50:605–616.
5. Schevon CA, Ng SK, Cappell J, Goodman RR, McKhann G Jr, Waziri A, Branner A, Sosunov A, Schroeder CE, Emerson RG. Microphysiology of epileptiform activity in human neocortex. *J Clin Neurophysiol*. 2008;25:321–330.
6. Roopun AK, Simonotto JD, Pierce ML, Jenkins A, Schofield I, Kaiser M, Whittington MA, Traub RD, Cunningham MO. A non-synaptic mechanism underlying interictal discharges in human epileptic neocortex. *Proc Natl Acad Sci USA*. 2010;107:338–343.
7. Traub RD, Whittington MA, Buhl EH, LeBeau FEN, Bibbig A, Boyd S, Cross H, Baldeweg T. A possible role for gap junctions in generation of very fast EEG oscillations preceding the onset of, and perhaps initiating, seizures. *Epilepsia*. 2001;42:153–170.
8. Traub RD, Duncan R, Russell AJC, Baldeweg T, Tu Y, Cunningham MO, Whittington MA. Spatiotemporal patterns of electrocorticographic very fast oscillations (>80 Hz) consistent with a network model based on electrical coupling between principal neurons. *Epilepsia*. 2010;51:1587–1597.
9. Fisher RS, Webber WRS, Lesser RP, Arroyo S, Uematsu S. High-frequency EEG activity at the start of seizures. *J Clin Neurophysiol*. 1992;9:441–448.
10. Grenier F, Timofeev I, Steriade M. Neocortical very fast oscillations (ripples, 80–200 Hz) during seizures: intracellular correlates. *J Neurophysiol*. 2003;89:841–852.
11. Jirsch JD, Urrestarazu E, LeVan P, Olivier A, Dubeau F, Gotman J. High-frequency oscillations during human focal seizures. *Brain*. 2006;129:1593–1608.
12. Worrell GA, Parish L, Cranstoun SD, Jonas R, Baltuch G, Litt B. High-frequency oscillations and seizure generation in neocortical epilepsy. *Brain.* 2004;127:1496–1506.
13. Traub RD, Whittington MA. *Cortical Oscillations in Health and Disease*. New York: Oxford University Press; 2010.
14. MacVicar BA, Dudek FE. Electrotonic coupling between pyramidal cells: a direct demonstration in rat hippocampal slices. *Science.* 1981;213:782–785.
15. Mercer A, Bannister AP, Thomson AM. Electrical coupling between pyramidal cells in adult cortical regions. *Brain Cell Biol*. 2006;35:13–27.
16. Church J, Baimbridge KG. Exposure to high-pH medium increases the incidence and extent of dye coupling between rat hippocampal CA1 pyramidal neurons in vitro. *J Neurosci*. 1991;11:3289–3295.
17. Gutnick MJ, Lobel-Yaakov R, Rimon G. Incidence of neuronal dye-coupling in neocortical slices depends on the plane of section. *Neuroscience*. 1985;15:659–666.
18. Schmitz D, Schuchmann S, Fisahn A, Draguhn A, Buhl EH, Petrasch-Parwez RE, Dermietzel R, Heinemann U, Traub RD. Axo-axonal coupling: a novel mechanism for ultrafast neuronal communication. *Neuron*. 2001;31:831–840.
19. Hamzei-Sichani F, Kamasawa N, Janssen WGM, Yasamura T, Davidson KGV, Hof PR, Wearne SL, Stewart MG, Young SR, Whittington MA, Rash JE, Traub RD. Gap junctions on hippocampal mossy fiber axons demonstrated by thin-section electron microscopy and freeze-fracture replica immunogold labeling. *Proc Natl Acad Sci USA*. 2007;104:12548–12553.
20. Kamasawa N, Hamzei-Sichani F, Yasamura T, Janssen WGM, Davidson KGV, Wearne SL, Hof PR,

Traub RD, Rash JE. Ultrastructural evidence for mixed synapses in hippocampal principal neurons using thin-section and freeze-fracture replica immunogold (FRIL) electron microscopy. *Soc Neurosci Abstr* 2007;581.12.
21. Draguhn A, Traub RD, Schmitz D, Jefferys JGR. Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro. *Nature*. 1998;394: 189–192.
22. Maier N, Nimmrich V, Draguhn A. Cellular and network mechanisms underlying spontaneous sharp wave-ripple complexes in mouse hippocampal slices. *J Physiol.* 2003;550:873–887.
23. Hormuzdi SG, Pais I, LeBeau FEN, Towers SK, Rozov A, Buhl EH, Whittington MA, Monyer H. Impaired electrical signaling disrupts gamma frequency oscillations in connexin 36-deficient mice. *Neuron*. 2001;31:487–495.
24. Nimmrich V, Maier N, Schmitz D, Draguhn A. Induced sharp wave-ripple complexes in the absence of synaptic inhibition in mouse hippocampal slices. *J Physiol*. 2005;563:663–670.
25. Ylinen A, Bragin A, Nádasdy Z, Jandó G, Szabó I, Sik A, Buzsáki G. Sharp wave-associated high frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *J Neurosci*. 1995;15:30–46.
26. Traub RD, Bibbig A. A model of high-frequency ripples in the hippocampus, based on synaptic coupling plus axon-axon gap junctions between pyramidal neurons. *J Neurosci.* 2000;20:2086–2093.
27. Traub RD, Cunningham MO, Gloveli T, LeBeau FEN, Bibbig A, Buhl EH, Whittington MA. GABA-enhanced collective behavior in neuronal axons underlies persistent gamma-frequency oscillations. *Proc Natl Acad Sci USA*. 2003;100:11047–11052.
28. Buzsáki G, Horváth Z, Urioste R, Hetke J, Wise K. High-frequency network oscillation in the hippocampus. *Science*. 1992;256:1025–1027.
29. Schwartzkroin PA, Prince DA. Penicillin-induced epileptiform activity in the hippocampal in vitro preparation. *Ann Neurol*. 1997;1:463–469.
30. Wong RKS, Traub RD. Synchronized burst discharge in the disinhibited hippocampal slice. I. Initiation in the CA2-CA3 region. *J Neurophysiol*. 1983;49:442–458.
31. Pais I, Hormuzdi SG, Monyer H, Traub RD, Wood IC, Buhl EH, Whittington MA, LeBeau FEN. Sharp wave-like activity in the hippocampus in vitro in mice lacking the gap junction protein connexin 36. *J Neurophysiol*. 2003;89:2046–2054.
32. Bragin A, Engel J Jr, Wilson CL, Fried I, Buzsáki G. High-frequency oscillations in the human brain. *Hippocampus*. 1999;9:137–142.
33. Staba RJ, Wilson CL, Bragin A, Jhung D, Fried I, Engel J Jr. High-frequency oscillations recorded in human medial temporal lobe during sleep. *Ann Neurol*. 2004;56:108–115.
34. Traub RD, Schmitz D, Jefferys JGR, Draguhn A. High-frequency population oscillations are predicted to occur in hippocampal pyramidal neuronal networks interconnected by axoaxonal gap junctions. *Neuroscience.* 1999;92:407–426.
35. Cunningham MO, Pervouchine D, Racca C, Kopell NJ, Davies CH, Jones RSG, Traub RD, Whittington MA. Neuronal metabolism governs cortical response state. *Proc Natl Acad Sci USA.* 2006;103:5597–5601.
36. Spencer WA, Kandel ER. Electrophysiology of hippocampal neurons IV. Fast prepotentials. *J Neurophysiol*. 1961;24:272–285.
37. Epsztein J, Lee AK, Chorev E, Brecht M. Impact of spikelets on hippocampal CA1 pyramidal cell activity during spatial exploration. *Science*. 2010;327: 474–477.
38. Roopun AK, Traub RD, Baldeweg T, Cunningham MO, Whittaker RG, Trevelyan A, Duncan R, Russell AJC, Whittington MA. Detecting seizure origin using basic, multiscale population dynamic measures: preliminary findings. *Epilepsy Behav*. 2009;14(suppl 1): 39–46.

Chapter 23

Computer Modeling of Epilepsy

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Robert J. Morgan
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There are 50 million people worldwide afflicted with epilepsy, and for roughly 15 million of them, existing epilepsy treatments are not sufficient.[1] Such stark facts spur clinicians and researchers to consider dramatically different approaches to treatment, such as an implantable device that could characterize electrical activity in real time, immediately detect when the brain reaches a preictal state, and apply a counteracting current waveform, averting the seizure before it starts (see the next section),[2] or an individualized, detailed model of the patient's brain—complete with patient-specific details such as genetic mutations or head trauma—to which doctors could administer virtual drugs to determine the best treatment regimen for that person. Such a tool would complement existing therapies by reducing the likelihood of patients being subjected to treatments to which they are unresponsive.

Notably, both the implantable device and the individualized brain model use computer modeling. The device employs computer modeling in its development and in the algorithms it uses to detect the seizure and formulate an appropriate response, whereas the individualized model would use an interactive, large-scale, biologically realistic computational model.

And then there are the 2.4 million people worldwide who will develop epilepsy over the next year due to a variety of causes.[1] Ideally, we could prevent epileptogenesis in these people entirely, and computer modeling can help us achieve this goal. For example, in people who have experienced head trauma, an ideal therapy might be a drug that could be administered to prevent the development of epileptogenic alterations without disrupting the brain further. Computational models could help us determine which of the alterations are most

clinically significant and also help characterize the therapeutic effects and side effects of any proposed treatment. In patients with gene mutations, a method to induce expression of a "therapeutic" ion channel could counteract the epileptic effects of a mutated ion channel.[3] Again, an individualized computational model of the brain could help by predicting the likelihood of epileptogenesis (and therefore the need for treatment) in a person exposed to a particular environmental or genetic factor.

Such ideas may sound impractical, even outlandish, given the current state of epilepsy therapy. However, work has already begun on projects using ideas similar to those described above. For example, the Blue Brain Project has created a detailed 10,000-neuron model of a rat neocortical column[4] and aims to build a detailed, biologically realistic model of the human brain.[5] Also impressive are several implantable devices undergoing clinical trials, some of which use algorithms that can be individually tuned for each subject by his or her doctor to detect and respond to seizures.[2]

Clearly, there is a role for computer modeling in the development and operation of epilepsy therapies. Not only can it be applied directly to therapies, as in the examples above, but it can also guide researchers in choosing and designing experiments and can provide a framework for organizing experimental results. In fact, computational models have already advanced our understanding of disorders such as schizophrenia,[6] Parkinson's disease,[7] stroke,[8] and, of course, epilepsy.[9] Within the field of computational epilepsy modeling, approaches include single-cell[10–12] models (simple or detailed) and network models ranging from mean-field[13,14] to large-scale, detailed models[15–19] (Fig. 23–1).

This range of approaches is not surprising, given that epilepsy is a dynamic disorder that can be characterized at multiple levels of detail.[20] Though there exists a wealth of data

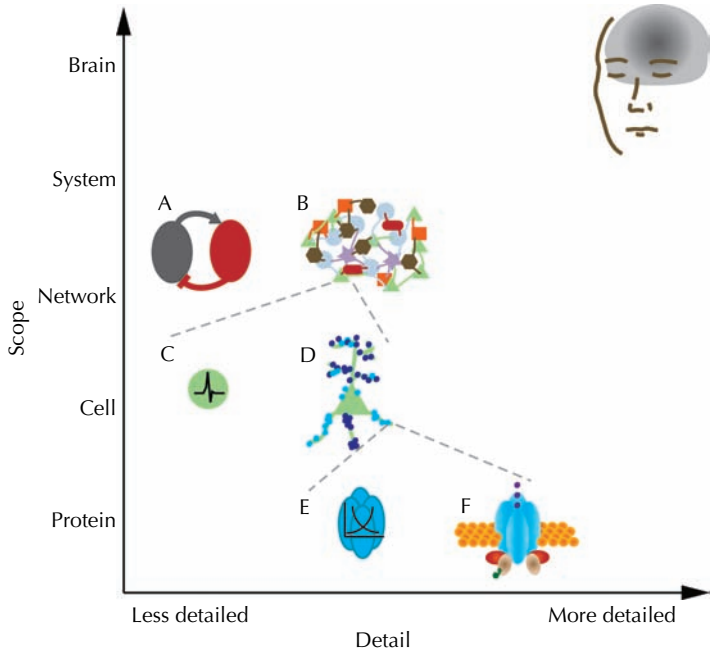

Figure 23–1. Functional computational models can be partially organized by their scope and detail. The icons above represent a few possible modeling approaches mentioned in this chapter. **A.** Macroscopic, mean-field network models, which usually incorporate at least an excitatory and an inhibitory neuronal population. **B.** Detailed network models, which include individual cells modeled at variable levels of detail. Note that both **A** and **B** can describe networks or even systems that can perform higher-level functions. **C, D.** Model cells at variable levels of detail, from integrate-and-fire models (**C**) to cells with simple morphology and mechanisms representing ion channels, receptors, and gap junctions (**D**). **E, F.** Ion channels and other subcellular mechanisms can also be represented at variable levels of detail, from their conductances in each state (**E**) to detailed protein structures that capture conformational changes and binding sites (**F**).

at each of these levels, the challenge of drawing connections across levels stands in the way of developing greater understanding and new treatments for the disorder. For example, it is difficult to understand how high-level dynamics such as aggregate electrical activity during a seizure could be predicted from low-level structural and functional factors like altered connectivity between cells or mutated ion channels. Furthermore, the many causes of epilepsy and its variable manifestations limit our ability to apply knowledge about one epilepsy model to other models. The challenges of integrating experimental research about epilepsy through multiple levels of detail and across multiple subcategories of the disorder can be addressed with computer modeling.

While a thorough survey of modeling techniques in the epilepsy field requires an entire book,[9] much can be learned from a tour of recently developed models. Here, we first discuss computer modeling to benefit patients who already have epilepsy and those at risk for developing epilepsy. Then we illustrate how computer modeling can complement experimental research. After the reader has been introduced to several models, we describe the various approaches one may take for computer modeling of epilepsy. We conclude by assessing the future of computer modeling in epilepsy and suggest resources available to those interested in using computers to model epilepsy.

COMPUTER MODELING TO PREVENT SEIZURES

Computational modeling is not just useful for clinical applications indirectly via research; it is already directly applicable to clinical practice. Aside from the well-known application of computer modeling to the discovery and development of pharmaceutical agents,[21,22] it has also been instrumental in designing therapies to prevent or reduce seizures, some of which have already been used successfully in human patients. For the purpose of this chapter, such therapies can be broadly classified as dynamic, which exert their effects only in response to seizures, and static, which exert their effects continuously (or continually, in some cases).

Dynamic Therapies

There have been promising developments in devices capable of online analysis of epileptic patients for the detection of and response to seizures. At least one device, the RNS™ device designed by NeuroPace, has shown a significant reduction in seizure frequency during clinical trials, with an acceptable level of side effects.[2] An application for premarket approval (PMA) for the RNS™ device was recently submitted to the Food and Drug Administration (FDA).[23] Though the seizure detection and response algorithm used in the NeuroPace RNS™ device is proprietary, we can review other computational models to highlight some of the computational issues inherent in seizure detection and response.

Detection of seizures requires a reliable method of characterizing seizure and even preseizure activity, one that is robust enough to detect each seizure that occurs, despite known variability in individual seizure dynamics. A variety of neural properties have been proposed as suitable for monitoring to detect seizures, including electroencephalographic (EEG) traces[24] and localized glutamate transmission.[25] One such method of characterization is matching pursuit, in which a signal (such as an EEG) is broken down into *atoms,* smaller and simpler signal elements.[26] Using this analysis, researchers were able to show considerable similarity among onset of seizures originating from the same foci in the same patient.[26]

Once a seizure has been detected, a variety of approaches can be used to correct the abnormal activity. In one approach, Lopour and Szeri[14] produced a mean-field model that showed how a control system could analyze the electrode signals from a seizing patient and apply a charge-balanced potential to nearby electrodes to terminate the seizure activity. Using a charge-balanced correction signal, where the net charge over time approaches 0, is thought to be less harmful to cortical tissue and is an important step toward the eventual goal of using such a controller therapeutically.[14]

With online detection of seizures, much emphasis is placed on how quickly the seizure can be detected; the earlier the stereotyped seizure pattern can be recognized, the more useful such information becomes. Ideally, the events leading to the clinical onset of the seizure could also be characterized well enough

to predict the onset of the seizure within a clinically meaningful time window. Seizure prediction cannot be covered in sufficient detail here, but other publications provide insight and discuss the controversies associated with computational modeling for prediction.[27,28]

Even if seizure dynamics could be characterized well enough that detection theoretically could be reliably performed early in the seizure, analysis speed is a challenge. We note that the Lopour-Szari model[14] required 5 min of computation time to analyze seizure dynamics and then compute and deliver a charge-balanced correction signal to end the seizure. However, most seizures self-terminate well within 5 min of onset.[29] For such an approach to be helpful, the detection and analysis should finish in just a few seconds, a speed increase of two orders of magnitude. The speed could be increased by running the analysis at a lower resolution[14] and more computational resources could be devoted to the task, but some technical innovation will be required for this algorithm to be incorporated in a portable seizure detection device.

Even with a therapeutic device in the FDA approval process, novel approaches to seizure detection and intervention are still relevant. Not all patients respond to the RNS™ device,[30] and for those who do, the therapeutic effects are not immediate. Tuning these devices to properly detect seizures currently takes many months, even for patients with relatively frequent seizures (i.e., at least three per month).[31] For patients with less frequent seizures, it is possible that the detection tuning process could take over a year.

Because seizure dynamics vary so widely among patients, it seems that a calibration period will be unavoidable for any detection device. However, as the field of seizure detection advances, calibrating the detection algorithm will likely require fewer seizures and fewer hours of physicians' time. Ideally, devices would be able to calibrate themselves. Current devices are already monitoring the patient's response continuously, even when the patient is receiving the therapeutic correction signal produced by the device in response to a seizure. As we develop a better understanding of seizure dynamics, that knowledge can be translated into intelligent devices capable of analyzing and correcting their own performance.

Static Therapies

In contrast to devices that detect and respond to seizures, there are also devices that deliver scheduled pulses, that is, their activity is defined by a preset pattern (periodic pacing), independently of when the patient seizes. Such devices have also shown a promising reduction in seizure frequency and do not require an extended calibration period, as they do not need to detect seizures.[2]

Other therapies that exert their effects independently of the patient's state include pharmacological treatments.[21,22] These agents affect subcellular mechanisms, such as ion channels, transporters, pumps, and receptors. Computational models that incorporate these subcellular mechanisms can help identify therapeutic targets and show how their modulation may influence dynamics at the cellular and network levels.

Here, we will introduce a computational model used by Dyhrfjeld-Johnsen et al.[38] to illustrate the effect of a channelopathy on cellular excitability. Channelopathies—pathological changes in the expression or function of ion channels—are of great interest in the field of epilepsy because they have been linked to both inherited and acquired epilepsies. For those inherited epilepsies whose mutations have been discovered, the mutations have been predominantly channelopathies.[32]

One common channelopathy occurring in epileptic animal models affects the hyperpolarization-activated, cyclic nucleotide-gated (HCN) channel responsible for an inward, mixed-cation current (I_h). Most experimental paradigms report that seizures reduce I_h,[33–37] a reduction thought to increase hyperexcitability[33] because it lessens the shunting effect associated with I_h. However, in an experimental febrile seizure model, the hyperexcitable dendrites of CA1 pyramidal cells show a marked increase in I_h.[38] Computer modeling provided an explanation for these seemingly contradictory results.

Using a computational model, Dyhrfjeld-Johnsen et al.[38] altered the h-current and examined the effects on the excitability of three detailed pyramidal cell models (Fig. 23–2). The models indicated that an increased I_h can contribute to hyperexcitability (Fig. 23–2A). Analysis of the mechanism by which I_h enhanced excitability showed that the upregulated I_h

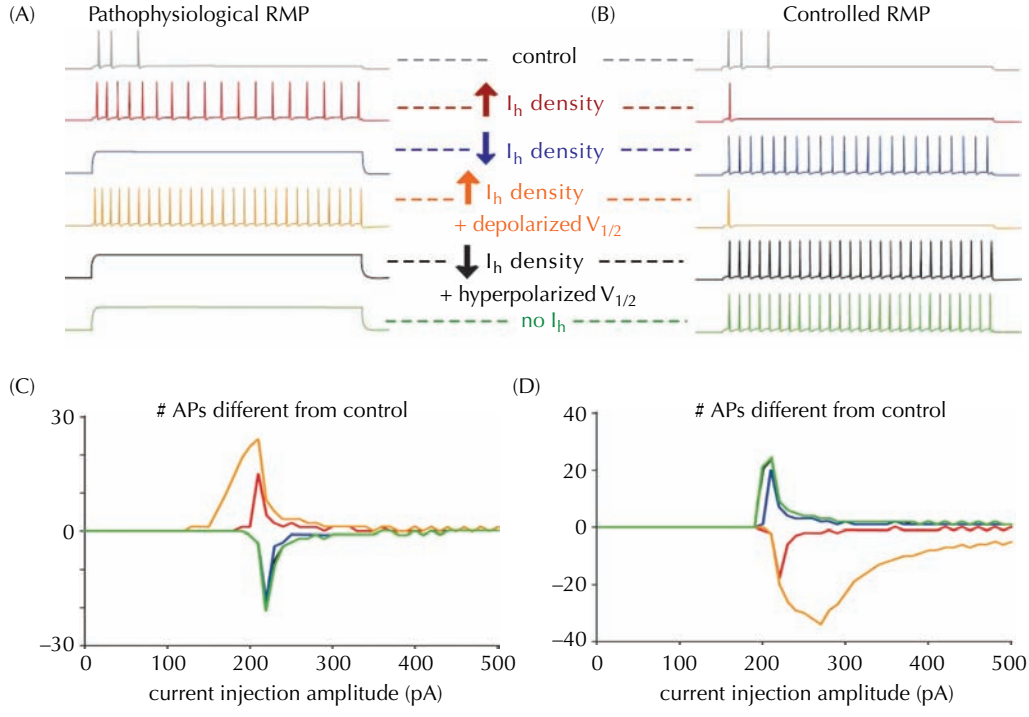

Figure 23–2. Altered h-current changes neuronal excitability. Both the modified control CA1 pyramidal neuron model[38,68] and models with altered I_h are subjected to a 1000 ms depolarizing current injection, with the membrane potential allowed to vary from control resting membrane potential (left column) or clamped at control resting membrane potential (right column). **A, B.** Example traces of simulated neuronal behaviors in response to depolarizing current injection (+210 pA) in the cases of pathologically depolarized (**A**) and controlled (**B**) resting membrane potential (RMP). **C, D.** For each amplitude of current injection, the activity of the models with altered I_h is contrasted with that of control models by comparing the number of action potentials fired. A positive difference means that the pathophysiological model has become more excitable than the control model; a negative difference indicates that the pathophysiological model is less excitable. The resting membrane potential of the pathophysiological model is set to either the pathophysiological, depolarized resting potential (**C**) or the controlled resting potential (**D**). Adapted from ref. 69.

depolarized the dendritic resting membrane potential, shifting it closer to threshold. When cells with increased I_h were held at the control resting membrane potential, no increase in excitability was observed (Fig. 23–2B). These results highlight the complex relationship between I_h and neuronal excitability; perhaps functional effects cannot be predicted by looking at only the direction of changes in I_h.

Knowing that the hyperexcitability seen in increased I_h is related to the more positive resting membrane potential, we can then propose possible therapeutic interventions. For example, a pharmacological agent that can counteract the more depolarized membrane potential[38] with minimal disturbance to physiological functions, perhaps mediated by the M-current,[38–40] should be sufficient to reduce the I_h-associated network hyperexcitability seen in the above-mentioned febrile seizure model.[38]

COMPUTER MODELING TO PREVENT EPILEPTOGENESIS

Understanding how the healthy brain becomes capable of seizing requires knowledge of the detailed changes associated with epileptogenesis. These alterations include cell loss, changes in network connectivity, mutated ion channels, and altered gene expression profiles. Quite often, multiple changes occur together, obscuring each factor's contribution to the seizure-prone brain. In this scenario, untangling the role of each factor may

be far easier in computational models than in experimental ones.

Head trauma is one model of epilepsy in which multiple changes are known to occur. These alterations affect, among other brain regions, the dentate gyrus, an area of the hippocampal formation thought to gate network activity in the healthy brain. Epileptogenic changes seen in the dentate gyrus after head trauma include mossy fiber sprouting and hilar cell loss. Mossy fiber sprouting, that is, the development of new axonal branches by granule cells, accounts for recurrent excitation among granule cells, consequent to the establishment of reciprocal synaptic contacts that are not normally present in the healthy dentate gyrus. Hilar cell loss not only affects inhibitory interneurons, but also reduces the number of excitatory mossy cells. The effect of reducing the number of excitatory mossy cell synapses onto the granule cells is not well understood.

It is quite challenging to separate these alterations in experimental models of epilepsy, but computer modeling provides a flexible way to study the changes in isolation. Santhakumar et al.[19], Dyhrfjeld-Johnsen et al.,[17] Morgan and Soltesz,[18] studied, in progressively more detail, the effects of structural changes in the dentate circuitry on hyperexcitability. The first of these studies, by Santhakumar et al.,[19] played a major role in demonstrating the promise of large-scale, data-driven models in epilepsy research, as it detailed the construction of the dentate gyrus model, containing four biophysically realistic cell types, each connected via realistic synapses using connection probabilities derived from in vivo and in vitro experiments. Using the 500-cell model that was thus constructed, Santhakumar et al.[19] were able to show that structural changes alone can predispose the dentate network to hyperexcitability.

Dyhrfjeld-Johnsen et al.[17] expanded the functional model of Santhakumar et al.[19] to over 50,000 cells, a scale of 1:20 compared to the full-size rat dentate gyrus. Their experiments also used a complete 1:1 scale structural model of the dentate gyrus to determine the graph-theoretical characteristics of the dentate network. The results of the structural model showed that the dentate gyrus is a small-world network. In a small-world network, cells make many connections to their close neighbors, yet the number of cells along a path from a given cell to any distant cell remains quite small due to the presence of a few long-distance connections. By combining the structural model results with those of the functional model at varying levels of dentate injury, Dyhrfjeld-Johnsen et al.[17] showed that head injury causes substantial alterations to the small-world structure of the dentate gyrus, resulting in a hyperexcitability profile closely correlated with structural alterations.

Because the specific pattern of new granule cell connections that occurs with mossy fiber sprouting is not known, Morgan and Soltesz[18] studied the pathological alterations in the dentate gyrus circuitry in even more detail. They questioned whether the hyperexcitability of the sprouted network depends on the specific pattern of new connections. To answer the question, Morgan and Soltesz compared nonrandom patterns of connectivity to a control network in which the granule cells were connected together randomly (Fig. 23–3A). In each case, the model had the same number of connections among granule cells, but the connections were distributed differently among the cells.

Morgan and Soltesz found that simply rewiring the granule cell network in certain ways could markedly increase excitability (Fig. 23–4). In particular, if the connections were redistributed so that most granule cells had few granule cell connections but some rare granule cells (*hubs*) had many connections (Fig. 23–3B), network activity increased greatly. These highly connected cells made the dentate network significantly more excitable without changing the total excitatory drive of the network, demonstrating the potential importance of hub cells in seizures (compare Fig. 23–4A and 23–4B). Experimental work has supported the presence of hub cells in the dentate gyrus after injury, thus beginning to validate the model's prediction and pointing to the importance of understanding the microcircuit connectivity in seizure-prone networks.[41] This work suggests that therapeutic efforts to prevent mossy fiber sprouting could reduce epileptogenesis in people who have experienced head trauma.

Epileptogenic changes are not limited to neurons and their connections. Glia dysfunction has been implicated in epileptogenesis, and therefore deserves consideration as a possible therapeutic target.[42–44] Its significance arises

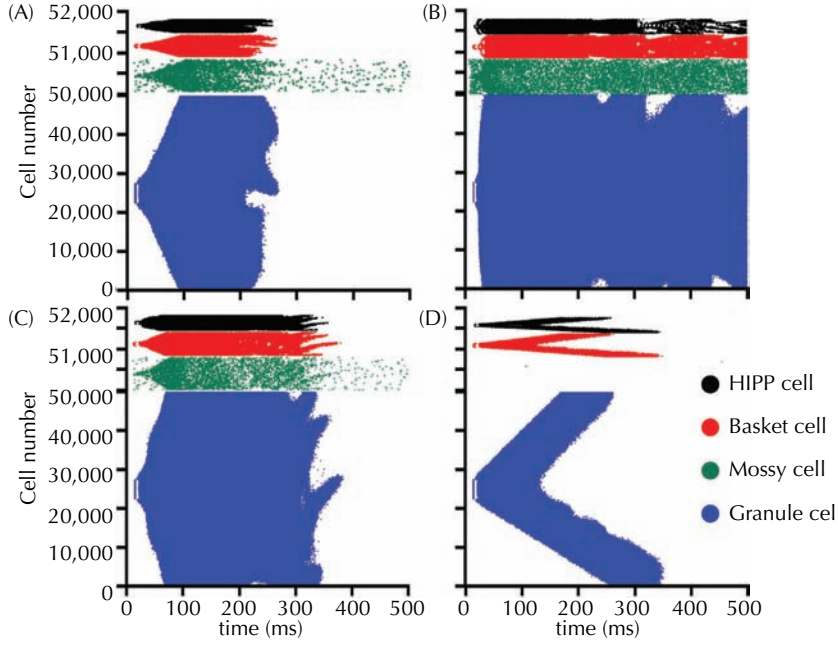

Figure 23–3. Reconnecting the granule cell network. In temporal lobe epilepsy, granule cells in the dentate gyrus sprout axon collaterals that synapse onto other granule cells, creating a recurrent excitatory circuit. **A.** In the control network, connections were modeled as random, constrained only by the extent of the granule cells' axonal arbors.[17] **B.** In the fluid percussion injury (FPI) network model, the same total number of connections are introduced, but the connectivity is different. A few cells are highly connected, while the rest remain sparsely connected. Note that the experimental network depicted here is only a visual guide to understanding the general connectivity of the network.

especially because of the glial role in regulating the concentration of extracellular potassium and because of the ability of glial cells to interfere with inhibition,[45] to release adenosine triphosphate (ATP),[39] and to affect the glutamate concentration at nearby synapses.[11,44,46] Cressman et al. and Ullah et al. investigated the role of ion concentrations and glia in epileptic networks using a model of a single cell[11] and a network.[44]

In this work, Cressman et al. first examined the effects of intracellular and extracellular ion concentrations on neuronal excitability and seizure frequency with a mathematical model of a neuron and its extracellular space, including the glia surrounding it.[11] Using the model, they showed that this single cell was capable of producing abnormal bursts of action potentials, as may be seen during seizures. The model also illustrated how increased extracellular potassium could lead to depolarization block, during which the cell is depolarized but unable to spike. Some studies have implicated depolarization block in inhibitory interneurons during seizure-like events in brain slices, which could free excitatory cells to spike excessively in bursts.[11,47]

Then, to examine the role of glia directly, Ullah et al. extended the single-cell model to a 200-cell network model consisting of pyramidal and inhibitory cells and accounting for glial function, ion pumps, and diffusion. Their model illustrated how glial dysfunction could cause neural networks to be less resilient to small perturbations such that a network exhibiting the normal persistent activity associated with higher-level functions could suddenly transition to seizure-like activity.[44] For example, they showed that the rearrangement of astrocytes associated with epileptogenesis leads to an increased extracellular potassium concentration and a reduced ability to buffer the extracellular potassium, as well as increased strength of excitatory synapses.[44] The behavior of their model was consistent with several experimental results,[44] and it underscores the potential of glia as therapeutic targets.

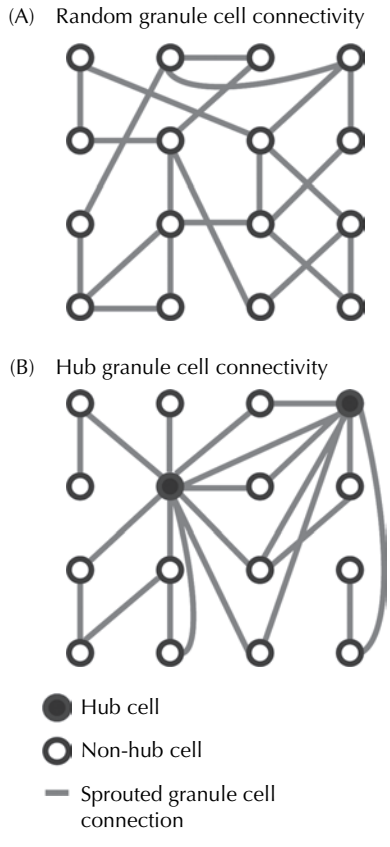

Figure 23–4. Highly interconnected granule cell hubs greatly augment network excitability. Both **A** and **B** represent raster plots of granule cell firing in a network with 50% of maximal granule cell-to-granule cell connections, where stimulation was delivered via the perforant path to 1% of the granule cells. Both networks have the same number of added connections, just distributed differently. Representative firing patterns of cells in the network are shown on the right of the raster plots. **A.** The sprouted connections are randomly distributed throughout the network. **B.** The sprouted connections are distributed such that 5% of granule cells make 210 more connections than the average granule cell and thus serve as hubs. Adapted from ref. 18.

COMPUTER MODELING TO ASSIST RESEARCHERS

Computer modeling and experimentation are complementary in research and the development of therapeutics. Each approach provides unique benefits and is improved by contributions of the other. Though definitive confirmation of scientific fact requires experimental results, modeling serves the field by enabling a flexibility and efficiency not always possible in experiments, and it can help researchers design more focused experiments.[39]

One example of a scenario in which computer modeling allowed researchers to extend their experimental observations occurred during an electrophysiological study of hippocampal mossy cells (see also above) after head injury. These excitatory neurons, located in the dentate hilus, are among the most vulnerable neurons in the entire mammalian brain[48]; factors including strong excitatory inputs, a sustained response to excitatory input, and a high level of spontaneous activity render them particularly susceptible to excitotoxicity.[49] Significant mossy cell loss is observed in human epileptic tissue and in animal models after experimental head trauma.[50–52] However, several lines of evidence suggest that some mossy cells survive trauma[49,50,52] and that these surviving mossy cells may spread or amplify dentate network hyperexcitability.

Howard et al.[53] used a rat fluid percussion injury model to study the changes in surviving mossy cells after head trauma, comparing their *I-F* (current versus spike frequency) and *I-V* (current versus membrane potential) curves before and after trauma. Surprisingly, they found no significant difference. Yet, on closer inspection, extensive, opposing alterations were found in various membrane currents and properties that together resulted in the unchanged *I-F* and *I-V* relationships observed in mossy cells after head trauma. The resting membrane potential was depolarized, the tetraethylammonium (TEA)-sensitive potassium current was decreased, and the voltage-dependent sodium channel required more depolarized potentials to activate.

Howard et al.[53] then used computer modeling to understand whether each one of these changes would affect the dentate network when considered separately. Notably, when modeled alone, each of these alterations dramatically affected network excitability (Fig. 23–5), despite canceling each other when occurring together. In this case, the computational model complemented the experimental observations by vividly demonstrating the importance of potentially homeostatic mechanisms[54] in regulating overall levels of neuronal activity in large networks.

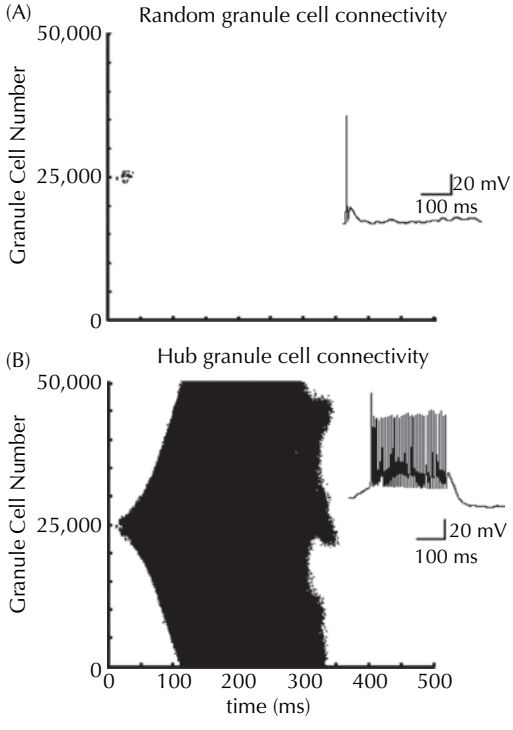

Figure 23–5. Individual posttraumatic changes to intrinsic properties of mossy cells have robust effects on dentate gyrus network hyperexcitability. Dentate gyrus network activity in response to a single simulated perforant path stimulation at $t = 5$ ms is plotted as cell number versus time. Each dot represents an action potential in one cell (blue: granule cell; green: mossy cell; red: basket cell; black: hilar-perforant path associated (HIPP) cell). Note that the scale for granule cell numbers is smaller than that for other cells. **A.** Network activity of the dentate gyrus in the FPI model with biologically realistic levels of mossy fiber sprouting and hilar cell loss. All subsequent simulations were performed with these anatomical changes in place. **B.** Network hyperexcitability is increased when mossy cell V_m is depolarized by 3 mV in the FPI model. **C.** Hyperexcitability of the network is also increased when the model is implemented with an increased mossy cell-to-granule cell synaptic conductance, representing the effect of the increased mossy cell action potential width due to change in tetraethylammonium (TEA)-sensitive potassium current. **D** Network hyperexcitability is decreased significantly when the activation curve of I_{Na} in mossy cells is shifted by 5 mV. Note that only 2 of the 750 mossy cells fire during the 500 ms simulation. Figure adapted from ref. 53.

SUMMARY OF APPROACHES TO MODELING EPILEPSY

The models described above vary in their level of detail and scope (see Fig. 23–1), and there are valid reasons for the different approaches. Researchers seeking an overall description of seizure dynamics would not necessarily want it to be in terms of the activity of thousands of neurons. For them, a higher-level approach, based on the assumption that the neural network can be characterized in aggregate terms, would be more appropriate. These aggregate, macroscopic models, such as the Lopour-Szeri model described above,[14] are often called *mean-field models* (Fig. 23–1A), indicating that their base components are entire populations of neurons similar enough to be grouped together and described in terms of average properties. Most such approaches are based on a computational model developed by Wilson and Cowan[55] that had two interconnected neuronal populations, one excitatory and one inhibitory. Though the cells are described as populations, using an average rather than individually, the average properties are still informed by physiological data. The models often produce results in the form of EEG traces, directly comparable to patient data.

Such models may also make predictions about the role of a cell population in seizure dynamics, such as one model that split the inhibitory population into separate fast and slow components while maintaining a single excitatory population.[13] The model was fit to real patient EEG data taken from preictal, preonset, and ictal time periods, and it could produce realistic EEG waveforms. The model made several experimentally verifiable predictions about the role of excitatory and inhibitory populations in seizures, including—paradoxically—that slow inhibition increases prior to seizure onset but contributes to overall excitation by inhibiting mainly the fast inhibition component.[13]

These macroscopic models have an advantage in describing seizure dynamics because they require relatively few computational resources. Their smaller computational requirements enable them to simulate the longer time periods required to model transitions into and out of the seizure state. Understanding what prompts the transition from nonepileptiform activity to the seizure state is crucial for seizure detection and intervention, and high-level models are well suited to describe such system-level dynamics.[56]

In contrast, there are questions that call for much lower-level, detailed computational models. For example, researchers interested in how a particular ion channel mutation

affects network hyperexcitability would need a highly detailed, biologically realistic model that could incorporate well-characterized ion channel properties and output precise spike times for each cell in the network (Fig. 23–1B). Such computational models are also useful for exploring the altered connectivity and cell loss seen in most models of epilepsy.[19] The various models from the Soltesz lab described above are examples of large-scale, highly detailed, biologically realistic network models.[17–19,38,53]

Even within network models that include multiple distinct cells, there is a wide variety of approaches. Neurons can be modeled at various levels of detail, ranging from morphologically detailed models with over 1000 compartments or morphologically simplified cells with detailed subcellular mechanisms (Fig. 23–1D) to integrate-and-fire neurons (Fig. 23–1C) and even simpler representations.[34] The level of sophistication at which a neuron should be modeled depends on the complexity of the computation it is expected to perform. While integrate-and-fire neurons may be sufficient to produce expected spiking patterns in some cases,[19] specific distributions of ion channels and synapses along dendrites may be necessary to model more complicated functions performed by neurons, such as coincidence detection.[34] The Cressman et al. and Ullah et al. models described above[11,44] employed mathematical neurons with subcellular and extracellular mechanisms described by a few equations. This level of detail was sufficient to answer the questions they posed in the model and enabled them to run the longer simulations necessary to characterize the dynamics in the ion concentrations. To model the astrocytic rearrangement more concretely, as they propose for future work, a more detailed model that incorporated three-dimensional cell positions would probably be necessary.[44]

As mentioned in passing above, computer modeling is limited by time and resource availability. If a model contains very detailed components (such as numerous ion channel types or detailed cell morphology), it may be necessary to remove complexity from another area of the model, either the scope (number of cells) or the length of simulation time (whether the model simulates activity occurring over a few milliseconds or several days). For this reason, often the more detailed a model is, the smaller its scope and simulation time.

Because of the above limits, computational models used in epilepsy generally describe only part of the disorder,[20] such as aggregate electrical dynamics during seizures[13] or altered neural connectivity found in the affected areas of the epileptic brain.[19] While partial models can provide much insight, there is significantly more explanatory power in a model that answers questions covering multiple levels simultaneously, such as: how do the altered firing patterns of cell type A influence the EEG signal after seizure onset?

THE FUTURE OF COMPUTER MODELING OF EPILEPSY

Given the rapid rate at which technology is developed, it is easy to imagine that computers will soon be fast enough to support a detailed, biologically realistic model of the entire human brain that could answer questions such as the one above.[5] Today modelers must choose between modeling seizure dynamics and transitions (which require lengthy simulation times) or detailed biological structures and mechanisms; in the future, it will be possible to do both at once. Such a powerful model would represent a significant tool for understanding overall seizure and transition dynamics in terms of network and cell-level activity, an area of epilepsy research that needs significant development.[47]

Already, advances in neural simulation tools allow them to run simulations in parallel (on multiple computers at once) in a fraction of the time it would take to run the same simulation on a single processor. These parallel models can support far more neurons and detail so that, for example, network models focusing on altered connectivity in epilepsy can be scaled up to full size to enable a more realistic picture of neural connectivity. Additionally, detailed network models of particular brain regions could be made bilateral. Both computational models and in vitro experiments generally approach epilepsy "unilaterally," without regard to which side of the brain is being observed or any effects of the contralateral hemisphere. However, the two sides of the brain vary remarkably in ways relevant to epilepsy. For example, there is known to be significant asymmetry between the bilateral connections of the hippocampal

formation.[57] Bilateral models could address the effects of contralateral connections and asymmetry[57] on seizures and seizure generalization.

The computational models all described so far have been simulations, in which software is configured to produce the model. However, as we create models with increasing numbers of cells, it is worth noting that a model of the whole human brain would be quite resource-intensive. For example, the Blue Brain project mentioned above uses 8192 processors to model 10,000 highly detailed multicompartmental neurons with 100 million synapses.[5,58] A model of the whole human brain would need to include 100 billion neurons and 10 quadrillion synapses, an expensive endeavor that is projected to require millions of processors and roughly 100 megawatts of power.[58]

To overcome these limitations, another approach to modeling has been developed. Rather than configure software, some modelers are configuring hardware to emulate neural networks, such as the recent Neurogrid project.[58] The resulting neuromorphic chips can be combined to produce a device capable of emulating 1 million neurons and 6 billion synapses in real time. Though these neurons are less detailed than those on the Blue Brain computer, they still contain multiple compartments and realistic ion channels. Highly detailed neurons can be modeled as well if the overall number of neurons is decreased; models with hundreds of compartments and any number of distinct ion channel types are not unfeasible.[58]

One of the main arguments against hardware modeling is its lack of flexibility. However, significant improvements have been made to address this limitation. The current Neurogrid device can support 16 different cell types, each having its own combination of ion channels.[58] The connections between cells are programmed using random access memory (RAM) and can be changed on demand. The results of the neuromorphic simulations can be viewed on an interactive display in real time at various levels, from the spike patterns of a single cell to the activity of a whole cortical layer. The applications of such a tool to epilepsy research are apparent: the ability to quickly model such a large number of neurons over a long simulation time is just what we need to characterize seizure dynamics in terms of cell and ion channel activity.

COMPUTER MODELING RESOURCES

Computer modeling is a useful research tool available to almost everyone. The monetary investment in computer modeling can be small, requiring resources available to anyone with a computer and Internet access or, for more intensive modeling, resources readily accessible at research institutions or through multipurpose shared resources such as the National Science Foundation's TeraGrid.[59]

Researchers and clinicians interested in computer modeling are urged to explore the many computational models freely available online at the ModelDB Web site.[60] In most cases, the software used to create such models is also freely available online (e.g., the neural simulators NEURON[61] and GENESIS[62]) or through network licenses at academic institutions (MATLAB[63]). The time required to learn these programs is comparable to the time required to learn various experimental techniques, and results can be obtained quickly. Importantly, the researcher has far more control over the factors important to obtaining results from models than is possible with experiments.

The large-scale, detailed, biologically realistic models referenced above employed NEURON, but many successful models have employed GENESIS as well. For example, van Drongelen et al.[64] produced a detailed, 656 cell neocortical network with GENESIS. The model included two types of pyramidal cells, basket cells, and chandelier cells, with small-world connectivity. The cells contained realistic ion channels and gap junctions. The authors used this model to explore the effect of synapse weights on the ability of the cortical network to form and sustain seizure-like oscillations and to observe the activity displayed by subpopulations of neurons during these oscillations. Surprisingly, among other results, they found that a weakening of excitatory connections in the neocortex may enable the propagation of seizure-like activity, and they concluded that strong excitatory connections are not always necessary for a network to produce seizures.[64]

Both NEURON and GENESIS can be run in parallel, which allows for a near-linear speed increase in modeling time; to run a model for the same simulation on two processors will take roughly half the time taken to run the model on

one processor. The authors recently ran a 1000 ms simulation of over 1 million detailed cells in less than 2 hour, using parallel NEURON on 608 processors.[65] MATLAB can be run in parallel as well, using its Parallel Computing Toolbox.

All three of these software programs have extensive online documentation, tutorials, and user forums supported by the software developers. Both NEURON and MATLAB regularly hold in-person workshops as well. While both NEURON and GENESIS have graphical user interfaces (GUIs), these GUIs are generally most useful when building single-cell models. For network models, there is a simulation software-independent tool called NeuroConstruct, which provides an interface to NEURON, GENESIS, and other programs for those who prefer not to work with code directly.[66]

In addition to software-specific resources, there are some excellent books on computational neuroscience in general[67] and computational modeling of epilepsy in particular.[9] They are useful for the potential modeler or for anyone wanting a deeper understanding of computer modeling in neuroscience and epilepsy.

CONCLUSION

A detailed model of the entire human brain, which could be accessed at any level of detail and be personalized for individualized patient treatment analysis, no longer seems outlandish. Likewise, it appears that an implantable seizure detection and intervention device will be available as a standard treatment option before long. Such exciting advances made possible through computer modeling should inspire researchers and clinicians to be even more creative in their approaches to epilepsy therapies. We look forward to the advances the next decade will bring in computer modeling of epilepsy.

NOTES

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DISCLOSURE STATEMENT

The authors have no conflicts of interests to disclose.

REFERENCES

1. World Health Organization. Epilepsy. [Epilepsy fact sheet]. http://www.who.int/mediacentre/factsheets/fs999/ en/index.html. Accessed August 1 2010.
2. Jobst BC, Darcey TM, Thadani VM, Roberts DW. Brain stimulation for the treatment of epilepsy. *Epilepsia.* 2010;51:88–92.
3. Glasscock E, Qian J, Yoo JW, Noebels JL. Masking epilepsy by combining two epilepsy genes. *Nat Neurosci.* 2007;10(12):1554–1558.
4. Hines M, Markram H, Schürmann F. Fully implicit parallel simulation of single neurons. *J Comput Neurosci.* 2008;25(3):439–448.
5. Markram H. The Blue Brain Project. *Nat Rev Neurosci.* 2006;7(2):153–160.
6. Rolls ET, Loh M, Deco G, Winterer G. Computational models of schizophrenia and dopamine modulation in the prefrontal cortex. *Nat Rev Neurosci.* 2008;9(9): 696–709.
7. Moustafa AA, Gluck MA. A neurocomputational model of dopamine and prefrontal–striatal interactions during multicue category learning by Parkinson's patients. *J Cogn Neurosci.* 2011;23(1):151–167.
8. Reinkensmeyer DJ, Iobbi MG, Kahn LE, Kamper DG, Takahashi CD. Modeling reaching impairment after stroke using a population vector model of movement control that incorporates neural firing-rate variability. *Neural Comput.* 2003;15(11):2619–2642.
9. Soltesz I, Staley KJ. *Computational Neuroscience in Epilepsy*. New York: Elsevier; 2008.
10. Poirazi P, Brannon T, Mel BW. Arithmetic of subthreshold synaptic summation in a model CA1 pyramidal cell. *Neuron.* 2003;37(6):977–987.
11. Cressman J, Ullah G, Ziburkus J, Schiff S, Barreto E. The influence of sodium and potassium dynamics on excitability, seizures, and the stability of persistent states: I. Single neuron dynamics. *J Comput Neurosci.* 2009;26(2):159–170.
12. Poolos NP, Migliore M, Johnston D. Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. *Nat Neurosci.* 2002;5(8): 767–774.
13. Wendling F, Hernandez A, Bellanger J-J, Chauvel P, Bartolomei F. Interictal to ictal transition in human temporal lobe epilepsy: insights from a computational model of intracerebral EEG. *J Clin Neurophysiol.* 2005;22(5):343–356.
14. Lopour B, Szeri A. A model of feedback control for the charge-balanced suppression of epileptic seizures. *J Comput Neurosci.* 2010;28(3):375–387.
15. Brunel N. Dynamics of networks of randomly connected excitatory and inhibitory spiking neurons. *J Physiol (Paris).* 2000;94(5–6):445–463.
16. Brunel N, Wang X-J. What determines the frequency of fast network oscillations with irregular neural discharges? I. Synaptic dynamics and excitation-inhibition balance. *J Neurophysiol.* 2003;90(1):415–430.

17. Dyhrfjeld-Johnsen J, Santhakumar V, Morgan RJ, Huerta R, Tsimring L, Soltesz I. Topological determinants of epileptogenesis in large-scale structural and functional models of the dentate gyrus derived from experimental data. *J Neurophysiol.* 2007;97(2): 1566–1587.
18. Morgan RJ, Soltesz I. Nonrandom connectivity of the epileptic dentate gyrus predicts a major role for neuronal hubs in seizures. *Proc Natl Acad Sci USA.* 2008;105(16):6179–6184.
19. Santhakumar V, Aradi I, Soltesz I. Role of mossy fiber sprouting and mossy cell loss in hyperexcitability: a network model of the dentate gyrus incorporating cell types and axonal topography. *J Neurophysiol.* 2005;93(1):437–453.
20. Lytton WW. Computer modelling of epilepsy. *Nat Rev Neurosci.* 2008;9(8):626–637.
21. Weaver DF. Principles and practice of computer-aided drug design as applied to the discovery of antiepileptic agents. In: Soltesz I, Staley K, eds. *Computational Neuroscience in Epilepsy*. San Diego, CA: Academic Press; 2008:515–529.
22. Jorgensen WL. The many roles of computation in drug discovery. *Science.* 2004;303(5665):1813–1818.
23. NeuroPace. NeuroPace submits PMA application for FDA approval of novel investigational device for epilepsy. http://www.neuropace.com/about/news/20100708.html. Accessed August 2010.
24. Echauz J, Wong S, Smart O, Gardner A, Worrell G, Litt B. Computation applied to clinical epilepsy and antiepileptic devices. In: Soltesz I, Staley K, eds. *Computational Neuroscience in Epilepsy*. San Diego, CA: Academic Press; 2008:530–558.
25. Stephens ML, Spencer DD, Cavus I, et al. Microelectrode-based epilepsy therapy: a hybrid neural prosthesis incorporating seizure prediction and intervention with biomimetic maintenance of normal hippocampal function. In: Soltesz I, Staley K, eds. *Computational Neuroscience in Epilepsy*. San Diego, CA: Academic Press; 2008:559–586.
26. Jouny CC, Bergey GK. Dynamics of epileptic seizures during evolution and propagation. In: Soltesz I, Staley K, eds. *Computational Neuroscience in Epilepsy*. San Diego, CA: Academic Press; 2008:457–470.
27. Lehnertz K, Mormann F, Osterhage H, et al. State-of-the-art of seizure prediction. *J Clin Neurophysiol.* 2007;24(2):147–153.
28. Lai Y-C, Osorio I, Frei MG, Harrison MAF. Are correlation dimension and Lyapunov exponents useful tools for prediction of epileptic seizures? In: Soltesz I, Staley K, eds. *Computational Neuroscience in Epilepsy*. San Diego, CA: Academic Press; 2008:471–495.
29. Afra P, Jouny CC, Bergey GK. Duration of complex partial seizures: an intracranial EEG study. *Epilepsia.* 2008;49(4):677–684.
30. Sun FT, Morrell MJ, Wharen RE Jr. Responsive cortical stimulation for the treatment of epilepsy. *Neurotherapeutics.* 2008;5(1):68–74.
31. NeuroPace. Clinical trials. 2010; http://www.neuropace.com/trials/overview.html. Accessed August 1 2010.
32. Steinlein OK. Genetic mechanisms that underlie epilepsy. *Nat Rev Neurosci.* 2004;5(5):400–408.
33. Jung S, Jones TD, Lugo JN Jr, et al. Progressive dendritic HCN channelopathy during epileptogenesis in the rat pilocarpine model of epilepsy. *J Neurosci.* 2007;27(47):13012–13021.
34. Marcelin B, Chauvière L, Becker A, Migliore M, Esclapez M, Bernard C. h Channel-dependent deficit of theta oscillation resonance and phase shift in temporal lobe epilepsy. *Neurobiol Dis.* 2009;33(3):436–447.
35. Shah MM, Anderson AE, Leung V, Lin X, Johnston D. Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. *Neuron.* 2004;44(3):495–508.
36. Shin M, Brager D, Jaramillo TC, Johnston D, Chetkovich DM. Mislocalization of h channel subunits underlies h channelopathy in temporal lobe epilepsy. *Neurobiol Dis.* 2008;32(1):26–36.
37. Zhang K, Peng B-W, Sanchez RM. Decreased I_H in hippocampal area CA1 pyramidal neurons after perinatal seizure-inducing hypoxia. *Epilepsia.* 2006;47(6): 1023–1028.
38. Dyhrfjeld-Johnsen J, Morgan RJ, Foldy C, Soltesz I. Upregulated H-current in hyperexcitable CA1 dendrites after febrile seizures. *Front Cell Neurosci.* 2008;2:1–8.
39. Ascoli GA, Gasparini S, Medinilla V, Migliore M. Local control of postinhibitory rebound spiking in CA1 pyramidal neuron dendrites. *J Neurosci.* 2010;30(18): 6434–6442.
40. George MS, Abbott LF, Siegelbaum SA. HCN hyperpolarization-activated cation channels inhibit EPSPs by interactions with M-type K^+ channels. *Nat Neurosci.* 2009;12(5):577–584.
41. Walter C, Murphy BL, Pun RYK, Spieles-Engemann AL, Danzer SC. Pilocarpine-induced seizures cause selective time-dependent changes to adult-generated hippocampal dentate granule cells. *J Neurosci.* 2007;27(28):7541–7552.
42. Schwarcz R. Early glial dysfunction in epilepsy. *Epilepsia.* 2008;49:1–2.
43. Barres BA. The mystery and magic of glia: a perspective on their roles in health and disease. *Neuron.* 2008;60(3):430–440.
44. Ullah G, Cressman J Jr, Barreto E, Schiff S. The influence of sodium and potassium dynamics on excitability, seizures, and the stability of persistent states: II. Network and glial dynamics. *J Comput Neurosci.* 2009;26(2):171–183.
45. Ortinski PI, Dong J, Mungenast A, et al. Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nat Neurosci.* 2010;13(5): 584–591.
46. Somjen G, Kager H, Wadman W. Computer simulations of neuron-glia interactions mediated by ion flux. *J Comput Neurosci.* 2008;25(2):349–365.
47. Schiff SJ, Cressman JR, Barreto E, Ziburkus J. Towards a dynamics of seizure mechanics. In: Soltesz I, Staley K, eds. *Computational Neuroscience in Epilepsy*. San Dieg, CA: Academic Press; 2008:496–512.
48. Sloviter RS, Zappone CA, Harvey BD, Bumanglag AV, Bender RA, Frotscher M. "Dormant basket cell" hypothesis revisited: relative vulnerabilities of dentate gyrus mossy cells and inhibitory interneurons after hippocampal status epilepticus in the rat. *J Comp Neurol.* 2003;459(1):44–76.
49. Ratzliff AH, Santhakumar V, Howard A, Soltesz I. Mossy cells in epilepsy: rigor mortis or vigor mortis? *Trends Neurosci.* 2002;25(3):140–144.
50. Blumcke I, Suter B, Behle K, et al. Loss of hilar mossy cells in Ammon's horn sclerosis. *Epilepsia.* 2000;41(suppl 6):S174–S180.

51. Lowenstein D, Thomas M, Smith D, McIntosh T. Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. *J. Neurosci*. 1992;12(12):4846–4853.
52. Toth Z, Hollrigel GS, Gorcs T, Soltesz I. Instantaneous perturbation of dentate interneuronal networks by a pressure wave-transient delivered to the neocortex. *J Neurosci.* 1997;17(21):8106–8117.
53. Howard AL, Neu A, Morgan RJ, Echegoyen JC, Soltesz I. Opposing modifications in intrinsic currents and synaptic inputs in post-traumatic mossy cells: evidence for single-cell homeostasis in a hyperexcitable network. *J Neurophysiol.* 2007;97(3): 2394–2409.
54. Turrigiano G. Homeostatic signaling: the positive side of negative feedback. *Curr Opin Neurobiol.* 2007;17(3):318–324.
55. Wilson HR, Cowan JD. Excitatory and inhibitory interactions in localized populations of model neurons. *Biophys J.* 1972;12(1):1–24.
56. Suffczynski P, Kalitzin S, Lopes da Silva FH. Dynamics of non-convulsive epileptic phenomena modeled by a bistable neuronal network. *Neuroscience.* 2004;126(2): 467–484.
57. Shinohara Y, Hirase H, Watanabe M, Itakura M, Takahashi M, Shigemoto R. Left-right asymmetry of the hippocampal synapses with differential subunit allocation of glutamate receptors. *Proc Natl Acad Sci USA.* 2008;105(49):19498–19503.
58. Silver R, Boahen K, Grillner S, Kopell N, Olsen KL. Neurotech for neuroscience: unifying concepts, organizing principles, and emerging tools. *J Neurosci.* 2007;27(44):11807–11819.
59. Beckman PH. Building the TeraGrid. *Philos Trans R Soc A: Math, Phys Eng Sci.* 2005;363(1833):1715–1728.
60. Hines M, Morse T, Migliore M, Carnevale NT, Shepherd GM. ModelDB: a database to support computational neuroscience. *J Comput Neurosci.* 2004;17:7–11.
61. Carnevale NT, Hines ML. *The NEURON Book*. Cambridge: Cambridge University Press; 2006.
62. Bower JM, Beeman D. *The Book of GENESIS: Exploring Realistic Neural Models with the GEneral NEural SImulation System*. 2nd ed. New York: Springer-Verlag; 1998.
63. Wallisch P, Lusignan M, Benayoun M, Baker T, Dickey A, Hatsopoulos N. *Matlab for Neuroscientists: An Introduction to Scientific Computing in Matlab*. Academic Press; 2008.
64. van Drongelen W, Lee HC, Stevens RL, Hereld M. Propagation of seizure-like activity in a model of neocortex. [Review]. *J Clin Neurophysiol.* 2007;24(2):182–188.
65. Case MJ, Schneider CJ, Soltesz I. Parallel computing enables full-scale modeling of the rat dentate gyrus. Poster presented at the Joint Symposium on Neural Computation. University of California at Los Angeles, 2010.
66. Gleeson P, Steuber V, Silver RA. neuroConstruct: a tool for modeling networks of neurons in 3D space. *Neuron.* 2007;54(2):219–235.
67. Trappenberg T. *Fundamentals of Computational Neuroscience*. 2nd ed. New York: Oxford University Press; 2009.
68. Golding NL, Kath WL, Spruston N. Dichotomy of action-potential backpropagation in CA1 pyramidal neuron dendrites. *J Neurophysiol.* 2001;86(6):2998–3010.
69. Dyhrfjeld-Johnsen J, Morgan RJ, Soltesz I. Double trouble? Potential for hyperexcitability following both channelopathic up- and downregulation of I(h) in epilepsy. *Front Neurosci.* 2009;3(1):25–33.

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SECTION 3

Mechanisms of Seizures Susceptibility and Epileptogenesis

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Chapter 24

Traumatic Brain Injury and Posttraumatic Epilepsy

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Isabel Parada
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The epidemiology of posttraumatic epilepsy (PTE) has been extensively analyzed and reviewed in a number of studies of both civilian and military brain injuries[1,2] (reviewed in ref. 3). Several conclusions from this research are relevant to considerations of the potential mechanisms and prophylaxis of PTE. Results clearly show that the incidence of PTE is related to the severity of injury, and is therefore significantly higher in the military during wartime than in the civilian population, ranging up to 53% with penetrating wounds[1,2] (reviewed in ref. 3). Both the increased incidence at older ages and the potential development of PTE by the large number of individuals who have survived severe concussive injury during recent conflicts suggest that the size of the affected population will increase in coming years, emphasizing the need for understanding the underlying pathophysiological processes and the development of prophylactic strategies.[4,5] Although initial seizures in those who develop epilepsy most commonly have a focal origin in neocortex, both partial neocortical and temporal lobe epilepsy can follow traumatic brain injury (TBI) in humans.[6] One remarkable feature of PTE is the variable, often very prolonged latency from injury to epilepsy, which can range from weeks to years.[1,2,6] This provides a possible window for prophylactic intervention once more information regarding the underlying pathophysiological processes

and strategies for modifying them is available. However, the long latency also represents a potential therapeutic problem, particularly in the absence of reliable biomarkers of epileptogenesis in progress. This chapter will focus on examples of aberrant excitatory and inhibitory processes in injured epileptogenic cortex and potential approaches to prevention of epileptogenesis that are focused on these pathophysiological mechanisms. Some of the challenges for the development of prophylactic therapies are also discussed. Readers are referred to a number of reviews and papers published very recently that deal with various aspects of the basic mechanisms, pathogenesis, and potential prophylaxis of PTE and complement the areas covered in this chapter.[6–18]

SPECTRUM OF POTENTIAL EPILEPTOGENIC MECHANISMS INDUCED BY TBI

A large number of alterations in gene expression[19] and a variety of pathophysiological processes occur in parallel following a brain injury[20–22] (reviewed in refs. 7 and 23), making it unlikely that an intervention focused on any one of these, in isolation, will emerge as a prophylactic "silver bullet." The situation is further complicated by the likelihood that variables such as the level of brain maturation, site and distribution of injury (focal vs. multifocal vs. diffuse), type of trauma (e.g., concussive vs. penetrating), presence or absence of significant bleeding, and other factors may affect the underlying type and sequence of epileptogenic events and the optimal timing of a potentially successful intervention in a given individual. Do different combinations of pathophysiological mechanisms underlie human epileptogenesis that follows different types of cortical injuries, such as those due to stroke with cortical infarction, penetrating versus closed concussive head injuries, focal infections, or other etiologies? The same question is relevant to potential similarities or differences in events underlying chronic epileptogenesis in various models of TBI, such as fluid percussion injury (FPI)[24] versus controlled cortical impact (CCI)[25] versus neocortical partial isolation or "undercut."[26] Are underlying mechanisms in these models in neocortex the same as those in posttraumatic temporal lobe epilepsy models, or when hippocampal damage is induced by status epilepticus rather than direct trauma? These are critical questions because they bear on potential prophylactic therapies and, unfortunately, the detailed data required for answers are incomplete.

A survey of the limited cellular results from neocortical injury models, and from animals whose temporal lobes are injured in the course of experimental status epilepticus, as well as from available human material, indicates that two pathophysiological processes are prominent in focal epileptogenesis, namely, enhanced excitatory connectivity[10,27–34,] and alterations in GABAergic inhibitory mechanisms.[33,35–40] But even within these broad categories, different types of abnormalities may be present that will require different prophylactic or therapeutic approaches. For example, disinhibition might involve alterations in gama-aminobutyric acid A ($GABA_A$) receptor subunits,[41,42] decreases in voltage-dependent calcium channels[40] or Na^+/K^+ adenosine triphosphatase (ATPase) at inhibitory terminals,[43,44] shifts in the chloride gradient due to changes in expression of chloride transporters KCC2 or NKCC,[45–47] loss of inhibitory connectivity due to structural changes in interneurons,[16,48] or actual loss of interneurons of various subtypes.[36,38]

Subsets of abnormalities can also affect the mechanisms controlling excitation, such as alterations in the probability of release (Pr) at terminals,[49] burst firing in axons[50] (reviewed in ref. 51), receptor efficacy or number,[52–56] and dysfunction of ion or transmitter transport.[57–62] In addition to alterations in inhibitory efficacy and enhanced excitation, many other potentially epileptogenic changes are present following injury, such as alterations in voltage-dependent ion channels,[63–67] blood-brain barrier disturbances,[68] inflammatory responses and release of cytokines,[21,69] alterations in glia,[70,71] and so on. In terms of evaluation of therapeutic trials of potential prophylactic agents, this plethora of abnormalities raises a difficult issue: a single agent may fail to prevent PTE even though it is effective at its intended target, that is, a false-negative result may be obtained due to the presence of other epileptogenic mechanisms acting in parallel.

CHOICE OF MODELS FOR RESEARCH ON PTE

There is no perfect model of human PTE. The advantages and disadvantages of acute and chronic models of epilepsy have recently been reviewed in detail.[72] Fluid percussion injury, CCI, and undercut models each have their place in advancing our understanding of PTE. Valuable information has also been obtained from status epilepticus temporal lobe injury models, although direct traumatic injury is not present and the resulting epileptogenesis represents a different epilepsy syndrome that may involve a somewhat different spectrum of underlying mechanisms. Discussions about the merits of one model versus another thus are only useful in the context of the particular pathophysiological process or event to be investigated. Obviously, to determine whether a drug will be prophylactic against seizures in vivo, a model in which there might be extensive injury and an expected high incidence of electrographic and behavioral posttraumatic seizures at relatively short latency after injury (i.e., high throughput) would be most practical and desirable.[73–75] However, this might not be the model of choice for investigation of the details of functional or structural alterations in neocortical GABAergic interneurons or pyramidal cells that occur at a site of stereotyped restricted epileptogenic focal injury and the potential prophylactic effects induced by the same drug on these alterations. Such a question would be better addressed with a more reductionistic approach using a model that would facilitate detailed cellular in vitro experiments and avoid the complications of widespread damage and variability.[30,32,76] Both kinds of experiments are critical for progress, and fitting the preparation used to the question posed is certainly not a new concept in neurobiological research. There is no one best approach to unraveling the mechanisms underlying the pathogenesis and prophylaxis of PTE.

PARTIAL NEOCORTICAL ISOLATION (UNDERCUT) MODEL

The authors' familiarity with this model, the significant amount of anatomical and cellular electrophysiological data available (references below and in ref. 77), and the fact that this is the first case in which prophylaxis of epileptogenesis after local cortical injury has been demonstrated (discussion below), has led us to focus on the undercut model in this review. The advantages of this model have been detailed elsewhere.[77] Most important is the relatively short interval between injury and epileptiform activity that is present in a high proportion of neocortical slices cut through the damaged area and maintained in vitro[78–80] (Fig.24–1C,D). This has allowed the detailed examination of epileptogenic cellular structural and functional alterations in pyramidal (Pyr) cells and GABAergic interneurons detailed below. We have also obtained in vivo video/electroencephalographic (EEG) recordings that show electrographic and behavioral seizures beginning with a focal discharge in the undercut cortex, spreading across the cortex on the injured side, and propagating contralaterally (Fig. 2 in ref. 77).

Partially isolated neocortical islands with intact pial circulation (*undercuts*, discussed below) are an established in vivo and in vitro model for development of chronic posttraumatic hyperexcitability and epileptogenesis,[32,78,79,81,82] and partially isolated neocortex is also epileptogenic in humans[83] (Fig. 24–1A). The undercut cortex retains normal laminations (Fig. 24–1B), although it becomes thinner with modest cell loss and obvious structural alterations in deep-lying Pyr cells[78,84] (K Graber and DA Prince, unpublished findings). Disinhibition, increases in neuronal membrane excitability, and increases in excitatory synaptic coupling have been suggested as potential mechanisms in this chronic epilepsy model.[32,78,85–87] The undercut cortex becomes progressively more epileptogenic over several weeks,[82,88] and spontaneous interictal discharges can persist for at least 1 year in the monkey.[26] The time of onset of epileptogenesis, or the "critical period" in rats, occurs during the first 3 days after injury,[89] and recent data suggest that epileptogenic activity is already present 3 days after the undercut (DK Takahashi and DA Prince, unpublished results). Isolated islands of neocortical gray matter, with neuropathological evidence of substantial axonal reorganization, are also present in postmortem specimens from epileptic children who developed extensive underlying

white matter lesions as infants.[90] Interictal epileptiform activity can be recorded within partially isolated cortex of anesthetized rats, and c-*fos* immunoreactivity (IR) is increased for weeks in the injured cortex, suggesting ongoing abnormal activity.[91] Behavioral and electrographic seizures occur in in vivo experiments on monkeys, cats, and rodents in this model (see above and references in ref. 77). Areas of partially isolated cortex with underlying loss of white matter are also present in the cortical contusion (Fig. 2 in ref. 25 and FPI models (Fig.1D in ref. 23), although it is not clear whether seizure activity originates from the cortex above these sites. Of interest are reports of chronic electrographic and clinical seizures in humans as a complication of psychosurgery in which connections from portions of frontal lobes were severed, essentially producing large, partially isolated neocortical slabs[92,93] (Fig. 24–1A).

Abnormalities in Excitatory Mechanisms in Undercut Cortex

The capacity of injured brain to make new connections has been known since the groundbreaking anatomical studies of Cajal,[94] who described sprouting of injured Pyr cell axons

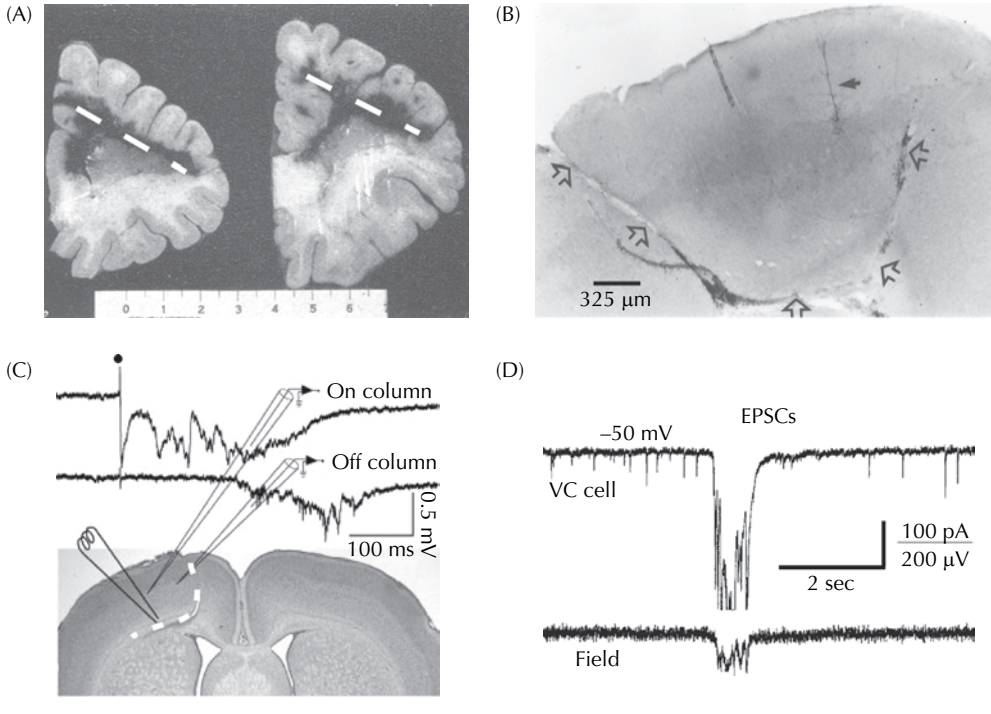

Figure 24–1. Undercut cortex in rats and humans. **A.** Coronal section of human brain in a patient who underwent undercutting surgery of the right frontal lobe for intractable pain. Dashed white lines are drawn through the undercut here and in **C**. **B.** Fixed coronal section cut through rat sensorimotor cortex containing a partial cortical isolation made 3 weeks earlier. The black arrow points to a layer V pyr cell filled with biocytin. Open arrows mark the edge of the undercut that extends from the pial surface to the white matter. **C.** Upper: Evoked epileptiform field potentials recorded simultaneously by two electrodes (On column, Off column) in layer V of an in vitro slice 3 weeks after partial isolation. Lower: Nissl-stained section from the same slice showing the approximate site of the undercut lesion and electrode positions. Stim: stimulation electrode. All-or-none prolonged polyphasic epileptiform activity was evoked by a stimulus in layer VI/white matter just above the undercut margin. An epileptiform burst was initiated by On-column stimulation and propagated across cortex to the Off-column electrode. **D.** Representative voltage clamp recording (upper trace) from layer V pyr cell in the undercut during a spontaneous epileptiform event. Vhold: –50 mV close to E_{Cl}; inward current downward. Random EPSCs were followed by a large, spontaneous epileptiform event consisting of summed polysynaptic EPSCs coincident with the epileptiform field potential burst in the bottom trace. Field: negativity down; current peak clipped). The evoked epileptiform field potential and the cell recording were similar to those evoked by extracellular stimuli (not shown). Slice perfused with standard artificial cerebralspinal fluid (ACSF). **A** modified from ref 92. **C** from ref. 89. **D** from ref. 32.

in neocortex. Maladaptive axonal sprouting and establishment of new excitatory connections occur in mature undercut neocortex[32] and as early as 2 days after injury in isolated immature neocortex.[95] Excitatory sprouting also occurs following injury to the hippocampus in other animal models of epileptogenesis[27,28,96–102] and in epileptic human temporal lobes.[103–107] The ubiquitous nature of this phenomenon is shown by its occurrence in other models of cortical trauma such as stroke,[108,109] theromoischemic lesions,[110] and FPI,[111] where it may begin hours after the trauma.[112] The onset of an axonal reaction and sprouting, as signaled by increases in immunoreactivity for growth-associated protein (GAP) 43, may begin as early as 12 h after lesions in culture, and these alterations are well established by 3 days after injury.[30,113] Sprouting occurs 3–4 days after injury to dissociated neocortical neurons in culture.[114] Hyperexcitability due to synaptic innervation by sprouted axons has been shown in experiments in hippocampus[29,30,76] and neocortex.[34] Activation of brain-derived neurotrophic factor (BDNF) may be an important mechanism underlying injury-induced sprouting and hyperactivity in hippocampus.[115,116]

Results from whole cell recordings of layer V Pyr neurons done 2–3 weeks after injury in the undercut cortex model support the conclusion that there is enhanced synaptic excitatory connectivity by showing (1) an increased frequency of miniature (m) excitatory postsynaptic currents (EPSCs); (2) a steeper input–output relationship for evoked EPSCs; and (3) an increased probability of release of glutamate from excitatory terminals.[33,49] The last finding suggests intrinsic abnormalities in the terminals of Pyr cells. In addition, anatomical studies of biocytin-filled layer V Pyr cell axons showed evidence of significant sprouting, mainly in layer V,[32] where the epileptogenic field potentials were initiated.[78,79] These functional and structural abnormalities presumably contribute to the large polysynaptic excitatory currents in Pyr cells that occur synchronously with field potential epileptiform bursts (Fig. 24–1D) and propagate across the cortex (Fig. 24–1C).

More recently, laser scanning photostimulation of caged glutamate in epileptogenic slices from undercuts allowed detailed mapping of excitatory and inhibitory connectivity.[34,48] Results showed that the excitatory "map" was significantly expanded, particularly in layer V, and that both Pyr cells and fast-spiking inhibitory interneurons were targets of presumed sprouted axons and terminals from other nearby Pyr neurons.[32] These alterations in excitatory synaptic connectivity and strength, together with abnormalities in inhibitory circuits discussed below, may contribute to the development and increased conduction of epileptiform activity across cortex in the in vivo undercut model in cat[117] (reviewed in ref. 118).

It is interesting that hyperexcitability closely resembling that recorded in layer V of undercut cortex has also been shown in neocortical slices from the FPI model. Rats from both models have epileptiform seizures in vivo,[77,119] and there is also a similarity in the morphology of field potentials that are evoked or occur spontaneously in slices versus those in in vivo EEG recordings in these two models. Also, recent recordings from another TBI model studied in vitro clearly show repetitive bursts of EPSCs that coincide with epileptiform field potentials that would be termed *ictal* EEG discharges if they occurred in vivo.[10]

Abnormalities in GABAergic Inhibitory Mechanisms in Undercut Cortex

A variety of structural and electrophysiological evidence shows that GABAergic inhibition is compromised in undercut cortex. Recent experiments in the cat suggest that glutamic acid decarboxylase (GAD)- or gamma-aminobutyric acid (GABA)- positive neocortical interneurons are selectively and progressively reduced in density in cat undercut cortex.[84] Although our initial cell counts in undercut rat cortex have not shown a selective decrease in the density of parvalbumin (PV)-immunoreactive interneurons,[120] we have found significant structural changes in biocytin-filled fast-spiking PV-containing cells, including marked decreases in axonal lengths and dendritic volume (Fig. 24–2), giving them an appearance similar to that seen in immature PV interneurons (compare Fig. 3A of ref. 121 with Fig. 24–2B). Further, the axons of these interneurons in the undercut cortex have a significant increase in the proportion of small (<1 μm in diameter) boutons and a decrease in the number of larger (>1 μm in diameter)

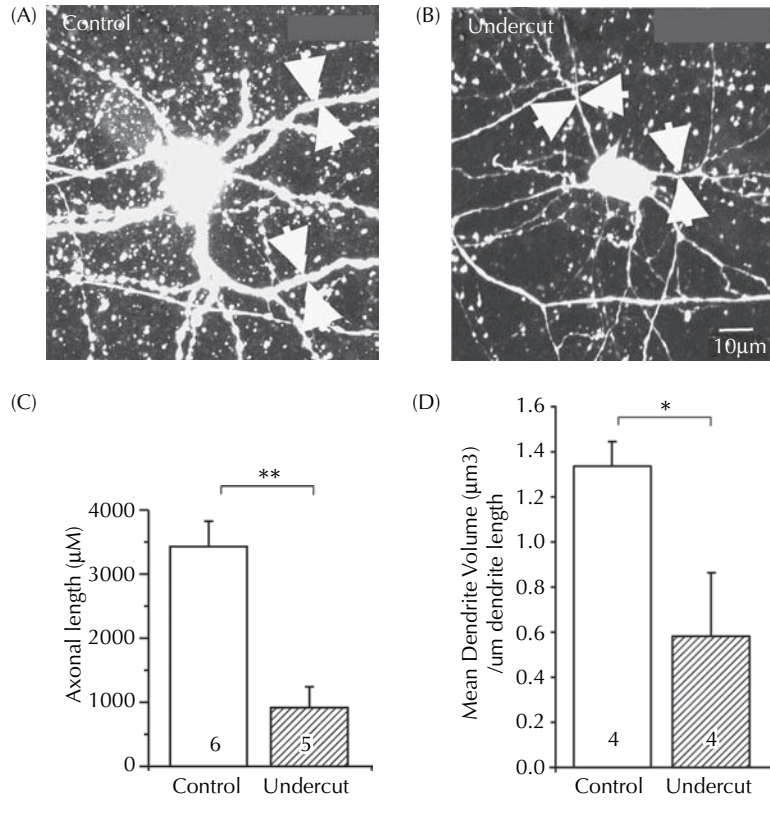

Figure 24–2. Structural alterations in fast-spiking interneurons in undercut cortex. **A,B.** Images of single layer V fast-spiking interneurons filled with biocytin and processed in control (**A**) and undercut slices (**B**). The cell from the undercut slice has thinner dendrites (arrowheads) and a less dense axonal arbor. Calibration in **B**: 10 μm for **A,B**. **C,D.** Graphs show significant decreases in axonal length (**C**) and mean dendritic volume (**D**) in undercut (hatched bars) versus control cells (white bars). The number of cells analyzed is shown in each column. Measurements were obtained from stacks of confocal images. Mean ± SD axonal length for control: 3429.8 ± 968.1 μm and for undercut: 726.9 ± 325.1 μm. $^{**}p < .001$; $^{*}p < .01$. **A,B,D** from I Parada, DA Prince, unpublished. **C** modified from ref. 16.

boutons (see Fig. 5 in ref. 16), changes that would be associated with altered pre- and postsynaptic structures at GABAergic synapses and with less effective inhibitory transmission.[122,123]

Results of several electrophysiological experiments confirm the decreased efficacy of GABAergic inhibitory transmission in the undercut cortex. Whole cell recordings in rat undercut slices showed a decreased frequency of mIPSCs in Pyr cells,[33] and quantitative electron microscopic experiments confirmed a decreased density of symmetrical (inhibitory) synapses on somata of layer V Pyr cells (J Wenzel, PA Schwartzkroin, and DA Prince, unpublished results) as one potential mechanism for decreased miniature inhibitory postsynaptic current (mIPSC) frequency. More recently, we have also shown that the axonal terminals of layer V interneurons in undercuts are abnormal in that they have a decreased probability of GABA release and an increased failure rate[40] due in part to a downregulation of N-type calcium channels in terminals (LC Faria and DA Prince, unpublished results). Dual recordings from synaptically coupled FS-Pyr or FS-spiny stellate pairs in layer IV of undercuts showed a decrease in Pr, a large reduction in the amplitude of unitary IPSCs, increased coefficient of variation and increased failures, indicating alterations in presynaptic terminals of the largest subgroup of GABAergic neurons in cortex, FS cells (J. Ma and DA Prince, unpublished results). Neuronal injury can also decrease the efficacy of postsynaptic inhibition by decreasing expression of KCC2 and impairing outward chloride transport.[124,125] In the undercut, there are also decreases in KCC2 and in the outward transport of chloride in postsynaptic Pyr cells

that would make GABAergic inhibition less effective at times of high-frequency activity.[126] Recent results, obtained with laser scanning photostimulation of caged glutamate in combination with whole cell recordings, have shown that the net effect of some of the above-mentioned anatomical and electrophysiological abnormalities is to reduce the spatial extent of inhibitory inputs onto both Pyr cells and FS interneurons in the chronic undercut.[127]

Fast-spiking interneurons in neocortex normally have a high density of Na^+-K^+ ATPase (*sodium pump*) in their membranes[44] and particularly in their axonal terminals surrounding Pyr cell somata[43,128] (Fig. 24–3A). Sodium pump activation would be important in fast-firing neurons to prevent excessive increases in $[Na^+]_i$ that might depolarize terminals and decrease GABA release. There is a significant loss of Na pump immunoreactivity in undercut cortex surrounding Pyr cells (Fig. 24–3B), similar to that previously found in the freeze-microgyrus model of epileptogenesis,[43] suggesting another potential mechanism that would lead to terminal dysfunction and decreased GABA release.

WHEN DOES POSTTRAUMATIC EPILEPTOGENESIS BEGIN?

Answers to this critical question would influence decisions about the timing of potential antiepileptogenic treatment. From the available data, it appears likely that processes eventually leading to hyperexcitability in cortical networks and to seizures may be set in motion at the time of the TBI, although the latency to the first behavioral spontaneous seizure is highly variable. Seizures in the first week after injury are usually not followed by epilepsy in humans; however, they are associated with an increased statistical risk of subsequent epilepsy,[3] indicating that, at least in some individuals, an epileptogenic process is initiated early. Epileptiform activity may be initially undetectable by surface EEG, making this an unreliable marker of the onset of epileptogenesis.[129] There is evidence for early emergence of epileptogenesis in a variety of experimental data. In models of acute neocortical trauma, epileptiform activity may develop within minutes or hours after injury in vitro,[130,131] and results from acute partial isolation experiments done under ketamine anesthesia in cats do show that acute epileptiform discharges are generated in cortex near the isolation.[132] However, the underlying mechanisms in these early seizures may be different from those in more chronic models in that they might involve acute alterations such as release of excitatory amino acids,[131] spreading depression, large increases in $[K^+]_o$, and blood-brain barrier disruption.

Recently, we recorded in vitro from undercut slices obtained 3 days after the injury and found that they generate robust, prolonged, spontaneous and evoked epileptiform field potentials associated with large-amplitude EPSCs. Confocal images obtained after immunocytochemical processing for GAP43, vesicular glutamate transporter 1 (vGLUT1), and postsynaptic density (PSD) 95 suggest

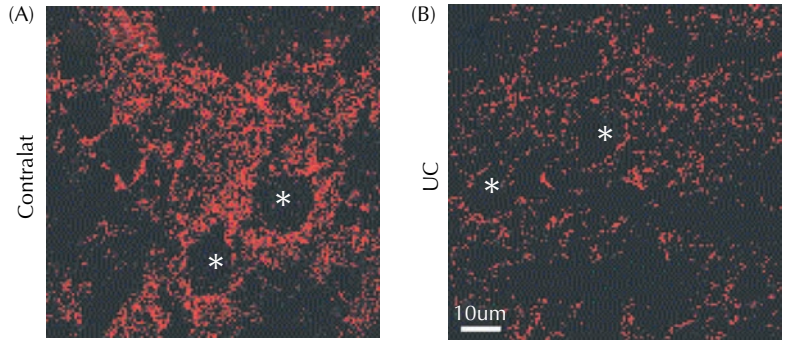

Figure 24–3. Loss of perisomatic α3Na^+K^+ ATPase in undercut cortex. **A.** Immunoreactivity (IR) for α3Na^+K^+ATPase in layer V of control cortex contralateral and homotopic to the undercut on a rat 21 days after lesioning. The IR is localized around the somata of pyr cells (asterisks in **A,B**), suggesting that it is in terminals of FS interneurons that target somata. Dual staining with GAD65 (not shown) confirmed this conclusion.[43] **B.** Undercut side from the rat in **A** shows significant downregulation of α3Na^+K^+ATPase-IR. From ref. 128.

that there is significant sprouting of excitatory axons onto Pyr somata within days after the undercut is placed. Other electrophysiological data suggest that these sprouted terminals are functional and contribute to epileptogenesis (DK Takahashi and DA Prince, unpublished results).

Axonal sprouting and excitatory synapse formation occur in parallel with a number of other pathophysiological events following TBI (e.g., alterations in GABAergic inhibition discussed above, intrinsic changes in membrane excitability[78]), so it is difficult to determine whether hyperconnectivity alone would be sufficient to induce posttraumatic epileptogenesis. A potential answer to this question comes from recent results in C1q knockout mice that have behavioral and electrographic seizures resulting from failure to prune excitatory cortical synapses during development.[133] In vitro slices from these animals are epileptogenic due to increased excitatory connectivity without apparent alterations in inhibitory events.

In experiments using CCI, epileptiform activity and electrographic seizures are present in vitro the first week after injury[10] and appear to progress, with generation of ictal discharges lasting for many seconds by the second week. In other experiments, such as those involving kainate kindling, epileptogenic activity is present early in hippocampus but goes undetected in in vivo recordings from the usual skull electrodes.[134] Our previous results using the undercut model provided the first proof in principle that posttraumatic epileptogenesis, as gauged by the occurrence of epileptiform activity in in vitro slices, begins shortly after injury and can be prevented.[80] However, this prophylaxis was effective only if the treatment, namely, topical exposure of the injured cortex to tetrodotoxin (TTX) in a slow-release resin, was administered for the first 3 days after injury. Later applications were ineffective in limiting the proportion of slices that were epileptogenic.[89] Thus, the results revealed a brief critical period of a few days beginning after the partial isolation when the seeds for subsequent epileptogenesis are sown in the undercut cortex model. Tetrodotoxin has also been effective in decreasing axonal sprouting and rhythmic neocortical burst discharges that begin by the second day after development of thermocoagulation lesions in rat neocortex, although the relationship to later epileptogenesis is unclear.[110]

These results with TTX appear contrary to the hypothesis that the enhanced excitability and epileptogenesis in the undercut may be due to activation of homeostatic increases in excitatory neurotransmission due to deafferentation.[118,135] It is possible that homeostatic compensatory increases in alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors do occur, but they are offset by decreases in innervation of postsynaptic targets induced by the TTX treatment. Immunocytochemical analysis of undercut cortex shows that TTX blockade of activity downregulates anatomical markers of the axonal and terminal sprouting response that are evident as early as 3 days after injury (Fig. 24–4A–C) and are long-lasting (Fig. 24–4D–F). Other as yet unexplored results of silencing injured cortex may account for the blockade of hyperexcitability in the undercut that outlasts the TTX treatment by many months (KD Graber and DA Prince, unpublished results). It is important to note that TTX may produce quite different (opposite) effects on epileptogenesis when given during early development in hippocampus,[136] a result that emphasizes the difficulty of generalizing results from one model to another in terms of potential prophylactic approaches.

PROPHYLAXIS OF POSTTRAUMATIC EPILEPTOGENESIS

Potential approaches to modification of the increased excitatory sprouting and synapse formation and the decreased GABAergic interneuronal structure/function are suggested by results of experiments dealing with normal development of excitatory synapses and interneurons.

Limiting Excitatory Connectivity

Reactive astrogliosis is a ubiquitous pathological finding following TBI and is present in all of the models discussed above, including the undercut. Release of thrombospondins (TSPs) by astrocytes provides an important signal for excitatory synapse formation early in development[137,138] and following injury to the mature central nervous system (CNS).[139,140]

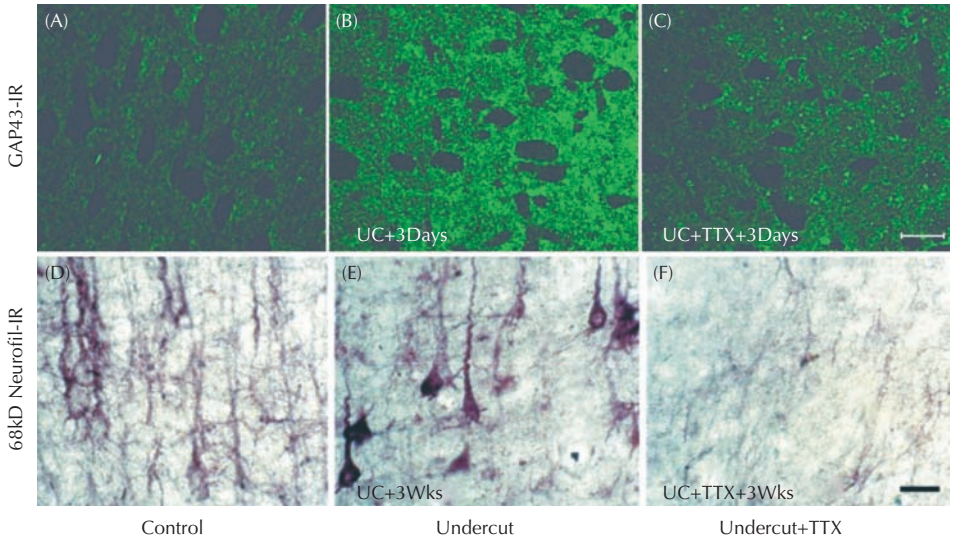

Figure 24–4. Immunoreactivity (IR) of axons and terminals in partially isolated neocortex.
A–C. Sections through layer V of rat sensorimotor cortex reacted with growth-associated protein (GAP) 43 antibody. **D–F.** Comparable sections from rats reacted with antibody for 68 kDa neurofilaments. A,D. Control from layer V of hemisphere contralateral to the undercut. B,E. GAP43-IR (B) and 68 kDa neurofilament-IR (E) in layer V of undercuts made 3 days earlier, contralateral to A and D, respectively. C,F. Representative sections from undercuts of two other rats in which Elvax impregnated with TTX was placed subdurally over the undercut area at the time of surgery. Immunocytochemistry was done after 3 days in **A–C** and after 3 weeks in **D–F**. The TTX treatment reduced IR for both GAP43 and neurofilament in the undercuts. Calibrations in C and F: 50 μm for **A–C** and **D–F**, respectively. From ref. 16.

The α2δ-1 voltage-gated calcium channel subunit, which is upregulated in peripheral pain models and after brain injury, is the receptor for the antiallodynic/antiepileptic drug gabapentin (GBP)[141,142] (reviewed in refs. 143 and 144) and is also the receptor for TSPs.[137] Gabapentin can block excitatory synapse formation in the developing retinogeniculate pathway by interfering with TSP actions.[137] The increased axonal sprouting and synapse formation are reduced in TSP knockout animals in a stroke model, leading to the hypothesis that GBP would have similar actions and might be an antiepileptogenic agent in the undercut model. In recent experiments, GBP, given by subcutaneous infusion or intraperitoneally for 2–3 days up to 14 days following the day of the undercut, decreased the proportion of slices that subsequently generated evoked epileptiform activity (Fig. 24–5A–C; H Li, KD Graber, and DA Prince, unpublished results). In addition, dual immunocytochemical processing of sections from the animals treated with GBP showed significantly fewer presumptive excitatory synapses (i.e., close appositions between pre- [vGLUT1] and postsynaptic markers [PSD95] (Fig. 24–5D–F)). Gabapentin also reduced expression of 200 kD neurofilament-IR and the number of neurons stained with fluorojade C (not shown), suggesting potential neuroprotective effects.

Preventing Structural/Functional Alterations in GABAergic Interneurons

The above structural changes in FS interneurons gave them an appearance that resembled, in some respects, that seen in immature GABAergic cells,[121] prompting us to assess expression of BDNF in neurons of the undercut, as this trophic factor is a key molecule in regulating the development and maintenance of both interneuronal and Pyr cell structure and function[121,135,145–147] (reviewed in ref. 145). Immunoreactivity for BDNF in Pyr cells and its TrkB receptor on PV-containing interneurons and the associated mRNAs were significantly downregulated as early as 3 days after the undercut, suggesting that supplying this or another trophic factor after injury

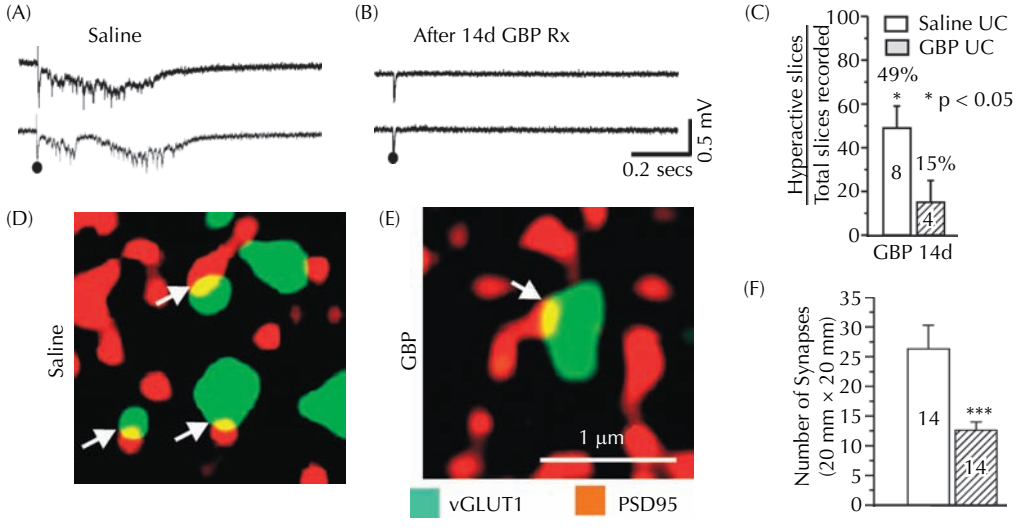

Figure 24–5. Gabapentin (GBP) in vivo reduces epileptogenesis and excitatory synapse density in undercut slices. **A,B.** Field potentials evoked in layer V of an undercut slice by stimuli in partial cortical isolations 14 days after injury. **A.** A rat was treated with an intraperitoneal (ip) infusion of saline for 14 days, followed by a slice experiment. Single stimuli within isolation evoke typical epileptiform discharges consisting of slow potentials lasting for ~400–500 ms with superimposed extracellular unit bursts. **B.** Representative nonepileptiform responses to stimulation of a slice from a rat treated for 14 days with an ip infusion of ~8 mg/day GBP via Alzet pump. Recordings were done 1 day after termination of GBP infusion. **C.** Group data showing the percentage of epileptogenic slices 14 days after undercuts in GBP- versus saline-treated animals. UC: undercut in both groups. Numbers in bars: numbers of animals. Average of 4.3 slices/rat. Gabapentin significantly reduced epileptogenesis in these experiments. **D,E.** Confocal images of neocortical layer V from undercut rats treated with ip saline (**D**) or GBP (**E**; ~8 mg/kg × 7days) after the undercut. Sections were immunoreacted with antibodies for postsynaptic (PSD95, red) and presynaptic markers (vGlut1, green). Sites of putative synapses shown by close appositions (yellow; arrows) were fewer in sections from GBP-treated rats (**E** vs. **D**). **F.** Group data from saline- (white bar) and GBP-treated animals (hatched bar). Numbers in bars: total number of sections examined. Three images were taken from each section and two to three sections were taken from each of the five rats in each group. $^{***}p < .001$. From H Li, KD Graber, DA Prince, unpublished.

(for example, ref. 148) might be an approach to prevention of trauma-induced alterations in these cells (Fig. 6 in ref. 16; see also ref. 17). Brain-derived neurotrophic factor has many potential actions[149], including both enhancement of network excitation and inhibition,[150,151] so it is unclear whether the net effect of BDNF or other TrkB receptor agonists will be anti- or pro-epileptogenic. Variables such as dose level and timing or choice of the mimetic molecule might allow differentiation of beneficial versus detrimental effects.

IMPORTANT UNRESOLVED ISSUES AFFECTING APPLICATION OF ANTIEPILEPTOGENIC THERAPIES FOR PTE

1. The question of adaptive versus maladaptive changes in connectivity following injury is a key one that must be considered in approaching potential preventive treatments that decrease epileptogenic sprouting. A number of reports implicate axonal sprouting and new connections as major adaptive plastic events in recovery of function after development of cortical lesions.[108,109] In recent experiments in a stroke model where middle cerebral artery occlusion induced expression of TSPs in astrocytes, TSP1–2 knockout mice showed significant defects in axonal sprouting and synaptic density compared to wild-type animals, together with defects in functional recovery.[140] The poststroke incidence of epilepsy was not studied in these experiments; however, the results, and those in the above references, provide a cautionary note.
2. A number of pathophysiological processes occur in parallel after a serious

epileptogenic brain injury. Although any one of these in isolation might not induce seizure activity, in combination their effects on excitability would summate and epileptogenesis could result. Thus, a single prophylactic approach might be ineffective and a "prophylactic cocktail" might be required.

3. Two key elements in developing epileptogenesis in a variety of injury models are reduction in functional GABAergic inhibition and enhanced new excitatory connectivity. Although attempts to reverse such alterations may be effective, the relationships between both excitatory and inhibitory circuit function, circuit repair, and epileptiform activity are complex. GABAergic synchronization of cortical networks occurs in epileptogenic cortical lesions[152] and in both acute[153] and genetic models of epileptiform discharge.[154] Also, depolarizing GABA responses due to altered chloride gradients occur in excitatory cells during development[155] and after injury.[125,156] These factors make the net effect of enhanced interneuronal output hard to predict. Antiepileptogenesis, through decreases in excitatory circuit activities, might also have obverse effects such as decreased activation of interneurons[157] (but see refs. 158–160) or reduced activity-dependent axonal sprouting, pathfinding, and circuit repair.[110,161,162]
4. As more becomes known about the processes controlling excitation and inhibition during cortical development or following injury, it is possible that prophylactic therapies selectively affecting maladaptive processes might be applied. One important obstacle at this time is the unavailability of a reliable biological marker that would select for individuals who will go on to develop PTE, although it is clear that the incidence increases with the severity of brain injury (reviewed in ref. 163).
5. We know little about the temporal extent of critical periods in humans when prophylactic intervention would be effective or how to identify epileptogenesis in progress. The latent period may be very long between injury and expression of behavioral seizures[1]; however, the critical period for intervention could closely follow the injury.[89,164]
6. Finally, multiple offsetting potential effects of a given intervention are possible, such as enhancement of excitatory connectivity together with "rescue" of inhibitory interneurons by TrkB receptor agonists (e.g., refs. 165, 166).

The chapters in this volume suggest that significant progress is being made in understanding the basic mechanisms leading to epilepsy, and that we may have potential prophylactic therapies available in the years to come, provided that some of the issues mentioned above are settled by detailed basic and clinical investigations.

DISCLOSURE STATEMENT

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REFERENCES

1. Salazar AM, Jabbari B, Vance SC, Grafman J, Amin D, Dillon JD. Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam Head Injury Study. *Neurology.* 1985;35:1406–1414.
2. Annegers JF, Hauser WA, Coan SP, Rocca WA. A population-based study of seizures after traumatic brain injuries. *N Engl J Med.* 1998;338:20–24.
3. Frey LC. Epidemiology of posttraumatic epilepsy: a critical review. *Epilepsia.* 2003;44(suppl 10):11–17.
4. Garga N, Lowenstein DH. Posttraumatic epilepsy: a major problem in desperate need of major advances. *Epilepsy Curr.* 2006;6(1):1–5.
5. Dichter MA. Posttraumatic epilepsy: the challenge of translating discoveries in the laboratory to pathways to a cure. *Epilepsia.* 2009;50(Suppl 2):41–45.
6. Raymont V, Salazar AM, Lipsky R, Goldman D, Tasick G, Grafman J. Correlates of posttraumatic epilepsy 35 years following combat brain injury. *Neurology.* 2010;75(3):224–229.
7. Giblin KA, Blumenfeld H. Is epilepsy a preventable disorder? New evidence from animal models. *Neuroscientist.* 2010;16(3):253–275.
8. Hunt RF, Scheff SW, Smith BN. Regionally localized recurrent excitation in the dentate gyrus of a cortical contusion model of posttraumatic epilepsy. *J Neurophysiol.* 2010;103(3):1490–1500.
9. Kharatishvili I, Pitkanen A. Posttraumatic epilepsy. *Curr Opin Neurol.* 2010;23(2):183–188.
10. Yang L, Afroz S, Michelson HB, Goodman JH, Valsamis HA, Ling DS. Spontaneous epileptiform

activity in rat neocortex after controlled cortical impact injury. *J Neurotrauma.* 2010;27(8):1541–1548.
11. D'Ambrosio R, Hakimian S, Stewart T, Verley DR, Fender JS, Eastman CL, et al. Functional definition of seizure provides new insight into post-traumatic epileptogenesis. *Brain.* 2009;132(pt 10):2805–2821.
12. Dichter MA. Emerging concepts in the pathogenesis of epilepsy and epileptogenesis. *Arch Neurol.* 2009;66(4):443–447.
13. Israelsson C, Wang Y, Kylberg A, Pick CG, Hoffer B, Ebendal T. Closed head injury in a mouse model results in molecular changes indicating inflammatory responses. *J Neurotrauma.* 2009;26:1307–1314.
14. Lowenstein DH. Epilepsy after head injury: an overview. *Epilepsia.* 2009;50(Suppl 2):4–9.
15. Pitkanen A, Lukasiuk K. Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. *Epilepsy Behav.* 2009;14(suppl 1):16–25.
16. Prince DA, Parada I, Scalise K, Graber K, Jin X, Shen F. Epilepsy following cortical injury: cellular and molecular mechanisms as targets for potential prophylaxis. *Epilepsia.* 2009;50(suppl 2):30–40.
17. Paradiso B, Marconi P, Zucchini S, Berto E, Binaschi A, Bozac A, et al. Localized delivery of fibroblast growth factor-2 and brain-derived neurotrophic factor reduces spontaneous seizures in an epilepsy model. *Proc Natl Acad Sci USA.* 2009;106(17):7191–7196.
18. Temkin NR. Preventing and treating posttraumatic seizures: the human experience. *Epilepsia.* 2009;50(suppl 2): 10–13.
19. Raghavendra R, Dhodda VK, Song G, Bowen KK, Dempsey RJ. Traumatic brain injury-induced acute gene expression changes in rat cerebral cortex identified by GeneChip analysis. *J Neurosci Res.* 2003;71(2): 208–219.
20. David Y, Cacheaux LP, Ivens S, Lapilover E, Heinemann U, Kaufer D, et al. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *J Neurosci.* 2009;29: 10588–10599.
21. Vezzani A. Innate immunity and inflammation in temporal lobe epilepsy: new emphasis on the role of complement activation. *Epilepsy Curr.* 2008;8(3):75–77.
22. Hicks RR, Martin VB, Zhang L, Seroogy KB. Mild experimental brain injury differentially alters the expression of neurotrophin and neurotrophin receptor mRNAs in the hippocampus. *Exp Neurol.* 1999;160(2): 469–478.
23. Pitkanen A, Immonen RJ, Grohn OH, Kharatishvili I. From traumatic brain injury to posttraumatic epilepsy: what animal models tell us about the process and treatment options. *Epilepsia.* 2009;50(suppl 2):21–29.
24. McIntosh TK, Vink R, Yamakami I, Faden AI. Magnesium protects against neurological deficit after brain injury. *Brain Res.* 1989;482(2):252–260.
25. Feeney DM, Boyeson MG, Linn RT, Murray HM, Dail WG. Responses to cortical injury: I. Methodology and local effects of contusions in the rat. *Brain Res.* 1981;211:67–77.
26. Echlin FA, Battista A. Epileptiform seizures from chronic isolated cortex. *Arch Neurol.* 1963;9:154–170.
27. Tauck DL, Nadler JV. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid treated rats. *J Neurosci.* 1985;5:1016–1022.
28. Cronin J, Dudek FE. Chronic seizures and collateral sprouting of dentate mossy fibers after kainic acid treatment in rats. *Brain Res.* 1988;474(1):181–184.
29. Molnar P, Nadler JV. Mossy fiber-granule cell synapses in the normal and epileptic rat dentate gyrus studied with minimal laser photostimulation. *J Neurophysiol.* 1999;82(4):1883–1894.
30. McKinney RA, Debanne D, Gahwiler BH, Thompson SM. Lesion-induced axonal sprouting and hyperexcitability in the hippocampus in vitro: implications for the genesis of post-traumatic epilepsy. *Nature Med.* 1997;3:990–996.
31. Esclapez M, Hirsch JC, Ben Ari Y, Bernard C. Newly formed excitatory pathways provide a substrate for hyperexcitability in experimental temporal lobe epilepsy. *J Comp Neurol.* 1999;408(4):449–460.
32. Salin P, Tseng GF, Hoffman S, Parada I, Prince DA. Axonal sprouting in layer V pyramidal neurons of chronically injured cerebral cortex. *J Neurosci.* 1995;15:8234–8245.
33. Li H, Prince DA. Synaptic activity in chronically injured, epileptogenic sensory-motor neocortex. *J Neurophysiol.* 2002;88(1):2–12.
34. Jin X, Prince DA, Huguenard JR. Enhanced excitatory synaptic connectivity in layer v pyramidal neurons of chronically injured epileptogenic neocortex in rats. *J Neurosci.* 2006;26(18):4891–4900.
35. Ribak CE, Bradburne M, Harris AB. A preferential loss of GABAergic, symmetric synapses in epileptic foci: a quantitative ultrastructural analysis of monkey neocortex. *J Neurosci.* 1982;2:1725–1735.
36. Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, De Felipe J, Ben Ari Y, et al. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. *Nat Neurosci.* 2001;4(1):52–62.
37. Magloczky Z, Freund TF. Impaired and repaired inhibitory circuits in the epileptic human hippocampus. *Trends Neurosci.* 2005;28:334–340.
38. Rosen GD, Jacobs KM, Prince DA. Effects of neonatal freeze lesions on expression of parvalbumin in rat neocortex. *Cereb Cortex.* 1998;8(8):753–761.
39. Kumar SS, Buckmaster PS. Hyperexcitability, interneurons, and loss of GABAergic synapses in entorhinal cortex in a model of temporal lobe epilepsy. *J Neurosci.* 2006;26(17):4613–4623.
40. Faria LC, Prince DA. Presynaptic inhibitory terminals are functionally abnormal in a rat model of posttraumatic epilepsy. *J Neurophysiol.* 2010;104(1):280–290.
41. Kharlamov EA, Downey KL, Jukkola PI, Grayson DR, Kelly KM. Expression of $GABA_A$ receptor alpha1 subunit mRNA and protein in rat neocortex following photothrombotic infarction. *Brain Res.* 2008;1210:29–38.
42. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell $GABA_A$ receptor subunit expression and function in temporal lobe epilepsy. *Nature Med.*1998;4:1166–1172.
43. Chu Y, Parada I, Prince DA. Temporal and topographic alterations in expression of the alpha3 isoform of Na^+, $K(^+)$-ATPase in the rat freeze lesion model of microgyria and epileptogenesis. *Neuroscience.* 2009;162(2):339–348.
44. Anderson TR, Huguenard JR, Prince DA. Differential effects of Na^+/K^+ ATPase blockade on cortical layer V neurons. *J Physiol* (*Lond*). 2010;588.22:4401–4414.
45. Aronica E, Boer K, Redeker S, Spliet WG, van Rijen PC, Troost D, et al. Differential expression patterns of chloride transporters, Na^+-K^+-2Cl–cotransporter and K^+-Cl–cotransporter, in epilepsy-associated malformations of cortical development. *Neuroscience.* 2007;145(1):185–196.

46. Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, et al. NKCC1 transporter facilitates seizures in the developing brain. *Nat Med.* 2005;11(11):1205–1213.
47. Shimizu-Okabe C, Okabe A, Kilb W, Sato K, Luhmann HJ, Fukuda A. Changes in the expression of cation-Cl- cotransporters, NKCC1 and KCC2, during cortical malformation induced by neonatal freeze-lesion. *Neurosci Res.* 2007;59(3):288–295.
48. Jin X, Huguenard JR, Prince DA. Reorganization of inhibitory synaptic circuits in rodent chronically injured epileptogenic neocortex. *Cereb Cortex.* 2011:21(5):1094–1104.
49. Li H, Bandrowski AE, Prince DA. Cortical injury affects short-term plasticity of evoked excitatory synaptic currents. *J Neurophysiol.* 2005;93(1):146–156.
50. Gutnick MJ, Prince DA. Thalamocortical relay neurons: antidromic invasion of spikes from cortical epileptogenic focus. *Science.* 1972;176:424–426.
51. Pinault D. Backpropagation of action potentials generated at ectopic axonal loci: hypothesis that axon terminals integrate local environmental signals. *Brain Res Rev.* 1995:21(1):42–92.
52. De Lanerolle NC, Eid T, Von Campe G, Kovacs I, Spencer DD, Brines M. Glutamate receptor subunits GluR1 and GluR2/3 distribution shows reorganization in the human epileptogenic hippocampus. *Eur J Neurosci.* 1998;10(5):1687–1703.
53. Blumcke I, Beck H, Scheffler B, Hof PR, Morrison JH, Wolf HK, et al. Altered distribution of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor subunit GluR2(4) and the *N*-methyl-D-aspartate receptor subunit NMDAR1 in the hippocampus of patients with temporal lobe epilepsy. *Acta Neuropathol (Berl).* 1996;92:576–587.
54. Chen HX, Xiang H, Roper SN. Impaired developmental switch of short-term plasticity in pyramidal cells of dysplastic cortex. *Epilepsia.* 2007;48(1):141–148.
55. Wong RK, Bianchi R, Taylor GW, Merlin LR. Role of metabotropic glutamate receptors in epilepsy. *Adv Neurol.* 1999;79:685–698.
56. Gold SJ, Hennegriff M, Lynch G, Gall CM. Relative concentrations and seizure-induced changes in mRNAs encoding three AMPA receptor subunits in hippocampus and cortex. *J Comp Neurol.* 1996;365:541–555.
57. Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, et al. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science.* 1997;276:1699–1702.
58. Campbell SL, Hablitz JJ. Decreased glutamate transport enhances excitability in a rat model of cortical dysplasia. *Neurobiol Dis.* 2008;32(2):254–261.
59. Boulland JL, Ferhat L, Tallak ST, Ferrand N, Chaudhry FA, Storm-Mathisen J, et al. Changes in vesicular transporters for gamma-aminobutyric acid and glutamate reveal vulnerability and reorganization of hippocampal neurons following pilocarpine-induced seizures. *J Comp Neurol.* 2007;503(3):466–485.
60. Brines ML, Tabuteau H, Sundaresan S, Kim J, Spencer DD, De Lanerolle N. Regional distributions of hippocampal Na^+,$K^{(+)}$-ATPase, cytochrome oxidase, and total protein in temporal lobe epilepsy. *Epilepsia.* 1995;36(4):371–383.
61. Grisar T. Glial and neuronal Na^+-K^+ pump in epilepsy. *Ann Neurol.* 1984;16(suppl):S128–S134.
62. Vaillend C, Mason SE, Cuttle MF, Alger BE. Mechanisms of neuronal hyperexcitability caused by partial inhibition of Na^+-K^+-ATPases in the rat CA1 hippocampal region. *J Neurophysiol.* 2002;88(6):2963–2978.
63. Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I. Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat Med.* 2001;7(3):331–337.
64. Beck H, Steffens R, Elger CE, Heinemann U. Voltage-dependent Ca^{2+} currents in epilepsy. *Epilepsy Res.* 1998;32(1–2):321–332.
65. Kohling R. Voltage-gated sodium channels in epilepsy. *Epilepsia.* 2002;43(11):1278–1295.
66. Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, et al. A potassium channel mutation in neonatal human epilepsy. *Science.* 1998;279:403–406.
67. Smart SL, Lopantsev V, Zhang CL, Robbins CA, Wang H, Chiu SY, et al. Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. *J Physiol (Lond).* 1998;20:809–819.
68. Ivens S, Kaufer D, Flores LP, Bechmann I, Zumsteg D, Tomkins O, et al. TGF-beta receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. *Brain.* 2007;130:535–547.
69. Marcon J, Gagliardi B, Balosso S, Maroso M, Noe F, Morin M, et al. Age-dependent vascular changes induced by status epilepticus in rat forebrain: implications for epileptogenesis. *Neurobiol Dis.* 2009;34(1):121–132.
70. Takahashi DK, Vargas JR, Wilcox KS. Increased coupling and altered glutamate transport currents in astrocytes following kainic-acid-induced status epilepticus. *Neurobiol Dis.* 2010;40(3):573–585.
71. Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, et al. An astrocytic basis of epilepsy. *Nat Med.* 2005;11:973–981.
72. Pitkanen A, McIntosh TK. Animal models of post-traumatic epilepsy. *J Neurotrauma.* 2006;23(2):241–261.
73. Kharatishvili I, Nissinen JP, McIntosh TK, Pitkanen A. A model of posttraumatic epilepsy induced by lateral fluid-percussion brain injury in rats. *Neuroscience.* 2006;140(2):685–697.
74. Kharlamov EA, Jukkola PI, Schmitt KL, Kelly KM. Electrobehavioral characteristics of epileptic rats following photothrombotic brain infarction. *Epilepsy Res.* 2003;56(2–3):185–203.
75. D'Ambrosio R, Fender JS, Fairbanks JP, Simon EA, Born DE, Doyle DL, et al. Progression from frontal-parietal to mesial-temporal epilepsy after fluid percussion injury in the rat. *Brain* 2005;128(pt 1):174–188.
76. Dudek FE, Staley KJ. Laser scanning photostimulation: new evidence for enhanced recurrent excitation in a model of posttraumatic epilepsy. *Epilepsy Curr.* 2006;6(6):215–216.
77. Graber KD, Prince DA. Chronic partial cortical isolation. In: Pitkanen A, Spa MS, eds. *Models of Seizures and Epilepsy.* San Diego, CA: Elsevier Academic Press; 2006:477–493.
78. Prince DA, Tseng G-F. Epileptogenesis in chronically injured cortex: in vitro studies. *J Neurophysiol.* 1993;69:1276–1291.
79. Hoffman SN, Salin PA, Prince DA. Chronic neocortical epileptogenesis in vitro. *J Neurophysiol.* 1994;71:1762–1773.

80. Graber KD, Prince DA. Tetrodotoxin prevents posttraumatic epileptogenesis in rats. *Ann Neurol.* 1999;46(2):234–242.
81. Halpern LM. Chronically isolated aggregates of mammalian cerebral cortical neurons studied in situ. In: Purpura DP, Penry JK, Tower D, Woodbury DM, Walter R, eds. *Experimental Models of Epilepsy—A Manual for the Laboratory Worker.* New York: Raven Press; 1972:197–221.
82. Sharpless SK, Halpern LM. The electrical excitability of chronically isolated cortex studied by means of permanently implanted electrodes. *Electroencephalogr Clin Neurophysiol.* 1962;14:244–255.
83. Echlin FA, Arnett V, Zoll J. Paroxysmal high-voltage and rhythmic low-voltage discharges from isolated and partially isolated human cortex. *AMA Arch Neurol Psychiatry.* 1952;67(5):692–693.
84. Avramescu S, Nita DA, Timofeev I. Neocortical posttraumatic epileptogenesis is associated with loss of GABAergic neurons. *J Neurotrauma.* 2009;26(5): 799–812.
85. Ribak CE, Reiffenstein RJ. Selective inhibitory synapse loss in chronic cortical slabs: a morphological basis for epileptic susceptibility. *Can J Physiol Pharmacol.* 1982;60(6):864–870.
86. Bush PC, Prince DA, Miller KD. Increased pyramidal excitability and NMDA conductance can explain posttraumatic epileptogenesis without disinhibition: a model. *J Neurophysiol.* 1999;82(4):1748–1758.
87. Prince DA. Epileptogenic neurons and circuits. *Adv Neurol.* 1999;79:665–684.
88. Grafstein B, Sastry P. Some preliminary electrophysiological studies on chronically neuronally isolated cerebral cortex. *Electroencephalogr Clin Neurophysiol.* 1957;9:723–725.
89. Graber KD., Prince DA. A critical period for prevention of posttraumatic neocortical hyperexcitability in rats. *Ann Neurol.* 2004;55(6):860–870.
90. Marin-Padilla M. Developmental neuropathology and impact of perinatal brain damage. II: white matter lesions of the neocortex. *J Neuropathol Exp Neurol.* 1997;56(3):219–235.
91. Jacobs KM, Parada I, Prince DA. Enhanced c-*fos* staining in two post-lesional models of cortical hyperexcitability: neonatal freeze lesions and partial cortical isolations [abstract] *Epilepsia*. 2001;42(suppl 7), 221.
92. Scoville WB. Selective cortical undercutting as a means of modifying and studying frontal lobe function in man; preliminary report of 43 operative cases. *J Neurosurg.* 1949;6(1):65–73.
93. Scoville WB. Late results of orbital undercutting. Report of 76 patients undergoing quantitative selective lobotomies. *Am J Psychiatry.* 1960;117:525–532.
94. Cajal SRY. Study of traumatic degeneration in the cerebral cortex. In: May RM, ed. *Degeneration and Regeneration of the Nervous System*. London: Oxford University Press; 1928:656–692.
95. Purpura DP, Housepian EM. Morphological and physiological properties of chronically isolated immature neocortex. *Exp Neurol.* 1961;4:377–401.
96. Sutula T, He XX, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science.* 1988;239:1147–1150.
97. Mello LE, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, et al. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia.* 1993;34:985–995.
98. Represa A, Jorquera I, Le Gal la Salle G, Ben-Ari Y. Epilepsy induced collateral sprouting of hippocampal mossy fibers: does it induce the development of ectopic synapses with granule cell dendrites? *Hippocampus.* 1993;3:257–268.
99. Perez Y, Morin F, Beaulieu C, Lacaille JC. Axonal sprouting of CA1 pyramidal cells in hyperexcitable hippocampal slices of kainate-treated rats. *Eur J Neurosci.* 1996;8:736–748.
100. Buckmaster PS, Zhang GF, Yamawaki R. Axon sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. *J Neurosci.* 2002;22(15):6650–6658.
101. Buckmaster PS, Dudek FE. Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol.* 1997;385(3):385–404.
102. Smith BN, Dudek FE. Network interactions mediated by new excitatory connections between CA1 pyramidal cells in rats with kainate-induced epilepsy. *J Neurophysiol.* 2002;87(3):1655–1658.
103. Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF. Synaptic reorganization by mossy fibers in human epileptic fascia dentata. *Neuroscience.* 1991;42:351–363.
104. Babb TL, Pretorius JK, Kupfer WR, Mathern GW, Crandall PH, Levesque MF. Aberrant synaptic reorganization in human epileptic hippocampus: evidence for feedforward excitation. *Dendron.* 1992;1:7–25.
105. Masukawa LM, Uruno K, Sperling M, O'Connor MJ, Burdette LJ. The functional relationship between antidromically evoked field responses of the dentate gyrus and mossy fiber reorganization in temporal lobe epileptic patients. *Brain Res.* 1992;579:119–127.
106. Isokawa M, Levesque MF, Babb TL, Engel J Jr. Single mossy fiber axonal systems of human dentate granule cells studied in hippocampal slices from patients with temporal lobe epilepsy. *J Neurosci.* 1993;13:1511–1522.
107. Marco P, DeFelipe J. Altered synaptic circuitry in the human temporal neocortex removed from epileptic patients. *Exp Brain Res.* 1997;114:1–10.
108. Dancause N, Barbay S, Frost SB, Plautz EJ, Chen D, Zoubina EV, et al. Extensive cortical rewiring after brain injury. *J Neurosci.* 2005;25(44):10167–10179.
109. Lee JK, Kim JE, Sivula M, Strittmatter SM. Nogo receptor antagonism promotes stroke recovery by enhancing axonal plasticity. *J Neurosci.* 2004;24(27): 6209–6217.
110. Carmichael ST, Chesselet MF. Synchronous neuronal activity is a signal for axonal sprouting after cortical lesions in the adult. *J Neurosci.* 2002;22: 6062–6070.
111. Christman CW, Salvant JB Jr, Walker SA, Povlishock JT. Characterization of a prolonged regenerative attempt by diffusely injured axons following traumatic brain injury in adult cat: a light and electron microscopic immunocytochemical study. *Acta Neuropathol* (*Berl*). 1997;94:329–337.
112. Povlishock JT, Kontos HA. Continuing axonal and vascular change following experimental brain trauma. *Cent Nerv Syst Trauma.* 1985;2(4):285–298.
113. Dickson TC, Adlard PA, Vickers JC. Sequence of cellular changes following localized axotomy to

cortical neurons in glia-free culture. *J Neurotrauma.* 2000;17(11):1095–1103.
114. Chuckowree JA, Vickers JC. Cytoskeletal and morphological alterations underlying axonal sprouting after localized transection of cortical neuron axons in vitro. *J Neurosc.i* 2003;23:3715–3725.
115. Dinocourt C, Gallagher SE, Thompson SM. Injury-induced axonal sprouting in the hippocampus is initiated by activation of trkB receptors. *Eur J Neurosci.* 2006;24(7):1857–1866.
116. Scharfman HE, Goodman JH, Sollas AL, Croll SD. Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. *Exp Neurol.* 2002;174(2):201–214.
117. Nita DA, Cisse Y, Timofeev I, Steriade M. Waking-sleep modulation of paroxysmal activities induced by partial cortical deafferentation. *Cereb Cortex.* 2007;17(2):272–283.
118. Timofeev I, Bazhenov M, Avramescu S, Nita DA. Posttraumatic epilepsy: the roles of synaptic plasticity. *Neuroscientist.* 2010;16(1):19–27.
119. D'Ambrosio R, Perucca E. Epilepsy after head injury. *Curr Opin Neurol.* 2004;17:731–735.
120. Graber KD, Kharazia VN, Parada I, Prince DA. Parvalbumin-containing interneurons are spared in the undercut model of posttraumatic epileptogenesis. [Abstract]. *Epilepsia*. 1999;40(suppl 7):31.
121. Jin X, Hu H, Mathers PH, Agmon A. Brain-derived neurotrophic factor mediates activity-dependent dendritic growth in nonpyramidal neocortical interneurons in developing organotypic cultures. J Neurosci. 2003;23(13):5662–5673.
122. Harris KM, Sultan P. Variation in the number, location and size of synaptic vesicles provides an anatomical basis for the nonuniform probability of release at hippocampal CA1 synapses. *Can J Physiol Pharmacol.* 1995;34(11):1387–1395.
123. Pierce JP, Lewin GR. An ultrastructural size principle. *Neuroscience.* 1994;58(3):441–446.
124. Nabekura J, Ueno T, Okabe A, Furuta A, Iwaki T, Shimizu-Okabe C, et al. Reduction of KCC2 expression and $GABA_A$ receptor-mediated excitation after in vivo axonal injury. *J Neurosci.* 2002;22(11): 4412–4417.
125. van den Pol AN, Obrietan K, Chen G. Excitatory actions of GABA after neuronal trauma. *J Neurosci.* 1996;16:4283–4292.
126. Jin XM, Huguenard JR, Prince DA. Impaired Cl^- extrusion in layer V pyramidal neurons of chronically injured epileptogenic neocortex. *J Neurophysiol.* 2005;93:2117–2126.
127. Jin X, Huguenard JR, Prince DA. Reorganization of inhibitory synaptic circuits in rodent chronically injured epileptogenic neocortex. *Cereb Cortex*. 2011;21:1094–1104.
128. Lee L, Parada I, Prince DA. Downregulation of the $\alpha 3$ subunit of NA^+K^+ ATPAse in a model of post-traumatic epileptogenesis. *Epilepsia.* 2006;47:3.058.
129. Spencer SS, Sperling MR, Shewmon DA, Khane P. Intracranial electrodes. In: Engel T Jr, Pedley TA, eds. *Epilepsy, a Comprehensive Textbook*. *Second Edition*. Philadelphia: Lippincott Williams & Wilkins; 2010:1791–1815.
130. Yang L, Benardo LS. Epileptogenesis following neocortical trauma from two sources of disinhibition. *J Neurophysiol.* 1997;78:2804–2810.
131. Nilsson P, Ronne-Engström E, Flink R, Ungerstedt U, Carlson H, Hillered L. Epileptic seizure activity in the acute phase following cortical impact trauma in rat. *Brain Res.* 1994;637:227–232.
132. Topolnik L, Steriade M, Timofeev I. Partial cortical deafferentation promotes development of paroxysmal activity. *Cereb Cortex.* 2003;13(8):883–893.
133. Chu Y, Jin X, Parada I, Pesic A, Stevens B, Barres B, et al. Enhanced synaptic connectivity and epilepsy in C1q knockout mice. *Proc Natl Acad Sci USA.* 2010;107(17):7975–7980.
134. Hellier JL, Patrylo PR, Dou P, Nett M, Rose GM, Dudek FE. Assessment of inhibition and epileptiform activity in the septal dentate gyrus of freely behaving rats during the first week after kainate treatment. *J Neurosci.* 1999;19(22):10053–10064.
135. Turrigiano GG. Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci.* 1999;22:221–227.
136. Galvan CD, Hrachovy RA, Smith KL, Swann JW. Blockade of neuronal activity during hippocampal development produces a chronic focal epilepsy in the rat. *J Neurosci.* 2000;20:2904–2916.
137. Eroglu C, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, et al. Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell.* 2009;139(2):380–392.
138. Christopherson KS, Ullian EM, Stokes CCA, Mullowney CE, Hell JW, Agah A, et al. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell.* 2005;120(3): 421–433.
139. Lin TN, Kim GM, Chen JJ, Cheung WM, He YY, Hsu CY. Differential regulation of thrombospondin-1 and thrombospondin-2 after focal cerebral ischemia/reperfusion. *Stroke.* 2003;34(1):177–186.
140. Liauw J, Hoang S, Choi M, Eroglu C, Choi M, Sun GH. et al. Thrombospondins 1 and 2 are necessary for synaptic plasticity and functional recovery after stroke. *J Cereb Blood Flow Metab.* 2008;28(10): 1722–1732.
141. Luo ZD, Chaplan SR, Higuera ES, Sorkin LS, Stauderman KA, Williams ME, et al. Upregulation of dorsal root ganglion (alpha)2(delta) calcium channel subunit and its correlation with allodynia in spinal nerve-injured rats. *J Neurosci.* 2001;21(6): 1868–1875.
142. Luo ZD, Calcutt NA, Higuera ES, Valder CR, Song YH, Svensson CI, et al. Injury type-specific calcium channel alpha 2 delta-1 subunit up-regulation in rat neuropathic pain models correlates with antiallodynic effects of gabapentin. *J Pharmacol Exp Ther.* 2002;303(3):1199–1205.
143. Maneuf YP, Luo ZD, Lee K. Alpha2delta and the mechanism of action of gabapentin in the treatment of pain. *Semin Cell Dev Biol.* 2006;17(5):565–570.
144. Field MJ, Cox PJ, Stott E, Melrose H, Offord J, Su TZ, et al. Identification of the alpha2-delta-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. *Proc Natl Acad Sci USA.* 2006;103(46): 17537–17542.
145. McAllister AK, Katz LC, Lo DC. Neurotrophins and synaptic plasticity. *Annu Rev Neurosci.* 1999;22: 295–318.

146. Marty S, Berzaghi M, Berninger B. Neurotrophins and activity-dependent plasticity of cortical interneurons. *Trends Neurosci.* 1997;20(5):198–202.
147. Croll SD, Wiegand SJ, Anderson KD, Lindsay RM, Nawa H. Regulation of neuropeptides in adult rat forebrain by the neurotrophins BDNF and NGF. *Eur J Neurosci.* 1994;6:1343–1353.
148. Burkhalter J, Fiumelli H, Allaman I, Chatton JY, Martin JL. Brain-derived neurotrophic factor stimulates energy metabolism in developing cortical neurons. *J Neurosci.* 2003;23(23):8212–8220.
149. Massa SM, Yang T, Xie Y, Shi J, Bilgen M, Joyce JN, et al. Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. *J Clin Invest.* 2010;120(5):1774–1785.
150. Danzer SC, McNamara JO. Localization of brain-derived neurotrophic factor to distinct terminals of mossy fiber axons implies regulation of both excitation and feedforward inhibition of CA3 pyramidal cells. *J Neurosci.* 2004;24(50):11346–11355.
151. Koyama R, Ikegaya Y. To BDNF or not to BDNF: that is the epileptic hippocampus. *Neuroscientist.* 2005;11(4):282–287.
152. D'Antuono M, Louvel J, Kohling R, Mattia D, Bernasconi A, Olivier A, et al. $GABA_A$ receptor-dependent synchronization leads to ictogenesis in the human dysplastic cortex. *Brain.* 2004;127(pt 7): 1626–1640.
153. Marchionni I, Maccaferri G. Quantitative dynamics and spatial profile of perisomatic GABAergic input during epileptiform synchronization in the CA1 hippocampus. *J Physiol.* 2009;587(pt 23):5691–5708.
154. Klaassen A, Glykys J, Maguire J, Labarca C, Mody I, Boulter J. Seizures and enhanced cortical GABAergic inhibition in two mouse models of human autosomal dominant nocturnal frontal lobe epilepsy. *Proc Natl Acad Sci USA.* 2006;103(50):19152–19157.
155. Tyzio R, Minlebaev M, Rheims S, Ivanov A, Jorquera I, Holmes GL, et al. Postnatal changes in somatic gamma-aminobutyric acid signalling in the rat hippocampus. *Eur J Neurosci.* 2008;27(10):2515–2528.
156. Pathak HR, Weissinger F, Terunuma M, Carlson GC, Hsu FC, Moss SJ, et al. Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. *J Neurosci.* 2007;27(51):14012–14022.
157. Sloviter RS. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the "dormant basket cell" hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus.* 1991;1: 41–66.
158. Zhang W, Yamawaki R, Wen X, Uhl J, Diaz J, Prince DA, et al. Surviving hilar somatostatin interneurons enlarge, sprout axons, and form new synapses with granule cells in a mouse model of temporal lobe epilepsy. *J Neurosci.* 2009;29(45):14247–14256.
159. Halabisky B, Parada I, Buckmaster PS, Prince DA. Excitatory input onto hilar somatostatin interneurons is increased in a chronic model of epilepsy. *J Neurophysiol.* 2010;104:2214–2223.
160. Sloviter RS, Zappone CA, Harvey BD, Bumanglag AV, Bender RA, Frotscher M. "Dormant basket cell" hypothesis revisited: relative vulnerabilities of dentate gyrus mossy cells and inhibitory interneurons after hippocampal status epilepticus in the rat. *J Comp Neurol.* 2003;459(1):44–76.
161. Katz LC, Shatz CJ. Synaptic activity and the construction of cortical circuits. *Science.* 1996;274(5290): 1133–1138.
162. Ming G, Henley J, Tessier-Lavigne M, Song H, Poo M. Electrical activity modulates growth cone guidance by diffusible factors. *J Physiol* (*Lond*). 2001;29(2): 441–452.
163. Chen JW, Ruff RL, Eavey R, Wasterlain CG. Posttraumatic epilepsy and treatment. *J Rehabil Res Dev.* 2009;46(6):685–696.
164. Yang L, Benardo LS. Valproate prevents epileptiform activity after trauma in an in vitro model in neocortical slices. *Epilepsia.* 2000;41(12):1507–1513.
165. Binder DK, Croll SD, Gall CM, Scharfman HE. BDNF and epilepsy: too much of a good thing? *Trends Neurosci.* 2001;24:47–53.
166. Reibel S, Depaulis A, Larmet Y. BDNF and epilepsy—the bad could turn out to be good. *Trends Neurosci.* 2001;24(6):318–319.

Chapter 25

Head Trauma and Epilepsy

Asla Pitkänen
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Traumatic brain injury (TBI) refers to a brain injury caused by an external mechanical force such as a blow to the head, concussive forces, acceleration-deceleration forces, blast injury, or a projectile missile such as a bullet.[1] According to the World Health Organization, TBI will surpass many diseases as a major cause of death and disability by the year 2020.[2] With an estimated 10 million people affected annually by TBI, the burden of mortality and morbidity that this condition imposes on society makes TBI a serious public health and medical problem.

Traumatic brain injury is a heterogeneous disorder that can be classified based on the injury mechanism.[3] Depending on the characteristics of the mechanical force (amplitude, duration, velocity, acceleration), injury can be static or dynamic. Static loading occurs when the head is exposed to a heavy weight—for example, if the head is trapped underneath a car. Dynamic loading is a more common type of mechanical input causing TBI, and can be divided into two types: (1) impact loading, which occurs when a blunt object strikes the head and can initiate both contact and inertial forces, and (2) impulsive loading, which occurs during the rapid acceleration or deceleration of the head.

The 15-point Glasgow Coma Scale (GCS) is a rating commonly used to evaluate the severity of brain injury. It assesses three major parameters: verbal, motor, and eye-opening reactions to stimuli.[4] A GCS score of 13 or above indicates mild TBI, 9–12 is moderate TBI, and ≤8 is severe TBI. It is important to note that the same injury severity may represent different clinical endophenotypes that depend on the injury mechanism (e.g., static vs. dynamic) or the distribution and type of damage (e.g., gray vs. white matter).

POSTTRAUMATIC EPILEPSY IN HUMANS

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological,

cognitive, psychological, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one spontaneous (i.e., unprovoked) seizure.[5] After TBI, the occurrence of seizures has been categorized as immediate (<24 h), early (1–7 days), or late seizures (>1 week after TBI).[6] The immediate and early seizures are considered to reflect the severity of the injury itself, whereas the late seizures result from maturation of epileptogenic pathology. Thus, TBI associated with one unprovoked late seizure qualifies for the diagnosis of posttraumatic epilepsy (PTE).

Epidemiological studies show that a 30-year cumulative incidence of epilepsy is 2.1% for mild, 4.2% for moderate, and 16.7% for severe injuries.[7] As discussed by Herman,[8] TBI accounts for 20% of symptomatic epilepsy in the general population and 5% of all epilepsy. It can be estimated that in the United States and the European Union (EU), with a total population of about 800 million, at least 0.5 million surviving individuals live with PTE. Data on the economic burden of PTE are unavailable, but some idea is provided by the lifetime cost of TBI on average, which in the United States is about $200,000 per case when scaled to 2004 price levels.[9] Thus, in addition to the personal burden, the economic burden caused by PTE is also substantial, and treatments to prevent posttraumatic epileptogenesis would benefit the individual as well as society.

The risk factors for PTE obtained in studies of civilian or military populations include old age, penetrating injuries, injury severity (GCS <10), biparietal or multiple contusions, intracranial hemorrhage, frontal or temporal location of the lesion, brain midline shift >5 mm, duration of coma >24 h, loss of consciousness >24 h, prolonged period of posttraumatic amnesia, multiple intracranial procedures, and the occurrence of early posttraumatic seizures.[7,10–15] These data are mostly derived from epidemiological studies that were conducted before magnetic resonance imaging (MRI) became available. Apparently related to methodology, many of the identified risk factors directly or indirectly reflect the severity of brain injury, and they strengthen the view that the risk for PTE increases with the severity of TBI. However, these risk factors give little indication of the neurobiological characteristics of the injury that are most significantly associated with epileptogenesis.

A recent study investigated the civilian population with TBI using MRI analysis performed 1–360 months (median, 12 months) post-TBI; the study reported an association of PTE with contusions but not with an isolated diagnosis of diffuse axonal injury (DAI).[16] This study found no association between micro bleeds and PTE, which challenges the data from previous epidemiology studies. Further information about the endophenotypes of TBI patients that are associated with the highest risk of PTE would be of great value for attempts to model PTE in clinically relevant ways. Such information would also guide the search of mechanisms and biomarkers (or surrogate markers) for posttraumatic epileptogenesis.

Some information is available on the epileptogenic process. After the first late seizure, 86% of patients were reported to develop a second seizure within 2 years, suggesting the establishment of an epileptogenic process.[17] Further, recent magnetoencephalography (MEG) studies revealed that some patients without reported seizures after mild TBI have epileptiform spikes when assessed at 12–140 months post-TBI.[18] Some studies have also investigated the persistence of seizures after TBI. For example, Eftekhar et al.[19] reported that 75% of patients with penetrating TBI suffered during the Iraq-Iran war continue to have seizures after a 21-year follow-up, even when data were adjusted for antiepileptic drug (AED) treatment. Interestingly, no association was found between the persistence of epilepsy and lesion size.

Data on genetic risk factors for the outcome of patients with TBI are emerging, but very few studies have assessed the linkage of genes to PTE.[20] Some data are available from genes encoding ApoE or haptoglobin. The *APOE* gene, encoding for a cholesterol carrier lipoprotein, exists in three common isoforms: ε2, ε3, and ε4. One study found that the ApoE4 allele is associated with a 2.4-fold increased risk of late posttraumatic seizures after moderate to severe TBI.[21] Interestingly, an ApoE4 allele has been identified as a susceptibility gene for Alzheimer's disease, which is another condition with an increased risk for epilepsy.[22–25] Linkage of ApoE4 to an elevated risk of PTE was not, however, confirmed by another study.[26] Another gene of interest encodes haptoglobin. Previous studies have shown that serum and cerebrospinal fluid haptoglobin concentrations

are increased after TBI.[27] Sadrzadeh et al.[28] reported that the HP2–2 allele was the risk factor for PTE. However, this observation was also not verified in another study.[26]

EXPERIMENTAL MODELS OF PTE

In vivo long-term video-electroencephalographic (EEG) monitoring studies demonstrate that several models commonly used to investigate the molecular and cellular mechanisms of TBI have chronically lowered seizure thresholds or even spontaneous seizures (Table 25–1). Chronically increased seizure susceptibility to chemoconvulsants or electroshock has been demonstrated in weight-drop, fluid-percussion, controlled cortical impact, and closed skull models of TBI (Fig. 25–1). Chronic spontaneous seizures were reported after fluid-percussion- and controlled cortical impact-induced TBI in both rats and mice. Acute epileptiform activity (up to 3 days postinjury) has been reported in a rat model of penetrating ballistic injury, but whether late spontaneous seizures develop remains to be studied.[49] No data on hyperexcitability are available from blast-induced TBI in rats.[50]

The following discussion focuses on models of TBI with spontaneous late seizures.

Epilepsy in the Fluid-Percussion Model

Lateral fluid-percussion (FP)-induced TBI has become the most extensively utilized animal model of TBI, producing both focal and diffuse (mixed) brain injury.[51] It reproduces several aspects of human TBI, including focal contusion, petechial intraparenchymal and subarachnoid hemorrhages, tissue tears, and traumatic axonal injury. The sequelae include blood-brain barrier (BBB) disruption, white matter damage, neuronal loss, gliosis, altered cerebral metabolism, altered cerebral blood flow, and altered brain electrical activity. The damage appears to be most severe in the ipsilateral cortex, hippocampus, and thalamus, but milder lesions can also be detected contralaterally. More chronic network alterations include neurogenesis with axonal and dendritic plasticity. The molecular and cellular changes can continue for weeks or months, and they seem not only to underlie the acute and chronic behavioral impairments but also to contribute to the recovery process.[51]

Kharatishvili et al.[38] monitored adult Sprague-Dawley rats with lateral FP injury over a period of 12 months with periodic 24/7 video-EEG and demonstrated that 50% of the rats developed PTE (Fig. 25–2). The seizures were partial or secondarily generalized and lasted for about 60–110 s.[52] A substantial proportion of animals had lowered seizure thresholds, and it remains to be determined whether some of these animals will develop epilepsy over a longer follow-up period. Rostral parasagittal FP was also reported to trigger epileptogenesis and the occurrence of spontaneous seizures.[31] Our preliminary data show that lateral FP can trigger increased seizure susceptibility in the pentylenetetrazole (PTZ) test, as well as spontaneous seizures, in mice.[42]

Epilepsy in the Controlled Cortical Impact Model

The controlled cortical impact (CCI) injury to the lateral cortex causes a focal brain injury that is associated with a spectrum of contusion injuries including intraparenchymal petechial hemorrhages that are accompanied by epidural and subdural hematomas. Histological analysis reveals widespread cortical gray matter damage as well as axonal injury in the adjacent white matter, corpus callosum, and capsula interna. Degeneration is present not only in the cortex but also in the hippocampus and thalamus. Damage can also be observed in all of the major afferent pathways and in the visual cortex. These anatomical changes are associated with a spectrum of cognitive and motor deficits.[53] Recently, elegant studies from Hunt and colleagues[45,46] demonstrated that CCI-induced TBI in the lateral cortex triggers the development of PTE in CD-1 mice. Behavioral seizures were observed in 20% of animals with mild injury and 36% of those with severe injury when observed after 42–71 days postinjury.[45] Our preliminary observations also show increased susceptibility to PTZ-induced seizures and the occurrence of electrographic spontaneous seizures after lateral CCI injury in C57BL/6J mice.[47] Statler et al.[44] monitored rats with lateral CCI at postnatal day 17 with

Table 25–1 **Summary of *In Vivo* Recorded Changes in Excitability in Different Models of TBI (Only the Data Collected at Least 1 Week Post-Injury Are Included)**

Model	Species, Age, Strain, Preparation	Seizure Susceptibility *In Vivo*	Epilepsy					Reference
			Animals with Epilepsy (%)	Latency to Spontaneous Seizure	Seizure Frequency	Seizure Duration (s)	Epileptiform Spiking or EDs in EEG	
Weight-drop	Rat, adult	Increased susceptibility to PTZ-induced seizures 15 weeks post-TBI	n.d.	n.d.	n.d.	n.d.	n.d.	Golarai et al. [29]
Central FPI	Rat, adult	No difference in PTZ kindling, started 24 h post-TBI	n.d.	n.d.	n.d.	n.d.	n.d.	Hamm et al. [30]
Parasagittal FPI	Rat, P32–35	n.d.	100% (follow-up: 7 months)	~2 weeks	Up to 7 seizures/h	Ictal episodes ≤10 s (up to 99 s)	n.d.	D'Ambrosio et al. [31,32]
	Rat, adult	Increased susceptibility to PTZ-induced seizures 12 weeks post-TBI	n.d.	n.d.	n.d.	n.d.	n.d.	Atkins et al. [33]
	Rat, adult	Increased susceptibility to PTZ-induced seizures 2 weeks post-TBI	No	n.d.	n.d.	n.d.	n.d.	Bao et al. [34]
Lateral FPI	Rat, adult	Granule cell hyperexcitability 1 week post-TBI	n.d.	n.d.	n.d.	n.d.	n.d.	Lowenstein et al. [35]
	Rat, adult	Increased inhibition in dentate gyrus 15 days post-TBI	n.d.	n.d.	n.d.	n.d.	n.d.	Reeves et al. [36]
	Rat, P19	No change in PTZ seizure threshold 20 weeks post-TBI	0% (behavioral observation)	n.d.	n.d.	n.d.	n.d.	Gurkoff et al.[37]
	Rat, adult	Increased susceptibility to PTZ-induced seizures 12 month post-TBI	50% (follow-up: 12 months)	4–11 weeks	0.3/day	104	80%	Kharatishvili et al. [38,39]
	Rat, P21–P22	Increased susceptibility to kainate-induced seizures 6 weeks post-TBI	n.d.	n.d.	n.d.	n.d.	n.d.	Echegoyen et al. [40]
	Rat, adult	No change in susceptibility to fluorothyl-induced seizures 3 and 6 weeks post-TBI	n.d.	n.d.	n.d.	n.d.	n.d.	Schwarztkroin et al.[41]

	Mouse, adult	Increased susceptibility to PTZ-induced seizures 6 month post-TBI	6% (follow-up: 9 months)	n.d.	0.1/d	91		Bolkvadze et al.[42]
CCI	Rat, P16–P18	Unchanged threshold for tonic hind limb extension or minimal clonic seizures in electroconvulsive seizure threshold test, testing on P34–P40 Reduced threshold for minimal clonic seizures, testing on P60–P63	n.d.	n.d.	n.d.	n.d.	n.d.	Statler et al. [43]
	Rat, P17	n.d.	1 of 8 (13%) (follow-up: 11 months)	n.d.	n.d.	45–60 s	88% had epileptiform spiking	Statler et al. [44]
	Mouse, adult	n.d.	20%(mild)–36% (severe injury)	n.d. (monitoring 42 –71 days post-TBI)	n.d	~90 s (behavioral assessment)	n.d.	Hunt et al. [45]
	Mouse, adult	n.d.	40%	6.5 ± 1.3 weeks	n.d.	n.d.	n.d.	Hunt et al. [46]
	Mouse, adult	Increased susceptibility to PTZ-induced seizures 6 months post-TBI	9% (follow-up: 9 months)	n.d.	0.2/day	50		Bolkvadze et al. [47]
Closed skull TBI	Mouse, adult	Increased seizure susceptibility to ECS-induced seizures 7 days post-TBI	n.d.	n.d.	n.d.	n.d.	n.d.	Chrzaszcz et al. [48]

Abbreviations: CCI, controlled cortical impact; ECS, electroconvulsive shock; FPI, fluid-percussion injury; n.d., no data; P, postnatal day; PTZ, pentylenetetrazol; sz, seizure; TBI, traumatic brain injury.

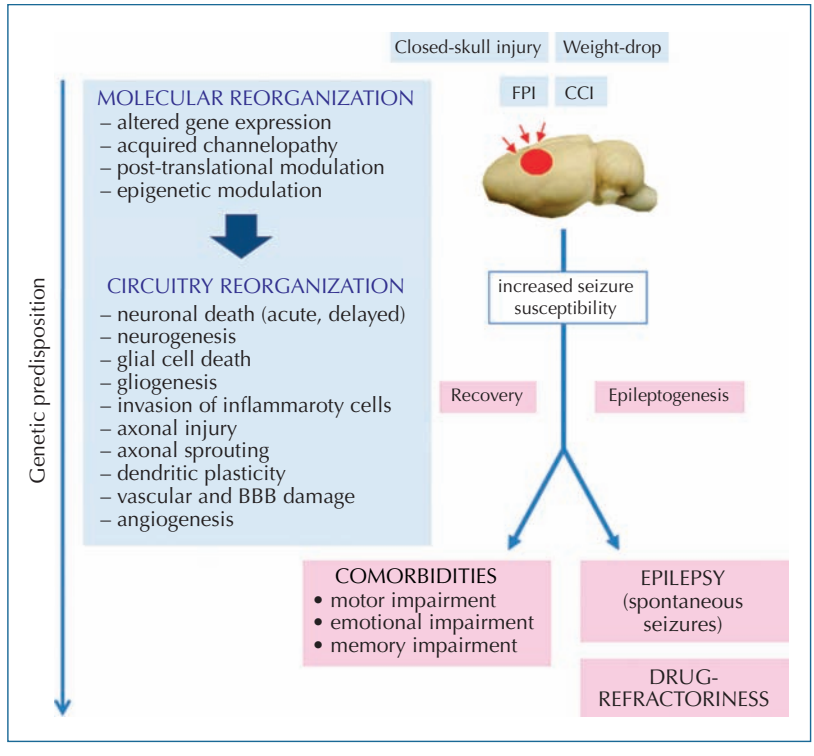

Figure 25–1. A schematic drawing of posttraumatic epileptogenesis. So far, increased seizure susceptibility in *in vivo* testing has been demonstrated in weight-drop injury, closed-skull injury, fluid percussion (FP) injury (both lateral and parasagittal), and controlled cortical impact (CCI) models of TBI. Spontaneous seizures have been shown in FP and CCI models. Traumatic brain injury triggers myriad of molecular changes at transcriptional, posttranslational, and epigenetic levels, some of which likely underlie the consequent circuitry reorganization. Recent data also demonstrate the development of acquired channelopathy after TBI that can contribute to increased excitability. Functionally, epileptogenesis and recovery progress in parallel. The outcomes can include both epilepsy and comorbidities. The epilepsy phenotype may progress and become drug-refractory. All the different aspects are under the influence of genetic factors. See text for references.

video-EEG over a period of 4–11 months post-TBI. They reported that 88% of rats developed epileptiform spiking and 13% of them (one of eight) had spontaneous recurrent seizures. Recently, Yang et al.[54] recorded spontaneous epileptiform activity in cortical slices from rats that had experienced CCI injury 14–16 days earlier at postnatal day 24.

MOLECULAR AND CELLULAR REORGANIZATION DURING POSTTRAUMATIC EPILEPTOGENESIS

The aftermath of TBI is a complex process and is considered to consist of several phases, including primary injury, evolution of the primary injury, secondary injury, and regeneration (Fig. 25–1).[55] Primary injury occurs at the moment of trauma and is accompanied by massive disturbance of cellular ion homeostasis, release of excitatory neurotransmitters, and exacerbation of excitotoxicity. The secondary injury occurs in the hours and days following the primary injury and is an indirect result of the insult. It includes a complex set of molecular changes and cellular processes, some of which may also be relevant to posttraumatic epileptogenesis. However, very few reports have specifically linked the observed postinjury molecular changes with epileptogenesis. Further, none of the studies have tried to address the question of whether the molecular cascades or network alterations contributing to epileptogenesis are separate or overlapping compared to those relevant to postinjury recovery.

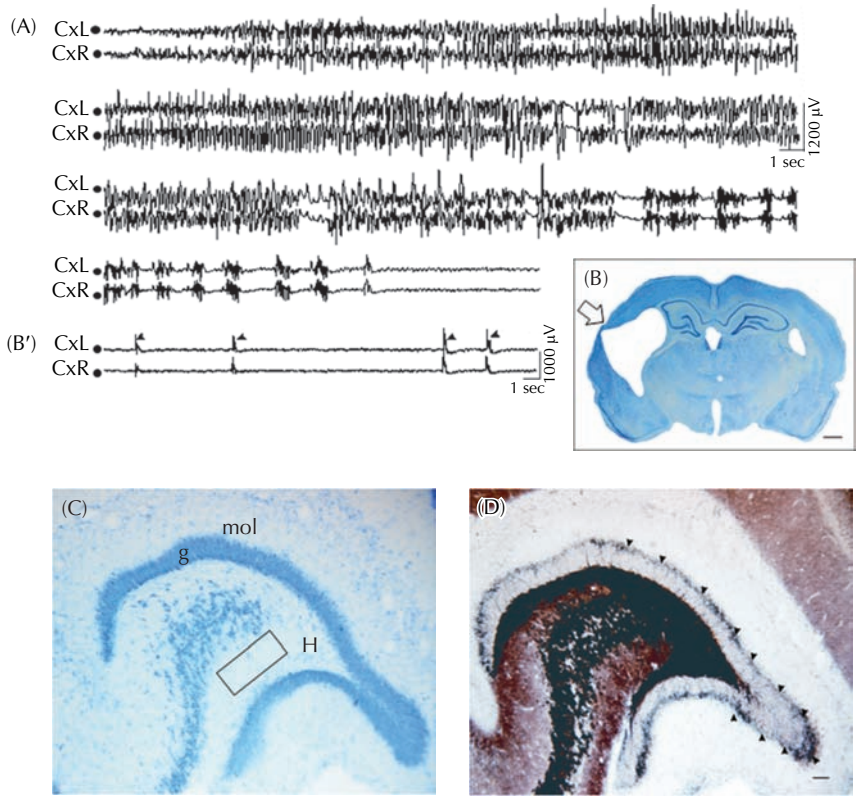

Figure 25–2. A. A representative example of a spontaneous seizure that was recorded at 6 months after lateral FP injury and was seen in both the ipsilateral and contralateral leads. The duration of the electrographic seizure was 94 s and its behavioral severity was 5 on Racine's scale.[72] **A'** shows epileptiform spiking in the same rat. **B.** A thionin-stained coronal section from the same rat subjected to lateral FP brain injury 9 months earlier. The open arrow indicates the injury site. **C.** A higher-magnification photomicrograph from a thionin-stained section of the septal end of the hippocampus of the same rat. Note the loss of hilar cells (open square). **D.** An adjacent Timm-stained section. Note the sprouting of mossy fibers into the inner molecular layer of the dentate gyrus (arrowheads). Abbreviations: C×L, left parietal cortex; C×R, right parietal cortex; g, granule cell layer; H, hilus; mol, molecular layer. The scale bar equals 1 mm in panel **B** and 100 μm in panels **C** and **D**.

Currently, *period of epileptogenesis* and *latency period* are used synonymously as operational terms referring to a time period that begins after the insult occurs (e.g., TBI or stroke) or even during the insult (prolonged febrile seizure, status epilepticus [SE], or encephalitis) and ends when the first spontaneous seizure appears. It refers to a dynamic process that progressively alters neuronal excitability, establishes critical interconnections, and perhaps requires intricate structural changes before the first spontaneous seizure appears.[56] These changes can include neurodegeneration, neurogenesis, gliosis, axonal damage or sprouting, dendritic plasticity, BBB damage, recruitment of inflammatory cells into brain tissue, reorganization of the extracellular matrix, and reorganization of the molecular architecture of individual neuronal cells.[57] Interestingly, all of these changes have been reported to occur after experimental TBI.[58] Further, they are not unique for the aftermath of TBI; they have also been described after SE, which is another common injury type used to trigger epileptogenesis in rodents.[57]

Even though data from human PTE are meager, available studies show hippocampal neurodegeneration as well as mossy fiber sprouting.[59] Approximately 53% of patients with posttraumatic temporal lobe epilepsy (TLE) have mesial temporal lobe sclerosis on MRI; in some patients, the MRI abnormalities can be bilateral and associated with multifocal injury.[60,61] Interestingly, a recent study by

Vespa et al.[62] suggests that hippocampal atrophy detected during the chronic postinjury phase could be caused by (prolonged) seizures during the acute post-TBI phase.

What are the molecular changes underlying circuitry reorganization during epileptogenesis? We recently conducted a meta-analysis of published gene array data after TBI and SE.[63] When the lists of genes regulated during post-TBI and post-SE epileptogenesis were compared, only 46 out of a total of 624 regulated genes were found to have abnormal regulation in more than one study. The commonly upregulated genes were involved in inflammatory and immune responses, response to wounding, complement activation, synaptic transmission, ion transport, regulation of apoptosis, protein phosphorylation, and proteolysis. Functions of commonly downregulated genes were related to synaptic transmission, ion transport, and calcium binding. Seventeen of 46 genes (40%) were regulated in both SE and TBI models, indicating similarity in molecular events during epileptogenesis between different etiologies. The genes regulated by both SE and TBI were *CALM3, CAMK2B, CTSB, CTSS, DBI, DNAJC3, DNAJC5, GABRD, GFAP, GRN, HPCA, IL6R, NPC2, NPTX2, PTPN6, S100B*, and *SPARC*. In addition to changes in transcription, posttranslational modifications and epigenetic changes have been described after TBI.[63, 64] One interesting subcategory of molecular reorganization is the development of various types of channelopathies, which to date have been demonstrated to affect gamma-aminobutyric acid A ($GABA_A$) receptors as well as sodium and potassium channels.[63] Further studies are needed to reveal whether any of these genes form a target to combat posttraumatic epileptogenesis.

SEARCH OF BIOMARKERS FOR POSTTRAUMATIC EPILEPTOGENESIS

Recent long-term MRI studies have provided new insight into the progression of brain damage during epileptogenesis. These studies indicate that the progression of pathology has a different temporal course in the cortex, hippocampus, and thalamus.[65] Moreover, these studies suggest that in the lateral FP model the contralateral hippocampus also undergoes changes, although to a lesser extent than the ipsilateral hippocampus. In addition to apparent neurodegeneration, long-term alterations include changes in axons/myelin as well as vasculature.[58,66] Importantly, not only epileptogenesis, but also the extent and temporal progression of neuropathological changes, vary among animals.

Little information is available concerning how each of these changes contributes to epileptogenesis and to the parallel recovery processes. So far, correlations have be found between the increased seizure susceptibility and diffusion changes or reduced cerebral blood flow (arterial spin labeling) in MRI in the hippocampus.[39,66] The development of new *in vivo* MRI techniques will hopefully enable researchers to follow specific molecular or network changes related to epileptogenesis, which in turn could advance surrogate marker identification for posttraumatic epileptogenesis. One example of this approach is the monitoring of axonal plasticity in the hippocampus by using manganese-enhanced MRI or diffusion tensor imaging (DTI).[39,65,67] It is likely that the first applications of MRI surrogate markers will soon be available for preclinical use. They will be useful for the identification of animals at risk for epilepsy after TBI as well as for the follow-up of treatment effects on epileptogenesis in individual animals.

PREVENTION OF EPILEPTOGENESIS AFTER TBI

A large number of preclinical trials have been conducted to improve motor and cognitive recovery from TBI, but none of these studies have assessed seizure susceptibility or epilepsy as an outcome measure.[68,69] Recently, however, four studies have made attempts to prevent posttraumatic epileptogenesis in experimental models (Table 25–2). The first one was the study conducted by Soltesz and colleagues, who induced epileptogenesis by lateral FP-induced TBI and administered SR141716A (Rimonabant®) as a single injection at 2 min postinjury.[40] The threshold for kainate-induced seizures was assessed at 6 weeks post-TBI. The TBI-associated reduction in the latency to kainate-induced seizures was prevented by

Table 25–2 **Effect of Treatment on Epileptogenesis after TBI in Experimental Models**

Treatment	Model	Treatment Protocol	Time of Analysis (Postinjury)	Seizure Susceptibility	Reference
Rimonabant	Lateral FP	2 or 20 min post-TBI single dose	6 weeks	⇓	Echegoyen et al.[40]
Minozac	Closed-skull midline impact	3 and 6 h post-TBI two doses	7 days	⇓	Chrzaszcz et al.[48]
Ketogenic diet	Lateral FP	3 weeks post-TBI	3 or 6 weeks	±0	Schwartzkroin et al.[41]
Hypothermia	Parasagittal FP	30 min post-TBI for 4 h	12 weeks	⇓	Atkins et al.[33]

Abbreviations: FP, fluid percussion; TBI, traumatic brain injury; ±0, no effect.

SR141716A. Also, the total time spent in seizures after kainate administration was reduced in the SR141716A group compared to the vehicle group. Importantly, no positive effect of treatment was found if SR141716A was administered 20 min post-TBI.

Another compound with favorable effects is minozac, which was discovered by using a molecular target-independent discovery paradigm.[70] The goal of this discovery program was to find a small molecule that suppressed the increased production of proinflammatory cytokines in glial cultures, as assessed using disease-relevant endpoints rather than designing a compound targeting a specific molecular pathway. This approach was combined with hierarchical biological screens for oral bioavailability, toxicity, brain penetrance, and stability before testing for efficacy in animal models of brain disorders. Chrzaszcz et al.[48] used the closed-skull midline impact model of TBI in mice and administered minozac at 3 or 6 h after injury. After 1 week post-TBI, minozac-treated mice showed less susceptibility to electroconvulsive shock-induced seizures than sham-operated rats. Whether minozac treatment prevents the long-term increase in seizure susceptibility and the occurrence of late seizures remains to be explored.

Schwartzkroin and colleagues[41] triggered lateral FP injury to 8-week-old rats and administered a ketogenic diet for 3 weeks postinjury. Seizure susceptibility to flurothyl was no different from that in rats on a standard diet when assessed 3 and 6 weeks after discontinuation of the ketogenic diet. It should be noted, however, that TBI had no effect on seizure susceptibility to flurothyl (seizure threshold, seizure duration) when injured and sham-operated animals on the standard diet were compared.

Hypothermia is considered to be a promising therapy that improves structural and functional outcome measures after experimental and clinical TBI.[71] Atkins et al.[33] induced moderate parasagittal FP injury in adult rats. Animals were kept under normothermic or moderate hypothermic temperatures for 4 h starting at 30 min postinjury. Susceptibility to PTZ-induced seizures was tested at 12 weeks post-TBI. Behavioral analysis of the data indicated a reduced number of induced seizures during the 60 min period after PTZ injection. The behavioral severity of seizures was not affected.

Taken together, there is evidence that treatments with different mechanisms of action can modulate post-TBI seizure susceptibility. Whether reduction in seizure susceptibility predicts antiepileptogenesis remains to be studied.

SUMMARY

Recent advances in model development provide a platform for studies that are aimed at understanding the post-TBI molecular

and cellular alterations leading to epilepsy. Undoubtedly, the search for surrogate markers that identify subjects with the highest risk for posttraumatic epileptogenesis will benefit from novel possibilities for following candidate epileptogenic changes in neuronal circuits *in vivo* using structural and functional imaging. Some proof-of-principle trials already suggest that post-TBI seizure susceptibility can be favourably modified.

DISCLOSURE STATEMENT

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REFERENCES

1. Maas AI, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. *Lancet Neurol*. 2008;7(8):728–741.
2. Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G, Kobusingye OC. The impact of traumatic brain injuries: a global perspective. *Neuro Rehabil*. 2007;22(5):341–353.
3. Graham DI, McIntoshTK, Maxwell WL, Nicoll JA. Recent advances in neurotrauma. *J Neuropathol Exp Neurol*. 2000;59(8):641–651.
4. Marion DW. Management of traumatic brain injury: past, present, and future. *Clin Neurosurg*. 1999;45: 184–191.
5. Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J Jr. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia*. 2005;46(4):470–472.
6. Frey LC. Epidemiology of posttraumatic epilepsy: a critical review. *Epilepsia*. 2003;10:11–17.
7. Annegers JF, Hauser A, Coan SP, Rocca WA. A population-based study of seizures after traumatic brain injuries. *N Engl J Med.* 1998;338:20–24.
8. Herman ST. Epilepsy after brain insult: targeting epileptogenesis. *Neurology*. 2002;59:21–26.
9. Berg J, Tagliaferri F, Servadei F. Cost of trauma in Europe. *Eur J Neurol.* 2005;12(supp1):85–90.
10. Annegers JF, Grabow JD, Groover RV, Laws ER Jr, Elveback LR, Kurland LT. Seizures after head trauma: a population study. *Neurology*. 1980;30: 683–689.
11. Brandvold B, Levi L, Feinsod M, George ED. Penetrating craniocerebral injuries in the Israeli involvement in the Lebanese conflict, 1982–1985. Analysis of a less aggressive surgical approach. *J Neurosurg*. 1990;72(1):15–21.
12. Asikainen I, Kaste M, Sarna S. Early and late posttraumatic seizures in traumatic brain injury rehabilitation patients: brain injury factors causing late seizures and influence of seizures on long-term outcome. *Epilepsia.* 1999;40(5):584–589.
13. Englander J, Bushnik T, Duong TT, Cifu DX, Zafonte R, Wright J, Hughes R, Bergman W. Analyzing risk factors for late posttraumatic seizures: a prospective, multicenter investigation. *Arch Phys Med Rehabil*. 2003;84(3):365–373.
14. Messori A, Polonara G, Carle F, Gesuita R, Salvolini U. Predicting posttraumatic epilepsy with MRI: prospective longitudinal morphologic study in adults. *Epilepsia*. 2005;46(9):1472–1481.
15. Skandsen T, Ivar Lund T, Fredriksli O, Vik A. Global outcome, productivity and epilepsy 3–8 years after severe head injury. The impact of injury severity. *Clin Rehabil*. 2008;22(7):653–662.
16. Scheid R, von Cramon DY. Clinical findings in the chronic phase of traumatic brain injury: data from 12 years' experience in the Cognitive Neurology Outpatient Clinic at the University of Leipzig. *Dtsch Arztebl Int.* 2010;107(12):199–205.
17. Haltiner AM, Temkin NR, Dikmen SS. Risk of seizure recurrence after the first late posttraumatic seizure. *Arch Phys Med Rehabil*. 1997;78(8):835–840.
18. Lewine JD, Davis JT, Bigler ED, Thoma R, Hill D, Funke M, Sloan JH, Hall S, Orrison WW. Objective documentation of traumatic brain injury subsequent to mild head trauma: multimodal brain imaging with MEG, SPECT, and MRI. *J Head Trauma Rehabil*. 2007;22(3):141–155.
19. Eftekhar B, Sahraian MA, Nouralishahi B, Khaji A, Vahabi Z, Ghodsi M, Araghizadeh H, Soroush MR, Esmaeili SK, Masoumi M. Prognostic factors in the persistence of posttraumatic epilepsy after penetrating head injuries sustained in war. *J Neurosurg.* 2009;110(2):319–326.
20. Dardiotis E, Fountas KN, Dardioti M, Xiromerisiou G, Kapsalaki E, Tasiou A, Hadjigeorgiou GM. Genetic association studies in patients with traumatic brain injury. *Neurosurg Focus.* 2010;28(1):E9.
21. Diaz-Arrastia R, Gong Y, Fair S, Scott KD, Garcia MC, Carlile MC, Agostini MA, Van Ness PC. Increased risk of late posttraumatic seizures associated with inheritance of *APOE* epsilon4 allele. *Arch Neurol*. 2003;60(6):818–822.
22. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993;261(5123): 921–923.
23. Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology.* 1993; 43(8):1467–1472.
24. Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, Schmechel D, Saunders AM, Goldgaber D, Roses AD. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc Natl Acad Sci USA*. 1993;90(17):8098–8102.
25. Palop JJ, Mucke L. Epilepsy and cognitive impairments in Alzheimer disease. *Arch Neurol.* 2009;66(4):435–440.

26. Anderson GD, Temkin NR, Dikmen SS, Diaz-Arrastia R, Machamer JE, Farhrenbruch C, Miller JW, Sadrzadeh SM. Haptoglobin phenotype and apolipoprotein E polymorphism: relationship to posttraumatic seizures and neuropsychological functioning after traumatic brain injury. *Epilepsy Behav*. 2009;16(3):501–506.
27. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. *Clin Chem*. 1996;42:1589–1600.
28. Sadrzadeh SM, Bozorgmehr J. Haptoglobin phenotypes in health and disorders. *Am J Clin Pathol*. 2004;121:97–104.
29. Golarai G, Greenwood AC, Feeney DM, Connor JA. Physiological and structural evidence for hippocampal involvement in persistent seizure susceptibility after traumatic brain injury. *J Neurosci*. 2001;21(21):8523–8537.
30. Hamm RJ, Pike BR, Temple MD, O'Dell DM, Lyeth BG. The effect of postinjury kindled seizures on cognitive performance of traumatically brain-injured rats. *Exp Neurol*. 1995;36(2):143–148.
31. D'Ambrosio R, Fairbanks JP, Fender JS, Born DE, Doyle DL, Miller JW. Post-traumatic epilepsy following fluid percussion injury in the rat. *Brain*. 2004;127:304–314.
32. D'Ambrosio R, Fender JS, Fairbanks JP, Simon EA, Born DE, Doyle DL, Miller JW. Progression from frontal-parietal to mesial-temporal epilepsy after fluid percussion injury in the rat. *Brain*. 2005;128(pt 1): 174–188.
33. Atkins CM, Truettner JS, Lotocki G, Sanchez-Molano J, Kang Y, Alonso OF, Sick TJ, Dietrich WD, Bramlett HM. Post-traumatic seizure susceptibility is attenuated by hypothermia therapy. *Eur J Neurosci*. 2010;32(11):1912–1920.
34. Bao YH, Bramlett HM, Atkins CM, Truettner JS, Lotocki G, Alonso OF, Dietrich WD. Post-traumatic seizures exacerbates histopathological damage after fluid-percussion brain injury. *J Neurotrauma*. 2011;28(1):35–42.
35. Lowenstein DH, Thomas MJ, Smith DH, McIntosh TK. Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. *J Neurosci*. 1992;12(12):4846–4853.
36. Reeves TM, Lyeth BG, Phillips LL, Hamm RJ, Povlishock JT. The effects of traumatic brain injury on inhibition in the hippocampus and dentate gyrus. *Brain Res*. 1997;757(1):119–132.
37. Gurkoff GG, Giza CC, Shin D, Auvin S, Sankar R, Hovda DA. Acute neuroprotection to pilocarpine-induced seizures is not sustained after traumatic brain injury in the developing rat. *Neuroscience*. 2009;164(2): 862–876.
38. Kharatishvili I, Nissinen JP, McIntosh TK, Pitkänen A. A model of posttraumatic epilepsy induced by lateral fluid-percussion brain injury in rats. *Neuroscience*. 2006;140(2):685–697.
39. Kharatishvili I, Immonen R, Gröhn O, Pitkänen A. Quantitative diffusion MRI of hippocampus as a surrogate marker for post-traumatic epileptogenesis. *Brain*. 2007;130:3155–3168.
40. Echegoyen J, Armstrong C, Morgan RJ, Soltesz I. Single application of a CB1 receptor antagonist rapidly following head injury prevents long-term hyperexcitability in a rat model. *Epilepsy Res*. 2009;85(1): 123–127.
41. Schwartzkroin PA, Wenzel HJ, Lyeth BG, Poon CC, Delance A, Van KC, Campos L, Nguyen DV. Does ketogenic diet alter seizure sensitivity and cell loss following fluid percussion injury? *Epilepsy Res*. 2010;92(1):74–84.
42. Bolkvadze T, Nissinen J, Kharatishvili I, Pitkänen A. Development of lowered seizure threshold after lateral fluid-percussion brain injury in mouse. *Epilepsia*. 2008;49(7):462.
43. Statler KD, Swank S, Abildskov T, Bigler ED, White HS. Traumatic brain injury during development reduces minimal clonic seizure thresholds at maturity. *Epilepsy Res*. 2008;80(2–3):163–170.
44. Statler KD, Scheerlinck P, Pouliot W, Hamilton M, White HS, Dudek FE. A potential model of pediatric posttraumatic epilepsy. *Epilepsy Res*. 2009;86(2–3): 221–223.
45. Hunt RF, Scheff SW, Smith BN. Posttraumatic epilepsy after controlled cortical impact injury in mice. *Exp Neurol*. 2009;215(2):243–252.
46. Hunt RF, Scheff SW, Smith BN. Regionally localized recurrent excitation in the dentate gyrus of a cortical contusion model of posttraumatic epilepsy. *J Neurophysiol*. 2010;103(3):1490–1500.
47. Bolkvadze T, Nissinen J, Kharatishvili I, Pitkänen A. Development of post-traumatic epilepsy in C57BL/6 mice after controlled cortical impact injury. *J Neurotrauma*. 2009;26(8):A75.
48. Chrzaszcz M, Venkatesan C, Dragisic T, Watterson DM, Wainwright MS. Minozac treatment prevents increased seizure susceptibility in a mouse "two-hit" model of closed skull traumatic brain injury and electroconvulsive shock-induced seizures. *J Neurotrauma*. 2010;27:1283–1295.
49. Lu XC, Hartings JA, Si Y, Balbir A, Cao Y, Tortella FC. Electrocortical pathology in a rat model of penetrating ballistic-like brain injury. *J Neurotrauma*. 2011;28(1): 71–83.
50. Cheng J, Gu J, Ma Y, Yang T, Kuang Y, Li B, Kang J. Development of a rat model for studying blast-induced traumatic brain injury. *J Neurol Sci*. 2010;294(1–2):23–28.
51. Thompson HJ, Lifshitz J, Marklund N, Grady MS, Graham DI, Hovda DA, McIntosh TK. Lateral fluid percussion brain injury: a 15-year review and evaluation. *J Neurotrauma*. 2005;22:42–75.
52. Kharatishvili I, Pitkänen A. Posttraumatic epilepsy. *Curr Opin Neurol*. 2010;23(2):183–188.
53. Morales DM, Marklund N, Lebold D, Thompson HJ, Pitkanen A, Maxwell WL, Longhi L, Laurer H, Maegele M, Neugebauer E, Graham DI, Stocchetti N, McIntosh TK. Experimental models of traumatic brain injury: do we really need to build a better mousetrap? *Neuroscience*. 2005;136:971–989.
54. Yang L, Afroz S, Michelson HB, Goodman JH, Valsamis HA, Ling DS. Spontaneous epileptiform activity in rat neocortex after controlled cortical impact injury. *J Neurotrauma*. 2010;27(8):1541–1548.
55. Cernak I. Animal models of head trauma. *NeuroRx*. 2005;2(3):410–422.
56. Engel J Jr, Pedley TA. What is epilepsy? In: Engel J Jr, Pedley TA, eds. *Epilepsy: A Comprehensive Textbook*. Philadelphia: Lippincott-Raven; 2005:1–11.
57. Pitkänen A, Lukasiuk K. Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. *Epilepsy Behav*. 2009;1:16–25.

58. Pitkänen A, McIntosh TK. Animal models of post-traumatic epilepsy. *J Neurotrauma*. 2006;23(2): 241–261.
59. Swartz BE, Houser CR, Tomiyasu U, Walsh GO, DeSalles A, Rich JR, Delgado-Escueta A. Hippocampal cell loss in posttraumatic human epilepsy. *Epilepsia*. 2006;47(8):1373–1382.
60. Hudak AM, Trivedi K, Harper CR, Booker K, Caesar RR, Agostini M, Van Ness PC, Diaz-Arrastia R. Evaluation of seizure-like episodes in survivors of moderate and severe traumatic brain injury. *J Head Trauma Rehabil*. 2004;19(4):290–295.
61. Diaz-Arrastia R, Agostini MA, Frol AB, Mickey B, Fleckenstein J, Bigio E, Van Ness PC. Neurophysiologic and neuroradiologic features of intractable epilepsy after traumatic brain injury in adults. *Arch Neurol*. 2000;57(11):1611–1616.
62. Vespa PM, McArthur DL, Xu Y, Eliseo M, Etchepare M, Dinov I, Alger J, Glenn TP, Hovda D. Nonconvulsive seizures after traumatic brain injury are associated with hippocampal atrophy. *Neurology*. 2010;75(9):792–798.
63. Pitkänen A, Lukasiuk K. Mechanisms of epileptogenesis and potential treatment targets. *Lancet Neurol*. 2011;10(2):173–186.
64. Lai Y, Chen Y, Watkins SC, Nathaniel PD, Guo F, Kochanek PM, Jenkins LW, Szabó C, Clark RS. Identification of poly-ADP-ribosylated mitochondrial proteins after traumatic brain injury. *J Neurochem*. 2008;104(6):1700–1711.
65. Immonen RJ, Kharatishvili I, Gröhn H, Pitkänen A, Gröhn OH. Quantitative MRI predicts long-term structural and functional outcome after experimental traumatic brain injury. *Neuroimage*. 2009;45(1):1–9.
66. Hayward NM, Ndode-Ekane XE, Kutchiashvili N, Gröhn O, Pitkänen A. Elevated cerebral blood flow and vascular density in the amygdala after status epilepticus in rats. *Neurosci Lett*. 2010;484(1):39–42.
67. Laitinen T, Sierra A, Pitkänen A, Gröhn O. Diffusion tensor MRI of axonal plasticity in the rat hippocampus. *Neuroimage*. 2010;51(2):521–530.
68. Pitkänen A, Longhi L, Marklund N, Morales D, McIntosh TK. Mechanisms of neuronal death and neuroprotective strategies after traumatic brain injury. *Drug Discovery Today: Dis Mech*. 2005;2(4):409–418.
69. Marklund N, Bakshi A, Castelbuono DJ, Conte V, McIntosh TK. Evaluation of pharmacological treatment strategies in traumatic brain injury. *Curr Pharm Res*. 2006;12(13):1645–1680.
70. Wing LK, Behanna HA, Van Eldik LJ, Watterson DM, Ralay Ranaivo H. De novo and molecular target-independent discovery of orally bioavailable lead compounds for neurological disorders. *Curr Alzheimer Res*. 2006;3(3):205–214.
71. Dietrich WD, Bramlett HM. The evidence for hypothermia as a neuroprotectant in traumatic brain injury. *Neurotherapeutics*. 2010;7(1):43–50.
72. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol*. 1972;32:281–294.

Chapter 26

Fever, Febrile Seizures, and Epileptogenesis

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INTRODUCTION

Febrile seizures (FS) are the most common type of convulsions in infants and young children, occurring in 2%–6% of children.[1,2] They are defined as seizures arising during fever, not caused by an infection of the central nervous system. However, their definition does not exclude children with preexisting neurological deficits, a fact that might confound studies on the outcome of these seizures. Although there is limited evidence for adverse outcomes of simple (defined as short, with no focal motor phenomena) FS on the immature brain, complex FS, particularly long-duration FS or febrile status epilepticus (defined as seizures lasting for more than 30 min), have been associated with subsequent limbic epilepsy, as indicated by both prospective and retrospective studies as well as by recent data in animal models.[3–14] Some investigators have suspected that long-duration FS might result in cognitive defects in a subset of children.[15–17] Understanding the basic mechanisms of FS, and the potential epileptogenesis that follows them, requires animal models that enable direct examination of the causal mechanisms for the generation and consequences of these seizures. The mechanisms by which fever leads to FS, the outcome of FS measured as the risk of epilepsy, the properties

of FS associated with limbic epilepsy, and the mechanisms of epileptogenesis are discussed in the following sections.

HOW DOES FEVER CAUSE SEIZURES?

Genetic Susceptibility to FS

Febrile seizures occur sporadically and also run in families, suggesting a genetic contribution to their onset.[18] In immature rodent studies, including mice and rats of many different strains, all the animals developed hyperthermia-induced seizures, indicating that genetic susceptibility is not necessarily required for their generation.[12,19–21] Conversely, the fact that seizure-temperature thresholds (a measure of excitability)[22,23] vary among mouse strains with differing genetic makeup suggests the involvement of genotype in these seizures. Recently, increased susceptibility to FS has been found to result from mutations of several genes, including sodium channels,[24–26] gamma-aminobutyric acid A ($GABA_A$) receptors,[27–30] and hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels.[31] Other single-gene mutations, such as in the interleukin gene promoter, might predispose individuals to FS.[32] In addition, several genes might interact to promote the occurrence of these seizures in a more complex manner.

Increased Brain Temperature as a Mechanism of FS

Temperature influences neuronal properties.[33] The functions of specific ions channels (e.g., transient receptor potential vanilloid, 1 and 4) are regulated by brain temperature in the physiological and fever ranges.[34,35] Therefore, elevating the brain temperature should increase neuronal firing culminating in a seizure. Notably, hyperthermia induced by hot baths[36] or an overdose of hyperthermia-inducing medications (anticholinergics)[37] can provoke seizures in children, indicating that increased temperature itself can generate seizures. However, the exact mechanisms by which increased temperature might lead to the generation of FS have not been fully elucidated.

Fever Mediators Lead to Seizures

Fever not only increases brain temperature but also involves the release of inflammatory mediators, particularly cytokines[38,39] such as interleukin-1β (IL-1β) within the brain. Interleukin-1β contributes to neuronal hyperexcitability long term, in part by increasing the ceramide-induced Src family of tyrosine kinase function.[40] Acute changes in brain excitability might derive from the enhanced calcium permeability of glutamate receptors.[41] In addition, IL-1β exacerbates convulsant-induced seizures.[42,43] The involvement of endogenous IL-1β in the generation of FS was supported by the increased threshold temperature required to elicit experimental FS in mice lacking the IL-1β receptor type 1[22] (Fig. 26–1). Interestingly higher levels of this cytokine have been detected in individuals with FS with a mutation in the IL-1β gene promoter[32]—although the significance of this finding has been debated.[44] It is intriguing that fever of specific infectious etiologies, and specifically human herpesvirus 6, might influence the probability of FS generation.[45,46] Whether this virus, compared with other pathogens, leads to higher levels of cytokines in the child's brain has not been studied.

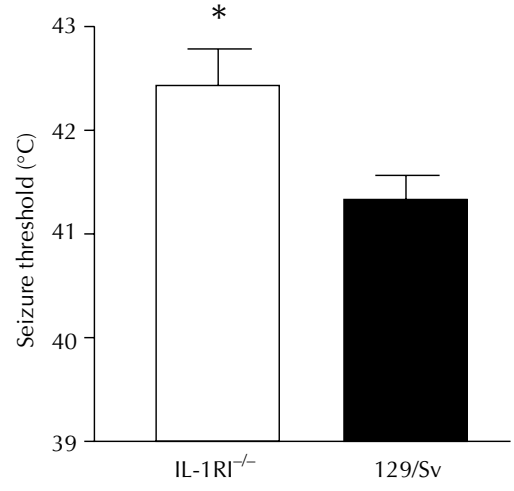

Figure 26–1. Mice lacking the IL-1 receptor type 1 ($IL\text{-}1RI^{-/-}$) are more resistant to the generation of experimental FS than wild-type controls of a similar genetic background (129/Sv). Threshold temperatures for the onset of these seizures were significantly higher in $IL\text{-}1RI^{-/-}$ mice (42.4 ± 0.3°C, $n = 26$) compared with the wild types (41.3 ± 0.2°C, $n = 21$). $^*p < .05$. From ref. 22.

Hyperthermia-Induced Hyperventilation and Alkalosis

Hyperthermia-induced hyperventilation and the consequent alkalosis have been suggested as important mechanisms for the generation of FS. Brain alkalosis has been shown to promote neuronal excitability[47,48] and may contribute to seizure pathophysiology in models with a long delay from hyperthermia to seizure onset.[49] However, it should be noted that the severe alkalosis found during prolonged crying, and that can exist for weeks in babies with pyloric stenosis, has not been correlated with a higher incidence of seizures (fever-induced or otherwise).

DO FS CAUSE EPILEPSY?

There is little evidence for an enduring adverse impact of short-duration FS on the developing brain.[4,50,51] However, prolonged or focal FS have been associated with the development of limbic (temporal lobe) epilepsy.[3,6,7,52] What is the neurobiological basis of this statistical correlation? Do long-duration FS cause temporal lobe epilepsy (TLE)? Are FS a marker of preexisting pathology? Is there a common denominator or cause that independently leads to FS and TLE or that alters the probability that FS would result in TLE?

A causal relationship between long-duration FS and the development of TLE is difficult to demonstrate or refute in humans because of the diverse genetic makeup of children and other uncontrolled preexisting factors. Single-gene (e.g., ion channel; see above) mutations, or a combination of multiple susceptibility genes, might predispose certain individuals to FS or to the development of epilepsy following FS.[25,27,28,31] In addition, the previous history of a child might render him or her more vulnerable to FS or their pro-epileptic or cognitive consequences. Controlling for these genetic and environmental/acquired factors is very difficult.

Therefore, to facilitate investigation of these seizures and their consequences, several animal models of prolonged FS[11,14,19–21,49,53,54] have been developed. In recent years, these models have led to fundamental discoveries about the mechanisms of these seizures, their effects on neuronal excitability, and their relationship to epilepsy.[8,12,55–59] We have devised and characterized a model of FS in infant rats and mice. Because fever cannot be reliably induced in suckling rodents,[60] we employed hyperthermia. Core and brain temperatures were raised to the range of 39.5 to 41.5°C for 30 min or ~60 min (re-creating febrile status epilepticus) in 10-to 11-day-old rats,[12,21] an age when hippocampal development approximates that of human infants.[61] In this model, core body measurements provide an adequate approximation of brain threshold temperature for experimental FS.[62] As described above, hyperthermia and fever utilize common mechanisms to elicit the seizures because hyperthermia leads to the release of cytokines.

Using this model, we determined if long-duration FS, induced at the stage of hippocampal development that corresponds to that of human infants,[61] leads to the development of limbic epilepsy. We demonstrated that experimental FS cause spontaneous recurrent seizures in a significant proportion of animals (35%) later in life.[57] These later seizures, resulting from an early 20 min FS, consisted of sudden freezing and typical limbic automatisms (stage 1)[63] that were coupled with polyspike/sharp-wave trains with increasing amplitude and slowing frequency on electroencephalogram (EEG). In addition, interictal epileptiform discharges were recorded in 88.2% of the experimental FS group. Interictal activity was never detected in normothermic control rats or in hyperthermic control rats (animals that sustained hyperthermia with no seizures).[57] Because, as described above, predisposing factors, both genetic and acquired, have largely been excluded in this model, these studies directly supported a causal relationship of long-duration FS and the development of TLE and set the stage for investigating the mechanisms by which these seizures promote epileptogenesis.

WHICH FS CAUSE EPILEPSY?

Febrile seizures occur in 2%–6% of children[2,64,65] and are prolonged in ~15% of this group.[1,66–68] As mentioned above, a key issue about FS is the clinical question of whether long-duration FS cause epilepsy,

and if so, why the process takes place only in some individuals. To address this question, we tested the hypothesis that seizure duration is a key parameter that governs epileptogenesis, as suggested by clinical studies.[4,7] We found that experimental FS lasting for ~20 min resulted in limbic epilepsy in ~35% of rats.[57] In contrast, when we increased the duration of seizures to ~60 min, we found that ~45% of rats developed limbic epilepsy.[13] Importantly, whereas the spontaneous seizures that resulted from FS lasting for ~20 min were mild (Racine[63] stage 1; duration average ~8 s), the duration of seizures constituting the limbic epilepsy provoked by febrile status epilepticus (FSE; 60 min FS) was much longer: 137 s on average (Fig. 26–2). The behavioral manifestations of the epileptic seizures were also more pronounced and included head bobbing, alternating or bilateral clonus, rearing, and falling (Racine stages 2–5).[63] The interictal activity recorded from cortical and hippocampal electrodes varied according to the duration of the inciting FS. Interictal activity was found in 84% of rats sustaining a ~60 min inciting FS, and individual interictal activity bouts were significantly longer and more robust than those after a ~20 min seizure.[13] However, the presence and duration of the interictal events did not predict the development of epilepsy. Thus, using an animal model enabled us to define the duration of FS as an important predictor of the severity of the TLE that develops after FS. This finding, in line with suggestive clinical literature,[69–72] carries enormous implications for the clinical management of FS.

HOW DO FS CAUSE EPILEPSY?

The mechanisms by which long-duration FS might contribute to the development of TLE are not fully resolved. Here we review several mechanisms that have been implicated in epileptogenesis and discuss their role in the epileptogenic process that follows long-duration experimental FS.

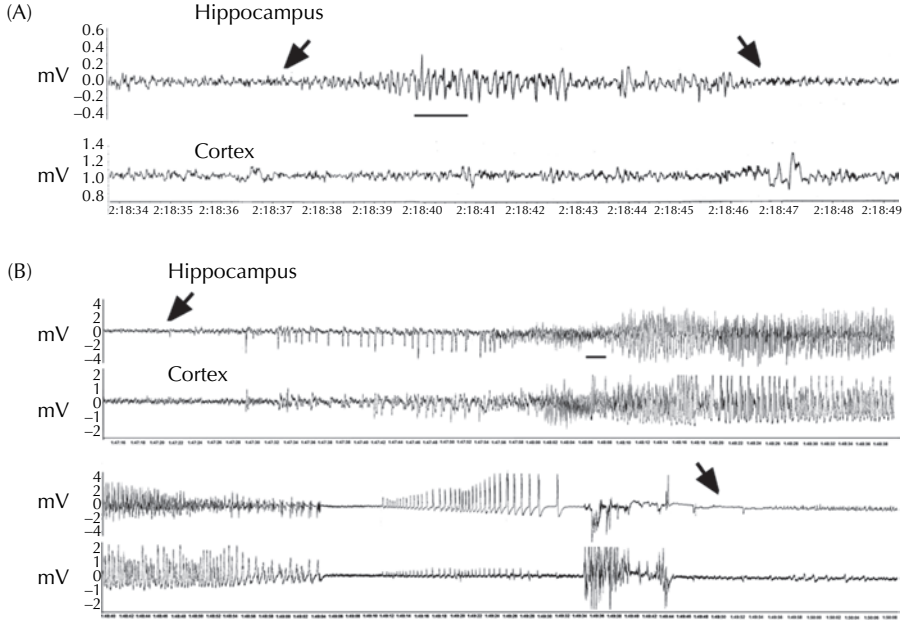

Figure 26–2. Examples of typical spontaneous electrographic seizures recorded from hippocampal and cortical bipolar electrodes in adult rats that had experienced (**A**) a 20 min or (**B**) a 60 min experimental FS. In the 60 min epilepsy model, spontaneous seizures commenced first in the hippocampus and propagated to the cortex (**B**), where epileptiform cortical discharges were apparent. In epilepsy generated by a 20 min FS (**A**), the epileptic seizure propagation to the cortex consisted of suppression. Note that the duration of the seizures was significantly longer after a 60 min FS compared to a 20 min FS (mean: 137 and 8 s, respectively; median duration: 91 and 7 s, respectively). Arrows point to the onset and end of epileptiform discharges. Calibration: 1 s. From ref. 13.

Inflammation and Cell Loss

As mentioned above, the inflammatory cytokine IL-1β contributes to the generation of experimental (and probably human) FS. However, whether or not IL-1β contributes to the epileptogenic process that is triggered by FS remains unclear. We found that IL-1β expression was induced in reactive astrocytes for at least 24 h after a ~60 min FS and returned to basal levels within 72 h.[13] Interestingly, when FS-sustaining rats that became epileptic were compared to those in which the inciting FS did not lead to spontaneous seizures, hippocampal IL-1β levels were higher only in the rats that developed epilepsy.[13]

Cytokines and other inflammatory mediators have been shown to contribute to neuronal injury and death.[73–75] In the mature brain, limbic seizures lead to the loss of vulnerable hippocampal cell populations,[76–79] and this loss is considered by many to be required for epileptogenesis.[76,78,79] In the FS model described above, a model that has been adopted by several other groups, appreciable neuronal death has not been found.[13,54,57,80] The seizures did provoke neuronal injury in the same cell distribution experiencing cell damage in human mesial temporal sclerosis (MTS); yet, the involved neurons did not seem to die.[21] Neurogenesis was also not detected after these seizures, and mossy fiber sprouting was minimal.[14,54,80,81] Therefore, such structural alterations are unlikely to account for the epileptogenic process that follows long-duration FS.

Role of Changes in the Expression of Gene Sets, Including Ion Channels, in FS-Induced Epileptogenesis

The likely mechanisms of epileptogenesis that follow experimental (and perhaps human) long-duration FS involve profound and persistent molecular changes in hippocampal neurons that induce alterations of their intrinsic excitability and network properties. Numerous molecular changes have been described after experimental FS (reviewed in ref. 82), and many are not yet known. Lasting changes in the expression of specific genes such as ion channels and endocannabinoid receptors have been explored. For example, experimental long-duration FS led to altered expression of ion channels conducting I_h, a hyperpolarization-triggered cationic current that contributes to the maintenance of neuronal membrane potential, subthreshold oscillations, and dendritic integration.[83–85] This change in I_h promoted frequency-dependent rebound depolarization in response to hyperpolarizing input; this rebound depolarization was augmented after the seizures.[8,55] At the molecular level, these changes were a result of long-lasting reduction in the expression of the HCN1 isoform, leading to an increased HCN2/HCN1 ratio and augmented heteromerization of these two isoforms.[86,87] Remarkably, reduction of HCN1 expression has since emerged as a fairly general principle in a number of models of acquired TLE.[88–92] HCN1 channel expression was found to be altered in a subset of resected hippocampi from patients with TLE and MTS, often with a history of early-life seizures.[93] As mentioned above, mutations in HCN channel genes have recently been discovered in individuals with epilepsy,[31] further supporting the role of these channels in the epileptogenic process. The precise mechanisms by which alterations in HCN channels and I_h contribute to human epileptogenesis are not fully understood.

An additional mechanism by which long-duration FS may promote hyperexcitability involves an increase in a short-term plasticity phenomenon known as *depolarization-induced suppression of inhibition* (DSI).[56] Depolarization-induced suppression of inhibition is mediated by cannabinoid receptors (CB1).[94,95] Following experimental FS, increased release of endocannabinoids upon postsynaptic depolarization of hippocampal pyramidal cells led to an increase in the number of presynaptic cannabinoid type 1 receptors, promoting increased retrograde inhibition of GABA release, which resulted in a reduction of inhibition in the hippocampal circuit.[9,56]

Activation of specific transcription factors by seizures, including experimental FS, has been recently found,[96] suggesting that rather than changes in single genes (even those governing important properties of the neuron), a coordinated change in the expression of gene clusters may be an important mechanism in the transformation of normal neurons and neuronal circuits into epileptic ones. One can only speculate that, in addition to the mechanisms described

above, many other alterations in gene expression will be found—after experimental and human prolonged FS—that will contribute to the epileptogenic process.

RELATIONSHIP BETWEEN FS, MTS, AND TLE

The absence of cell loss in rats that became epileptic following long-duration FS and FSE raises questions regarding the causal relationship of cell loss to epileptogenesis in children sustaining these seizures. One of the structural hallmarks of patients with mesial TLE with a history of long-duration FS is a specific pattern of cell loss in hippocampus, that is, MTS. It has been widely hypothesized that FS cause MTS and that the development of TLE is a consequence of MTS.[69,71,97] An alternative view is also supported by clinical data: MTS results from the recurrent seizures in individuals with TLE after FS.[98–100] The absence of cell loss in epileptic rats in our model is more consistent with the latter view.

In addition, the increased T2 relaxation time seen on magnetic resonance imaging (MRI) was reported in children with FSE and interpreted as indicating MTS.[69,71,97] In our animal model, we found abnormally elevated T2 relaxation times in the hippocampus in a subpopulation of FS-experiencing rats[13,17] (Fig. 26–3). These T2 changes on MRI were not associated with increased water content (CM Dubé, unpublished observations) and were not accompanied by neuronal loss in the hippocampus. These data suggest that acute MRI changes after long-duration FS should be interpreted with caution. Interestingly, the MRI changes observed in the hippocampus of a subset of rats following long-duration FS were predictive of hippocampal dysfunction manifesting as cognitive defects.[17]

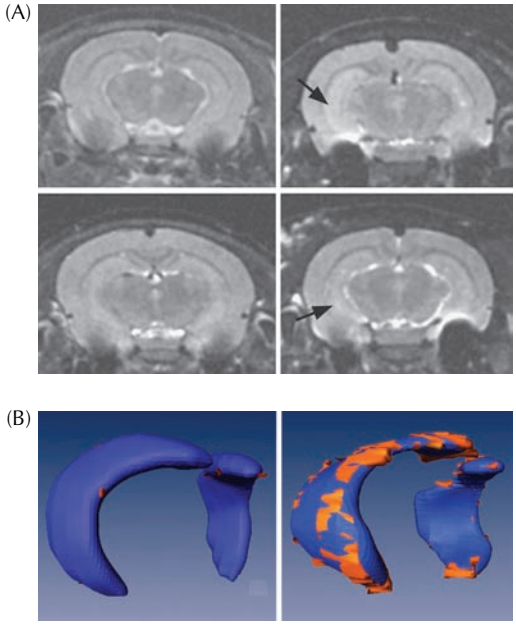

Figure 26–3. A. Examples of T2-weighted images obtained 1 month after a 60 min FS: the T2 signal (arrows) increased in the hippocampus of a subset of rats that experienced long-duration FS (right panels) compared with control rats (left panels). Absolute T2 relaxation time values (ms) were calculated on a pixel-by-pixel basis from the T2-weighted images, and T2 maps were generated. The FS rats segregated into two groups: one group with hippocampal T2 relaxation time values significantly higher compared to the mean values of the controls and a second group with T2 values similar to those of the controls. **B.** Examples of three-dimensional reconstruction of the hippocampi of two rats that sustained FS: pixels with significantly higher hippocampal T2 values compared to the pixel values (in blue) found in controls are noted in orange. **A** from ref. 13.

FUTURE DIRECTIONS

Febrile seizures are common and important, and we have only a partial understanding of their generation and consequences. Whereas short-duration (simple) FS are benign, more work is needed to tease out the neurobiological basis of the consequences of long-duration FS and FSE—both cognitive changes as well as epileptogenesis.

Among the key remaining goals in this field is the development of early and effective biomarkers that will define which child with long-duration FS is at risk for epilepsy or cognitive sequelae. Such surrogate markers of the disease process that culminate in the development of TLE and/or cognitive deficits should provide powerful tools for testing potential interventions aimed at stopping or reversing the epileptogenesis.

An additional important goal is the elucidation of the complex mechanisms underlying epileptogenesis that follows long-duration FS. Current data suggest that persistent changes

in the expression and function of gene sets that regulate neuronal and network activity (including cytokines and ions channels) might be involved. Identifying the key genes that contribute to the development of these sequelae, and delineating the mechanisms (e.g., epigenetic) that regulate these genes, may provide molecular targets for the development of novel preventive and therapeutic strategies for preventing the development of TLE after long-duration FS.[96]

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REFERENCES

1. Nelson KB, Ellenberg JH. Predictors of epilepsy in children who have experienced febrile seizures. *N Engl J Med*. 1976;295:1029–1033.
2. Stafstrom CE. The incidence and prevalence of febrile seizures. In: Baram TZ, Shinnar S, eds. *Febrile Seizures*. San Diego, CA: Academic Press; 2002:1–25.
3. Nelson, KB, Ellenberg JH. Prognosis in children with febrile seizures. *Pediatrics*. 1978;61:720–727.
4. Annegers JF, Hauser WA, Shirts SB, Kurland LT. Factors prognostic of unprovoked seizures after febrile convulsions. *N Engl J Med*. 1987;316:493–498.
5. Cendes F, Andermann F, Gloor P, Lopes-Cendes I, Andermann E, Melanson D, Jones-Gotman M, Robitaille Y, Evans A, Peters T. Atrophy of mesial structures in patients with temporal lobe epilepsy: cause or consequence of repeated seizures? *Ann Neurol*. 1993;34:795–801.
6. French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, Spencer DD. Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. *Ann Neurol*. 1993;34:774–780.
7. Berg AT, Shinnar S. Unprovoked seizures in children with febrile seizures: short-term outcome. *Neurology*. 1996;47:562–568.
8. Chen K, Baram TZ, Soltesz I. Febrile seizures in the developing brain result in persistent modification of neuronal excitability in limbic circuits. *Nat Med*. 1999;5:888–894.
9. Chen K, Neu A, Howard AL, Földy C, Echegoyen J, Hilgenberg L, Smith M, Mackie K, Soltesz I. Prevention of plasticity of endocannabinoid signaling inhibits persistent limbic hyperexcitability caused by developmental seizures. *J Neurosci*. 2007;27:46–58.
10. Theodore WH, Bhatia S, Hatta J, Fazilat S, DeCarli C, Bookheimer SY, Gaillard WD. Hippocampal atrophy, epilepsy duration, and febrile seizures in patients with partial seizures. *Neurology*. 1999;52:132–136.
11. Heida JG, Pittman QJ. Causal links between brain cytokines and experimental febrile convulsions in the rat. *Epilepsia*. 2005;46:1906–1913.
12. Dubé C, Chen K, Eghbal-Ahmadi M, Brunson K, Soltesz I, Baram TZ. Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long-term. *Ann Neurol*. 2000;47:336–344.
13. Dubé CM, Ravizza T, Hamamura M, Zha Q, Keebaugh A, Fok K, Andres AL, Nalcioglu O, Obenaus A, Vezzani A, Baram TZ. Epileptogenesis provoked by prolonged experimental febrile seizures: mechanisms and biomarkers. *J Neurosci*. 2010;22:7484–7494.
14. Lemmens EM, Lubbers T, Schijns OE, Beuls EA, Hoogland G. Gender differences in febrile seizure-induced proliferation and survival in the rat dentate gyrus. *Epilepsia*. 2005;46:1603–1612.
15. Baram TZ, Shinnar S. Do febrile seizures improve memory? *Neurology*. 2001;57:7–8.
16. Chang YC, Guo NW, Wang ST, Huang CC, Tsai JJ. Working memory of school-aged children with a history of febrile convulsions: a population study. *Neurology*. 2001;57:37–42.
17. Dubé CM, Zhou JL, Hamamura M, Zhao Q, Ring A, Abrahams J, McIntyre K, Nalcioglu O, Shatskih T, Baram TZ, Holmes GL. Cognitive dysfunction after experimental febrile seizures. *Exp Neurol*. 2009;215:167–177.
18. Berg AT, Shinnar S, Levy SR, Testa FM. Childhood-onset epilepsy with and without preceding febrile seizures. *Neurology*. 1999;53:1742–1748.
19. Holtzman D, Obana K, Olson J. Hyperthermia-induced seizures in the rat pup: a model for febrile convulsions in children. *Science*. 1981;213:1034–1036.
20. Morimoto T, Nagao H, Sano N, Takahashi M, Matsuda H. Electroencephalographic study of rat hyperthermic seizures. *Epilepsia*. 1991;32:289–293.
21. Toth Z, Yan XX, Haftoglou S, Ribak CE, Baram TZ. Seizure-induced neuronal injury: vulnerability to febrile seizures in an immature rat model. *J Neurosci*. 1998;18:4285–4294.
22. Dubé C, Vezzani A, Behrens M, Bartfai T, Baram TZ. Interleukin-ß contributes to the generation of experimental febrile seizures. *Ann Neurol*. 2005;57:152–155.
23. van Gassen KL, Hessel EV, Ramakers GM, Notenboom RG, Wolterink-Donselaar IG, Brakkee JH, Godschalk TC, Qiao X, Spruijt BM, van Nieuwenhuizen O, de Graan PN. Characterization of febrile seizures and febrile seizure susceptibility in mouse inbred strains. *Genes Brain Behav*. 2008;5:578–586.
24. Wallace RH, Wang DW, Singh R, Scheffer IE, George AL Jr, Phillips HA, Saar K, Reis A, Johnson EW, Sutherland GR, Berkovic SF, Mulley JC. Febrile seizures and generalized epilepsy associated with a mutation in the Na^+-channel beta1 subunit gene *SCN1B*. *Nat Genet*. 1998;19:366–370.

25. Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A. Mutations of *SCN1A*, encoding a neuronal sodium channel, in two families with GEFS+2. *Nat Genet*. 2000;24:343–345.
26. Martin MS, Dutt K, Papale LA, Dubé CM, Dutton SB, de Haan G, Shankar A, Tufik S, Meisler MH, Baram TZ, Goldin AL, Escayg A. Altered function of the *SCN1A* voltage-gated sodium channel leads to gamma-aminobutyric acid-ergic (GABAergic) interneuron abnormalities. *J Biol Chem*. 2010;285:9823–9834.
27. Harkin LA, Bowser DN, Dibbens LM, Singh R, Phillips F, Wallace RH, Richards MC, Williams DA, Mulley JC, Berkovic SF, Scheffer IE, Petrou S. Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am J Hum Genet*. 2002;70:530–536.
28. Audenaert D, Schwartz E, Claeys KG, Claes L, Deprez L, Suls A, Van Dyck T, Lagae L, Van Broeckhoven C, Macdonald RL, De Jonghe P. A novel *GABRG2* mutation associated with febrile seizures. *Neurology*. 2006;67:687–690.
29. Kang JQ, Shen W, Macdonald RL. Why does fever trigger febrile seizures? $GABA_A$ receptor gamma2 subunit mutations associated with idiopathic generalized epilepsies have temperature-dependent trafficking deficiencies. *J Neurosci*. 2006;26:2590–2597.
30. Kang JQ, Shen W, Macdonald RL. The *GABRG2* mutation, *Q351X*, associated with generalized epilepsy with febrile seizures plus, has both loss of function and dominant-negative suppression. *J Neurosci*. 2009;29:2845–2856.
31. Dibbens LM, Reid CA, Hodgson B, Thomas EA, Phillips AM, Gazina E, Cromer BA, Clarke AL, Baram TZ, Scheffer IE, Berkovic SF, Petrou S. Augmented currents of an HCN2 variant in patients with febrile seizure syndromes. *Ann Neurol*. 2010;67: 542–546.
32. Virta M, Hurme M, Helminen M. Increased frequency of interleukin-1beta (-511) allele 2 in febrile seizures. *Pediatr Neurol*. 2002;26:192–195.
33. Hodgkin AL, Katz B. The effect of temperature on the electrical activity of the giant axon of the squid. *J Physiol*. 1949;109:240–249.
34. Shibasaki K, Suzuki M, Mizuno A, Tominaga M. Effects of body temperature on neural activity in the hippocampus: regulation of resting membrane potentials by transient receptor potential vanilloid 4. *J Neurosci*. 2007;27:1566–1575.
35. Kim JA, Kauer JA, Connors BW. Hyperthermia increases intrinsic excitability and spontaneous synaptic activity of hippocampal CA3 pyramidal cells and interneurons. Abstract, Society for Neuroscience meeting, Washington DC, November 15–19, 38th meeting. *Neuroscience* 2008, #123.
36. Fukuda M, Morimoto T, Nagao H, Kida K. Clinical study of epilepsy with severe febrile seizures and seizures induced by hot water bath. *Brain Dev*. 1997;19: 212–216.
37. Olson KR, Kearney TE, Dyer JE, Benowitz NL, Blanc PD. Seizures associated with poisoning and drug overdose. *Am J Emerg Med*. 1994;12:392–395.
38. Alheim K, Bartfai T. The interleukin-1 system: receptors, ligands, and ICE in the brain and their involvement in the fever response. *Ann NY Acad Sci.* 1998;840:51–58.
39. Cartmell T, Luheshi GN, Rothwell NJ. Brain sites of action of endogenous interleukin-1 in the febrile response to localized inflammation in the rat. *J Physiol*. 1999;518:585–594.
40. Balosso S, Maroso M, Sanchez-Alavez M, Ravizza T, Frasca A, Bartfai T, Vezzani A. A novel non-transcriptional pathway mediates the proconvulsive effects of interleukin-1beta. *Brain*. 2008;131:3256–3265.
41. Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, Binaglia M, Corsini E, Di Luca M, Galli CL, Marinovich M. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J Neurosci*. 2003;23:8692–8700.
42. Vezzani A, Conti M, De Luigi A, Ravizza T, Moneta D, Marchesi F, De Simoni MG. Interleukin-1beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: functional evidence for enhancement of electrographic seizures. *J Neurosci*. 1999;19:5054–5065.
43. Vezzani A, Moneta D, Conti M, Richichi C, Ravizza T, De Luigi A, De Simoni MG, Sperk G, Andell-Jonsson S, Lundkvist J, Iverfeldt K, Bartfai T. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. *Proc Natl Acad Sci USA*. 2000;97:11534–11539.
44. Tan NC, Mulley JC, Berkovic SF. Genetic association studies in epilepsy: "the truth is out there." *Epilepsia*. 2004;45:1429–1442.
45. Barone SR, Kaplan MH, Krilov LR. Human herpesvirus-6 infection in children with first febrile seizures. *J Pediatr*. 1995;127:95–97.
46. Zerr DM, Meier AS, Selke SS, Frenkel LM, Huang ML, Wald A, Rhoads MP, Nguy L, Bornemann R, Morrow RA, Corey L. A population-based study of primary human herpesvirus 6 infection. *N Engl J Med*. 2005;352:768–776.
47. Aram JA, Lodge D. Epileptiform activity induced by alkalosis in rat neocortical slices: block by antagonists of *N*-methyl-D-aspartate. *Neurosci Lett*. 1987;83: 345–350.
48. Balestrino M, Somjen CG. Concentration of carbon dioxide, interstitial pH and synaptic transmission in hippocampal formation of the rat. *J Physiol*. 1988;396:247–266.
49. Schuchmann S, Schmitz D, Rivera C, Vanhatalo S, Salmen B, Mackie K, Sipilä ST, Voipio J, Kaila K. Experimental febrile seizures are precipitated by a hyperthermia-induced respiratory alkalosis. *Nat Med*. 2006;12:817–823.
50. Camfield P, Camfield C. Febrile seizures. In: Shinnar S, Amir N, Branski D, eds. *Childhood Seizures*. Basel: Karger; 1995:32–38.
51. Shinnar S. Do febrile seizures lead to temporal lobe epilepsy? Prospective and epidemiological studies. In: Baram TZ, Shinnar S, eds. *Febrile Seizures*. San Diego, CA: Academic Press; 2002:87–101.
52. Cendes F, Andermann F. Do febrile seizures promote temporal lobe epilepsy? Retrospective studies. In: Baram TZ, Shinnar S, eds. *Febrile Seizures*. San Diego, CA: Academic Press; 2002:77–86.
53. Germano IM, Zhang YF, Sperber EF, Moshé SL. Neuronal migration disorders increase susceptibility

to hyperthermia-induced seizures in developing rats. *Epilepsia.* 1996;37:902–910.
54. Jiang W, Duong TM, de Lanerolle NC. The neuropathology of hyperthermic seizures in the rat. *Epilepsia*. 1999;40:5–19.
55. Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I. Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat Med.* 2001;7:331–337.
56. Chen K, Ratzliff A, Hilgenberg L, Gulyás A, Freund TF, Smith M, Dinh TP, Piomelli D, Mackie K, Soltesz I. Long-term plasticity of endocannabinoid signaling induced by developmental febrile seizures. *Neuron.* 2003;39:599–611.
57. Dubé C, Richichi C, Bender RA, Chung G, Litt B, Baram TZ. Temporal lobe epilepsy after experimental prolonged febrile seizures: prospective analysis. *Brain*. 2006;129:911–922.
58. Chang YC, Huang AM, Kuo YM, Wang ST, Chang YY, Huang CC. Febrile seizures impair memory and cAMP response-element binding protein activation. *Ann Neurol*. 2003;54:706–718.
59. Kamal A, Notenboom RG, de Graan PN, Ramakers GM. Persistent changes in action potential broadening and the slow afterhyperpolarization in rat CA1 pyramidal cells after febrile seizures. *Eur J Neurosci*. 2006;23:2230–2234.
60. Heida JG, Boissé L, Pittman QJ. Lipopolysaccharide-induced febrile convulsions in the rat: short-term sequelae. *Epilepsia*. 2004;45:1317–1329.
61. Avishai-Eliner S, Brunson KL, Sandman CA, Baram TZ. Stressed-out, or in (utero)? *Trends Neurosci*. 2002;25:518–524.
62. Dubé C, Brunson KL, Eghbal-Ahmadi M, Gonzalez-Vega R, Baram TZ. Endogenous neuropeptide Y prevents recurrence of experimental febrile seizures by increasing seizure threshold. *J Mol Neurosci*. 2005;25: 275–284.
63. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol*. 1972;32:281–294.
64. Hauser W, Hersdorffer D. *Epilepsy: Frequency, Causes and Consequences*. New York: Demos; 1990.
65. Berg AT, Shinnar S, Hauser WA, Alemany M, Shapiro ED, Salomon ME, Crain EF. A prospective study of recurrent febrile seizures. *N Engl J Med*. 1992;327:1122–1127.
66. Sillanpää M, Camfield PR, Camfield CS, Aromaa M, Helenius H, Rautava P, Hauser WA. Inconsistency between prospectively and retrospectively reported febrile seizures. *Dev Med Child Neurol*. 2008;50: 25–28.
67. Berg, AT, Shinnar S. Complex febrile seizures. *Epilepsia.* 1996;37:126–133.
68. Hesdorffer DC, Chan S, Tian H, Allen Hauser W, Dayan P, Leary LD, Hinton VJ, Gertrude H. Are MRI-detected brain abnormalities associated with febrile seizure type? *Epilepsia*. 2008;49:765–771.
69. VanLandingham KE, Heinz ER, Cavazos JE, Lewis DV. Magnetic resonance imaging evidence of hippocampal injury after prolonged focal febrile convulsions. *Ann Neurol*. 1998;43:413–426.
70. Natsume J, Bernasconi N, Miyauchi M, Naiki M, Yokotsuka T, Sofue A, Bernasconi A. Hippocampal volumes and diffusion-weighted image findings in children with prolonged febrile seizures. *Acta Neurol Scand Suppl*. 2007;186:25–28.
71. Provenzale JM, Barboriak DP, VanLandingham K, MacFall J, Delong D, Lewis DV. Hippocampal MRI signal hyperintensity after febrile status epilepticus is predictive of subsequent mesial temporal sclerosis. *Am J Roentgenol*. 2008;190:976–983.
72. Shinnar S, Hesdorffer DC, Nordli DR Jr, Pellock JM, O'Dell C, Lewis DV, Frank LM, Moshé SL, Epstein LG, Marmarou A, Bagiella E, FEBSTAT Study Team. Phenomenology of prolonged febrile seizures: results of the FEBSTAT study. *Neurology*. 2008;71:170–176.
73. Allan SM, Tyrrell PJ, Rothwell NJ. Interleukin-1 and neuronal injury. *Nat Rev Immunol*. 2005;5:629–640.
74. Vezzani A, Baram TZ, French J, Bartfai T. Brain inflammation in epilepsy. *Nat Rev Neurol.* 2011;7:31–40.
75. Vezzani A, Baram TZ. New roles for interleukin-1 beta in the mechanisms of epilepsy. *Epilepsy Curr*. 2007;7:45–50.
76. Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience*. 1985;14: 375–403.
77. Sutula T, He XX, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science.* 1988;239:1147–1150.
78. Sloviter RS. The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy. *Ann Neurol*. 1994;35:640–654.
79. Pitkänen A, Nissinen J, Nairismägi J, Lukasiuk K, Grohn OH, Miettinen R, Kaupinen R. Progression of neuronal damage after status epilepticus and during spontaneous seizures in a rat model of temporal lobe epilepsy. *Prog Brain*. 2002;135:67–83.
80. Bender RA, Dubé C, Gonzalez-Vega R, Mina EW, Baram TZ. Mossy fiber plasticity and enhanced hippocampal excitability, without hippocampal cell loss or altered neurogenesis, in an animal model of prolonged febrile seizures. *Hippocampus*. 2003;13:399–412.
81. Baram TZ, Eghbal-Ahmadi M, Bender RA. Is neuronal death required for seizure-induced epileptogenesis in the immature brain? *Prog Brain Res.* 2002;135: 365–375.
82. Dubé CM, Brewster AL, Richichi C, Zha Q, Baram TZ. Fever, febrile seizures and epilepsy. *Trends Neurosci*. 2007;30:490–496.
83. Magee JC. Dendritic lh normalizes temporal summation in hippocampal CA1 neurons. *Nat Neurosci.* 1999;2:508–514.
84. Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: from molecules to physiological function. *Annu Rev Physiol*. 2003;65:453–480.
85. Santoro B, Baram TZ. The multiple personalities of h-channels. *Trends Neurosci*. 2003;26:550–554.
86. Brewster AL, Bender RA, Chen Y, Dube C, Eghbal-Ahmadi M, Baram TZ. Developmental febrile seizures modulate hippocampal gene expression of hyperpolarization-activated channels in an isoform- and cell-specific manner. *J Neurosci*. 2002;22:4591–4599.
87. Brewster AL, Bernard JA, Gall CM, Baram TZ. Formation of heteromeric hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in the

hippocampus is regulated by developmental seizures. *Neurobiol Dis*. 2005;19:200–207.
88. Jung S, Jones TD, Lugo JN Jr, Sheerin AH, Miller JW, D'Ambrosio R, Anderson AE, Poolos NP. Progressive dendritic HCN channelopathy during epileptogenesis in the rat pilocarpine model of epilepsy. *J Neurosci*. 2007;27:13012–13021.
89. Dugladze T, Vida I, Tort AB, Gross A, Otahal J, Heinemann U, Kopell NJ, Gloveli T. Impaired hippocampal rhythmogenesis in a mouse model of mesial temporal lobe epilepsy. *Proc Natl Acad Sci USA*. 2007;104:17530–17535.
90. Powell KL, Ng C, O'Brien TJ, Xu SH, Williams DA, Foote SJ, Reid CA. Decreases in HCN mRNA expression in the hippocampus after kindling and status epilepticus in adult rats. *Epilepsia*. 2008;49: 1686–1695.
91. Marcelin B, Chauvière L, Becker A, Migliore M, Esclapez M, Bernard C. h channel-dependent deficit of theta oscillation resonance and phase shift in temporal lobe epilepsy. *Neurobiol Dis*. 2009;33: 436–447.
92. Santoro B, Lee JY, Englot DJ, Gildersleeve S, Piskorowski RA, Siegelbaum SA, Winawer MR, Blumenfeld H. Increased seizure severity and seizure-related death in mice lacking HCN1 channels. *Epilepsia*. 2010;51:1624–1627.
93. Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ. Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. *J Neurosci*. 2003;23:6826–6836.
94. Chevaleyre V, Takahashi KA, Castillo PE. Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu Rev Neurosci*. 2006;29:37–76.
95. Monory K, Massa F, Egertová M, Eder M, Blaudzun H, Westenbroek R, Kelsch W, Jacob W, Marsch R, Ekker M, Long J, Rubenstein JL, Goebbels S, Nave KA, During M, Klugmann M, Wölfel B, Dodt HU, Zieglgänsberger W, Wotjak CT, Mackie K, Elphick MR, Marsicano G, Lutz B. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron*. 2006;51:455–466.
96. McClelland S, Flynn C, Dubé CM, Richichi C, Zha Q, Gesthem A, Esclapez M, Bernard C, Baram TZ. Neuron-restrictive silencer factor-mediated cyclic nucleotide gated channelopathy in experimental temporal lobe epilepsy. *Ann Neurol*. 2011, In press.
97. Scott RC, King MD, Gadian DG, Neville BG, Connelly A. Hippocampal abnormalities after prolonged febrile convulsion: a longitudinal MRI study. *Brain*. 2003;126:2551–2557.
98. Shinnar S. Febrile seizures and mesial temporal sclerosis. *Epilepsy Curr*. 2003;3:115–118.
99. Salmenperä T, Könönen M, Roberts N, Vanninen R, Pitkänen A, Kälviäinen R. Hippocampal damage in newly diagnosed focal epilepsy: a prospective MRI study. *Neurology*. 2005;64:62–68.
100. Kapur J. Is mesial temporal sclerosis a necessary component of temporal lobe epilepsy? *Epilepsy Curr*. 2006;6:208–209.

Chapter 27

Role of Blood-Brain Barrier Dysfunction in Epileptogenesis

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Focal epilepsy typically arises from neuronal tissue either within or adjacent to a cortical lesion. About 30% of epilepsies are caused by acquired etiologies such as traumatic brain injury, stroke, infection, or prolonged febrile seizures.[1] Injury-related acquired epilepsy is frequently resistant to medications and may be associated with other neurological impairments.

In most animal models of acquired epilepsy (similar to the situation in humans), a period of days to weeks is required for the development of seizures.[2,3] Typically, the initial insult is followed by a latent interval, referred to as *epileptogenesis*, in which cellular and structural reorganization occurs that ultimately leads to chronic recurrent epileptic seizures. While the molecular, anatomical, and electrophysiological activities in the epileptic focus have been described in great details (e.g., in refs. 4–8), the critical changes occurring following injury and *before* epileptic activity develops are mostly unknown. A better understanding of the molecular and physiological events during epileptogenesis is essential for the targeted development of preventive therapeutic approaches that are presently unavailable.[1]

BRAIN INSULTS AND INJURY TO THE BLOOD-BRAIN BARRIER

A plethora of data accumulating in recent years has indicated a central role for vascular integrity, specifically the permeability of the blood-brain barrier (BBB), as an important mediator of brain damage, including the delayed appearance of neuronal dysfunction and death.[9–14] Dysfunction of the BBB (which may be long-lasting,[2,15]) is frequently associated with myriad neurological pathologies and has thus attracted growing attention as a novel and important target for intervention in the setting of brain injury. Specifically, clinical and animal studies have presented evidence to support the

hypothesis that opening of the BBB triggers a chain of events leading to epilepsy.[12,13,16–23]

Both clinical data and research on animal models indicate that BBB dysfunction is a typical finding following traumatic, ischemic, or infectious brain insults, and may last for several days to weeks and even years after the acute event.[12,24,25] Opening of the BBB following experimental brain injury is usually considered to be biphasic.[26–28] The onset of the early phase is rapid, and permeability of the BBB typically reaches a maximum within a few hours, after which time it declines. The onset of the second phase is delayed, starting 3 to 7 days following injury, and is probably part of the brain's response to the injury. Clinical data suggest that for most patients, BBB permeability returns to normal within days to weeks following the insult; however, there is lack of repeated data from the same animals on BBB functions and there are no quantitative data on the extent of BBB damage in relation to the mechanism, location, and severity of the insult. The mechanisms of BBB breakdown and repair are not well understood. Breakdown of the BBB may be a direct result of the insult (trauma or ischemia to endothelial vessels—*primary BBB damage*), but it may also be secondary to the abnormal brain activity, astrocytic dysfunction, inflammation, and metabolic disturbances that typically take place as part of the brain's response to injury.

Mechanisms Underlying BBB Damage

Direct injury to endothelial cells—as occurs in traumatic shear injuries, in ischemic and metabolic insults, and in infectious and inflammatory vasculature pathologies—leads to impaired regulation of the BBB, of cerebral blood flow (CBF), and of metabolic demands, which in turn lead to metabolic imbalance and to the formation of an "ischemic" zone. The hypoxic tissue contributes locally to dysfunction of the BBB.[29] Secondary BBB damage is usually initiated within hours or days after injury; it is considered to be treatable and to have a considerable influence on the long-range clinical outcome. A number of mechanisms have been proposed for the emergence of secondary damage following BBB disruption, all of which are a consequence of either the disruption of the intricate relationship between the cellular elements of the neurovascular unit (which involves the blood vessels and particularly capillaries with astrocytic endfeet from surrounding astrocytes and—at least at larger vessels—nerve terminals impinging on blood vessels) or the impairment of the structural integrity within the tight-junction complex of the BBB (see below). Processes such as edema, inflammation, and cell death may further contribute to BBB dysfunction, independently or synergistically. Other mechanisms, such as vasospasm,[30] CBF autoregulatory failure,[31] irregularities in nitric oxide secretion[32] and coagulopathies[33] may coincide with the impairment of BBB structural integrity[34] and contribute to the resultant ischemic state and secondary loss of BBB functions. The underlying molecular changes leading to BBB dysfunction are not completely clear, but may involve vasogenic edema due to penetration of blood protein into the brain extracellular space due to increased endothelial caveolae, leading to transcytosis of plasma proteins,[35,36] decreased expression of junctional adhesion as well as tight junction proteins,[37,38] and increased expression of matrix metalloproteases.[39] Reactive cellular activity in the neurovascular junction has also been observed, including increased migratory activity of pericytes[40] and the proliferation of blood vessels due to upregulation of vascular endothelial growth factor (VEGF).[41] In addition, BBB opening itself leads to exposure of the brain tissue to serum-derived (normally nonpermeable) molecules, which serve as signaling mediators for brain repair mechanisms but which also facilitate BBB breakdown.

BBB DYSFUNCTION FOLLOWING SEIZURES AND STATUS EPILEPTICUS

While seizures and vascular changes are often found at the same time, the causal relationship between the two phenomena remains a matter of intensive research. In experimental animals, induction of seizures,[42] especially status epilepticus (SE),[13,43–48] leads to a rapid increase in BBB permeability lasting for several days to weeks. Studies in human epileptic patients are consistent with the animal data, showing evidence of a frequent increase in BBB permeability during seizures.[12,21,49–54] Ultrastructural research

on human epileptic tissue has confirmed the results of imaging studies, showing clear BBB abnormalities, including increased micropinocytosis and fewer mitochondria in endothelial cells, thickening of the basal membrane, and the presence of abnormal tight junctions[55–57] with evidence of barrier dysfunction such as markedly elevated levels of serum proteins within the epileptic tissue.[13,58] The accumulating data thus suggest that the increase in blood vessel permeability within the epileptic focus does not represent a transient seizure-related event, but rather a prominent vascular pathology within the epileptic tissue. However, the lack of a reliable quantitative method to measure BBB permeability in vivo precludes a large-scale human study to clarify this issue.

In addition to changes in BBB permeability, an increase in vessels density has been recently described in the hippocampus of patients suffering from drug-resistant temporal lobe epilepsy (TLE).[59] Notably, the vascular density, while associated with leaky vessels, is independent of seizure etiology, duration of the disease, or severity of damage or gliosis; it is, however, positively correlated with the frequency of seizures. The immature and tortuous aspect of microvessels and the loss of tight junction proteins indicate a pathological angiogenesis. This abnormality was further confirmed in the pilocarpine rat model of epilepsy (see also ref. 48), which showed progressive neovascularization during epileptogenesis, with high levels of VEGF in neurons and astrocytes and high levels of the VEGF receptor tyrosine kinase (VEGFR-2) in neurons and endothelial cells. Together, these human and animal studies strongly indicate lasting vascular and BBB damage in at least some forms of epilepsy, which calls for the understanding of the role of such vascular pathologies in epileptogenesis and seizure generation.

BBB DYSFUNCTION AND EPILEPTOGENSIS

The frequent finding of BBB dysfunction after SE, after seizures, and in epileptic patients led to the captivating hypothesis of a direct role for abnormal blood-brain communication in epileptogenesis,[20] supported by the observation of a positive correlation between the extent of BBB opening and the number of subsequent seizures in the pilocarpine model of TLE.[13] More direct evidence for the involvement of BBB dysfunction in epileptogenesis has been recently established by a series of animal studies in which a long-lasting opening of the BBB in the rat neocortex was shown to result in the delayed appearance of hypersynchronous epileptiform activity (reviewed in ref. 60; see also Fig. 27–1). Importantly, BBB dysfunction does not seem to induce epileptogenesis by provoking SE or immediate neuronal death, although under some conditions (e.g., intra-arterial mannitol) it can provoke seizures,[19] and it does reduce the seizure threshold in epileptic animals.[13] The mechanisms underlying the emergence of network hypersynchronicity under the BBB-disrupted brain are not entirely clear; however, experimental data support a role for serum proteins that normally are not found in the adult central nervous system, including albumin[17,22,23,61] and perhaps thrombin[62] via specific signaling pathways (see below). Epileptogenesis following BBB injury does not seem to be mediated by a significant loss of inhibitory interneurons,[23] but rather seems to involve early dysfunction of astrocytes (i.e., *reactive astrogliosis*) and activation of an inflammatory response (see below).

The Role of Astrocytes in Epileptogenesis Induced by BBB Dysfunction

In animal studies where BBB has been disrupted to induce a chronic epileptic focus, early dysfunction of astrocytes was observed prior to the occurrence of neuronal hypersynchronization.[17,61] These studies, as well as in vitro experiments, suggested the possible involvement of serum albumin in the regulation of astrocytic calcium signaling[63] and gene expression.[61,64] Changes in astrocytic gene expression, morphology, and function following insults to the brain are well documented,[65] and the potential role of astrocytes in seizure generation and epilepsy has been described in both experimental animals and epileptic patients.[66–69] Recent studies have indicated novel physiological roles for glial cells in the central nervous system,, such as modulation of synaptic transmission and plasticity.[70,71] It is

thus plausible that reactive (*transformed* or *activated*) glial cells play a significant role in neuronal network reorganization, hypersynchronicity, and hyperexcitability, leading to seizures.[61,72] Changes in astrocytic functions that are potentially involved in increased neuronal excitability and epileptogenesis include four alterations.

(1) *Reduced expression of potassium inward-rectifying channels* (*Kir4.1*) *and water channels* (*aquaporin 4, AQP4*): Both channels are colocalized most abundantly in astrocytic endfeet and are considered crucial for the regulation of the brain's extracellular potassium ($[K^+]_0$) and water content. Indeed, downregulation of Kir4.1 and AQP4 in genetically engineered mice results in impaired $[K^+]_0$ buffering and seizures.[73,74] Impaired $[K^+]_0$ buffering due to downregulation of Kir4.1 channels has also been reported in the hippocampus from pilocarpine-treated epileptic rats[75] and in the sclerotic hippocampus from TLE patients.[76,77] Impaired $[K^+]_0$ buffering, and specifically activity-dependent $[K^+]_0$ accumulation, will contribute to changes in the extracellular space and to neuronal excitability, resulting in a reduced firing threshold, slower neuronal repolarization, increased transmitter release, and facilitated activation of *N*-methyl-D-aspartate (NMDA) receptors, thus enhancing synchronization.[61,78]

(2) *Reduced gap junctions:* Gap junctions are functional channels between cells and consist of connexin proteins. Astrocytes are coupled via gap junctions to form large cellular networks that facilitate spatial buffering of small molecules (e.g., K^+). Connexin knockout mice show a mild decrease in the spatial buffering of $[K^+]_0$ and a decreased seizure threshold,[79] although most buffering capacity is maintained.

(3) *Impaired glutamate metabolism:* Glia cells may express glutamatergic receptors and release glutamate, and they are important for the uptake and metabolism of glutamate. In the hippocampal slice preparation from healthy mice, astrocytic glutamate release has been shown to contribute to a slow tetrodotoxin (TTX)-resistant, NMDA-sensitive neuronal inward current that may represent the typical *paroxysmal depolarization shift* (PDS) underlying the interictal burst.[80] It is not clear, however, to what extent such release contributes to epileptogenesis or to the propagating seizure activity in the epileptic brain. There is no direct evidence for increased glutamate release from astrocytes in chronic epileptic tissue, although upregulation of tumor necrosis factor alpha (TNFα; together with microglial activation) in the epileptic tissue may enhance glutamate release.[81,82] Transformed astrocytes may also affect extracellular glutamate levels by decreased uptake and metabolism. Astrocytes express two specific glutamate transporters, EAAT1 (also known as GLAST in rodents) and EAAT2 (GLT-1), which are responsible for most glutamate uptake in the brain. There is strong evidence to support the conclusion that significant impairment of astrocytic glutamate transporters is associated with the development of seizures; however, whether glutamate transporters are indeed downregulated in reactive astrocytes within the epileptic tissue is controversial, with reports demonstrating reduced, normal, and increased levels (reviewed in ref. 68). Astrocytes are also responsible for the conversion of the transported glutamate into glutamine by glutamine synthetase. Glutamine is transported back into neurons, where it is converted to glutamate by mitochondrial glutaminase and eventually to gamma-amino-butyric acid (GABA) in neurons that express the enzyme glutamate decarboxylase (GAD). Sclerotic brain tissue from TLE patients shows an approximately 40% reduction in the level of glutamine synthetase in astrocytes,[83] and glutamine synthetase inhibitors (80%–90% inhibition) cause seizures in experimental animals[84] (but see also ref. 85).

(4) *Increased release of inflammatory mediators by transformed astrocytes:* Astrocytes can produce many pro- and anti-inflammatory molecules, and these can be pro- and antiepileptogenic. Accumulating experimental evidence supports the role of interleukin 1-beta (IL-1β) in reducing the seizure threshold and in promoting epileptogenesis in the pilocarpine SE model of epilepsy.[86,87] Recent data suggest that other inflammatory mediators (e.g., cyclooxygenase-2) that are released from astrocytes may directly affect synaptic signaling,[88] plasticity,[89] and perhaps epileptogenesis.[90,91] It is still not yet clear to what extent and via which mechanisms the different inflammatory mediators contribute to alterations within the neuronal network and the generation of seizures; what

the role of inflammatory mediators might be in controlling BBB permeability; and what the role of leukocytes is in trafficking mechanisms in the inflammatory response, BBB permeability changes, and epileptogenesis.[92,93]

To summarize, experimental evidence unequivocally supports the notion that transformed (activated) astrocytes are prominent in the epileptic brain, and that these astrocytes have properties that are consistent with a reduced seizure threshold. However, it is important to distinguish between seizure generation and epileptogenicity—which involves a more complex and lasting changes in the network. To what extent do astrocytes have a direct role in such complex and long-term network changes? Hints for such a role come from experiments showing that in the SE-induced model of epilepsy, transformation of astrocytes starts within hours to days following SE, during the latent period of epileptogenesis.[94] Similarly, in the BBB disruption or albumin-exposure models, increased expression of the glial fibrillary acidic protein (GFAP), reduced expression of Kir4.1, and disturbed $[K^+]_0$ buffering precede the development of seizures,[17] strengthening the argument that transformation of astrocytes does not just reflect a hyperexcitable network, but rather contributes directly to its development. The possible involvement of BBB breakdown and albumin in the induction of astrocytic transformation is supported by studies of altered calcium signaling and DNA synthesis in cultured astrocytes exposed to serum albumin.[63] The observations that the action of serum albumin to induce astrocytic transformation is mediated via transforming growth factor beta receptor II (TGFβR2) highlight the involvement of specific signaling cascades in epileptogenesis following insult.

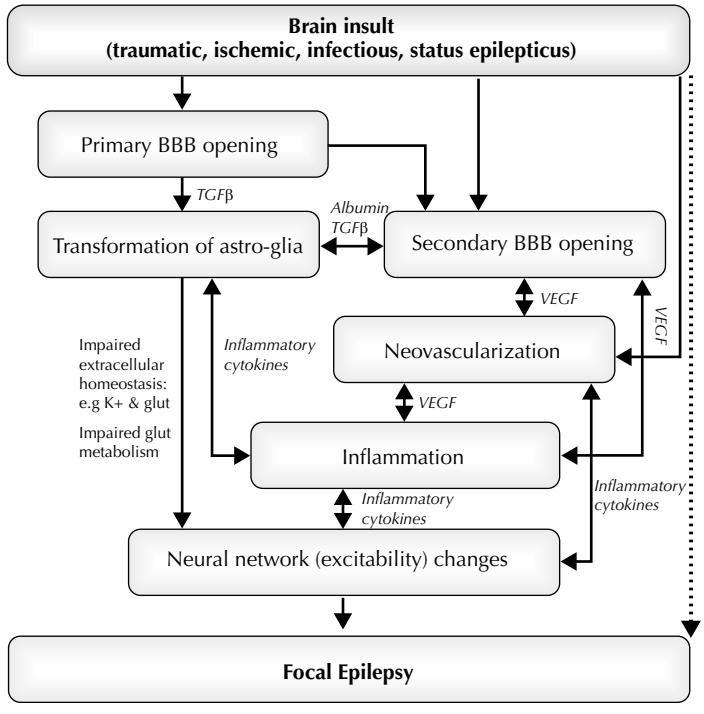

Figure 27–1. Proposed events during the development of an epileptic focus following brain injury. Prolonged seizures and ischemic, traumatic, or infectious insults induce primary and/or secondary opening of the BBB. Through serum proteins (e.g., albumin) and specific signaling cascades (e.g., TGFβ), BBB opening induces the transformation of astroglia into reactive or activated states, which initiates the release of inflammatory cytokines. In addition, vascular injury and BBB opening are associated with neovascularization with the generation of additional leaky vessels. Both the impaired buffering of extracellular potassium (K^+) and glutamate (glut) and the secreted inflammatory cytokines increase neuronal excitability and network connectivity to yield the epileptic network.

BBB Dysfunction and the Role of TGFβ Signaling in Epileptogenesis

Transforming growth factor beta (TGFβ) consists of pleiotropic cytokines that play a pivotal role in intercellular communication,[95,96] and their signaling pathways are frequently involved in cell growth, embryogenesis, differentiation, morphogenesis, wound healing, immune response, and apoptosis in a wide variety of cells.[97–99] The past decade has seen an explosion of information regarding the expression and action (sometimes contradictory) of TGFβ in the brain. Accumulating evidence unequivocally indicates that TGFβ is an injury-related cytokine that is upregulated in different forms of brain injury[100,101]; however, its exact actions are still under debate.[102] The potential involvement of TGFβ in epileptogenesis was first suggested on the basis of animal experiments documenting TGFβ upregulation in the brains of amygdala-kindled rats.[103] Aronica and colleagues showed TGFβ expression in astrocytes from the hippocampus of SE-experienced rats and suggested that it has a role in modulating glial functions in the course of epileptogenesis.[104] However, direct evidence for the role of TGFβ in cortical dysfunction and epileptogenesis is scarce. Following BBB opening, serum albumin has been shown to bind to TGFβR2, induce Smad2 phosphorylation, and activate its signaling cascades.[17,64] Indeed, BBB opening and brain exposure to albumin or TGFβ1 all induce a similar transcriptional modulation of genes, including those associated with the TGFβ pathway, astrocytic activation, inflammation, and reduced inhibitory transmission.[64] Future studies are required to confirm these promising results and the potential role of inhibition of this signaling pathway in the prevention of epileptogenesis.

In summary, data from human epileptic patients show clearly that vascular pathologies, and specifically increased BBB permeability, are a frequent finding in injury-related focal epilepsies. Studies in experimental animals are starting to reveal the mechanisms and signaling cascades leading from vascular changes to modifications of the neural network (Fig. 27–1). These studies stress the importance of interactions within different components of the neurovascular unit. Future developments in quantitative imaging of BBB permeability in human patients, together with individual and mechanism-targeted therapeutic approaches, may allow the development of new antiepileptogenic and antiepileptic treatments for specific populations.

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REFERENCES

1. Herman ST. Epilepsy after brain insult: targeting epileptogenesis. *Neurology.* 2002;59:21–26.
2. Hoffman SN, Salin PA, Prince DA. Chronic neocortical epileptogenesis in vitro. *J Neurophysiol.* 1994;71:1762–1773.
3. Prince DA, Tseng GF. Epileptogenesis in chronically injured cortex: in vitro studies. *J Neurophysiol.* 1993;69:1276–1291.
4. Huguenard JR, Chung JM, Prince DA. Excitability changes in thalamic and neocortical neurons after injury. *Epilepsy Res Suppl.* 1996;12:129–135.
5. Jacobs KM, Kharazia VN, Prince DA. Mechanisms underlying epileptogenesis in cortical malformations. *Epilepsy Res.* 1999;36:165–188.
6. Prince DA, Futamachi KJ. Intracellular recordings in chronic focal epilepsy. *Brain Res.* 1968;11:681–684.
7. Mody I, Lambert JD, Heinemann U. Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. *J Neurophysiol.* 1987;57:869–888.
8. Prince DA, Gutnick MJ. Neuronal activities in epileptogenic foci of immature cortex. *Trans Am Neurol Assoc.* 1971;96:88–91.
9. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci.* 2006;7:41–53.
10. Adelson PD, Whalen MJ, Kochanek PM, Robichaud P, Carlos TM. Blood brain barrier permeability and acute inflammation in two models of traumatic brain injury in the immature rat: a preliminary report. *Acta Neurochir Suppl.* 1998;71:104–106.
11. Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev.* 2005;57:173–185.
12. Tomkins O, Shelef I, Kaizerman I, Eliushin, A, Afawi Z, Misk A, Gidon M, Cohen A, Zumsteg D, Friedman A. Blood-brain barrier disruption in post-traumatic epilepsy. *J Neurol Neurosurg Psychiatry,* 2008;79:774–777.
13. van Vliet EA, da Costa AS, Redeker S, van SR, Aronica E, Gorter JA. Blood-brain barrier leakage may

lead to progression of temporal lobe epilepsy. *Brain.* 2007;130:521–534.
14. Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron.* 2008;57:178–201.
15. Tomkins O, Kaufer D, Korn A. Shelef I, Golan H, Reichenthal E, Soreq H, Friedman A. Frequent blood-brain barrier disruption in the human cerebral cortex. *Cell Mol Neurobiol.* 2001;21:675–691.
16. Avivi E, Tomkins O, Korn A, Pavlovsky L, Shelef I, Friedman A. Blood-brain-barrier disruption in humans: a window to neurodegenerative diseases. In: Silman I, Fisher A, Anglister L, Michaelson D, Soreq H, eds. *Cholinergic Mechanisms.* London: Martin Dunitz; 2004:423–429.
17. Ivens S, Kaufer D, Flores LP, Bechmann I, Zumsteg D, Tomkins O, Seiffert E, Heinemann U, Friedman A. TGF-beta receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. *Brain,* 2007;130:535–547.
18. Janigro D. Blood-brain barrier, ion homeostatis and epilepsy: possible implications towards the understanding of ketogenic diet mechanisms. *Epilepsy Res,* 1999;37:223–232.
19. Marchi N, Angelov L, Masaryk T, Fazio V, Granata T, Hernandez N, Hallene K, Diglaw T, Franic L, Najm I, Janigro D. Seizure-promoting effect of blood-brain barrier disruption. *Epilepsia.* 2007;48:732–742.
20. Oby E, Janigro D. The blood-brain barrier and epilepsy. *Epilepsia.* 2006;47:1761–1774.
21. Pavlovsky L, Seiffert E, Heinemann U, Korn A, Golan H, Friedman A. Persistent BBB disruption may underlie alpha interferon-induced seizures. *J Neurol.* 2005;252:42–46.
22. Tomkins O, Friedman O, Ivens S, Reiffurth C, Major S, Dreier JP, Heinemann U, Friedman A. Blood-brain barrier disruption results in delayed functional and structural alterations in the rat neocortex. *Neurobiol Dis.* 2007;25:367–377.
23S eiffert E, Dreier JP, Ivens S, Bechmann I, Tomkins O, Heinemann U, Friedman A. Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex. *J Neurosci,* 2004;24:7829–7836.
24. Korn A, Golan H, Melamed I, Pascual-Marqui R, Friedman A. Focal cortical dysfunction and blood-brain barrier disruption in patients with postconcussion syndrome. *J Clin Neurophysiol.* 2005;22:1–9.
25. Strbian D, Durukan A, Pitkonen M, Marinkovic I, Tatlisumak E, Pedrono E, Abo-Ramadan U, Tatlisumak T.. The blood-brain barrier is continuously open for several weeks following transient focal cerebral ischemia. *Neuroscience.* 2008;153:175–181.
26. Shapira Y, Setton D, Artru AA, Shohami E. Blood-brain barrier permeability, cerebral edema, and neurologic function after closed head injury in rats. *Anesth Analg.* 1993;77:141–148.
27. Baskaya MK, Rao AM, Dogan A, Donaldson D, Dempsey RJ. The biphasic opening of the blood-brain barrier in the cortex and hippocampus after traumatic brain injury in rats. *Neurosci Lett.* 1997; 226:33–36.
28. Huang ZG, Xue D, Preston E, Karbalai H, Buchan AM. Biphasic opening of the blood-brain barrier following transient focal ischemia: effects of hypothermia. *Can J Neurol Sci.* 1999;26:298–304.
29. Fischer S, Wobben M, Marti HH, Renz D, Schaper W. Hypoxia-induced hyperpermeability in brain microvessel endothelial cells involves VEGF-mediated changes in the expression of zonula occludens-1. *Microvasc Res.* 2002;63:70–80.
30. Lee JH, Martin NA, Alsina G, McArthur DL, Zaucha K, Hovda DA, Becker DP. Hemodynamically significant cerebral vasospasm and outcome after head injury: a prospective study. *J Neurosurg.* 1997;87:221–233.
31. Rangel-Castilla L, Gasco J, Nauta HJW, Okonkwo DO, Robertson CS. Cerebral pressure autoregulation in traumatic brain injury. *Neurosurg Focus.* 2008;25:E7.
32. Cherian L, Hlatky R, Robertson CS. Nitric oxide in traumatic brain injury. *Brain Pathol.* 2004;14:195–201.
33. Nekludov M, Antovic J, Bredbacka S, Blomback M. Coagulation abnormalities associated with severe isolated traumatic brain injury: cerebral arterio-venous differences in coagulation and inflammatory markers. *J Neurotrauma.* 2007;24:174–180.
34. Muellner A, Benz M, Ulrich C, Kloss A, Mautes A, Burggraf D, Hamann GF. Microvascular basal lamina antigen loss after traumatic brain injury in the rat. *J Neurotrauma.* 2003;20:745–754.
35. Nag S, Venugopalan R, Stewart DJ. Increased caveolin-1 expression precedes decreased expression of occludin and claudin-5 during blood-brain barrier breakdown. *Acta Neuropathol.* 2007;114:459–469.
36. Nag S, Manias JL, Stewart DJ. Expression of endothelial phosphorylated caveolin-1 is increased in brain injury. *Neuropathol Appl Neurobiol.* 2009;35: 417–426.
37. Zhao J, Moore AN, Redell JB, Dash PK. Enhancing expression of Nrf2-driven genes protects the blood brain barrier after brain injury. *J Neurosci.* 2007;27: 10240–10248.
38. Yeung D, Manias JL, Stewart DJ, Nag S. Decreased junctional adhesion molecule-A expression during blood-brain barrier breakdown. *Acta Neuropathol.* 2008;115:635–642.
39. Higashida T, Kreipke CW, Rafols JA, Peng C, Schafer S, Schafer P, Ding JY, Dornbos D 3rd, Li X, Guthikonda M, Rossi NF, Ding Y. The role of hypoxia-inducible factor-1alpha, aquaporin-4, and matrix metalloproteinase-9 in blood-brain barrier disruption and brain edema after traumatic brain injury. *J Neurosurg.* 2011;114:92–101.
40. Dore-Duffy P, Owen C, Balabanov R, Murphy S, Beaumont T, Rafols JA. Pericyte migration from the vascular wall in response to traumatic brain injury. *Microvasc Res.* 2000;60:55–69.
41. Nag S, Takahashi JL, Kilty DW. Role of vascular endothelial growth factor in blood-brain barrier breakdown and angiogenesis in brain trauma. *J Neuropathol Exp Neurol.* 1997;56:912–921.
42. Sokrab TE, Kalimo H, Johansson BB. Endogenous serum albumin content in brain after short-lasting epileptic seizures. *Brain Res.* 1989;489:231–236.
43. Zucker DK, Wooten GF, Lothman EW. Blood-brain barrier changes with kainic acid-induced limbic seizures. *Exp Neurol.* 1983;79:422–433.
44. Ruth RE. Increased cerebrovascular permeability to protein during systemic kainic acid seizures. *Epilepsia.* 1984;25:259–268.
45. Saija A, Princi P, Pisani A, Santoro G, De Pasquale R, Massi M, Costa G. Blood-brain barrier dysfunctions

following systemic injection of kainic acid in the rat. *Life Sci.* 1992;51:467–477.
46. Pont F, Collet A, Lallement G. Early and transient increase of rat hippocampal blood-brain barrier permeability to amino acids during kainic acid-induced seizures. *Neurosci Lett.* 1995;184:52–54.
47. Leroy C, Roch C, Koning E, Namer IJ, Nehlig A. In the lithium-pilocarpine model of epilepsy, brain lesions are not linked to changes in blood-brain barrier permeability: an autoradiographic study in adult and developing rats. *Exp Neurol.* 2003;182:361–372.
48. Ndode-Ekane XE, Hayward N, Grohn O, Pitkanen A. Vascular changes in epilepsy: functional consequences and association with network plasticity in pilocarpine-induced experimental epilepsy. *Neuroscience.* 2010;166:312–332.
49. Ivens S, Szendro G, Greenberg G, Friedman A, Shelef I. Blood-brain barrier breakdown as a novel mechanism underlying cerebral hyperperfusion syndrome. *J Neurol.* 2010;257:615–620.
50. Alvarez V, Maeder P, Rossetti AO. Postictal blood-brain barrier breakdown on contrast-enhanced MRI. *Epilepsy Behav.* 2010;17:302–303.
51. Lansberg MG, O'Brien MW, Norbash AM, Moseley ME, Morrell M, Albers GW. MRI abnormalities associated with partial status epilepticus. *Neurology.* 1999;52:1021–1027.
52. Hong KS, Cho YJ, Lee SK, Jeong SW, Kim WK, Oh EJ. Diffusion changes suggesting predominant vasogenic oedema during partial status epilepticus. *Seizure.* 2004;13:317–321.
53. Amato C, Elia M, Musumeci SA, Bisceglie P, Moschini M. Transient MRI abnormalities associated with partial status epilepticus: a case report. *Eur J Radiol.* 2001;38:50–54.
54. Yaffe K, Ferriero D, Barkovich AJ, Rowley H. Reversible MRI abnormalities following seizures. *Neurology.* 1995;45:104–108.
55. Cornford EM, Oldendorf WH. Epilepsy and the blood-brain barrier. *Adv Neurol.* 1986;44:787–812.
56. Cornford EM. Epilepsy and the blood brain barrier: endothelial cell responses to seizures. *Adv Neurol.* 1999;79:845–862.
57. Kasantikul V, Brown WJ, Oldendorf WH, Crandall PC. Ultrastructural parameters of limbic microvasculature in human psychomotor epilepsy. *Clin Neuropathol.* 1983;2:171–178.
58. Mihaly A, Bozoky B. Immunohistochemical localization of extravasated serum albumin in the hippocampus of human subjects with partial and generalized epilepsies and epileptiform convulsions. *Acta Neuropathol.* 1984;65:25–34.
59. Rigau V, Morin M, Rousset MC, de Bock F, Lebrun A, Coubes P, Picot MC, Baldy-Moulinier M, Bockaert J, Crespel A, Lerner-Natoli M. Angiogenesis is associated with blood-brain barrier permeability in temporal lobe epilepsy. *Brain.* 2007;130:1942–1956.
60. Friedman A, Kaufer D, Heinemann U. Blood-brain barrier breakdown-inducing astrocytic transformation: novel targets for the prevention of epilepsy. *Epilepsy Res.* 2009;85:142–149.
61. David Y, Cacheaux LP, Ivens S, Lapilover E, Heinemann U, Kaufer D, Friedman A. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *J Neurosci.* 2009;29:10588–10599.
62. Maggio N, Shavit E, Chapman J, Segal M. Thrombin induces long-term potentiation of reactivity to afferent stimulation and facilitates epileptic seizures in rat hippocampal slices: toward understanding the functional consequences of cerebrovascular insults. *J Neurosci.* 2008;28:732–736.
63. Nadal A, Fuentes E, Pastor J, McNaughton PA. Plasma albumin is a potent trigger of calcium signals and DNA synthesis in astrocytes. *Proc Natl Acad Sci USA.* 1995;92:1426–1430.
64. Cacheaux LP, Ivens S, David Y, Lakhter AJ, Bar-Klein G, Shapira M, Heinemann U, Friedman A, Kaufer D. Transcriptome profiling reveals TGF-beta signaling involvement in epileptogenesis. *J Neurosci.* 2009;29:8927–8935.
65. Floyd CL, Lyeth BG. Astroglia: important mediators of traumatic brain injury. In: John TWaA, I, ed. *Progress in Brain Research Neurotrauma: New Insights into Pathology and Treatment.* Elsevier; 2007:61–79.
66. Heinemann U, Gabriel S, Jauch R, Schulze K, Kivi A, Eilers A, Kovacs R, Lehmann TN. Alterations of glial cell function in temporal lobe epilepsy. *Epilepsia.* 2000;41(suppl 6):S185–S189.
67. Jabs R, Seifert G, Steinhauser C. Astrocytic function and its alteration in the epileptic brain. *Epilepsia.* 2008;49(suppl 2):3–12.
68. Wetherington J, Serrano G, Dingledine R. Astrocytes in the epileptic brain. *Neuron.* 2008;58:168–178.
69. Seifert G, Schilling K, Steinhauser C. Astrocyte dysfunction in neurological disorders: a molecular perspective. *Nat Rev Neurosci.* 2006;7:194–206.
70. Filosa A, Paixao S, Honsek SD, Carmona MA, Becker L, Feddersen B, Gaitanos L, Rudhard Y, Schoepfer R, Klopstock T, Kullander K, Rose CR, Pasquale EB, Klein R. Neuron-glia communication via EphA4/ephrin-A3 modulates LTP through glial glutamate transport. *Nat Neurosci.* 2009;12:1285–1292.
71. Henneberger C, Papouin T, Oliet SH, Rusakov DA. Long-term potentiation depends on release of D-serine from astrocytes. *Nature.* 2010;463:232–236.
72. Seifert G, Carmignoto G, Steinhauser C. Astrocyte dysfunction in epilepsy. *Brain Res Rev.* 2010;63:212–221.
73. Djukic B, Casper KB, Philpot BD, Chin LS, McCarthy KD. Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. *J Neurosci.* 2007;27:11354–11365.
74. Binder DK, Yao X, Zador Z, Sick TJ, Verkman AS, Manley GT. Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. *Glia.* 2006;53:631–636.
75. Gabriel S, Eilers A, Kivi A, Kovacs R, Schulze K, Lehmann TN, Heinemann U. Effects of barium on stimulus induced changes in extracellular potassium concentration in area CA1 of hippocampal slices from normal and pilocarpine-treated epileptic rats. *Neurosci Lett.* 1998;242:9–12.
76. Kivi A, Lehmann TN, Kovacs R, Eilers A, Jauch R, Meencke HJ, von Deimling A, Heinemann U, Gabriel S. Effects of barium on stimulus-induced rises of [K+]o in human epileptic non-sclerotic and sclerotic hippocampal area CA1. *Eur J Neurosci.* 2000;12:2039–2048.
77. Schroder W, Hinterkeuser S, Seifert G, Schramm J, Jabs R, Wilkin GP, Steinhäuser C. Functional and

molecular properties of human astrocytes in acute hippocampal slices obtained from patients with temporal lobe epilepsy. *Epilepsia.* 2000;41(suppl 6): S181–S184.
78. Lux HD, Heinemann U, Dietzel I. Ionic changes and alterations in the size of the extracellular space during epileptic activity. *Adv Neurol.* 1986;44:619–639.
79. Wallraff A, Kohling R, Heinemann U, Theis M, Willecke K, Steinhauser C. The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. *J Neurosci.* 2006;26:5438–5447.
80. Tian GF, Azmi H, Takano T, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M. An astrocytic basis of epilepsy. *Nat Med.* 2005;11: 973–981.
81. Bezzi P, Gundersen V, Galbete JL, Seifert G, Steinhäuser C, Pilati E, Volterra A. Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. *Nat Neurosci.* 2004;7: 613–620.
82. Bezzi P, Domercq M, Vesce S, Volterra A. Neuron-astrocyte cross-talk during synaptic transmission: physiological and neuropathological implications. *Prog Brain Res.* 2001;132:255–265.
83. Eid T, Thomas MJ, Spencer DD, Rundén-Pran E, Lai JC, Malthankar GV, Kim JH, Danbolt NC, Ottersen OP, de Lanerolle NC. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet.* 2004;363: 28–37.
84. Eid T, Ghosh A, Wang Y, Beckström H, Zaveri HP, Lee TS, Lai JC, Malthankar-Phatak GH, de Lanerolle NC. Recurrent seizures and brain pathology after inhibition of glutamine synthetase in the hippocampus in rats. *Brain.* 2008;131:2061–2070.
85. Sandow N, Zahn RK, Gabriel S, Heinemann U, Lehmann TN. Glutamine induces epileptiform discharges in superficial layers of the medial entorhinal cortex from pilocarpine-treated chronic epileptic rats in vitro. *Epilepsia.* 2009;50:849–858.
86. Ravizza T, Gagliardi B, Noe F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. *Neurobiol Dis.* 2008;29:142–160.
87. Vezzani A, Baram TZ. New roles for interleukin-1 beta in the mechanisms of epilepsy. *Epilepsy Curr.* 2007;7:45–50.
88. Yang H, Zhang J, Andreasson K, Chen C. COX-2 oxidative metabolism of endocannabinoids augments hippocampal synaptic plasticity. *Mol Cell Neurosci.* 2008;37:682–695.
89. Cowley TR, Fahey B, O'Mara SM. COX-2, but not COX-1, activity is necessary for the induction of perforant path long-term potentiation and spatial learning in vivo. *Eur J Neurosci.* 2008;27:2999–3008.
90. Zhang HJ, Sun RP, Lei GF, Yang L, Liu CX. Cyclooxygenase-2 inhibitor inhibits hippocampal synaptic reorganization in pilocarpine-induced status epilepticus rats. *J Zhejiang Univ Sci B.* 2008;9:903–915.
91. Cole-Edwards KK, Bazan NG. Lipid signaling in experimental epilepsy. *Neurochem Res.* 2005;30: 847–853.
92. Fabene PF, Mora GN, Martinello M, Rossi B, Merigo F, Ottoboni L, Bach S, Angiari S, Benati D, Chakir A, Zanetti L, Schio F, Osculati A, Marzola P, Nicolato E, Homeister JW, Xia L, Lowe JB, McEver RP, Osculati F, Sbarbati A, Butcher EC, Constantin G. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. *Nat Med.* 2008;14:1377–1383.
93. Fabene PF, Bramanti P, Constantin G. The emerging role for chemokines in epilepsy. *J Neuroimmunol.* 2010;224:22–27.
94. Shapiro LA, Wang L, Ribak CE. Rapid astrocyte and microglial activation following pilocarpine-induced seizures in rats. *Epilepsia.* 2008;49(suppl 2):33–41.
95. Massague J, Wotton D. Transcriptional control by the TGF-beta/Smad signaling system. *EMBO J.* 2000;19:1745–1754.
96. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell.* 2003;113:685–700.
97. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. *N Engl J Med.* 2000;342:1350–1358.
98. Flanders KC, Ludecke G, Engels S, Cissel DS, Roberts AB, Kondaiah P, Lafyatis R, Sporn MB, Unsicker K. Localization and actions of transforming growth factor-beta s in the embryonic nervous system. *Development.* 1991;113:183–191.
99. Gold LI, Parekh TV. Loss of growth regulation by transforming growth factor-beta (TGF-beta) in human cancers: studies on endometrial carcinoma. *Semin Reprod Endocrinol.* 1999;17:73–92.
100. Szelenyi J. Cytokines and the central nervous system. *Brain Res Bull.* 2001;54:329–338.
101. Vitkovic L, Maeda S, Sternberg E. Anti-inflammatory cytokines: expression and action in the brain. *Neuroimmunomodulation.* 2001;9:295–312.
102. Vivien D, Ali C. Transforming growth factor-beta signalling in brain disorders. *Cytokine Growth Factor Rev.* 2006;17:121–128.
103. Plata-Salaman CR, Ilyin SE, Turrin NP, Gayle D, Flynn MC, Romanovitch AE, Kelly ME, Bureau Y, Anisman H, McIntyre DC. Kindling modulates the IL-1beta system, TNF-alpha, TGF-beta1, and neuropeptide mRNAs in specific brain regions. *Brain Res Mol Brain Res.* 2000;75:248–258.
104. Aronica E, van Vliet EA, Mayboroda OA, Troost D, da Silva FH, Gorter JA. Upregulation of metabotropic glutamate receptor subtype mGluR3 and mGluR5 in reactive astrocytes in a rat model of mesial temporal lobe epilepsy. *Eur J Neurosci.* 2000;12:2333–2344.

Chapter 28

Cell Death and Survival Mechanisms after Single and Repeated Brief Seizures

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INTRODUCTION

While it is broadly accepted that status epilepticus can directly cause neuronal death, whether single or repeated brief seizures cause neuron loss is controversial. This is an important issue with both scientific and clinical implications. Patients may be concerned about whether their seizures are capable of causing brain damage, and clinicians make treatment decisions based

on an assumption that a few seizures are not really harmful. From a scientist's perspective, this issue is pertinent to the pathophysiology of epilepsy and the various mechanisms neurons employ to cope—or otherwise undergo cell death—in response to the repeated stress of frequent seizures. Research using animal models, and pathology and neuroimaging work in patients, show that single or repeated brief seizures under certain circumstances cause neuron loss, but they also indicate that neuron loss is not an inevitable consequence of a seizure. Recent human studies show that signaling pathways associated with apoptosis may be altered in the patient's brain, offering possible therapeutic opportunities to target seizure-induced neuronal death in different ways.

Hippocampal sclerosis is the most common pathological finding in temporal lobe epilepsy (TLE). However, there are patients with TLE with no apparent hippocampal damage and patients with hippocampal sclerosis without TLE. If hippocampal sclerosis causes TLE, then efforts to prevent this lesion's development are critical. If epileptic seizures cause neuron loss, however, therapeutic efforts to prevent all seizures from occurring become more important. This chapter describes cell death and survival mechanisms after single and repeated brief seizures in animal models and humans. What is the etiology of hippocampal and extrahippocampal cell loss in intractable TLE? Is there ongoing cell loss in refractory epilepsy? The question of whether single epileptic seizures damage the brain has been the subject of several previous reviews, to which the reader is referred.[1–3] The purpose of this chapter is to present the evidence for and against cell death after brief seizures and the molecular mechanisms that may underlie such an outcome. We omit discussion of other forms of neuronal damage (including reversible injury) that may also have significant behavioral or cognitive implications and the influence of repair mechanisms such as neurogenesis. Discussion of these issues can be found elsewhere.[4]

Evidence from Animal Models that Single or Repeated Evoked Seizures Cause Neuron Loss

Evidence that single or repeated brief seizures could cause neuronal death emerged from work in animals using electrical stimulation of various brain regions. While kindling paradigms are not ordinarily associated with permanent neuron loss,[4] papers published in the early 1990s, particularly by Sutula's laboratory, showed that kindling-induced seizures caused reductions in neuron numbers.[5] Cavazos et al. showed that repeated stimulation of the perforant path, olfactory bulb, or amygdala resulted in progressive decreases in neuronal density in multiple subfields of the hippocampus, including the hilus, CA1 and CA3, and parts of the entorhinal cortex.[6] The somatosensory cortex was unaffected, and changes were not attributable to tissue volume changes.[6]

Other studies using electrically evoked seizures have reported similar findings.[7,8] Not only is neuron loss progressive, but it may increase with secondarily generalized tonic-clonic seizures.[8] Reduced hippocampal neuron densities have also been reported after electroshock seizures,[9] and in addition to hippocampal neuron loss, a subpopulation of amygdala neurons may also be vulnerable.[10,11] Finally, recent studies by Sloviter and colleagues showed that sustained electrical stimulation of the perforant pathway leading to the hippocampus, which did not cause convulsive seizures or status epilepticus, produced extensive neuronal death and hippocampal sclerosis.[12] Thus, repeated brief seizures or subconvulsive stimulation of the hippocampus in certain models can reproduce patterns of neuron loss similar to those found in human hippocampal sclerosis (Table 28–1).

Evidence Against Single and Repeated Evoked Seizures Causing Neuron Loss

Studies in kindling models have shown that brief single seizures do not necessarily lead to cell loss. Thus, Bertram and Lothman reported reduced neuronal density after kindling but attributed this to tissue volume expansion.[13] The possible role of tissue volume changes and changes in neuronal morphology in reports of seizure-induced neuronal loss has been emphasized by numerous authors.[2,14,15] Brandt et al. also argued that neuronal density reductions after extended kindling were due to volume changes and not neuronal death.[16] Other groups also failed to detect neuronal death after

Table 28–1 **Summary of Findings on Neuron Loss after Single or Repeated Brief Seizures in Experimental Models**

Pathological Outcome	Findings
Neuron loss after repeated evoked brief seizures	Observed in many but not all models
Neuron death detected after repeated evoked brief seizures	Observed in some models
Neuron loss after seizures in spontaneously epileptic animals	Inconclusive
Neuron loss after seizures in animals with acquired epilepsy	Not currently supported by the evidence
Apoptosis-associated signaling	Modulation of Bcl-2 family genes, caspases

kindling in rats[17,18] and mice.[19] Thus, studies in which neuron counts were used as the principal measure of whether cell loss occurred are not in agreement as to whether brief seizures cause neuronal death (Table 28–1).

Detection of Acute Cell Death after Evoked Single and Repeated Brief Seizures

Direct evidence that brief seizures cause acute neuronal death has been provided by biochemical analyses. Bengzon et al. showed that a single seizure evoked by electrical stimulation of the hippocampus could cause hippocampal neurons to die, as detected by silver staining and staining of cells for irreversible DNA fragmentation using TUNEL (terminal deoxynucleotidyl transferase deoxyuridine triphsophate nick end labeling).[20] Notably, more stimulations caused proportionately more cells to die.[20] Using similar methods, other groups have also reported that repeated brief seizures cause hippocampal and extrahippocampal cell death.[11,21–23] These studies confirm that brief evoked seizures can cause neuronal death in animal models (Table 28–1).

Do Spontaneous Seizures in Epileptic Animals Cause Neuron Loss?

While brief evoked seizures in nonepileptic animals are useful models, they do not capture all aspects of the pathophysiology of spontaneous (i.e., epileptic) seizures. Is there evidence that spontaneous seizures in epileptic animals can cause neuron loss? This would be directly relevant to the etiology of progressive damage in human mesial temporal sclerosis. Two types of model have been studied in this context: animals that are spontaneously epileptic and animals that acquired epilepsy as the result of an initial precipitating injury. With the exception of certain genetically altered mice with active neurodegeneration, neuron loss does not appear to occur in spontaneously epileptic animals. For example, the hippocampus of spontaneously epileptic EL mice, which experience multiple complex partial seizures with secondary generalization on a weekly basis, shows no obvious neuron loss.[24] Evidence of subfield-specific seizure-induced hippocampal neuron loss has been reported in spontaneously epileptic rats,[25] although no acute cell death after a seizure or a biochemical marker thereof was detected.[25]

Studies in animal models of acquired epilepsy also suggest that spontaneous seizures do not cause further neuron loss. Pitkanen et al. reported that a longer duration of epilepsy was not associated with lower numbers of neurons in epileptic rats.[26] Moreover, no acutely degenerating neurons were found in any of the chronically epileptic animals, despite some of them experiencing more than 10 seizures per day.[26] Other studies appear to corroborate these data; hippocampal damage may continue for some time following status epilepticus, but neuron loss does not progress once animals are epileptic[27–29] (Table 28–1).

MOLECULAR MECHANISMS OF CELL DEATH FOLLOWING SINGLE AND REPEATED BRIEF SEIZURES

The molecular mechanisms underlying cell death following single and repeated brief

seizures are not as well researched as they have been in models of status epilepticus (reviewed in refs. 30–32). Glutamate-mediated excitotoxicity is the principal mechanism driving neuronal death after status epilepticus, whereby excessive glutamate release leads to intracellular calcium overload, oxidative stress, organelle swelling and rupture of intracellular membranes, activation of proteases, and necrosis.[33,34] Is glutamate-mediated toxicity the cause of neuron death after single or repeated brief seizures? We assume that it is, and necrosis has been detected after brief seizures,[11] but there have been no studies using appropriate pharmacological tools demonstrating that cell death can be prevented by glutamate receptor antagonists. Instead, there is biochemical and morphological evidence supporting cellular apoptosis occurring after brief seizures.[7,20,21] Notably, administration of the *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist MK801 (which is neuroprotective in models of status epilepticus) did not prevent cell death after brief seizures.[20] The pathophysiological changes caused by brief seizures are no doubt glutamate-driven and may feature perturbed intracellular calcium homeostasis,[35] but through other pathways. These might include non-NMDA receptor-gated calcium entry and disruption of endoplasmic reticulum or mitochondrial function. Thus, apoptosis, which may have overlapping mechanisms of activation with necrosis, contributes to cell death after single or repeated brief seizures.

Molecular Control of Apoptosis

Apoptosis is a form of programmed cell death used to dispose of unwanted or damaged cells in a controlled manner. Excess neurons are removed during brain development by apoptosis, and apoptosis also occurs after the developing or mature brain is exposed to, or deprived of, certain substances. For example, ethanol exposure triggers widespread apoptosis in the developing rat brain,[36] and adrenalectomy triggers apoptosis of dentate granule neurons.[37]

Two main molecular pathways control apoptosis—extrinsic and intrinsic.[38,39] The extrinsic pathway is triggered by surface-expressed death receptors of the tumor necrosis factor (TNF) superfamily on binding of their ligands (secreted cytokines such as TNFα). The intrinsic pathway is mitochondria-mediated, and is activated by an array of intracellular stressors including DNA damage and perturbation of intracellular calcium homeostasis or organelle function.[40,41] This pathway is regulated by members of the Bcl-2 gene family at the point of initiation. Both pathways result in the downstream activation of a group of enzymes called *caspases.*

CASPASES

The caspases are a family of cysteinyl aspartate-specific proteases expressed in healthy cells in an inactive zymogen form. Caspases share a common structure comprising an N-terminal pro-domain followed by a large ~20 kD subunit and a smaller ~10 kD subunit. Caspases regulating apoptosis are typically organized into two functional groups: The upstream *initiator* caspases have long pro-domains. Activation of these requires protein-protein binding interactions between the pro-domain and scaffolding molecules activated in response to pro-apoptotic stimuli. For example, the pro-domain of caspase-8 binds to regions on signaling molecules recruited to the intracellular side of activated death receptors, whereas the pro-domain of caspase-9 associates with the apoptotic protease activating factor 1 (Apaf-1) forming the so-called apoptosome in association with released cytochrome *c* from mitochondria.[42] Activated initiator caspases then cleave and remove the short pro-domain of apoptosis effector (or *executioner*) caspases, thereby activating them.[42] Caspase-3 and other effector caspases such as caspases-6 and -7 then cleave numerous proteins within the cell, including structural proteins (a full listing can be found at http://bioinf.gen.tcd.ie/casbah/). Collectively, the caspase system results in hallmark morphological changes, DNA fragmentation (which can be detected by TUNEL), and eventual dispersal of the cell within membrane-enclosed apoptotic "bodies" to be phagocytosed by surrounding cells.

Bcl-2 Family Proteins

Bcl-2 family proteins function as critical regulators of apoptosis by controlling release of intramitochondrial apoptogenic molecules via effects on outer mitochondrial membrane permeability. The Bcl-2 family comprises both

pro- and anti-apoptotic members that share one or more Bcl-2 homology (BH) domains. Anti-apoptotic members include Bcl-2 and Bcl-xL, which possess four BH domains in common and a transmembrane anchoring domain. The multidomain pro-apoptotic members include Bax and Bak, which only possess BH domains 1–3.[43]

BH3-only proteins are a subgroup of the pro-apoptotic Bcl-2 family. These function as upstream initiators of apoptosis by binding and either inactivating anti-apoptotic Bcl-2 family proteins or directly activating pro-apoptotic Bax/Bak. BH3-only proteins are highly heterogeneous. Some reside inactively in normal cells and require posttranslational modification to be active, while others require transcriptional upregulation by cell stress or damage. Bad, for example, is expressed in many cells (including neurons) but requires dephosphorylation and disengagement from a chaperone protein called 14–3-3 to be active. In contrast, the more potently pro-apoptotic members, such as Puma, require transcriptional upregulation, for example via DNA damage-sensing proteins such as p53.[43] Once activated, Bax/Bak trigger release of cytochrome *c* from mitochondria initiating the intrinsic apoptosis pathway, culminating in caspase-dependent or -independent cell death[41] (Fig. 28–1).

OTHER SURVIVAL PATHWAYS

In addition to the anti-apoptotic arm of the Bcl-2 family, other anti-apoptotic molecules have been identified. These include protein kinase B (Akt), which is activated downstream of phosphatidylinositol 3 (PI3) kinase, which itself lies downstream of certain cytokine and surface-expressed growth and survival factor receptors.[44] Activated Akt can block apoptosis by phosphorylating and inhibiting Bad or the

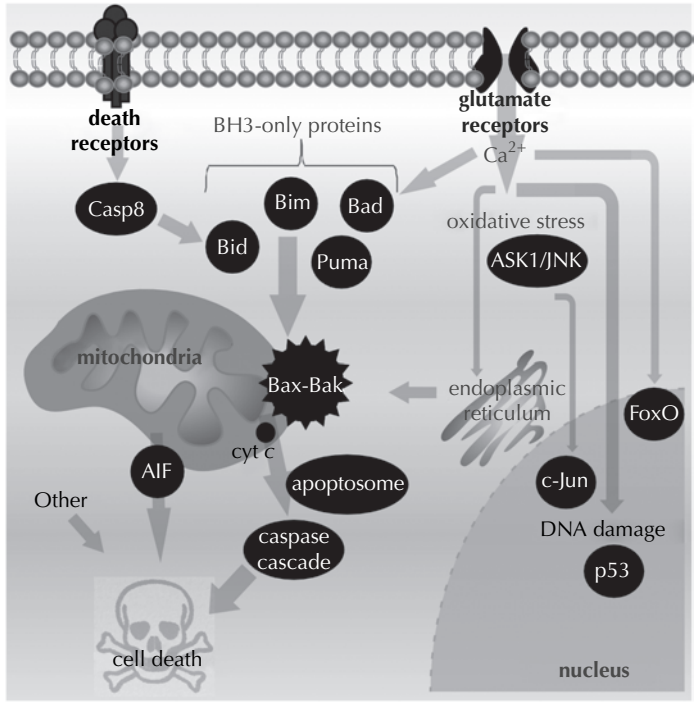

Figure 28–1. Pro-apoptotic signaling pathways. The diagram depicts major pro-apoptotic signaling pathways. BH3-only proteins function as upstream sentinels of cell stress. Bid is activated by caspase-8, which is itself activated downstream of death receptors. Bad is activated by calcium-dependent phosphatases, while Bim and Puma are upregulated by FoxO or p53, respectively. BH3-only proteins trigger Bax-Bak activation, and apoptogenic factors are released from mitochondria, including cytochrome *c* (cyt *c*), which activates the apoptosome and caspase cascade, and apoptosis-inducing factor (AIF). Ancillary pathways include induction of apoptosis via the ASK1/JNK/c-Jun pathway and other pathways, such as those downstream of endoplasmic reticulum stress.

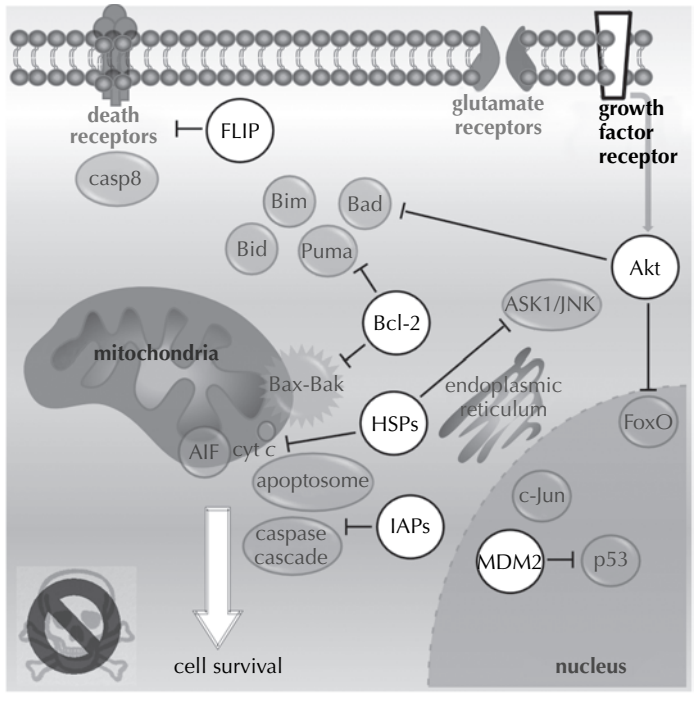

Figure 28–2. Anti-apoptotic signaling pathways. The diagram depicts key anti-apoptotic proteins that disrupt cell death at various points. FLIP (FLICE-like inhibitory protein) blocks the death receptor/caspase-8 pathway. Bcl-2 (plus the related Bcl-xL and Bcl-w) blocks pro-apoptotic Bcl-2 family proteins, Heat shock proteins (HSPs) inhibit mitochondrial apoptogenic proteins and the ASK1/JNK pathway. Akt downstream of various growth factor and other pathways inhibits the FoxO transcription factor and Bad. Inhibitor of apoptosis proteins (IAPs) function mainly by blocking caspases. Murine double minute 2 (MDM2) regulates p53 levels.

FoxO/Bim pathway.[45,46] The inhibitor of apoptosis protein (IAP) family functions mainly by direct inhibition of caspases and by targeting them for degradation by the proteasome[47] (Fig. 28–2).

Evidence of Apoptosis-Associated Signaling Pathways after Brief Seizures

Molecular evidence of apoptosis after brief seizures was first reported by Zhang et al., who detected an increase in *bax* mRNA, but unchanged *bcl-2,* expression in hilar neurons in the rat hippocampus following multiple kindling seizures.[21] Other studies have confirmed that kindling seizures cause hippocampal upregulation of *bax* as well as an increase in Bax protein.[48] Downregulation of anti-apoptotic Bcl-2 family proteins also occurs after repeated brief seizures.[48] The extrinsic apoptosis pathway may also be activated by brief seizures since kindling increases brain levels of TNFα.[49] Increased caspase-like enzyme activity and in situ staining of activated caspase-3 have been found in hippocampus after kindling seizures.[22,50]

Changes to Bcl-2 family protein expression have also been observed in models of electroshock-induced convulsions. Here, anti-apoptotic changes predominate, including downregulation of pro-apoptotic *bcl-xs* and Bim[51,52] and upregulation of anti-apoptotic Bcl-w.[53] This pattern supports protection rather than cell death.

There are no data on Bcl-2 family protein expression or function in epileptic animals. There is, however, evidence of caspase activity within the hippocampus of epileptic animals,[54,55] which may reflect ongoing cell death. The location of the active caspase signal within dendritic fields also supports caspase-mediated restructuring of neurons or other processes.[56]

It should be emphasized that the studies to date have not proven that apoptosis-associated gene changes are responsible for cell death in these models. This requires protein-protein interactions to be demonstrated and functional studies assessing, for example, damage in mice lacking specific genes. Evidence that genes associated with apoptosis can regulate seizure-induced neuronal death has been provided, however, from models of status epilepticus. Mice lacking the BH3-only proteins Bim and Puma are protected against status epilepticus-induced neuronal death,[57–59] and Bcl-2 and death receptor signaling protein complexes are formed in the hippocampus in these models.[52,60,61]

Summary: Animal Studies

Evoked brief seizures can cause neuronal death within the hippocampus and neocortex in animal models. The cell death has some of the biochemical and morphological features of apoptosis. In contrast, we do not have compelling evidence that spontaneous seizures in epileptic animals cause neuron loss. Both types of model show alterations in the expression of genes from the major families regulating apoptosis. Although we await evidence that this contributes to neuronal death after brief seizures, it has been demonstrated in models of prolonged seizures.

HUMAN CLINICOPATHOLOGICAL STUDIES: IS THERE DAMAGE PROGRESSION IN INTRACTABLE TLE?

Neuron loss, particularly within the hippocampus, is a widely observed pathological hallmark of refractory TLE in humans. Whether chronic epileptic seizures in patients cause neuron loss, however, or whether pathology arises independently of an initial precipitating injury or another (e.g., genetic) factor(s),[62] remains debated. In the next part of this chapter, we summarize various clinicopathological and neuroimaging studies that have provided evidence for and against neuron loss as a result of repeated brief seizures in humans.

Hippocampal Neuron Loss in Human TLE

There is a long history of studies identifying hippocampal neuron loss in TLE.[63–65] Most commonly, neuron loss is evident within the CA1 and endfolium or hilar region of the dentate gyrus.[66] Neuron loss and attendant gliosis are usually also evident in the CA3 subfield. While damage to the CA2 subfield and dentate gyrus tends to be less overt, neuron loss is evident in more severe cases.

A common source of evidence for seizures causing neuron loss in the human hippocampus is the association between longer seizure histories and greater neuron loss. For example, Mouritzen Dam, in a study of 20 patients with partial complex and generalized tonic-clonic seizures, found bilateral neuron loss that was most extensive in the end folium, CA3/4, and dentate granule cell layer.[67] The severity of neuron loss correlated with a longer duration of epilepsy, implying an effect of chronic seizures.[67] Similar conclusions were drawn from the large study by Mathern et al. of neuron densities in 572 hippocampal specimens from TLE patients.[68] Neuron counts decreased with longer seizure histories independent of TLE pathology or aging, implying that repeated seizures over many years (or factors linked to them) cause additional hippocampal neuron loss. However, the authors emphasized that their data showed hippocampal sclerosis to be an acquired pathology generated mainly by an initial precipitating injury, with a relatively small contribution from chronic seizures.[68] Similar conclusions were reached when hippocampal damage was examined in patients with TLE as a result of a temporal lobe mass[69] (Table 28–2).

Pathology in Nonhippocampal Regions

Extrahippocampal pathology within adjacent mesial limbic structures is present in a subset of patients with hippocampal sclerosis.[66] Affected structures include the amygdala, thalamus, and neocortex. Nevertheless, a majority of patients with TLE do not develop cortical neuron loss. A recent study reported that just 11% of surgically treated TLE cases also had neocortical neuron loss,[70] and no significant cortical neuron

Table 28–2 **Summary of Clinical Findings on Neuron Loss in Human Epilepsy**

Pathology	Neuron loss in hilus, CA1, CA3 >> CA2, granule neurons Cerebral cortex (layers II–III), cerebellum
"Cross-sectional neuroimaging" lines up with "Hippocampal volume loss. . . .".	Hippocampal volume loss proportional to duration of epilepsy
"Longitudinal imaging" should be aligned with "Mixed; evidence. . . ."	Mixed; evidence for and against progressive damage
"Acute cell death markers" aligned with "Temporal lobe. . ."	Temporal lobe TUNEL-stained cells in some but not all studies

loss was reported in another study.[71] The explanation for extrahippocampal/neocortical neuron loss in a subpopulation of patients is not yet known. It may be the result of more frequent or generalized seizures, or it may be due to an earlier initial precipitating injury[70] (Table 28–2).

Neuroimaging Evidence of Damage Progression in Intractable TLE

HIPPOCAMPUS

Many cross-sectional neuroimaging studies have reported lower hippocampal volumes in patients with epilepsy compared to controls and still lower volumes in patients with drug-resistant epilepsy.[72–84] Studies have also shown that hippocampal volume loss correlates with the number of epileptic seizures.[74,79] It should be noted, however, that not all studies explicitly state whether patients who experienced status epilepticus were excluded, and there are cross-sectional studies that failed to find an association between epileptic seizures and hippocampal volume reduction.[85] Twin studies using imaging have also contributed evidence that hippocampal sclerosis is an acquired lesion. For example, volumetric and T2 imaging of monozygotic twins by Jackson et al. determined that hippocampal sclerosis was present only in the twin with epilepsy.[86]

Longitudinal studies allow imaging of the same patients and controls over time, although they generally feature small cohorts and cover quite short periods of time.[87] Conclusions from such studies on whether epileptic seizures cause progressive hippocampal volume decline are mixed. Neuroimaging over periods of less than 4 years has detected hippocampal volume decline in relation to the number of generalized[88] and complex partial[89] seizures. However, several reports failed to detect reductions in hippocampal volume in epilepsy patients that exceeded those in controls over the same period.[83,84,90,91] Thus, current longitudinal studies have not resolved the question of progressive hippocampal atrophy in TLE (Table 28–2).

IMAGING: NONHIPPOCAMPAL REGIONS

Neuroimaging studies have reported extrahippocampal atrophy in patients with pharmacoresistant TLE. Regions affected include the entorhinal cortex and the amygdala ipsilateral to the seizure focus,[81,92–94] as well as frontal poles, lateral temporal and occipital regions,[95] and contralateral regions.[94] Some cortical decreases were found to relate to the duration of epilepsy, implying a role for repeated seizures in the changes.[95] Interestingly, extrahippocampal atrophy is more prominent in patients with left hemisphere TLE.[93]

We have few longitudinal imaging studies of nonhippocampal atrophy on which to base conclusions. Progressive atrophy involving orbitofrontal, insular, and angular regions has been reported in pharmacorefractory TLE patients.[94] Studies by Liu and colleagues, however, found that although patients with chronic epilepsy developed more neocortical volume loss compared to controls over a 3.5 year period, this was related to age and medication history rather than to an association with frequency of seizures.[96]

Summary: Imaging Evidence of Seizure-Induced Neuron Loss in Human Studies

Cross-sectional imaging studies support recurrent epileptic seizures as a cause of neuronal damage in the hippocampus of patients with TLE. There may also be seizure-induced neocortical neuron loss in some patients.

Nevertheless, major hippocampal atrophy probably results from an initial precipitating injury rather than recurring epileptic seizures. Longitudinal neuroimaging offers a better method for determining the effects of recurrent seizures in epilepsy patients, but findings to date are mixed. Taken together, neuroimaging studies suggest that structural damage is not an inevitable consequence of epileptic seizures in humans, in agreement with animal studies.

Histological Evidence of Acute Cell Death in Human TLE

Histological analyses of resected material have found evidence of acute cell death in patients with pharmacoresistant TLE. Henshall et al. detected TUNEL-positive cells in two of six neocortical resections from pharmacoresistant patients.[97] The same group, studying hippocampal sections, found TUNEL-positive cells in 9 out of 10 samples[52,98]; TUNEL-positive cells displayed features consistent with apoptosis.[98] However, the numbers were very low (ranging from zero to four per section) and did not differ statistically from those in controls.[52,98] TUNEL-positive cells were also reported to be higher in TLE sections compared to control sections in another study[99] but were not found to be elevated in three other reports[100–102] (Table 28–2). These studies suggest that there is at most very small-scale acute cell death in temporal lobe structures from pharmacoresistant epilepsy patients. Isolated dying cells may, however, be rapidly removed after seizures and difficult to detect; no study has yet undertaken an assessment of complete hippocampal resections, and counting has not been stereological.

MOLECULAR EVIDENCE OF APOPTOSIS-ASSOCIATED SIGNALING IN HUMAN TLE

The first studies to address whether programmed cell death/apoptosis signaling pathways were altered in the temporal lobe of patients experiencing frequent seizures emerged in the late 1990s. These descriptive reports noted increased Bcl-2 staining in astrocytes, although they found that Bcl-2 and Bcl-xL immunoreactivity in residual neurons of sclerotic hippocampi was similar to that in controls.[103] Glioneuronal hamartias, a form of cerebral dysgenesis, were strongly immunoreactive for Bcl-2.[103] Another early study noted that Bax immunoreactivity was stronger in TLE patients compared to control subjects and elderly drug-treated epileptics.[104]

Bcl-2 and Caspase Family Genes

The first study to apply quantitative measures of apoptosis-associated gene expression was done by Simon and colleagues at the University of Pittsburgh.[97] They reported data from 19 resected TLE patient temporal neocortex samples and 6 age- and gender-matched autopsy controls. Using Western blot analysis, they showed higher levels of Bcl-2 and Bcl-xL in patient brains (Tables 28–3 and 28–4). Immunohistochemistry showed that neurons were the main cell type expressing Bcl-2, while Bcl-xL stained mainly astrocytes.[97] The cleaved forms of caspase-1 and caspase-3 were also detected in TLE samples but not in the controls.[97] The elevated Bcl-2 and Bcl-xL levels might be molecular adaptations to inhibit cell death in surviving cells, while the activated caspases might be contributing to progressive pathology. Indeed, animal data show that overexpressing Bcl-2 or Bcl-xL is neuroprotective against excitotoxic insults,[105,106] while overexpression of caspase-3 enhances neurodegeneration after ischemia.[107] Caspase-1 knockout mice are refractory to kainic acid-induced

Table 28–3 **Expression of Pro-Apoptotic Proteins in Human TLE**

Increased
Pro-caspases-2, -3, -6, -7, and -9 (hippocampus or neocortex)
Cleaved caspases-1, -3, -7, -8, and -9 (hippocampus or neocortex)
Bax* (hippocampus, neocortex)
p53 (hippocampus)
Tumor necrosis factor receptor 1 (TNFR1)† (hippocampus)
Nuclear caspase-activated DNase (hippocampus)
Apoptosis signal-regulating kinase 1 (hippocampus)
Decreased/inhibited
BH3-only subgroup protein Bim (hippocampus)
FoxO transcription factors (hippocampus)

*Studies have also reported no changes to Bax.
†TNFR1 may have non-cell death-related functions.

Table 28–4 **Expression of Anti-Apoptotic Proteins in TLE Brain Tissue**

Increased
Bcl-2, Bcl-xL,* and Bcl-w (hippocampus or neocortex)
Akt phosphorylation (hippocampus)
X-linked IAP binding to caspase-7 (hippocampus)
Decreased
1. MDM2 (p53 negative regulator)

*Bcl-xL was increased in neocortex but not in hippocampus.

seizures,[108] so the presence of cleaved caspase-1 in human TLE might have pro-epileptic consequences in addition to, or instead of, a cell death-regulatory function.

Findings from Other Cohorts

Pro-apoptotic Bax expression has been reported to be moderately elevated in TLE hippocampi,[101] although this was not found in another study.[102] Several laboratories, using cohorts ranging from 12 to 24 patients, have also detected higher Bcl-2 levels in neurons and also glia in resected TLE hippocampi.[101,102,109] Levels of Bcl-w, another anti-apoptotic Bcl-2 family protein, are also elevated in resected TLE hippocampus.[53] Notably, Bcl-w expression in the hippocampus of mice is increased by exposure to repeated brief seizures.[53] This increase may protect hippocampus since overexpressing Bcl-w prevents excitotoxic (ischemic) injury in vivo,[110] while the absence of *bcl-w* increases neuron loss after status epilepticus.[53]

Another protective adaptation may be a reduction in levels of the BH3-only protein Bim in TLE hippocampus[52,111] (Table 28–3). Again, animal models of brief seizures have recapitulated this pattern,[52] which is very likely protective since the hippocampus of mice lacking *bim* are protected against status epilepticus.[59] Other BH3-only proteins may also be important; mice lacking the BH3-only protein Puma develop less hippocampal damage after status epilepticus.[57,58]

Other Caspases

Differences in the expression of many caspases have been detected in human TLE brain samples. Caspases-2, -3, -6, -7, and -9 have all been reported to be overexpressed and their active forms found,[55,102,111,112] and immunohistochemistry has localized cleaved caspases within neuron-like cells in TLE brain.[112,113] These data are evidence that caspase-mediated pro-apoptotic signaling occurs in human TLE. Caspases appear to localize within both the cell soma and dendrites,[55,111,113] supporting caspase-mediated cleavage of intracellular structural or synaptic proteins.[114]

Other Pro- and Anti-Apoptotic Proteins

Other putatively pro-apoptotic genes have been reported to be increased in human TLE tissue. There are increased nuclear levels of caspase-activated DNase, the enzyme responsible for the hallmark DNA laddering seen in apoptosis, in TLE samples.[113] Other putatively pro-apoptotic proteins showing higher expression in TLE include apoptosis signal-regulating kinase-1 (ASK1),[112,115] c-Jun,[116] Death-associated protein kinase,[98] Fas and its signaling components,[102, 115] p53,[102,117] and TNF receptor 1[115] (Table 28–3).

In addition to anti-apoptotic Bcl-2 family proteins, several other anti-apoptotic proteins are overexpressed in resected TLE tissue. These include protein kinase B (Akt),[52] heat shock protein 70,[116] endoplasmic reticulum stress-activated proteins such as glucose-regulated proteins 78/94,[111,112] and the cellular inhibitor of apoptosis protein-2 (cIAP-2)[118] (Table 28–4).

Summary: Molecular Evidence of Apoptosis in Human TLE

Alterations to apoptosis-associated signaling pathways are widely found in TLE tissue. Human findings probably reflect seizure-induced stress and the resulting adjustments to the molecular repertoire between adaptations that prevent neuron loss and, occasionally, signaling that ultimately results in cell death. Higher levels of anti-apoptotic Bcl-2 family proteins and related molecules may raise the threshold required for a seizure to cause cell death, thereby countering the influence of

pro-apoptotic molecules such as caspases. This may explain why so little acute cell death occurs in patients experiencing frequent seizures. This interpretation is consistent with animal data showing that changes to levels of apoptosis-associated genes can either prevent or exacerbate seizure-induced neuronal death.

MITOCHONDRIAL DNA DAMAGE IN EPILEPSY

Repeated seizures may result in other changes that enhance neuronal vulnerability over time. Mitochondrial function is critical for normal neuronal excitability, but mitochondria are also the primary sites in the cell for production of reactive oxygen species (ROS). Seizures increase ROS production, and studies suggest that this depletes cellular antioxidants, interferes with the function of electron transport chain enzymes, and causes DNA damage.[119] Indeed, mice overexpressing the mitochondrially localized superoxide dismutase 2 are protected against seizure-induced neuronal death.[120] Mitochondrial DNA (mtDNA) damage[121] and mtDNA copy number reductions[122,123] have also been reported in hippocampal tissue from epileptic rats, which has been suggested to contribute to reduced electron transport chain activity. Over time, neurons may become more susceptible to seizures and to their deleterious consequences, such as cationic overload.[119] Other groups, however, have not detected a reduction in electron transport chain enzymes in epileptic animals[124] or even found their expression to be increased.[125] Evidence of mitochondrial dysfunction has been reported in hippocampal tissue from TLE patients.[126,127] Together, cumulative mtDNA damage and compromised mitochondrial function may enhance neuronal vulnerability to seizures and contribute to epileptogenesis.

CHAPTER SUMMARY AND FUTURE QUESTIONS

Brief seizures can cause neuronal death in animal models. There is emerging evidence that apoptosis-associated signaling pathways are activated by these seizures, but so far we only have proof that these contribute to cell death in models of status epilepticus. There is little evidence that spontaneous seizures in epileptic animals cause acute cell death, but these animals nevertheless display alterations in apoptosis-associated pathways. In humans, there is evidence that recurrent seizures cause subtle or diffuse neuron loss in affected structures. Histopathological analyses have found a molecular signature of apoptosis-associated signaling in resected neocortical and hippocampal material from pharmocoresistant TLE patients.

Several questions remain to be answered. Is the frequency/clustering of seizures, or of particular seizure types—for example, secondarily generalized—more harmful? We await additional longitudinal neuroimaging studies specifically focused on comparing outcomes of different seizure types and severities. Future studies of pro- and anti-apoptotic signaling molecules should determine whether these occur in the same or different cells. Mouse models can make important contributions by allowing us to test which genes actually affect cell death following repeated brief seizures; in particular, they will allow us to test the influence of the particular genes in epileptic animals. This chapter has summarized the evidence for and against neuron loss after single and repeated brief seizures in animal models and human epilepsy and highlights the molecular pathways of apoptosis as a potential contributor to cell death and survival decisions.

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DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Cole AJ, Koh S, Zheng Y. Are seizures harmful? What can we learn from animal models? *Prog Brain Res.* 2002;135:13–23.
2. Sutula TP, Hagen J, Pitkanen A. Do epileptic seizures damage the brain? *Curr Opin Neurol.* 2003;16(2): 189–195.

3. Rocha LL, Lopez-Meraz ML, Niquet J, Wasterlain CG. Do single seizures cause neuronal death in the human hippocampus? *Epilepsy Curr.* 2007;7(3):77–81.
4. Morimoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog Neurobiol.* 2004;73(1):1–60.
5. Cavazos JE, Sutula TP. Progressive neuronal loss induced by kindling: a possible mechanism for mossy fiber synaptic reorganization and hippocampal sclerosis. *Brain Res.* 1990;527(1):1–6.
6. Cavazos JE, Das I, Sutula TP. Neuronal loss induced in limbic pathways by kindling: evidence for induction of hippocampal sclerosis by repeated brief seizures. *J Neurosci.* 1994;14(5 pt 2):3106–3121.
7. Sloviter RS, Dean E, Sollas AL, Goodman JH. Apoptosis and necrosis induced in different hippocampal neuron populations by repetitive perforant path stimulation in the rat. *J Comp Neurol.* 1996;366(3):516–533.
8. Kotloski R, Lynch M, Lauersdorf S, Sutula T. Repeated brief seizures induce progressive hippocampal neuron loss and memory deficits. *Prog Brain Res.* 2002;135:95–110.
9. Zarubenko, II, Yakovlev AA, Stepanichev MY, Gulyaeva NV. Electroconvulsive shock induces neuron death in the mouse hippocampus: correlation of neurodegeneration with convulsive activity. *Neurosci Behav Physiol.* 2005;35(7):715–721.
10. Callahan PM, Paris JM, Cunningham KA, Shinnick-Gallagher P. Decrease of GABA-immunoreactive neurons in the amygdala after electrical kindling in the rat. *Brain Res.* 1991;555(2):335–339.
11. Pretel S, Applegate CD, Piekut D. Apoptotic and necrotic cell death following kindling induced seizures. *Acta Histochem.* 1997;99(1):71–79.
12. Norwood BA, Bumanglag AV, Osculati F, Sbarbati A, Marzola P, Nicolato E, Fabene PF, Sloviter RS. Classic hippocampal sclerosis and hippocampal-onset epilepsy produced by a single "cryptic" episode of focal hippocampal excitation in awake rats *J Comp Neurol.* 2010;518:3381–3407.
13. Bertram EH 3rd, Lothman EW. Morphometric effects of intermittent kindled seizures and limbic status epilepticus in the dentate gyrus of the rat. *Brain Res.* 1993;603(1):25–31.
14. Guillery RW, August BK. Doubt and certainty in counting. *Prog Brain Res.* 2002;135:25–42.
15. West MJ. Design-based stereological methods for counting neurons. *Prog Brain Res.* 2002;135: 43–51.
16. Brandt C, Ebert U, Loscher W. Epilepsy induced by extended amygdala-kindling in rats: lack of clear association between development of spontaneous seizures and neuronal damage. *Epilepsy Res.* 2004;62(2–3):135–156.
17. Khurgel M, Switzer RC 3rd, Teskey GC, Spiller AE, Racine RJ, Ivy GO. Activation of astrocytes during epileptogenesis in the absence of neuronal degeneration. *Neurobiol Dis.* 1995;2(1):23–35.
18. Tuunanen J, Pitkanen A. Do seizures cause neuronal damage in rat amygdala kindling? *Epilepsy Res.* 2000;39(2):171–176.
19. Watanabe Y, Johnson RS, Butler LS, Binder DK, Spiegelman BM, Papaioannou VE, McNamara JO. Null mutation of c-*fos* impairs structural and functional plasticities in the kindling model of epilepsy. *J Neurosci.* 1996;16(12):3827–3836.
20. Bengzon J, Kokaia Z, Elmer E, Nanobashvili A, Kokaia M, Lindvall O. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *Proc Natl Acad Sci USA.* 1997;94(19): 10432–10437.
21. Zhang LX, Smith MA, Li XL, Weiss SR, Post RM. Apoptosis of hippocampal neurons after amygdala kindled seizures. *Brain Res Mol Brain Res.* 1998;55(2): 198–208.
22. Cole-Edwards KK, Musto AE, Bazan NG. c-Jun N-terminal kinase activation responses induced by hippocampal kindling are mediated by reactive astrocytes. *J Neurosci.* 2006;26(32):8295–8304.
23. Gawlowicz M, Reichert M, Wojcierowski J, Czuczwar SJ, Borowicz KK. Apoptotic markers in various stages of amygdala kindled seizures in rats. *Pharmacol Rep.* 2006;58(4):512–518.
24. Drage MG, Holmes GL, Seyfried TN. Hippocampal neurons and glia in epileptic EL mice. *J Neurocytol.* 2002;31(8–9):681–692.
25. Hanaya R, Sasa M, Sugata S, Tokudome M, Serikawa T, Kurisu K, Arita K. Hippocampal cell loss and propagation of abnormal discharges accompanied with the expression of tonic convulsion in the spontaneously epileptic rat. *Brain Res.* 2010;1328:171–180.
26. Pitkanen A, Nissinen J, Nairismagi J, Lukasiuk K, Grohn OH, Miettinen R, Kauppinen R. Progression of neuronal damage after status epilepticus and during spontaneous seizures in a rat model of temporal lobe epilepsy. *Prog Brain Res.* 2002;135:67–83.
27. Liu Z, Nagao T, Desjardins GC, Gloor P, Avoli M. Quantitative evaluation of neuronal loss in the dorsal hippocampus in rats with long-term pilocarpine seizures. *Epilepsy Res.* 1994;17(3):237–247.
28. Nairismagi J, Grohn OH, Kettunen MI, Nissinen J, Kauppinen RA, Pitkanen A. Progression of brain damage after status epilepticus and its association with epileptogenesis: a quantitative MRI study in a rat model of temporal lobe epilepsy. *Epilepsia.* 2004;45(9): 1024–1034.
29. Gorter JA, Goncalves Pereira PM, van Vliet EA, Aronica E, Lopes da Silva FH, Lucassen PJ. Neuronal cell death in a rat model for mesial temporal lobe epilepsy is induced by the initial status epilepticus and not by later repeated spontaneous seizures. *Epilepsia.* 2003;44(5):647–658.
30. Fujikawa DG. Prolonged seizures and cellular injury: understanding the connection. *Epilepsy Behav.* 2005;7(suppl 3):S3–S11.
31. Henshall DC, Simon RP. Epilepsy and apoptosis pathways. *J Cereb Blood Flow Metab.* 2005;25(12): 1557–1572.
32. Engel T, Henshall DC. Apoptosis, Bcl-2 family proteins and caspases: the ABCs of seizure-damage and epileptogenesis? *Int J Physiol Pathophysiol Pharmacol.* 2009;1:97–115.
33. Meldrum BS. Excitotoxicity and selective neuronal loss in epilepsy. *Brain Pathol.* 1993;3(4):405–412.
34. Meldrum BS. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *J Nutr.* 2000;130:1007S–1015S.
35. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol.* 2003;4(7):552–565.
36. Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, Price MT, Stefovska V, Horster F, Tenkova T, Dikranian K, Olney JW. Ethanol-induced

apoptotic neurodegeneration and fetal alcohol syndrome. *Science.* 2000;287(5455):1056–1060.
37. Sloviter RS, Dean E, Neubort S. Electron microscopic analysis of adrenalectomy-induced hippocampal granule cell degeneration in the rat: apoptosis in the adult central nervous system. *J Comp Neurol.* 1993;330(3)337–351.
38. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol.* 2008;9(3):231–241.
39. Hotchkiss RS, Strasser A, McDunn JE, Swanson PE. Cell death. *N Engl J Med.* 2009;361(16):1570–1583.
40. Demaurex N, Distelhorst C. Cell biology. Apoptosis—the calcium connection. *Science.* 2003;300(5616): 65–67.
41. Galluzzi L, Blomgren K, Kroemer G. Mitochondrial membrane permeabilization in neuronal injury. *Nat Rev Neurosci.* 2009;10(7):481–494.
42. Lavrik IN, Golks A, Krammer PH. Caspases: pharmacological manipulation of cell death. *J Clin Invest.* 2005;115(10):2665–2672.
43. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol.* 2008;9(1):47–59.
44. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet.* 2006;7(8):606–619.
45. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell.* 1997;91(2):231–241.
46. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell.* 1999;96(6): 857–868.
47. Salvesen GS, Duckett CS. IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol.* 2002;3(6): 401–410.
48. Akcali KC, Sahiner M, Sahiner T. The role of bcl-2 family of genes during kindling. *Epilepsia.* 2005;46(2): 217–223.
49. Shandra AA, Godlevsky LS, Vastyanov RS, Oleinik AA, Konovalenko VL, Rapoport EN, Korobka NN. The role of TNF-alpha in amygdala kindled rats. *Neurosci Res.* 2002;42(2):147–153.
50. Pavlova TV, Yakovlev AA, Stepanichev MY, Mendzheritskii AM, Gulyaeva NV. Pentylenetetrazole kindling induces activation of caspase-3 in the rat brain. *Neurosci Behav Physiol.* 2004;34(1):45–47.
51. Kondratyev A, Sahibzada N, Gale K. Electroconvulsive shock exposure prevents neuronal apoptosis after kainic acid-evoked status epilepticus. *Brain Res Mol Brain Res.* 2001;91(1–2):1–13.
52. Shinoda S, Schindler CK, Meller R, So NK, Araki T, Yamamoto A, Lan JQ, Taki W, Simon RP, Henshall DC. Bim regulation may determine hippocampal vulnerability after injurious seizures and in temporal lobe epilepsy. *J Clin Invest.* 2004;113(7):1059–1068.
53. Murphy B, Dunleavy M, Shinoda S, Schindler C, Meller R, Bellver-Estelles C, Hatazaki S, Dicker P, Yamamoto A, Koegel I, Chu X, Wang W, Xiong Z, Prehn J, Simon R, Henshall D. Bcl-w protects hippocampus during experimental status epilepticus. *Am J Pathol.* 2007;171(4):1258–1268.
54. Narkilahti S, Pitkanen A. Caspase 6 expression in the rat hippocampus during epileptogenesis and epilepsy. *Neuroscience.* 2005;131(4):887–897.
55. Narkilahti S, Jutila L, Alafuzoff I, Karkola K, Paljarvi L, Immonen A, Vapalahti M, Mervaala E, Kalviainen R, Pitkanen A. Increased expression of caspase 2 in experimental and human temporal lobe epilepsy. *Neuromolecular Med.* 2007;9(2):129–144.
56. Li Z, Jo J, Jia JM, Lo SC, Whitcomb DJ, Jiao S, Cho K, Sheng M. Caspase-3 activation via mitochondria is required for long-term depression and AMPA receptor internalization. *Cell.* 2010;141(5):859–871.
57. Engel T, Murphy BM, Hatazaki S, Jimenez-Mateos EM, Concannon CG, Woods I, Prehn JH, Henshall DC. Reduced hippocampal damage and epileptic seizures after status epilepticus in mice lacking proapoptotic Puma. *FASEB J.* 2010;24(3): 853–861.
58. Engel T, Hatazaki S, Tanaka K, Prehn JH, Henshall DC. Deletion of Puma protects hippocampal neurons in a model of severe status epilepticus. *Neuroscience.* 2010;168:443–450.
59. Murphy BM, Engel T, Paucard A, Hatazaki S, Mouri G, Tanaka K, Tuffy LP, Jimenez-Mateos EM, Woods I, Dunleavy M, Bonner HP, Meller R, Simon RP, Strasser A, Prehn JH, Henshall DC. Contrasting patterns of Bim induction and neuroprotection in Bim-deficient mice between hippocampus and neocortex after status epilepticus. *Cell Death Differ.* 2010;17: 459–468.
60. Henshall DC, Araki T, Schindler CK, Lan JQ, Tiekoter KL, Taki W, Simon RP. Activation of Bcl-2-associated death protein and counter-response of Akt within cell populations during seizure-induced neuronal death. *J Neurosci.* 2002;22(19):8458–8465.
61. Shinoda S, Skradski SL, Araki T, Schindler CK, Meller R, Lan JQ, Taki W, Simon RP, Henshall DC. Formation of a tumour necrosis factor receptor 1 molecular scaffolding complex and activation of apoptosis signal-regulating kinase 1 during seizure-induced neuronal death. *Eur J Neurosci.* 2003;17(10): 2065–2076.
62. Berkovic SF, Jackson GD. The hippocampal sclerosis whodunit: enter the genes. *Ann Neurol.* 2000;47(5): 557–558.
63. Bouchet C, Cazauvieilh M. De l'epilepsie consideree dans ses rapports avec l'alienation mentale. *Arch Gen Med.* 1825;9:510–542.
64. Sommer W. Erkrankung des ammonshorns als aetiologisches moment der epilepsie. *Arch Psychiatr Nervenkr.* 1880;10:631–675.
65. Margerison JH, Corsellis JA. Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain.* 1966;89(3):499–530.
66. Wieser HG. ILAE Commission Report. Mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia.* 2004;45(6):695–714.
67. Dam AM. Epilepsy and neuron loss in the hippocampus. *Epilepsia.* 1980;21(6):617–629.
68. Mathern GW, Adelson PD, Cahan LD, Leite JP. Hippocampal neuron damage in human epilepsy: Meyer's hypothesis revisited. *Prog Brain Res.* 2002;135: 237–251.

69. Fried I, Kim JH, Spencer DD. Hippocampal pathology in patients with intractable seizures and temporal lobe masses. *J Neurosurg.* 1992;76(5):735–740.
70. Thom M, Eriksson S, Martinian L, Caboclo LO, McEvoy AW, Duncan JS, Sisodiya SM. Temporal lobe sclerosis associated with hippocampal sclerosis in temporal lobe epilepsy: neuropathological features. *J Neuropathol Exp Neurol.* 2009;68(8):928–938.
71. Bothwell S, Meredith GE, Phillips J, Staunton H, Doherty C, Grigorenko E, Glazier S, Deadwyler SA, O'Donovan CA, Farrell M. Neuronal hypertrophy in the neocortex of patients with temporal lobe epilepsy. *J Neurosci.* 2001;21(13):4789–4800.
72. Van Paesschen W, Revesz T, Duncan JS, King MD, Connelly A. Quantitative neuropathology and quantitative magnetic resonance imaging of the hippocampus in temporal lobe epilepsy. *Ann Neurol.* 1997;42(5):756–766.
73. DeCarli C, Hatta J, Fazilat S, Gaillard WD, Theodore WH. Extratemporal atrophy in patients with complex partial seizures of left temporal origin. *Ann Neurol.* 1998;43(1):41–45.
74. Kalviainen R, Salmenpera T, Partanen K, Vainio P, Riekkinen P, Pitkanen A. Recurrent seizures may cause hippocampal damage in temporal lobe epilepsy. *Neurology.* 1998;50(5):1377–1382.
75. Salmenpera T, Kalviainen R, Partanen K, Pitkanen A. Hippocampal damage caused by seizures in temporal lobe epilepsy. *Lancet.* 1998;351(9095):35.
76. Jokeit H, Ebner A, Arnold S, Schuller M, Antke C, Huang Y, Steinmetz H, Seitz RJ, Witte OW. Bilateral reductions of hippocampal volume, glucose metabolism, and wada hemispheric memory performance are related to the duration of mesial temporal lobe epilepsy. *J Neurol.* 1999;246(10):926–933.
77. Tasch E, Cendes F, Li LM, Dubeau F, Andermann F, Arnold DL. Neuroimaging evidence of progressive neuronal loss and dysfunction in temporal lobe epilepsy. *Ann Neurol.* 1999;45:568–576.
78. Theodore WH, Bhatia S, Hatta J, Fazilat S, DeCarli C, Bookheimer SY, Gaillard WD. Hippocampal atrophy, epilepsy duration, and febrile seizures in patients with partial seizures. *Neurology.* 1999;52(1):132–136.
79. Salmenpera T, Kalviainen R, Partanen K, Pitkanen A. Hippocampal and amygdaloid damage in partial epilepsy: a cross-sectional MRI study of 241 patients. *Epilepsy Res.* 2001;46(1):69–82.
80. Bernasconi A, Tasch E, Cendes F, Li LM, Arnold DL. Proton magnetic resonance spectroscopic imaging suggests progressive neuronal damage in human temporal lobe epilepsy. *Prog Brain Res.* 2002;135:297–304.
81. Bernasconi N, Bernasconi A, Caramanos Z, Antel SB, Andermann F, Arnold DL. Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala and parahippocampal region. *Brain.* 2003;126(pt 2):462–469.
82. Kobayashi E, D'Agostino MD, Lopes-Cendes I, Berkovic SF, Li ML, Andermann E, Andermann F, Cendes F. Hippocampal atrophy and T2-weighted signal changes in familial mesial temporal lobe epilepsy. *Neurology.* 2003;60(3):405–409.
83. Holtkamp M, Schuchmann S, Gottschalk S, Meierkord H. Recurrent seizures do not cause hippocampal damage. *J Neurol.* 2004;251(4):458–463.
84. Liu RS, Lemieux L, Bell GS, Sisodiya SM, Bartlett PA, Shorvon SD, Sander JW, Duncan JS. Cerebral damage in epilepsy: a population-based longitudinal quantitative MRI study. *Epilepsia.* 2005;46(9):1482–1494.
85. Bower SP, Kilpatrick CJ, Vogrin SJ, Morris K, Cook MJ. Degree of hippocampal atrophy is not related to a history of febrile seizures in patients with proved hippocampal sclerosis. *J Neurol Neurosurg Psychiatry.* 2000;69(6):733–738.
86. Jackson GD, McIntosh AM, Briellmann RS, Berkovic SF. Hippocampal sclerosis studied in identical twins. *Neurology.* 1998;51(1):78–84.
87. Lemieux L. Causes, relationships and explanations: the power and limitations of observational longitudinal imaging studies. *Curr Opin Neurol.* 2008;21(4):391–392.
88. Briellmann RS, Berkovic SF, Syngeniotis A, King MA, Jackson GD. Seizure-associated hippocampal volume loss: a longitudinal magnetic resonance study of temporal lobe epilepsy. *Ann Neurol.* 2002;51:641–644.
89. Fuerst D, Shah J, Shah A, Watson C. Hippocampal sclerosis is a progressive disorder: a longitudinal volumetric MRI study. *Ann Neurol.* 2003;53(3):413–416.
90. Van Paesschen W, Duncan JS, Stevens JM, Connelly A. Longitudinal quantitative hippocampal magnetic resonance imaging study of adults with newly diagnosed partial seizures: one-year follow-up results. *Epilepsia.* 1998;39(6):633–639.
91. Liu RS, Lemieux L, Bell GS, Sisodiya SM, Bartlett PA, Shorvon SD, Sander JW, Duncan JS. The structural consequences of newly diagnosed seizures. *Ann Neurol.* 2002;52(5):573–580.
92. Bonilha L, Rorden C, Castellano G, Cendes F, Li LM. Voxel-based morphometry of the thalamus in patients with refractory medial temporal lobe epilepsy. *Neuroimage.* 2005;25(3):1016–1021.
93. Bonilha L, Rorden C, Halford JJ, Eckert M, Appenzeller S, Cendes F, Li LM. Asymmetrical extrahippocampal grey matter loss related to hippocampal atrophy in patients with medial temporal lobe epilepsy. *J Neurol Neurosurg Psychiatry.* 2007;78(3):286–294.
94. Bernhardt BC, Worsley KJ, Kim H, Evans AC, Bernasconi A, Bernasconi N. Longitudinal and cross-sectional analysis of atrophy in pharmacoresistant temporal lobe epilepsy. *Neurology.* 2009;72(20):1747–1754.
95. Lin JJ, Salamon N, Lee AD, Dutton RA, Geaga JA, Hayashi KM, Luders E, Toga AW, Engel J Jr, Thompson PM. Reduced neocortical thickness and complexity mapped in mesial temporal lobe epilepsy with hippocampal sclerosis. *Cereb Cortex.* 2007;17(9):2007–2018.
96. Liu RS, Lemieux L, Bell GS, Hammers A, Sisodiya SM, Bartlett PA, Shorvon SD, Sander JW, Duncan JS. Progressive neocortical damage in epilepsy. *Ann Neurol.* 2003;53(3):312–324.
97. Henshall DC, Clark RS, Adelson PD, Chen M, Watkins SC, Simon RP. Alterations in bcl-2 and caspase gene family protein expression in human temporal lobe epilepsy. *Neurology.* 2000;55(2):250–257.
98. Henshall DC, Schindler CK, So NK, Lan JQ, Meller R, Simon RP. Death-associated protein kinase expression in human temporal lobe epilepsy. *Ann Neurol.* 2004;55(4):485–494.

99. Yang T, Hsu C, Liao W, Chuang JS. Heat shock protein 70 expression in epilepsy suggests stress rather than protection. *Acta Neuropathol.* 2008;115(2):219–230.
100. Mathern GW, Leiphart JL, De Vera A, Adelson PD, Seki T, Neder L, Leite JP. Seizures decrease postnatal neurogenesis and granule cell development in the human fascia dentata. *Epilepsia.* 2002;43(suppl 5): 68–73.
101. Uysal H, Cevik IU, Soylemezoglu F, Elibol B, Ozdemir YG, Evrenkaya T, Saygi S, Dalkara T. Is the cell death in mesial temporal sclerosis apoptotic? *Epilepsia.* 2003;44:778–784.
102. Xu S, Pang Q, Liu Y, Shang W, Zhai G, Ge M. Neuronal apoptosis in the resected sclerotic hippocampus in patients with mesial temporal lobe epilepsy. *J Clin Neurosci.* 2007;14(9):835–840.
103. Yachnis AT, Powell SZ, Olmsted JJ, Eskin TA. Distinct neurodevelopmental patterns of bcl-2 and bcl-x expression are altered in glioneuronal hamartias of the human temporal lobe. *J Neuropathol Exp Neurol.* 1997;56(2):186–198.
104. Nagy Z, Esiri MM. Neuronal cyclin expression in the hippocampus in temporal lobe epilepsy. *Exp Neurol.* 1998;150(2):240–247.
105. Lawrence MS, Ho DY, Sun GH, Steinberg GK, Sapolsky RM. Overexpression of Bcl-2 with herpes simplex virus vectors protects CNS neurons against neurological insults in vitro and in vivo. *J Neurosci.* 1996;16(2):486–496.
106. Ju KL, Manley NC, Sapolsky RM. Anti-apoptotic therapy with a Tat fusion protein protects against excitotoxic insults in vitro and in vivo. *Exp Neurol.* 2008;210(2):602–607.
107. Kerr LE, McGregor AL, Amet LE, Asada T, Spratt C, Allsopp TE, Harmar AJ, Shen S, Carlson G, Logan N, Kelly JS, Sharkey J. Mice overexpressing human caspase 3 appear phenotypically normal but exhibit increased apoptosis and larger lesion volumes in response to transient focal cerebral ischaemia. *Cell Death Differ.* 2004;11(10):1102–1111.
108. Ravizza T, Lucas SM, Balosso S, Bernardino L, Ku G, Noe F, Malva J, Randle JC, Allan S, Vezzani A. Inactivation of caspase-1 in rodent brain: a novel anticonvulsive strategy. *Epilepsia.* 2006;47(7):1160–1168.
109. Yuzbasioglu A, Karatas H, Gursoy-Ozdemir Y, Saygi S, Akalan N, Soylemezoglu F, Dalkara T, Kocaefe YC, Ozguc M. Changes in the expression of selenoproteins in mesial temporal lobe epilepsy patients. *Cell Mol Neurobiol.* 2009;29(8):1223–1231.
110. Sun Y, Jin K, Clark KR, Peel A, Mao XO, Chang Q, Simon RP, Greenberg DA. Adeno-associated virus-mediated delivery of BCL-w gene improves outcome after transient focal cerebral ischemia. *Gene Therapy.* 2003;10:115–122.
111. Yamamoto A, Murphy N, Schindler CK, So NK, Stohr S, Taki W, Prehn JH, Henshall DC. Endoplasmic reticulum stress and apoptosis signaling in human temporal lobe epilepsy. *J Neuropathol Exp Neurol.* 2006;65(3):217–225.
112. Liu G, Guo H, Guo C, Zhao S, Gong D, Zhao Y. Involvement of IRE1alpha signaling in the hippocampus in patients with mesial temporal lobe epilepsy. *Brain Res Bull.* 2011;84(1):94–102.
113. Schindler CK, Pearson EG, Bonner HP, So NK, Simon RP, Prehn JH, Henshall DC. Caspase-3 cleavage and nuclear localization of caspase-activated DNase in human temporal lobe epilepsy. *J Cereb Blood Flow Metab.* 2006;26(4):583–589.
114. Chan SL, Mattson MP. Caspase and calpain substrates: roles in synaptic plasticity and cell death. *J Neurosci Res.* 1999;58(1):167–190.
115. Yamamoto A, Schindler CK, Murphy BM, Bellver-Estelles C, So NK, Taki W, Meller R, Simon RP, Henshall DC. Evidence of tumor necrosis factor receptor 1 signaling in human temporal lobe epilepsy. *Exp Neurol.* 2006;202:410–420.
116. Thom M, Seetah S, Sisodiya S, Koepp M, Scaravilli F. Sudden and unexpected death in epilepsy (SUDEP): evidence of acute neuronal injury using HSP-70 and c-Jun immunohistochemistry. *Neuropathol Appl Neurobiol.* 2003;29(2):132–143.
117. Engel T, Murphy BM, Schindler CK, Henshall DC. Elevated p53 and lower MDM2 expression in hippocampus from patients with intractable temporal lobe epilepsy. *Epilepsy Res.* 2007;77(2–3): 151–156.
118. Henshall DC, Simon RP. Molecular mechanisms of cell death after seizures. In: Schwartzkroin PA, ed. *Encyclopedia of Basic Epilepsy Research*. Vol. 1. San Diego, CA: Elsevier; 2009:119–124.
119. Waldbaum S, Patel M. Mitochondria, oxidative stress, and temporal lobe epilepsy. *Epilepsy Res.* 2010;88(1): 23–45.
120. Liang LP, Ho YS, Patel M. Mitochondrial superoxide production in kainate-induced hippocampal damage. *Neuroscience.* 2000;101(3):563–570.
121. Jarrett SG, Liang LP, Hellier JL, Staley KJ, Patel M. Mitochondrial DNA damage and impaired base excision repair during epileptogenesis. *Neurobiol Dis.* 2008;30(1):130–138.
122. Kudin AP, Kudina TA, Seyfried J, Vielhaber S, Beck H, Elger CE, Kunz WS. Seizure-dependent modulation of mitochondrial oxidative phosphorylation in rat hippocampus. *Eur J Neurosci.* 2002;15(7): 1105–1114.
123. Lin Y, Han Y, Xu J, Cao L, Gao J, Xie N, Zhao X, Jiang H, Chi Z. Mitochondrial DNA damage and the involvement of antioxidant defense and repair system in hippocampi of rats with chronic seizures. *Cell Mol Neurobiol.* 2010;30:947–954.
124. Nasseh IE, Amado D, Cavalheiro EA, Naffah-Mazzacoratti Mda G, Tengan CH. Investigation of mitochondrial involvement in the experimental model of epilepsy induced by pilocarpine. *Epilepsy Res.* 2006;68(3):229–239.
125. Yamada Y, Nakano K. Increased expression of mitochondrial respiratory enzymes in the brain of activated epilepsy-prone El mice. *Brain Res Mol Brain Res.* 1999;73(1–2):186–188.
126. Kunz WS, Kudin AP, Vielhaber S, Blumcke I, Zuschratter W, Schramm J, Beck H, Elger CE. Mitochondrial complex I deficiency in the epileptic focus of patients with temporal lobe epilepsy. *Ann Neurol.* 2000;48(5):766–773.
127. Vielhaber S, Niessen HG, Debska-Vielhaber G, Kudin AP, Wellmer J, Kaufmann J, Schonfeld MA, Fendrich R, Willker W, Leibfritz D, Schramm J, Elger CE, Heinze HJ, Kunz WS. Subfield-specific loss of hippocampal *N*-acetyl aspartate in temporal lobe epilepsy. *Epilepsia.* 2008;49(1):40–50.

Chapter 29

Programmed Necrosis after Status Epilepticus

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INTRODUCTION

In this review, we will discuss the most recent advances in the field of neuronal injury following epileptic seizures, with an emphasis on the mechanism of neuronal necrosis. The ultrastructure of cell death and the ubiquity of endogenous cell death programs were first described in the 1970s, and the two main categories of cell death, apoptosis and necrosis, were originally defined according to morphological criteria.[1] The role of glutamate and its analogues in excitotoxic cell death, and the concept of excitotoxicity—including its role in seizures—derived from the pioneering studies of John Olney and his disciples.[2–5] Multiple cell death factors and cell death programs have been identified in developmental and disease-induced neuronal apoptosis,[6–8] and there is mounting clinical and experimental evidence of their contribution to seizure-induced neuronal injury.[9–16] Because these death factors were originally identified in classic apoptosis, any form of cell death in which they are expressed is often called apoptotic, even if its morphology suggests necrosis. This has caused considerable confusion in the literature and should be discouraged.[17–20] In this chapter, we will discuss the involvement of cell death factors in morphologically defined necrosis, the main mode of status epilepticus (SE)-induced cell death in the adult and even in the developing brain. We find that necrosis is frequently an active form of neuronal death, requiring the

expression or activation of some of the same cell death factors usually identified with apoptosis. While this finding raises the hope that targeting common cell death pathways might have therapeutic benefits for both necrosis and apoptosis, the multiplicity and redundancy of cell death pathways for both modes of neuronal death also raise formidable problems when we consider the potential therapeutic applications of these mechanisms.

SEIZURE-INDUCED NEURONAL INJURY

Neuronal Injury and SE

Neuronal injury is widespread in the hippocampus and other brain regions of children or adults who died from SE,[21–24] or of patients with childhood-onset epilepsy who come to surgery for intractable seizures,[25] although epidemiological evidence of the deleterious effects of SE is lacking.[26] DeGiorgio et al. found decreased hippocampal neuronal densities in five patients who died after SE, compared to epileptic patients without SE and to controls.[27] Neuron-specific enolase, a marker of neuronal injury, is increased in the serum of patients with SE even when the seizures are nonconvulsive.[28,29] A number of anecdotal studies using magnetic resonance imaging (MRI) and other imaging techniques found cerebral edema acutely, and atrophy chronically, after nonconvulsive SE,[30–32] but other studies failed to find lesions.[33] Hippocampal atrophy has also been reported after complex or prolonged febrile seizures.[34,35] The presence of a normal brain MRI scan before nonconvulsive SE, evolving into brain atrophy after SE, has been documented,[36] and neuronal loss was found at autopsy in areas that became atrophic after nonconvulsive SE.[37] Atrophy (as seen on MRI) is strongly associated with areas of intense seizure activity in anecdotal cases,[38–40] and in one instance atrophy was shown at autopsy to correspond to neuronal necrosis and neuronal loss,[39] suggesting that neuronal loss and cerebral atrophy can result from SE in humans, although the incidence and severity of these complications after SE are not known.

Is Seizure-Associated Neuronal Injury Due to the Seizures Themselves or to Seizure-Associated Systemic Factors?

COMPLEX PARTIAL SE

As mentioned above, the limited evidence available shows elevation of cell death markers in the serum of patients after nonconvulsive complex partial SE in the absence of systemic complications. However, there is also anecdotal evidence that complex partial SE is not always followed by cerebral atrophy or behavioral deficits,[41] and there is little evidence of any type of brain damage or neurological deficit after petit mal absence SE.[42] This suggests that seizures per se can cause neuronal injury in humans, but that seizure type is a key determinant of injury.

EVIDENCE FROM ANIMAL MODELS

Meldrum and collaborators proved that seizures in paralyzed, ventilated monkeys caused neuronal loss.[43–45] The occurrence of neuronal injury in remote areas synaptically connected to the epileptic focus suggested an excitotoxic mechanism,[46] and Sloviter and Damiona showed that excessive neuronal firing by itself induced neuronal death.[47] In the immature brain, Thompson et al.[10,11] and Sankar et al.[12] demonstrated that severe, prolonged seizures cause neuronal death, although the extent of neuronal injury was highly model-dependent.[48] Recent evidence suggests that under circumstances approximating the clinical situation seen in patients with uncontrolled seizures, the mixture of the death-inducing action of severe seizures with the neuroprotective effect of seizure-associated preconditioning, in which mild seizure-associated injury mitigates the neuronal loss resulting from subsequent severe seizures, results in a neuropathological picture resembling hippocampal sclerosis.[49] Thus, the experimental evidence seems clear: systemic complications associated with seizures greatly aggravate mortality and morbidity from experimental seizures,[45,50] but in their absence, seizures per se are quite capable of causing neuronal injury and death, even in the immature brain,[10,11] and appears to do so by a variety of excitotoxic mechanisms.[16]

NECROSIS AND APOPTOSIS IN EXPERIMENTAL MODELS OF EPILEPSY

Necrosis and Apoptosis: A Morphological Definition

Necrosis and apoptosis were originally defined by morphological criteria.[1] In necrosis, early cytoplasmic changes, including severe mitochondrial and organelle swelling and rupture of the plasma membrane, precede late tigroid condensation of the nucleus with scattered, irregular, small chromatin clumps (Fig. 29–1). By contrast, in the early stage of apoptosis, chromatin condensation with loss of the nuclear membrane occurs in the presence of relatively preserved organelles and plasma/mitochondrial membranes. The advanced stages of apoptosis are characterized by the presence of multiple large, rounded chromatin masses. The picture can be complicated by the occurrence of both processes in the same cell. Following excitotoxic exposure, for example, apoptotic cells may undergo secondary necrosis, with severe swelling of the organelles and breakdown of the plasma membrane.

Following SE, Necrosis Is the Main Form of Neuronal Death in the Adult Brain…

In the adult rat brain, several models of SE show that necrosis is the dominant form of neuronal death. In models of SE induced by

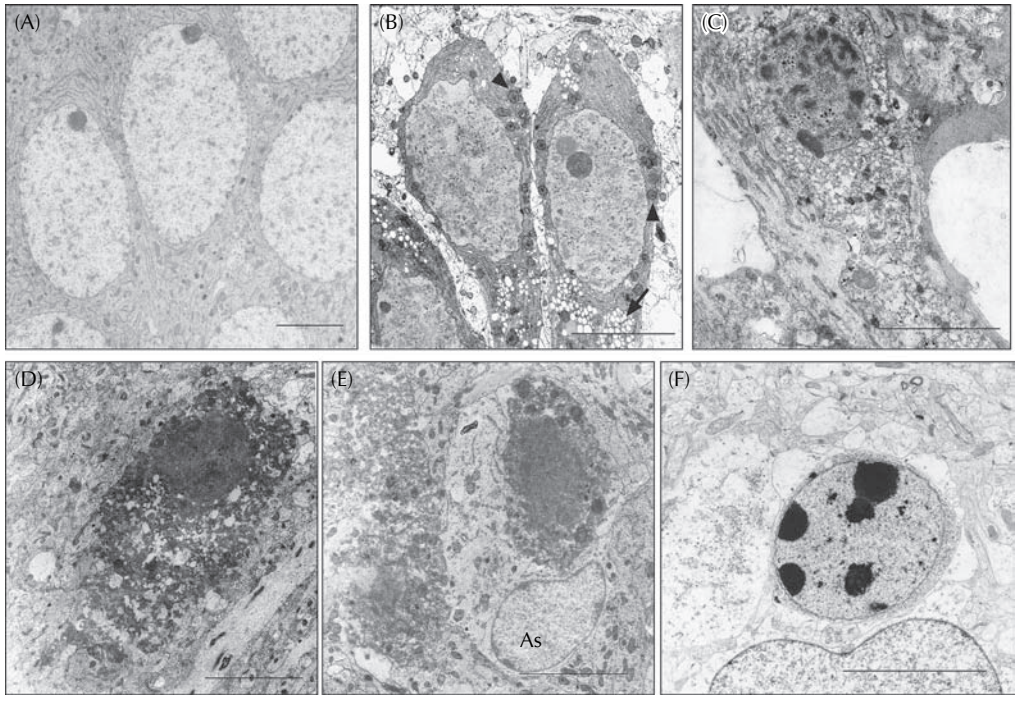

Figure 29–1. Example of necrotic and apoptotic morphologies in rat pup brain. Images **A–E** show the ultrastructure of CA1 neurons in control (**A**) and experimental animals 6 h (**B**), 24 h (**C**), and 72 h (**D,E**) following SE on day P14. Control neurons have healthy cigar-shaped mitochondria and intact nuclei (**A**). Mitochondrial swelling (arrowheads) and cytoplasmic vacuolization (arrow) are the first signs of cell damage 6 h following SE, suggesting early necrosis (**B**). Twenty-four hours following SE (**C**), this neuron displays mild, irregular chromatin fragmentation, while the cytoplasm, mitochondria, and endoplasmic reticulum show severe swelling. At 72 h (**D,E**), neurons exhibit advanced stages of necrosis, recognizable by severe organelle swelling and nuclear shrinkage (**D**), or are surrounded by astroglial cells (As, image **E**). **F**. Example of an apoptotic cell 24 h following MK-801 administration in the retrosplenial cortex from a P7 rat pup. Note the chromatin fragmentation into round clumps at a time when the cytosol is almost intact. Bars = 5 μm. From ref. 60.

lithium-pilocarpine or systemic injection of kainic acid (KA) in adult rats, Fujikawa et al.[17,18] and Tokuhara et al.[51] found necrosis in diverse areas of the brain, including the hippocampus, amygdala, and entorhinal, piriform, and frontal cortices 24 h to 7 days following SE. The apoptotic morphology could not be found even when SE duration was reduced to 60 min, suggesting that seizure severity is not a determining factor in the mode of death.[20] While necrosis is certainly the main form of seizure-associated death in the adult brain, apoptotic morphologies have been described in some models of SE, and their role cannot be disregarded.[52–54]

…And in the Developing Brain, Too

It is now well established that experimental seizures can induce neuronal injury in the developing brain.[12,55–58] Both apoptotic and necrotic morphologies have been clearly reported by light microscopy and electron microscopy (EM) in the developing brain following SE. On postnatal day 14 (P14) in rat pups, apoptosis was notably found in the inner granule cell layer of the dentate gyrus.[12,16] In the same rats, SE-induced neuronal injury was extensive in the CA1 pyramidal cell layer. Twenty-four hours after SE, EM observations showed that 47 of 50 injured CA1 neurons had a necrotic morphology (Fig. 29–1C). Seventy-two hours after SE, 50 of 50 injured neurons were necrotic[60] (Fig. 29–1D,E). In a model of lithium-pilocarpine SE in P12 rat pups, EM observations revealed that necrosis is the mode of death of injured neurons in the mediodorsal nucleus of the thalamus.[56] Altogether, these results show that necrosis is the main form of SE-induced neuronal death even in the immature brain.

Mechanisms of Seizure-Induced Neuronal Necrosis

THE TRADITIONAL VIEW OF NECROSIS AND APOPTOSIS

Traditionally, necrosis is viewed as a passive mechanism that does not require the activation of orderly cell death programs and/or synthesis of new proteins. However, many histological studies dispute this view. Following experimental SE, morphologically necrotic neurons display terminal deoxynucleotidyl deoxyuridine triphosphate nick end labeling (TUNEL) staining,[12,59,60] Bax immuoreactivity,[61] caspase expression,[16,60] or caspase-independent cell death programs.[62] Interestingly, nuclear expression of caspase-3 and nuclear translocation of Apoptosis-Inducing Factor (AIF) were found in injured neurons from patients with temporal lobe epilepsy.[63]

Are these *apoptotic* factors actively involved in the execution of necrosis? Alternatively, does their expression represent an epiphenomenon? The reduction of neuronal necrosis by caspase inhibitors in several models[16,64–66] supports the view that apoptotic factors play an important role in neuronal necrosis. Below, we will describe the experimental data suggesting the existence of active forms of necrosis.

By contrast, apoptotic neuronal death following brain insults is conceptualized as an active form of death that results from the execution of cellular programs that resemble those involved in developmentally programmed cell death. The best-characterized programs are those of the extrinsic and intrinsic pathways, which offer many alternative routes of activation of caspases, a family of cysteine proteases.[67] When the *extrinsic* pathway of programmed cell death is induced, the first step involves the activation of extracellular cell death receptors of the tumor necrosis factor (TNF) superfamily, which recruit other proteins to form a complex that activates caspase-8, which in turn activates caspase-3. This *executioner* caspase kills the cell through its widespread proteolytic effects, activating DNA breakdown, inactivating DNA repair enzymes, and attacking the cytoskeleton, among other activities. In the *intrinsic* pathway of programmed cell death, the mitochondrion plays a critical role by releasing cytochrome c from its intermembrane space to the cytosol, where, in association with apoptotic protease-activating factor-1 and deoxyadenosine triphosphate (dATP), it forms the apoptosome complex, activating caspase-9, which in turn activates caspase-3.[68] Below, we show evidence suggesting that these processes are not exclusively involved in apoptotic death but can also participate in necrotic forms of seizure-induced neuronal death.

EVIDENCE FOR CASPASE-DEPENDENT FORMS OF NEURONAL NECROSIS IN CULTURE

In primary neuronal cultures, chemical hypoxia or glutamate excitotoxicity can activate initiator caspase-9 and cell executioner caspase-3 in necrotic neurons. In the minutes to hours following the insult, cytochrome c is released from swollen mitochondria at a time when the nucleus is still intact (a sign of early stage of necrosis) and later colocalizes with active caspase-3, suggesting the activation of the intrinsic pathway. Caspase inhibitors reduce neuronal necrosis, showing that this caspase cascade is not an epiphenomenon but does contribute to neuronal necrosis. We have called this active form of necrosis *programmed necrosis*.[64–66]

Seizures and hypoxia/ischemia profoundly inhibit neuronal protein synthesis[69–72] and therefore inhibit processes requiring macromolecular synthesis (such as some forms of apoptosis). In opposition to classical apoptosis, programmed necrosis does not require protein synthesis. In primary neuronal cultures, the protein synthesis inhibitor cycloheximide reduced caspase-3 activation in staurosporine-induced apoptosis but had no effect on hypoxic neuronal necrosis.[64] Since the caspase-3 precursor is already expressed in control cultures, the execution of programmed necrosis does not require the energy-intensive process of expressing new genes, which is often necessary in classical apoptosis.

EVIDENCE FOR A CASPASE-DEPENDENT FORM OF NECROSIS INDUCED BY SE

In a lithium-pilocarpine model of SE in rat pups,[16] upregulation of initiator caspase-8

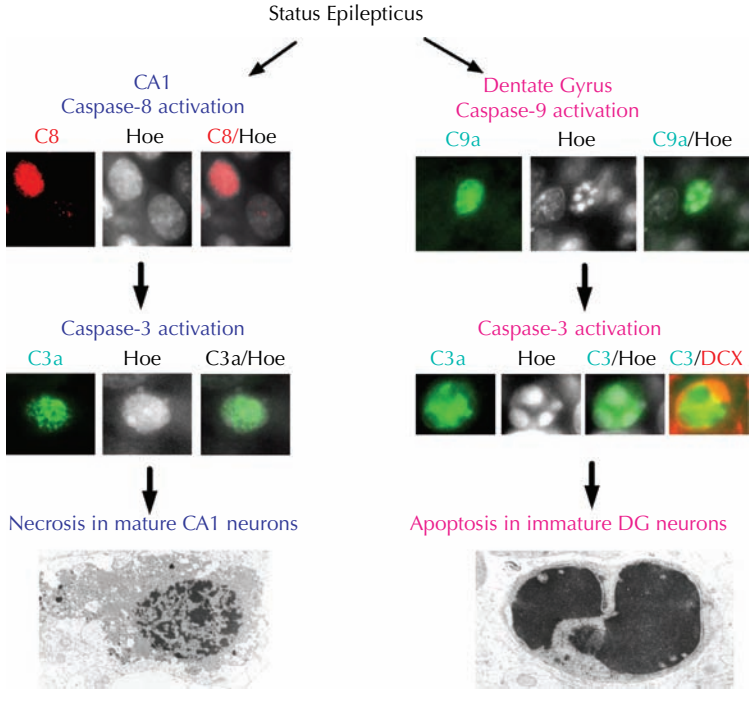

Figure 29–2. Putative neuronal death pathways induced by SE in the immature hippocampus. Fluorescent images show caspase-3a (green), caspase-8 (red), caspase-9a (green), and DCX (red) immunoreactivity and Hoechst staining (chromatin dye, white) in CA1 and the dentate gyrus following SE on P14. In CA1, images of caspase-8 and caspase-3 immunoreactivity were taken at 7 and 24 h, respectively. Images from dentate gyrus were taken 24 h following SE. At the bottom, EM micrographs obtained 24 h after SE show a CA1 necrotic neuron and a dentate gyrus apoptotic neuron. In CA1, seizures induce early and widespread activation of caspase-8, which subsequently activates the effector caspase-3 and leads to programmed necrosis. In dentate gyrus, seizures activate caspase-9 and caspase-3 and trigger apoptosis in immature neurons. From ref. 16.

precedes caspase-3 activation in CA1 (Fig. 29–2). Postembedding immunohistochemical studies show that caspase-3-immunoreactive CA1 neurons have a necrotic morphology. Pretreatment with a pan-caspase inhibitor reduces neuronal necrosis in CA1, showing that caspase activation is not an epiphenomenon but does contribute to SE-induced neuronal necrosis.[16] These results suggest that the caspase cascade of the extrinsic pathway (upregulation of initiator caspase-8 followed by caspase-3 activation) can also contribute to necrotic neuronal death.

There is indirect evidence suggesting that programmed necrosis can be found in other models of brain injury. In adult rats, KA-induced SE caused two types of pyramidal cell death in CA1: early necrosis (1 day after SE) and delayed TUNEL-positive and caspase-3-dependent programmed cell death (3–7 days after SE). Despite evidence of caspase-3 activation, apoptotic morphology could not be found at 1, 3, and 7 days following seizures, suggesting that necrosis is the main form of CA1 neuronal death.[51]

INFLUENCE OF CELL MATURITY AND INFLAMMATION ON PROGRAMMED NECROSIS

Following brain injury, apoptosis has been considered to be more likely to occur in the developing brain than in the adult one,[73] perhaps because of an age-dependent downregulation of apoptotic death factors such as caspases.[74,75] In the lithium-pilocarpine model of SE in rat pups described above, the same seizures trigger two distinct caspase pathways leading to two different modes of death: the extrinsic pathway leading to necrosis in CA1 (as described above) and the intrinsic pathway (caspase-9 and -3 activation) leading to apoptosis in the dentate gyrus (Fig. 29–2). One possible explanation for these results is the degree of cell maturity of these two neuronal populations. In the dentate gyrus, active caspase-3-immunoreactive cells had features of immature neurons: they expressed doublecortin (a marker of immature neurons[76]) and failed to express calbindin immunoreactivity (a marker of mature granule cells). Immature dentate gyrus cells may be more susceptible to death by apoptosis than CA1 pyramids, whose maturation occurs much earlier.[77–79] From these studies, we may speculate that the degree of cell maturity is more important than the level of maturation of the whole organism. Alternative explanations might involve environmental factors, that is, independent of the intrinsic properties of the neurons, such as inflammation or metabolic changes.

CASPASE-INDEPENDENT FORMS OF ACTIVE NECROSIS

Caspases are not the only apoptotic factors that may be involved in neuronal necrosis (Fig. 29–3). There is evidence that some forms of necrosis depend on the release of mitochondrial death factors, such as AIF and endonuclease G. Originally characterized for their role in apoptosis,[80–82] their release from mitochondria to cytosol and their translocation to the nucleus can induce DNA fragmentation in a caspase-independent manner. Translocation of AIF is involved in many models of neuronal injury often associated with a necrotic morphology. In cultured neurons, neurons with reduced AIF expression are more resistant to glutamate excitotoxicity or oxygen/glucose deprivation. Harlequin mice (expressing over 80% AIF expression reduction) exhibit less neuronal injury following ischemia or KA-induced seizures.[83–85] However, in the latter study,[84] seizure severity was not properly monitored and it is unclear whether Harlequin and control mice display the same susceptibility to seizures. In a model of lithium-pilocarpine SE in adult rats,[62] nuclear translocation of mitochondrial factors AIF and endonuclease G occur within 60 min of seizure onset (i..e., before signs of irreversible neuronal death), suggesting their involvement in nuclear pyknosis and DNA fragmentation. In the same study, nuclear translocation of other factors (cytochrome c, DNAse II) has been reported, suggesting the activation of various pathways leading to SE-neuronal necrosis.

The term *necroptosis* has been proposed for a kind of necrotic cell death that can be avoided by inhibiting the activity of the serine/threonine kinase RIP1, either through genetic or pharmacological methods.[86] Necrostatins, specific and potent inhibitors of RIP1, have been used as an operational definition of necroptosis.[87,88] By contrast with programmed necrosis, necroptosis involves caspase-independent pathways and other cell death factors have been implicated, including cyclophilin D, poly(ADP-ribose) polymerase 1 (PARP-1), and AIF (for review,

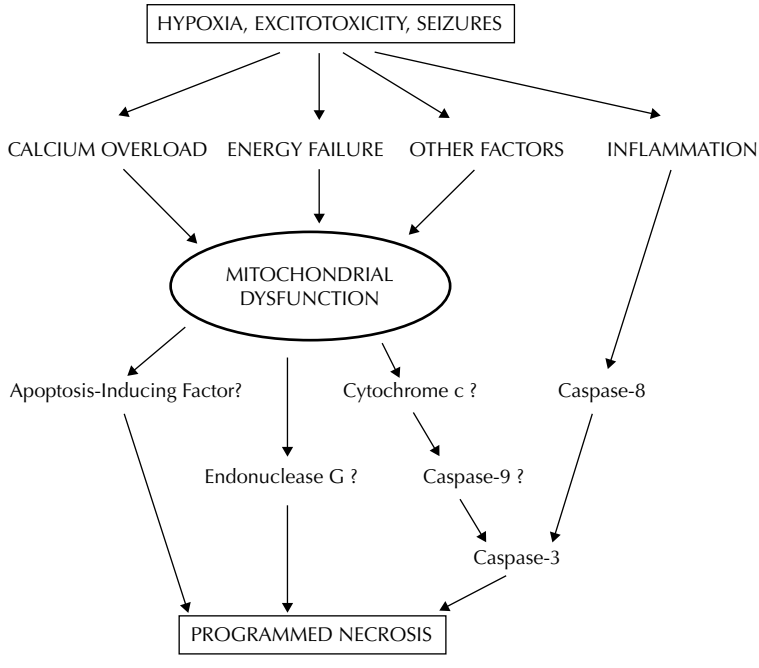

Figure 29–3. Putative mechanisms of programmed necrosis. Following hypoxia, excitotoxicity, and/or seizures, calcium entry into the mitochondria and/or energy failure contribute to mitochondrial swelling, outer membrane rupture, release of mitochondrial death factors, and activation of the intrinsic pathway. Alternatively, an inflammatory process may induce the activation of the extrinsic pathway. Adapted from ref. 6.

see ref. 89). Necroptosis has not been reported following SE, but it may contribute to *N*-methyl-D-aspartate (NMDA)-induced excitotoxicity in cultured cortical neurons.[90]

THERAPEUTIC IMPLICATION OF PROGRAMMED NECROSIS

Blocking neuronal necrosis is an important therapeutic goal. Plasma membrane rupture in the late stage of necrosis leads to release of cytoplasmic and/or nuclear material in the extracellular space, which may trigger an inflammatory response followed by secondary necrosis in neighboring neurons. The evidence of active forms of necrosis suggests the possibility of therapeutic interventions. An experimental model of SE showed that treatment with the cell-permeable, irreversible pan-caspase inhibitor Q-VD-OPH (Quinolyl-Val-Asp-Oph) can block active necrosis.[16] Intraperitoneal delivery of fusion proteins composed of an anti-apoptotic element and a transduction domain that enables the molecule to cross the blood-brain barrier has shown neuroprotective potential in models of SE.[91,92] Recent literature has also shown that cytokines, T-cells, and macrophages can add to the damage induced by seizures, and that targeting the innate and adaptive immune mechanisms may be a promising therapeutic approach.[93] However, the multiplicity of cell death pathways (caspase-dependent, caspase-independent) suggests that no magic bullet will be able to block simultaneously all forms of neuronal death.[94]

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*. 1972;26:239–257.
2. Olney JW, Rhee V, Ho OL. Kainic acid: a powerful neurotoxic analogue of glutamate. *Brain Res*. 1974;7: 507–512.
3. Olney JW. The toxic effects of glutamate and related compounds in the retina and the brain. *Retina*. 1982;2: 341–359.

4. Rothman S. Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. *J Neurosci*. 1984;4:1884–1891.
5. Choi DW. Glutamate neurotoxicity in cortical cell culture is calcium dependent. *Neurosci Lett.* 1985;58: 293–297.
6. Niquet J, Seo DW, Wasterlain CG. Mitochondrial pathways of neuronal necrosis. *Biochem Soc Trans*. 2006;34:1347–1351.
7. Ribe EM, Serrano-Saiz E, Akpan N, Troy CM. Mechanisms of neuronal death in disease: defining the models and the players. *Biochem J*. 2008;415: 165–182.
8. Lorz C, Mehmet H. The role of death receptors in neural injury. *Front Biosci*. 2009;14:583–595.
9. Penix LP, Thompson KW, Wasterlain CG. Selective vulnerability to perforant path stimulation: role of NMDA and non-NMDA receptors. *Epilepsy Res Suppl*. 1996;12:63–73.
10. Thompson KW, Wasterlain CG. Partial protection of hippocampal neurons by MK-801 during perforant path stimulation in the immature brain. *Brain Res.* 1997;751:96–101.
11. Thompson K, Holm AM, Schousboe A, Popper P, Micevych P, Wasterlain C. Hippocampal stimulation produces neuronal death in the immature brain. *Neuroscience*. 1998;82:337–348.
12. Sankar R, Shin DH, Liu H, Mazarati A, Pereira de Vasconcelos A, Wasterlain CG. Patterns of status epilepticus-induced neuronal injury during development and long-term consequences. *J Neurosci*. 1998;18:8382–8393.
13. Fujikawa DG. Prolonged seizures and cellular injury: understanding the connection. *Epilepsy Behav.* 2005;7: S3–S11.
14. Niquet J, Liu H, Wasterlain CG. Programmed neuronal necrosis and status epilepticus. *Epilepsia*. 2005;46:43–48.
15. Henshall DC, Murphy BM. Modulators of neuronal cell death in epilepsy. *Curr Opin Pharmacol*. 2008;8: 75–81.
16. Lopez-Meraz ML, Wasterlain CG, Rocha L, Allen S, Niquet J. Vulnerability of postnatal hippocampal neurons to seizures varies regionally with their maturational stage. *Neurobiol Dis*. 2010;37:394–402.
17. Fujikawa DG, Shinmei SS, Cai B. Lithium-pilocarpine-induced status epilepticus produces necrotic neurons with internucleosomal DNA fragmentation in adult rats. *Eur J Neurosci*. 1999;11:1605–1614.
18. Fujikawa DG, Shinmei SS, Cai B. Kainic acid-induced seizures produce necrotic, not apoptotic, neurons with internucleosomal DNA cleavage: implications for programmed cell death mechanisms. *Neuroscience*. 2000;98:41–53.
19. Fujikawa DG. Neuronal death in mesial temporal sclerosis: separating morphology from mechanism. *Epilepsia*. 2003;44:1607.
20. Fujikawa DG, Zhao S, Ke X, Shinmei SS, Allen SG. Mild as well as severe insults produce necrotic, not apoptotic, cells: evidence from 60-min seizures. *Neurosci Lett.* 2010;469:333–337.
21. Margerison JH, Corsellis JA. Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain*. 1966;89:499–530.
22. Corsellis JA, Bruton CJ. Neuropathology of status epilepticus in humans. *Adv Neurol*. 1983;34:129–139.
23. Sagar HJ, Oxbury JM. Hippocampal neuron loss in temporal lobe epilepsy: correlation with early childhood convulsions. *Ann Neurol*. 1987;22:334–340.
24. Sinha S, Satishchandra P, Mahadevan A, Bhimani BC, Kovur JM, Shankar SK. Fatal status epilepticus: A clinico-pathological analysis among 100 patients: from a developing country perspective. *Epilepsy Res*. 2010;91(2–3):193–204.
25. Babb TL, Brown WJ. Neuronal, dendritic, and vascular profiles of human temporal lobe epilepsy correlated with cellular physiology in vivo. *Adv Neurol*. 1986;44: 949–966.
26. Camfield PR. Recurrent seizures in the developing brain are not harmful. *Epilepsia.* 1997;38:735–737.
27. DeGiorgio CM, Tomyasu U, Gott PS, Treiman DM. Hippocampal pyramidal loss in human status epilepticus. *Epilepsia*. 33:1992;27–29.
28. Rabinowicz AL, Correale JD, Bracht KA, Smith TD, DeGiorgio CM. Neuron-specific enolase is increased after nonconvulsive status epilepticus. *Epilepsia.* 1995;36:475–479.
29. O'Regan ME, Brown JK. Serum neuron specific enolase: a marker for neuronal dysfunction in children with continuous EEG epileptiform activity. *Eur J Paediatr Neurol*. 1998;193–197.
30. Chu K, Kang DW, Kim JY, Chang KH, Lee SK. Diffusion-weighted magnetic resonance imaging in nonconvulsive status epilepticus. *Arch Neurol*. 2001;58:993–998.
31. Lansberg MG, O'Brien MW, Norbash AM, Moseley ME, Morrell M, Albers GW. MRI abnormalities associated with partial status epilepticus. *Neurology*. 1999;52:1021–1027.
32. Lazeyras F, Blanke O, Zimine I, Delavelle J, Perrig SH, Seeck M. MRI, (1)H-MRS, and functional MRI during and after prolonged nonconvulsive seizure activity. *Neurology*. 2000;55:1677–1682.
33. Salmenperä T, Kälviäinen R, Partanen K, Mervaala E, Pitkänen A. MRI volumetry of the hippocampus, amygdala, entorhinal cortex, and perirhinal cortex after status epilepticus. *Epilepsy Res*. 2000;40:155–170.
34. Wieshmann UC, Woermann FG, Lemieux L, Free SL, Bartlett PA, Smith SJ, Duncan JS, Stevens JM, Shorvon SD. Development of hippocampal atrophy: a serial magnetic resonance imaging study in a patient who developed epilepsy after generalized status epilepticus. *Epilepsia.* 1997;38:1238–1241.
35. VanLandingham KE, Heinz ER, Cavazos JE, Lewis DV. Magnetic resonance imaging evidence of hippocampal injury after prolonged focal febrile convulsions. *Ann Neurol*. 1998;43:413–426.
36. Pascual-Castroviejo I, Pascual-Pascual SI, Peña W, Talavera M. Status epilepticus-induced brain damage and opercular syndrome in childhood. *Dev Med Child Neurol*. 1999;41:420–423.
37. Nixon J, Bateman D, Moss T. An MRI and neuropathological study of a case of fatal status epilepticus. *Seizure*. 2001;10:588–591.
38. Freeman JL, Coleman LT, Smith LJ, Shield LK. Hemiconvulsion-hemiplegia-epilepsy syndrome: characteristic early magnetic resonance imaging findings. *J Child Neurol*. 2002;17:10–16.
39. Men S, Lee DH, Barron JR, Muñoz DG. Selective neuronal necrosis associated with status epilepticus: MR findings. *Am J Neuroradiol*. 2000;21:1837–1840.

40. Morimoto T, Fukuda M, Suzuki Y, Kusu M, Kida K. Sequential changes of brain CT and MRI after febrile status epilepticus in a 6-year-old girl. *Brain Dev*. 2002;24:190–193.
41. Neligan A, Shorvon SD. Frequency and prognosis of convulsive status epilepticus of different causes: a systematic review. *Arch Neurol*. 2010;67:931–940.
42. Thomas P, Valton L, Genton P. Absence and myoclonic status epilepticus precipitated by antiepileptic drugs in idiopathic generalized epilepsy. *Brain*. 2006;129: 1281–1292.
43. Meldrum BS, Brierley JB. Prolonged epileptic seizures in primates. Ischemic cell change and its relation to ictal physiological events. *Arch Neurol*. 1973;28:10–17.
44. Meldrum BS, Vigouroux RA, Rage P, Brierley JB. Hippocampal lesions produced by prolonged seizures in paralyzed artificially ventilated baboons. *Experientia*. 1973;29:561–563.
45. Meldrum BS, Vigouroux RA, Brierley JB. Systemic factors and epileptic brain damage. Prolonged seizures in paralyzed, artificially ventilated baboons. *Arch Neurol*. 1973;29:82–87.
46. Collins RC, Olney JW. Focal cortical seizures cause distant thalamic lesions. *Science*. 1982;218:177–179.
47. Sloviter RS, Damiano BP. On the relationship between kainic acid-induced epileptiform activity and hippocampal neuronal damage. *Neuropharmacology*. 1981;20:1003–1011.
48. Sankar R, Shin D, Liu H, Wasterlain C, Mazarati A. Epileptogenesis during development: injury, circuit recruitment, and plasticity. *Epilepsia*. 2002;43(Suppl5): 47–53.
49. Norwood BA, Bumanglag AV, Osculati F, Sbarbati A, Marzola P, Nicolato E, Fabene PF, Sloviter RS. Classic hippocampal sclerosis and hippocampal-onset epilepsy produced by a single "cryptic" episode of focal hippocampal excitation in awake rats. *J Comp Neurol*. 2010;518:3381–3407.
50. Wasterlain CG. Mortality and morbidity from serial seizures: an experimental study. *Epilepsia*. 1974;15:155–176.
51. Tokuhara D, Sakuma S, Hattori H, Matsuoka O, Yamano T. Kainic acid dose affects delayed cell death mechanism after status epilepticus. *Brain Dev*. 2007;29:2–8.
52. Sloviter RS, Dean E, Sollas AL, Goodman JH. Apoptosis and necrosis induced in different hippocampal neuron populations by repetitive perforant path stimulation in the rat. *J Comp Neurol*. 1996;366(3): 516–533.
53. Liu H, Cao Y, Basbaum AI, Mazarati AM, Sankar R, Wasterlain CG. Resistance to excitotoxin-induced seizures and neuronal death in mice lacking the preprotachykinin A gene. *Proc Natl Acad Sci USA*. 1999;96: 12096–12101.
54. Baille V, Clarke PG, Brochier G, Dorandeu F, Verna JM, Four E, Lallement G, Carpentier P. Soman-induced convulsions: the neuropathology revisited. *Toxicology*. 2005;215(1–2):1–24.
55. Thompson K, Wasterlain C. Lithium-pilocarpine status epilepticus in the immature rabbit. *Brain Res Dev Brain Res*. 1997;100:1–4.
56. Kubová H, Druga R, Lukasiuk K, Suchomelová L, Haugvicová R, Jirmanová I, Pitkänen A. Status epilepticus causes necrotic damage in the mediodorsal nucleus of the thalamus in immature rats. *J Neurosci*. 2001;21:3593–3599.
57. Silva AV, Regondi MC, Cipelletti B, Frassoni C, Cavalheiro EA, Spreafico R. Neocortical and hippocampal changes after multiple pilocarpine-induced status epilepticus in rats. *Epilepsia*. 2005;46:636–642.
58. Nairismagi J, Pitkanen A, Kettunen MI, Kauppinen RA, Kubova H. Status epilepticus in 12-day-old rats leads to temporal lobe neurodegeneration and volume reduction: a histologic and MRI study. *Epilepsia*. 2006;47: 479–488.
59. Charriaut-Marlangue C, Ben-Ari Y. A cautionary note on the use of the TUNEL stain to determine apoptosis. *Neuroreport*. 1995;7:61–64.
60. Niquet J, Auvin S, Archie M, Seo DW, Allen S, Sankar R, Wasterlain CW. Status epilepticus triggers caspase-3 activation and necrosis in the immature rat brain. *Epilepsia*. 2007;48:1203–1206.
61. Liu H, Cao Y, Basbaum AI, Mazarati AM, Sankar R, Wasterlain CG. Resistance to excitotoxin-induced seizures and neuronal death in mice lacking the preprotachykinin A gene. *Proc Natl Acad Sci USA*. 1999;96:12096–12101.
62. Zhao S, Aviles ER Jr, Fujikawa DG. Nuclear translocation of mitochondrial cytochrome c, lysosomal cathepsins B and D, and three other death-promoting proteins within the first 60 minutes of generalized seizures. *J Neurosci Res*. 2010;88:1727–1737.
63. Schindler CK, Pearson EG, Bonner HP, So NK, Simon RP, Prehn JH, Henshall DC. Caspase-3 cleavage and nuclear localization of caspase-activated DNase in human temporal lobe epilepsy. *J Cereb Blood Flow Metab*. 2006;26:583–589.
64. Niquet J, Baldwin RA, Allen SG, Fujikawa DG, Wasterlain CG. Hypoxic neuronal necrosis: protein synthesis-independent activation of a cell death program. *Proc Natl Acad Sci USA*. 2003;100:2825–2830.
65. Niquet J, Seo DW, Allen SG, Wasterlain CG. Hypoxia in presence of blockers of excitotoxicity induces a caspase-dependent neuronal necrosis. *Neuroscience*. 2006;14:77–86.
66. Seo DW, Lopez-Meraz ML, Allen S, Wasterlain CG, Niquet J. Contribution of a mitochondrial pathway to excitotoxic neuronal necrosis. *J Neurosci Res*. 2009;87: 2087–2094.
67. Liou AK, Clark RS, Henshall DC, Yin XM, Chen J. To die or not to die for neurons in ischemia, traumatic brain injury and epilepsy: a review on the stress-activated signaling pathways and apoptotic pathways. *Prog Neurobiol*. 2003;69(2):103–142.
68. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell*. 1997;91:479–489.
69. Wasterlain CG. Inhibition of cerebral protein synthesis by epileptic seizures without motor manifestations. *Neurology*. 1974;24:175–180.
70. Fando JL, Conn M, Wasterlain CG. Brain protein synthesis during neonatal seizures: an experimental study. *Exp Neurol*. 1979;63:220–228,.
71. Dwyer BE, Wasterlain CG. Regulation of the first step of the initiation of brain protein synthesis by guanosine diphosphate. *J Neurochem*. 1980;34:1639–1647.
72. Dwyer BE, Wasterlain CG. Selective focal inhibition of brain protein synthesis during generalized bicuculline

seizures in newborn marmoset monkeys. *Brain Res*. 1984;308:109–121.
73. Martin LJ, Al-Abdulla NA, Brambrink AM, Kirsch JR, Sieber FE, Portera-Cailliau C. Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: a perspective on the contributions of apoptosis and necrosis. *Brain Res Bull*. 1998;46:281–309.
74. Shimohama S, Tanino H, Fujimoto S. Differential expression of rat brain caspase family proteins during development and aging. *Biochem Biophys Res Commun*. 2001;289:1063–1066.
75. Yakovlev AG, Ota K, Wang G, Movsesyan V, Bao WL, Yoshihara K, Faden AI. Differential expression of apoptotic protease-activating factor-1 and caspase-3 genes and susceptibility to apoptosis during brain development and after traumatic brain injury. *J Neurosci*. 2001;21:7439–7446.
76. Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet MC, Friocourt G, McDonnell N, Reiner O, Kahn A, McConnell SK, Berwald-Netter Y, Denoulet P, Chelly J. Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron*. 1999;23:247–256.
77. Harris KM, Teyler TJ. Developmental onset of long-term potentiation in area CA1 of the rat hippocampus. *J Physiol*. 1984;346:27–48.
78. Bekenstein JW, Lothman EW. An in vivo study of the ontogeny of long-term potentiation (LTP) in the CA1 region and in the dentate gyrus of the rat hippocampal formation. *Brain Res Dev*. 1991;63:245–251.
79. Bekenstein JW, Lothman EW. A comparison of the ontogeny of excitatory and inhibitory neurotransmission in the CA1 region and dentate gyrus of the rat hippocampal formation. *Dev Brain Res*. 1991;63: 237–243.
80. Parrish J, Li L, Klotz K, Ledwich D, Wang X, Xue D. Mitochondrial endonuclease G is important for apoptosis in *C. elegans*. *Nature*. 2001;412:90–94.
81. Li LY, Luo X, Wang X. Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature*. 2001;412:95–99.
82. Candé C, Cecconi F, Dessen P, Kroemer G. Apoptosis-inducing factor (AIF): key to the conserved caspase-independent pathways of cell death? *J Cell Sci*. 2002;115:4727–4734.
83. Yu SW, Wang H, Dawson TM, Dawson VL. Poly(ADP-ribose) polymerase-1 and apoptosis inducing factor in neurotoxicity. *Neurobiol Dis*. 2003;14:303–317.
84. Cheung EC, Melanson-Drapeau L, Cregan SP, Vanderluit JL, Ferguson KL, McIntosh WC, Park DS, Bennett SA, Slack RS. Apoptosis-inducing factor is a key factor in neuronal cell death propagated by BAX-dependent and BAX-independent mechanisms. *J Neurosci*. 2005;25:1324–1334.
85. Culmsee C, Zhu C, Landshamer S, Becattini B, Wagner E, Pellechia M, Blomgren K, Plesnila N. Apoptosis-inducing factor triggered by poly(ADP-ribose) polymerase and Bid mediates neuronal cell death after oxygen-glucose deprivation and focal cerebral ischemia. *J Neurosci*. 2005;25:10262–10272.
86. Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH, Blagosklonny MV, El-Deiry WS, Golstein P, Green DR, Hengartner M, Knight RA, Kumar S, Lipton SA, Malorni W, Nuñez G, Peter ME, Tschopp J, Yuan J, Piacentini M, Zhivotovsky B, Melino G; Nomenclature Committee on Cell Death 2009. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ*. 2009;16(1):3–11.
87. Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, Cuny GD, Mitchison TJ, Moskowitz MA, Yuan J. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat Chem Biol*. 2005;1(2):112–119.
88. Degterev A, Hitomi J, Germscheid M, Ch'en IL, Korkina O, Teng X, Abbott D, Cuny GD, Yuan C, Wagner G, Hedrick SM, Gerber SA, Lugovskoy A, Yuan J. Identification of RIP1 kinase as a specific cellular target of necrostatins. *Nat Chem Biol*. 2008;4(5): 313–321.
89. Galluzzi L, Kroemer G. Necroptosis: a specialized pathway of programmed necrosis. *Cell*. 2008;135(7): 1161–1163.
90. Li Y, Yang X, Ma C, Qiao J, Zhang C. Necroptosis contributes to the NMDA-induced excitotoxicity in rat's cultured cortical neurons. *Neurosci Lett*. 2008;447(2–3):120–123.
91. Li T, Fan Y, Luo Y, Xiao B, Lu C. In vivo delivery of a XIAP (BIR3-RING) fusion protein containing the protein transduction domain protects against neuronal death induced by seizures. *Exp Neurol*. 2006;197(2):301–308.
92. Ju KL, Manley NC, Sapolsky RM. Anti-apoptotic therapy with a Tat fusion protein protects against excitotoxic insults in vitro and in vivo. *Exp Neurol*. 2008;210(2):602–607.
93. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol*. 2011;7(1):31–40.
94. Golstein P, Kroemer G. A multiplicity of cell death pathways. Symposium on Apoptotic and Non-Apoptotic Cell Death Pathways. *EMBO Rep*. 2007;8(9):829–833.

Chapter 30

Histopathology of Human Epilepsy

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INTRODUCTION

Histopathological lesions that are associated with seizures are described in several neurological disease states. Such histopathological lesions are found in developmental disorders, neoplasms, microbial diseases, cerebrovascular diseases, trauma, and immune-mediated disorders, as well as in disorders primarily related to seizures such as temporal lobe epilepsy (TLE). General descriptions of these lesions have been the subject of several reviews.[1,2] Temporal lobe epilepsy, a major seizure disorder that affects over 40 million people worldwide, has received the most study in relation to its histopathology, and the histological lesion is thus best understood in relation to epilepsy. This chapter will review the state of our knowledge in the histopathology of TLE.

Pierre Gloor provides an excellent review of the historical studies of TLE.[3] However, some of the more significant of these studies bear revisiting for our current appreciation of the histopathology of TLE. The earliest systematic attempt to understand the histopathology of TLE was made by Sommer.[4] He reviewed all previously published papers on the pathology of epilepsy and examined (macroscopically) a few of his own cases; he estimated that pathology in Ammon's horn was found in about 30% of cases and that the pathology was more often unilateral. Studying the histology of only one

case, he described diffuse cell loss limited to a sector of the hippocampus, field H1 of Rose (area CA1), which subsequently became known as the *Sommer sector*. This condition, which manifests as an increased hardening of the tissue, was first noted by Bouchet and Cazauvieilh[5] and was termed *Ammon's horn sclerosis*. Bratz,[6] studying autopsied brains of epilepsy patients, confirmed the loss of neurons in Ammon's horn in about 50% of the brains and also noted that such loss was predominantly unilateral. He provided a more complete description of a sclerotic hippocampus, with accompanying woodcuts to illustrate his findings. Bratz observed the following: almost complete pyramidal cell loss in CA1 and a sharp boundary with the subiculum, which showed no cell loss; preservation of spindle-shaped cells in the stratum oriens; less complete cell loss in the hilus (end folium) and dentate gyrus; preservation of pyramidal cells in area CA2; and an abundance of blood vessels in the atrophic sector (area CA1). These detailed observations have held up as an accurate description of the gross histology of a sclerotic hippocampus.[7,8]

Whether hippocampal pathology is specially associated with TLE is still a topic of considerable discussion. To date, several lines of evidence suggest that the hippocampus does play an important role in TLE. Positron emission tomography (PET) studies demonstrate that the most significant hypometabolism is seen in the lateral temporal and medial temporal regions, with the degree of hypometabolism correlating with the severity of hippocampal pathology.[9] Hippocampal cell densities are significantly related to the amount of hypometabolism in the thalamus and basal ganglia in TLE patients.[10] Further, anteromedial temporal lobectomy, with removal of the hippocampus and amygdala, controls seizures and leads to improvement of the preoperative hypometabolism in the remaining areas of the temporolimbic network (e.g., inferior frontal temporal lobe, ipsilateral temporal neocortex, and medial thalamus).[11] Stauder[12] was the first to associate hippocampal sclerosis with TLE. He made a list of ictal symptoms and signs attributable unequivocally to temporal lobe disease (olfactory and gustatory auras, sensory aphasia and paraphasia, vertigo, auditory hallucinations or illusions and dreamy states) and correlated these with the postmortem pathology of 53 cases of "genuine epilepsy." Sixty-eight percent of the brains showed hippocampal sclerosis, of which 80% had definite and another 11% probable symptoms of genuine epilepsy, whereas none of those without hippocampal sclerosis showed symptoms. Falconer,[13] who developed the technique of en bloc resection of the temporal lobe for treatment of drug-resistant epilepsy, found a high correlation of good surgical outcome with mesial temporal sclerosis in the resected temporal lobe. Further, intracranial electroencephalographic (EEG) recordings in patients with TLE associated with hippocampal sclerosis show that seizures originate in the sclerotic hippocampus.[14] More recently, a multicenter study of seizure outcome after medial temporal resective surgery reports that hippocampal atrophy (sclerosis) and a history of absence of tonic-clonic seizures were the sole predictors of remission of seizures 2 years after surgery.[15]

Bratz[6] suggested that the hippocampal pathology in TLE cannot be assumed to be a consequence of seizures but must play a pathogenetic role. Whether hippocampal sclerosis is the cause or a consequence of seizures has remained a topic of lively debate. Several more recent observations indicate that hippocampal sclerosis is probably not a consequence of seizures. Magnetic resonance imaging (MRI) volume measurements show that the degree of hippocampal atrophy is not correlated with the duration and severity of seizures.[16] Some patients with multiple daily seizures since infancy do not have hippocampal sclerosis.[17] Likewise, a study of postmortem hippocampi from patients with poorly controlled seizures has confirmed the presence of a subgroup with no significant neuronal loss despite decades of seizures.[18] Additionally, hippocampi surgically removed from a large series of patients with TLE show that in about 15% of them (paradoxical TLE) there is no hippocampal sclerosis or evidence of extrahippocampal mass lesions, though their seizure semiology and history are indistinguishable from those of patients with hippocampal sclerosis.[7] Thus, seizures themselves do not result in hippocampal sclerosis. A more detailed and careful investigation of the histopathology of the sclerotic hippocampus and its relationship to the pathogenesis of human TLE is important, and since the 1980s there has been resurgence in investigations of the histopathology of the hippocampus in TLE. This renewed effort was ignited by a wider use

of the technique of en bloc resection of the hippocampus by neurosurgeons[19,20] and the availability of new methods for histological analysis. This chapter will attempt to summarize this considerable body of recent literature, various aspects of which have been the subject of other detailed reviews.[7,21,22]

HISTOPATHOLOGICAL VARIATIONS IN TLE HIPPOCAMPUS

In their now classic study, Margerison and Corsellis[23] examined the brains of 55 epileptic patients postmortem, in which clinical and EEG assessments of seizures during life were available. The hippocampi of this group showed varying degrees of histological change. Thirty-five percent of the hippocampi showed no evidence of neuronal loss bilaterally, while the remainder showed varying degrees of hippocampal sclerosis, ranging from classical hippocampal sclerosis (40%) to end folium sclerosis (25%). With the advent of surgery for the control of intractable epilepsy, further analyses of excised hippocampi were undertaken. Bruton[24] identified a variety of hippocampal pathologies using neuronal and myelin stains.[23] More recently, in another surgical series, the use of neuronal cell counts with immunohistochemical stains for neurotransmitters has led to the identification of several variations in the histopathology of the hippocampus in patients with medically intractable TLE.[7,25] Based on conventional histopathological criteria, hippocampi were divided into two groups—those without classical hippocampal sclerosis (compared to autopsy controls from neurologically normal subjects) and those with hippocampal sclerosis (Fig. 30–1). Those without classical hippocampal sclerosis had a small loss (<25%) of neurons throughout the hippocampus, with hippocampal volumes similar to those of autopsy controls and none of the immunohistochemical changes observed in the sclerotic hippocampi. However, these nonsclerotic hippocampi are divisible into two subgroups based on epilepsy etiology: the mass-associated TLE group (MaTLE) had an extrahippocampal temporal lobe mass lesion (low-grade gliomas, cavernomas), as opposed to a group described as having "paradoxical" temporal lobe epilepsy (PTLE), in which such mass lesions were absent and there was no history of febrile seizure or other identifiable etiological factors. Distinctive patterns correlating neuronal densities of the dentate gyrus and all areas of Ammon's horn, electrophysiological measures, and power spectral densities further differentiated these sclerotic and nonsclerotic groups.[7,25,26] The hippocampi with hippocampal

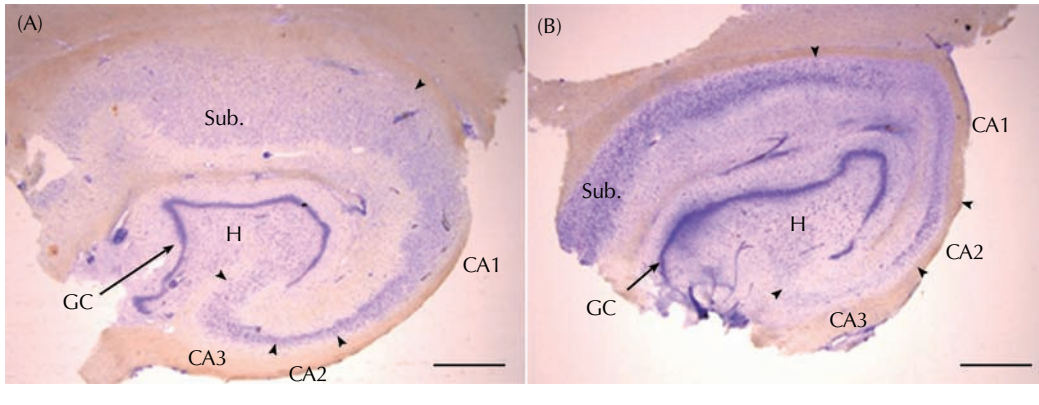

Figure 30–1. Photomicrographs of coronal sections of the hippocampus from a nonsclerotic and sclerotic (MTLE) hippocampus stained with Cresyl Violet. **A.** The nonsclerotic hippocampus is from a temporal lobe epilepsy patient with an extrahippocampal mass lesion in the temporal lobe. The hippocampus appears similar to that from a neurologically normal autopsy control, even through neuronal counts show approximately a 20% loss of neurons in all fields. **B.** This sclerotic hippocampus, also from a temporal lobe epilepsy patient, shows extensive neuronal loss in the hilus (H) and almost total neuronal loss in areas CA3 and CA1, with infiltration by glia. There is some preservation of neurons in area CA2. The neuronal density in the subiculum (Sub) is similar to that in the nonsclerotic hippocampus. The granule cell (GC) layer in **B** also shows significant neuronal loss. The arrowheads denote the limits of the respective CA fields. Bar = 50 μm.

sclerosis (mesial TLE [MTLE]) showed more extensive neuronal loss and some distinctive histopathological features. These features could be grouped into two broad categories: first, features associated with the neural reorganization of the dentate gyrus; and, second, the proliferation of astrocytes accompanying neuron loss in Ammon's horn (cornu ammonis [CA]), especially noticeable in area CA1. The details of these changes are described in the sections below.

REORGANIZATION OF THE DENTATE GYRUS IN SCLEROTIC HIPPOCAMPUS

Neuronal Changes in Dentate Gyrus

GRANULE CELLS

The principal neurons of the dentate gyrus are the granule cells, which form a densely packed C-shaped band of cells in a normal hippocampus. The granule cell has a cone-shaped tree of apical dendrites, which extend through the dentate molecular layer,[27] and in the human there are basal dendrites, which extend into the hilus as well as the molecular layer.[28] The granule cell axon, called the *mossy fiber*, extends into the hilus, where its collaterals synapse with hilar interneurons, continuing on to synapse on pyramidal neurons in area CA3.[27] Historically, the granule cells have been described as a band of cells that are relatively resistant to injury and loss in the sclerotic hippocampus. However, such resistance is visible only in a small proportion of TLE hippocampi, such as in a group of patients showing neuronal loss only in area CA1, as well as in MaTLE and PTLE groups.[7] In most of the MTLE hippocampi, there is loss of granular neurons resulting in a thinning of the layer and gaps in the cell band.[29] In other cases, there is a vertical spread or dispersion of the remaining granular neurons into the dentate molecular layer.[21] Reduced reelin mRNA levels are associated with granule cell dispersion, suggesting that the dispersion may be a developmental abnormality associated with granule cell migration.[30] Nevertheless, granule cell dispersion is not accompanied by enhanced neurogenesis in TLE patients.[31] Further, granule cell dispersion is not exclusive to hippocampi with sclerosis.[21,32] Granule cell dispersion seems more closely correlated with memory and learning changes than with seizures,[21] though some studies suggest that it is correlated with seizure frequency.[33] The surviving dentate granule cells in the sclerotic hippocampus show distinctive morphological alterations compared to those in nonsclerotic hippocampi. The apical dendrites of the granule cells in sclerotic hippocampi have a more limited spread in the longitudinal axis, a significant increase in the length of the portion of dendrites in the inner molecular layer, a decrease in the dendritic portion in the outer third, and an increase in spine density in both regions.[34] A proportion of the granule cells in sclerotic hippocampi also have axon collaterals from their mossy fibers extending into the molecular layer,[35] and these neurons have a significantly higher spine density in the proximal portion of the dendrite located in the inner molecular layer.[36]

Granule cells have a complex neurochemical profile. While glutamate is an important neurotransmitter produced by these cells, they also contain the neuropeptide dynorphin,[29] which increases in TLE hippocampi,[37] the calcium binding protein calbindin[38] and the hyperpolarization-activated cyclic nucleotide-gated cation channel 1 (HCN1), which is markedly increased when granule cell density is <50%.[39]

HILAR INTERNEURONS

The hilar region of the normal human hippocampus has many types of neurons. Among these are mossy cells, dentate pyramidal basket cells, and a variety of other interneurons characterized by their neurochemical content.[25] One of the early observations in relation to the histopathology of TLE is that interneurons mostly located in the subgranular region of the hilus and containing neuropeptide Y (NPY), somatostatin (SOM), and substance P (SP) were significantly reduced in number in the sclerotic hippocampus.[40] Many of these neurons also colocalize gamma-aminobutyric acid (GABA), and their loss may result in some loss of GABAergic inhibition. The dentate pyramidal basket cells, which are located on the hilar edge of the granule cell layer and are also GABAergic, are preserved in sclerotic hippocampi.[41]

The mossy cell is a neuron type of particular importance in TLE.[42] Large, complex spines

called *thorny excrescences*, which cover their proximal dendrites and from which the cell derives its name,[43] characterize these neurons, which are often triangular or multipolar in shape. These spines are the sites of termination of the granule cell mossy fiber axon. Mossy cells are excitatory glutamatergic neurons.[44] They can inhibit granule cells by directly exciting the pyramidal basket cells, which inhibit the granule cells.[45] In the rodent, the mossy cells are a very abundant cell type in the hilus. Mossy cells in primates (humans) and rodents show several differences. In the human, they are not the most abundant or largest cell type in the hilus, and the dendrites of most mossy cells penetrate into the dentate molecular layer and receive inputs from the perforant path.[46] In rodent models of seizures, the mossy cells are selectively vulnerable, and it is proposed that their death results in cellular reorganization favoring seizures.[42] In the human TLE hippocampus, mossy cells do not appear to be as vulnerable. Though the number of mossy cells is reduced, they have been identified in sclerotic hippocampi by increased expression of markers such as dynorphin,[47] GluR1 receptor,[48] and cocaine- and amphetamine-regulated transcript peptide.[49] In several sclerotic hippocampi, large numbers of mossy cells are observed even when most pyramidal neurons in CA1 and CA3 are lost.[47]

Plasticity of Neuronal Fibers

In addition to the loss of neurons in the TLE hippocampus, there is considerable evidence for the growth of new fiber systems, or *sprouting*. These changes are seen in the MTLE or sclerotic hippocampus. The most distinctive of these is the growth of recurrent collaterals from granule cell mossy axons into the inner molecular layer (IML). Such sprouting is recognized by the increased Timm stain, which visualizes the zinc contained in mossy fibers,[50,51] and by immunostaining for dynorphin (a peptide contained exclusively in dentate granule cells in nonsclerotic hippocampi).[40,52] These recurrent collaterals form synaptic contacts with the proximal portions of the apical dendrites of granule cells.[50,53] Enhanced immunoreactivity for dynorphin is also seen in surviving mossy cells, and their thorny excrescences are seen only in sclerotic hippocampi.[47] Four other examples of sprouting are observed in the MTLE hippocampus compared to those of other patient groups and autopsy controls.[25,40] The most striking of these examples is the increased density of NPY immunoreactive fibers throughout the dentate molecular layer. Somatostatin and SP fibers show a similar increase. Ultrastructural studies show that all of these fibers are axons, which form synaptic contacts with the granule cell soma and dendrites.[53] Acetylcholinesterase staining shows decreased staining in the IML and increased staining in the outer molecular layer (OML) of MTLE hippocampi compared to nonsclerotic hippocampi.[54]

Changes in Neurotransmitter Receptors

Associated with the reorganization of neurons and neural circuits in the dentate gyrus of the sclerotic hippocampus (MTLE) are several changes in the distribution of neurotransmitter receptors within the dentate gyrus compared to nonsclerotic surgically removed hippocampi (MaTLE and PTLE) and neurologically normal autopsy controls. These changes are summarized in Table 30–1. Several receptor subtypes that mediate glutamatergic transmission are changed. The alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) type receptor GluR 1 and GluR2/3 subunits are increased on the apical dendrites of the granule cells throughout the IML, while the GluR2/3 subunits are also increased on the granule cell bodies.[55] Such an increase in glutamate receptors may facilitate the excitation of granule cells through glutamatergic inputs such as the recurrent collaterals from mossy fibers or glutamatergic inputs from surviving hilar mossy cells (which terminate in the IML) and entorhinal inputs (which synapse on dendrites in the OML).[22] Upregulation of these AMPA-type receptor subunits on the thorny excrescences of mossy cells[56] suggests a facilitation of glutamatergic transmission from remaining granule cells through surviving mossy cells. The role of the metabotropic receptor subtypes (Table 30–1), which are also upregulated in the molecular layer and the granule cell layer,[57–59] is unclear at present; they may modulate excitatory and/or inhibitory effects.[60] Concomitant with upregulation of these glutamate receptors,

Table 30–1 **Neurotransmitter Receptor Localization in the Dentate Gyrus of the Sclerotic (MTLE) Hippocampus**

Receptor	GC	IML	OML	Hilus	Reference
GluR1 immunoreactivity				+ (Mossy cells)	55
GluR2/3 immunoreactivity	+	+	+	+ (Mossy cells)	55
[^{3}H] Kainate binding		+	+		144
mGluR1 immunoreactivity		+ (dendrites)	+		59
mGluR1α immunoreactivity	0				57
mGluR5 immunoreactivity		+ (dendrites/axon)	+	+	58
mGluR5 immunoreactivity	+		+		57
mGluR2/3 immunoreactivity		+ (terminals and dendrites)			58
mGluR4α immunoreactivity		+	+		58
mGluR8 immunoreactivity	+ (soma)				58
$GABA_A$ α1 immunoreactivity	+		+ (neurons)	+	61
$GABA_A$ α2 immunoreactivity	+ (soma)	++	+	+	61
$GABA_A$ α3 immunoreactivity	–				61
$GABA_A$ β2,3 γ2 immunoreactivity	+		+		61
$GABA_B$ binding				+	62
$GABA_B$ mRNA	+			+	63
[^{125}I]Tyr_0DTrp_8SRIF-14 and sst2 immunoreactivity	+	+	–		64
[^{125}I] Somatostatin	+	+	+	+	65
[^{125}I] Bolton-Hunter-labeled NPY	+	+		+	65
[^{125}I]{Pro^{34}}PYY-NPY Y1		–			66
[^{125}I]$PYY_{3–36}$-NPY Y2	+	+		+	66
3-Iodo-tyrosol-125]VIP	+			+	68
[^{125}I]Iodosulpride-D2	+	+			67

+ = increased, – = decreased, 0 = no change relative to nonsclerotic and/or autopsy controls. GC, granule cell layer; IML and OML, inner and outer molecular layer, respectively.

there is also evidence of upregulation of inhibitory receptors. Thus, the $GABA_A$ receptor subtypes α1 and α2,[61] along with $GABA_B$ receptors,[62,63] are upregulated on the granule cells somata, with the $GABA_A$ α2 subtype also significantly upregulated on the proximal portion of the granule cell dendrites in the IML.[61] $GABA_A$ and $GABA_B$ receptor subtypes are also upregulated in the hilus, though their exact cellular location is unclear.[61] The receptors for SOM are upregulated in the granule cell layer and the dentate molecular layer, particularly the IML as well as the hilus,[64,65] and are consistent with the sprouting of SOM-containing axons into these regions.[40] Likewise, the NPY-Y2 receptors are upregulated in the granule cell layer and the IML as well as the hilus,[65,66] consistent with the sprouting of NPY-positive axons in these regions. The dopamine D2 receptors are upregulated in the granule cell layer and IML,[67] while vasoactive intestinal polypeptide (VIP) receptors are upregulated in the granule cell layer and hilus.[68] Several of these receptor changes—in SOM, NPY-Y2, GABA, dopamine D2, and some of the mGluR receptors—may be involved in increasing the inhibition of the excitability of surviving granule cells.[69] It thus appears that in the sclerotic hippocampus, along with processes that facilitate hyperexcitability, there are also several reorganizational changes that seem to reestablish inhibition, though perhaps inadequately.

Implications of Reorganizational Changes in the Dentate Gyrus

The implications of this wide array of histopathological changes in the dentate gyrus for epileptogenesis or the maintenance of seizures in patients with TLE associated with hippocampal sclerosis are difficult to evaluate with any certainty, though several attempts have been made (see refs. 22, 70). However, though it is commonly thought that the dentate gyrus granule cells are relatively resistant to injury and thus play a role in seizure generation, the study of a large series of sclerotic hippocampi reveals cases in which there are extremely few granule cells remaining[29]—and so are unlikely to be of functional significance. Yet, these patients had intractable epilepsy. In an intermediate state of neuronal loss, it is likely that the dentate gyrus contributes to the epileptic state. Electrophysiological recordings from granule cells in such cases indicate that these neurons are hyperexcitable.[71] A more integrated understanding of these several changes, through experimental investigation, is needed.

CHANGES IN AMMON'S HORN AND THE ROLE OF ASTROCYTES

Neurons

Ammon's horn (the hippocampus proper) is divided into three distinct fields identified as CA3, CA2, and CA1. Viewed in cross section, it is composed of a single band of pyramidal cells (the stratum pyramidale) whose apical dendrites extend into a stratum radiatum. Between the pyramidal layer and the alveus is a network of small neurons forming the stratum oriens. Ammon's horn is a region most vulnerable to neuronal loss in TLE patients.[23,72,73] The pyramidal neurons are the cell type most prominently lost. The degree of neuronal loss can vary from patient to patient, and usually ranges from greater than 50% compared to neurologically normal controls[8] to almost complete loss. In some TLE patients, neuronal loss is seen only in Ammon's horn, with the dentate gyrus being intact (CA only; see ref. 7). In a small proportion of patients, neuronal loss is confined to the dentate hilus and area CA3, a condition called *end-folium sclerosis*.[23] In the nonsclerotic patient groups with MaTLE and PTLE (see above), Ammon's horn appears intact on visual inspection, but neuronal counts show about a 20% loss of neurons, as in the dentate gyrus, and these hippocampi are qualitatively indistinguishable from those of neurologically normal subjects.[7]

In the sclerotic hippocampi, even those with extensive pyramidal cell loss, several populations of neurochemically defined interneurons remain. These neurons are especially prominent in the stratum oriens/alvear region, where they appear as characteristic horizontal neurons. In MTLE hippocampi these neurons are immunoreactive for GABA, NPY, SOM, and SP (see Fig. 6 in ref. 74) and express glutamate receptor protein subunits GluR1, GluR2/3, mGluR1, and mGluR5.[57,75] These stratum oriens neurons, which appear to form a network throughout the stratum oriens, are also more strongly immunoreactive for the enzyme phosphate-activated glutaminase.[76] Stratum oriens neurons are characterized by having soma and dendrites that extend horizontally in the stratum oriens; their axonal arbors show distinctive projections, which serve as the basis on which they are subcategorized.[77] The physiological properties of these neurons have been studied extensively in normal animals, where they have been shown to modulate the excitability of Ammon's horn pyramidal cells.[78] While these neurons have been studied in animal models of epilepsy and have been reported to be even more excitable than pyramidal cells,[79] their functions in human sclerotic hippocampi are completely unknown.

Astrocytes

The most recognized feature in the histopathology of the sclerotic hippocampus is the proliferation of astrocytes in the neuron-depleted Ammon's horn.[4] These astrocytes exhibit many unusual properties compared to astrocytes from nonepileptic brain regions and have been the subject of previous reviews.[80,81] These changes are seen in their membrane proteins (receptors, transporters, ion channels), membrane physiology, enzymes, gene expression, and neurovascular relationships.

Astrocytes from sclerotic hippocampi have increased expression of the glutamatergic receptors mGluR2/3, mGluR4, and mGluR8.[82]

Activation of these receptors is known to lead to intracellular Ca^{2+} release and Ca^{2+} wave propagation, a phenomenon demonstrated in sclerotic astrocytes.[83] It is suggested that such Ca^{2+} release leads to glutamate release from astrocytes,[84] but this conclusion remains controversial. An elevated flip-to-flop mRNA ratio of the GluR1 subunit of the AMPA-type receptor has also been reported,[85,86] suggesting an increased responsiveness to glutamate. Patch- and voltage-clamp studies demonstrate increased density of membrane sodium channels.[87–89] There is also strong upregulation of the α_{1C} subunit of the voltage-dependent calcium channel.[90] The α_{1C} subunit contributes to L-type calcium currents, suggesting a change in the current characteristic of these cells. The Kir channel on astrocytes, which normally helps astrocytes remove extracellular K^+, is also defective in astrocytes from sclerotic tissue.[91,92] A large proportion (~60%) of astrocytes in primary cultures from sclerotic seizure foci are capable of generating action potential-like responses when depolarized[89] compared to those from nonsclerotic foci. This ability to produce action potentials may be related to their more depolarized resting membrane potential (~ –55 mV) and increased Na^+ channel densities.

Astrocytes possess some key enzymes not found in neurons. Astrocytes from sclerotic foci show changes in these enzymes as well. Most prominent is the downregulation of glutamine synthetase in astrocytes in the CA fields but not in the subiculum of the sclerotic hippocampus.[93,94] Glutamine synthetase catalyzes the conversion of glutamate, removed from the extracellular space, into glutamine in a process that utilizes ammonia. Indeed, astrocytes from a sclerotic hippocampus seem to have a reduced capacity for glutamine synthesis and ammonia detoxification,[95] and cellular glutamine[96,97] is reduced in the sclerotic hippocampus. Additionally, glutamate dehydrogenase (GDH) activity is increased while aspartate aminotransferase (AAT) activity is reduced in the sclerotic hippocampus.[98] Glutamate dehydrogenase catalyzes the interconversion of glutamate to α-ketoglutarate. Lactate dehydrogenase (LDH) activity levels, normalized to citrate synthase activity levels, are also decreased in the sclerotic hippocampus.[98]

The distribution of some transporter molecules on the astrocyte plasma membrane is changed in sclerotic hippocampi. Aquaporin 4 (AQP4) is a water transporter molecule on astrocyte membranes. These molecules are more densely located on the perivascular end feet than on the rest of the cell. In sclerotic hippocampi the density on the perivascular end feet is reduced, but it is unchanged on the rest of the cell membrane.[99] The glutamate transporters EAAT1 and EAAT2 are reported as downregulated by some[100,101] but not by others.[102] The degree of change does not seems to be an adequate explanation of the high extracellular glutamate levels observed in sclerotic seizure foci.[103] The expression of the GABA transporter GAT3 is increased on astrocytes in the sclerotic hippocampus.[104] GAT3 expression is confined to cells resembling protoplasmic astrocytes, which are located in regions of relative neuronal sparing such as the dentate gyrus and hilus. The increased expression of GAT3 may contribute to the increase in removal of GABA and thus to reduced extracellular levels during the ictal state.[105]

Astrocytes and the Microvasculature

It was reported over 100 years ago that there is a proliferation of the microvasculature in the sclerotic hippocampus,[6] a finding since confirmed.[106,107] It is also reported that the blood-brain–barrier (BBB) may become leaky during a seizure, releasing substances such as albumin from the blood vessels to the brain parenchyma.[108] Such albumin may, by binding to transforming growth factor beta (TGFβ) receptors on astrocytes, trigger transcriptional activation of downstream pathways, resulting in downregulation of inward-rectifying potassium channels (Kir 4.1), increased inflammatory responses, and reduced inhibitory transmission.[109,110]

Several changes in the expression of molecules at the BBB-astrocyte interface are also observed. The erythropoietin receptor (EPO-r) shows increased expression on the capillaries and perivascular astrocytic end feet of sclerotic hippocampi, particularly in regions of neuronal loss and gliosis (CA3 and CA1).[106] Likewise, the multiple drug resistance gene (*MDR1*) mRNA expression and the protein it encodes, P-glycoprotein, show increased expression in

the capillary endothelial cells in a majority of patients with intractable TLE.[111] Among the other molecules located on the astrocytic end feet, AQP4 and dystrophin are decreased in expression, whereas CD44 and Plectin 1 expression are increased.[112] These molecular changes suggest that there are functional changes in the BBB of sclerotic hippocampi. The significance of these changes for epileptogensis is poorly understood.

Astrocyte Types

In the sclerotic areas of the seizure foci, while many cells are strongly glial fibrillary acidic protein (GFAP)-positive reactive astrocytes, accumulating evidence suggests that the astrocytes may not constitute a homogeneous population. Two subtypes of cells have been distinguished: a GluR cell that is characterized by the presence of AMPA-type GluR receptors but does not express glutamate transporters, and a GluT cell that expresses glutamate transporters but does not express glutamate receptors.[113] These cells are recognized in cultures and in slices from hippocampi and are nonoverlapping populations. The GluR cells lack gap junctions, while the GluT cells are extensively coupled.[114] GluR cells seem identical to a cell type described as the Neuron-Glial cell (NG2 cell) or polydendrocyte.[115] In the sclerotic hippocampus, several lines of evidence suggest that in addition to GFAP-positive astrocytes, there is also a population of NG2 cells.[80] These NG2 cells appear to be those that have increased calcium oscillations and calcium waves, and are also those that can be depolarized to generate action potentials.[89] It is reported that there is an almost complete loss of GluT cells in the sclerotic hippocampus, with only the GluR cells remaining, and that it is these cells that have the flip isoform of GluR1.[85,91] There is, however, a population of GFAP-positive astrocytes in the sclerotic hippocampus that show downregulation of glutamine synthetase and defective Kir channels.[95]

Probable Roles of Astrocytes in a Seizure Focus

The foregoing changes in astrocytes at the sclerotic hippocampal seizure focus may influence epileptogenesis by contributing to high extracellular glutamate levels, to high extracellular K^+ levels, and/or to the spread of excitation through the hippocampus.

The downregulation of the enzyme glutamine synthetase may produce an increase in extracellular glutamate due to inadequate clearance of the glutamate released at synapses,[93] while the increase in GDH may produce an increase in the observed cellular glutamate.[116] Further, evidence of intracellular Ca^{2+} release and Ca^{2+} oscillations in NG2-like cells in the sclerotic focus (see above), along with evidence of upregulation of the synaptic vesicle protein SNAP 23,[112] suggests the possibility that the sclerotic hippocampus may have cells capable of Ca^{2+}-dependent exocytotic glutamate release.[117] Additionally, astrocytic swelling by accumulation of water—due to inadequate clearance because of changes in the membrane distribution of AQP4—may also facilitate astrocyte release of glutamate.[118]

A diminished capacity to buffer K^+ in the sclerotic hippocampus[119] may result from impaired Kir channels.[92] Since the buffering of K^+ by the Kir channel depends on a parallel flux of water through the plasma membranes of these cells, the loss of AQP4 from the perivascular astrocytic membrane could result in diminished extrusion of water from astrocytes and thus a concentration of extracellular K^+.

The probable presence of a population of NG2-like cells in the sclerotic hippocampus, in addition to their role in Ca^{2+}-dependent glutamate release, may directly contribute to the excitability of the seizure focus. These cells may contribute to the excitability of the seizure focus because they are capable of being depolarized to generate action potentials[89] and have GluR1 receptors with elevated flip-to-flop ratios that facilitate and prolong depolarization.[85] Such cells may facilitate the generation or spread of waves of depolarization from hyperexcitabe granule cells[120] to the subiculum without synaptic pathways between the two regions.[80] Alternatively, the NG2 cell action potentials may just mediate Na^+ ion influx, to increase astrocytic $[Na^+]_i$ that stimulates the activity of Na/K adenosine triphosphatase (ATPase). An increase in Na/K ATPase in astrocytes may play a role in buffering extracellular K^+, compensating to some degree for the decreased Kir function in these astrocytes, and so may decrease

excitability. The function of NG2 cells in the sclerotic hippocampus needs further study.

GENE EXPRESSION IN SEIZURE FOCUS

In recent years, several high-throughput gene expression analyses of hippocampal tissue from TLE patients have been reported.[112,121,122] The most interesting of these studies are those that have compared the expression patterns in sclerotic hippocampi with those in nonsclerotic hippocampi. Unbiased hierarchical cluster analysis of expression data reveals that the gene expression patterns of sclerotic hippocampi from TLE patients usually cluster closely together, suggesting that they have molecular characteristics distinguishable from those of other TLE subtypes[112,122] (Fig. 30–2). While there is variability among the studies on the specific genes that are selectively expressed, there is considerable agreement on some of the functional groups of genes that are differentially expressed in the sclerotic hippocampus. In our laboratory, we compared the CA1 region from TLE patients with hippocampal sclerosis (MTLE with sclerosis) with those from two groups of patients without sclerosis (MaTLE and PTLE; see ref. 7). A comparison of this study with other studies is published elsewhere.[112] On examination of the genes selectively expressed in all MTLE hippocampi, increased expression of genes belonging to three important categories is recognized—astrocyte-associated genes, immune and inflammatory response genes, and genes associated with the endothelial cell-astrocyte interface.

Among the astrocyte-related genes upregulated in MTLE are (1) those associated with astrocyte morphology: GFAP is associated with astrocyte process extension, paladin (KIAA0992) regulates astrocyte shape, and plectin 1 (PLEC1) serves as a structural protein; the genes *ezrin*, *radixin*, and *moesin*

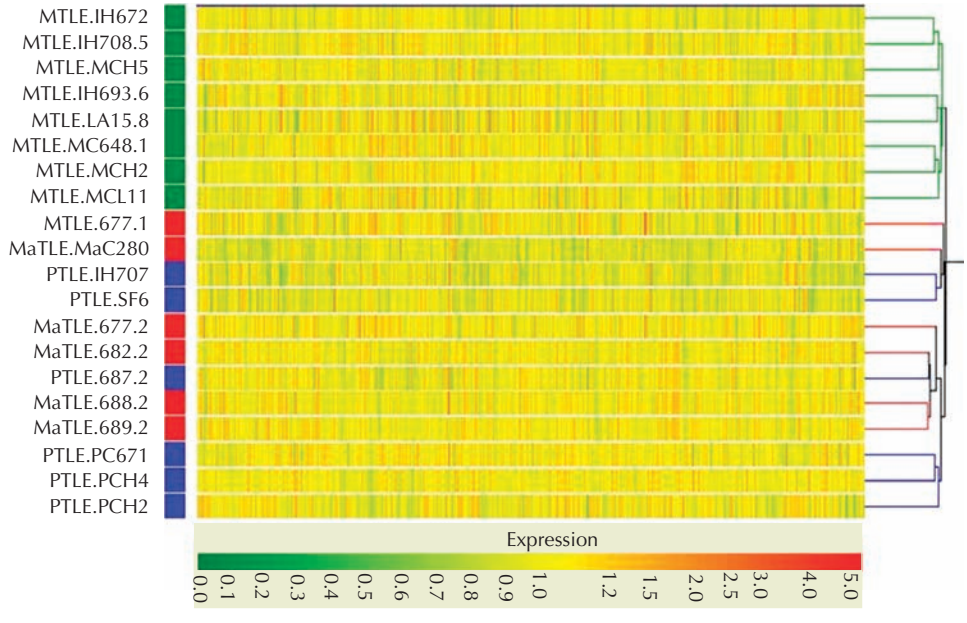

Figure 30–2. Comparison of the gene expression patterns in 20 hippocampi from patients with TLE. The dendrogram was obtained by unsupervised hierarchical cluster analysis using Gene Spring software. The analysis included all genes expressed in a microarray analysis using the Affymetrix GeneChip U133A. It shows a primary branching pattern, which separates the sclerotic MTLE (dark green) hippocampi as one cluster. The PTLE (blue) and MaTLE (red), both being nonsclerotic hippocampi, form a separate group. On the dendrogram, each horizontal color bar denotes the intensity of expression of a particular gene relative to the mean expression value of all samples. The color codes are shown in the color bar, where green represents transcript levels below the median and red represents transcript levels above it. Yellow is at the median. The more intense the color, the greater the level of trust it represents. MTLE, mesial temporal lobe epilepsy; MaTLE, mass-associated temporal lobe epilepsy; PTLE, paradoxical temporal lobe epilepsy. From ref. 112.

control three closely related proteins (ERM proteins), which constitute the machinery for association of actin filaments to the plasma membrane; (2) the CD44 family of surface glycoproteins, whose cytoplasmic domain is directly associated with the ERM proteins; (3) the extracellular matrix proteins tenascin (TNC) and chondroitin sulfate phosphoglycan 2 (CSPG2); (4) S100A6 and S100B—members of a family of proteins involved in Ca^{2+} regulation, with AHNAK being a target for S100B.

In addition to the molecules associated with the microvasculature described above (see the section "Astrocytes and the Microvasculature"), the ligands CCL3 and CCL2 for the chemokine receptors CCR1 and CCR2 found on the microvasculature[123] are increased in MTLE. CCL4 is also upregulated on astrocytes.[112,122] The binding of these ligands to their receptors on blood vessels may influence their permeability and perhaps leakage during seizures.

Several genes involved with immune and inflammatory responses are also upregulated. These include those regulating chemokines and their receptors, cytokines and their receptors, signal transduction proteins, transcription factors, transcription factor-related genes, and class II major histocompatibility complex genes. The particular genes belonging to these categories are listed in the article by Lee et al.[112] Many of these immune and inflammatory factors are probably produced by astrocytes.[124,125] Indeed, immunohistochemical localization of interleukin (IL)-1β and IL-1R in sclerotic hippocampi reveals their expression on astrocytes in areas of prominent gliosis and neuronal loss (CA1–CA3 and hilus), particularly in perivascular end feet.[126] Likewise, immunohistochemical localization of complement C1q, C3c, C3d, and C5b–C9 shows that C1q, C3c, and C3d are localized in astrocyte-like cells only in sclerotic hippocampi. C5–C9 are not localized on astrocytes. Experimental studies in animals indicate that molecules such as IL-1β and complement are closely associated with seizure generation.[127]

SUBICULUM

The subiculum is the region of the hippocampal formation from which major efferent pathways originate[128] and is involved in the spread of electrical activity from the hippocampus to other parts of the brain generating behavioral activity. Even in sclerotic hippocampi in MTLE patients, the subiculum remains largely intact, with no detectable neuron loss.[8,129,130] However, Cohen et al.[131] report that the subiculum of the sclerotic hippocampus initiates spontaneous interictal discharges that originate in a minority of subicular neurons, including interneurons and a subset of GABAergic pyramidal neurons in the subiculum and its transitional area with CA1. This paradoxical behavior of GABAergic neurons, which are normally inhibitory but behave as excitatory cells in the subiculum, has found an explanation in recent research. This switch of GABAergic neurons from inhibitory to excitatory relates to the relative expression of two Cl cotransporters—Na^+K^+, 2Cl cotransporters such as NKCC1, which accumulate Cl^- in the cell, and Cl^- extruding K^+, Cl cotransporters like KCC2. Alterations in the balance of NKCC1 and KCC2 determine the switch from hyperpolarizing to depolarizing effects of GABA. Immunohistochemical localization and quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of mRNA extracted from subiculum suggested that more than 20% of NKCC1-expressing pyramidal neurons in the sclerotic subicular/CA1 transitional zone not only lacked KCC2 but exhibited increased NKCC1. This pattern contrasts with about 95% colocalization in nonsclerotic patients.[132–134] Increased Cl^- in cells produces depolarization when GABA-mediated chloride channels are opened. Thus, it is suggested that altered Cl^- transport renders GABA neurons aberrantly excitable, contributing to the precipitation of seizures.[134] However, Wozny and coworkers[135] provide evidence, also obtained from slices of surgically removed hippocampal tissue, that interictal spontaneous activity is seen not only in sclerotic hippocampi but also in nonsclerotic hippocampi. Thus, the subiculum of the sclerotic hippocampus may not be involved in epileptogenesis only in sclerotic hippocampus. This region of the hippocampal formation may be a more generally excitable zone than other regions.

ENTORHINAL CORTEX

The entorhinal cortex (EC) is a principal source of efferents to the hippocampus, with

major projection to the dentate gyrus (perforant pathway) and CA1 (temporo-ammonic pathway). Abnormal epileptiform activity has been recorded from this region.[136,137] Histopathological studies of this region in TLE patients report neuronal loss and gliosis in layer III and, to a lesser extent, in layer II of the anterior portion of the medial EC of the sclerotic hippocampus.[138] However, other studies have reported a more variable pattern of neuronal loss[139] or no detectable loss.[140] Nevertheless, gliosis is a finding common to the EC in both sclerotic and nonsclerotic hippocampus.[140] Primary cultures from the EC (parahippocampus) of patients with a sclerotic hippocampus had increased Ca^{2+} release and Ca^{2+} oscillations in astrocytes.[83] If Ca^{2+} release is associated with glutamate release, these cells may trigger hyperexcitation of EC neurons and thus spread activity through the hippocampus. More needs to be learned about the role of these glial cells in the EC region.

PROBABLE PATHOPHYSIOLOGICAL MECHANISMS OF SEIZURE GENERATION

The probable pathophysiological mechanism of seizure generation must take into account the complex histopathological picture sketched above. Prominent features of this histopathology—a reorganized dentate gyrus, a neuronally depleted and gliotic Ammon's horn with an essentially intact and even spontaneously active subiculum—constitute the epileptogenic hippocampus.

Broadly, two sets of processes that have distinct histological substrates may be identified. The first set of processes are based in the dentate gyrus and may be important at least in the early genesis of the epileptic focus, before the loss of dentate granule cells is extensive. In this presumed early phase of pathogenesis, several cellular changes may underlie the development of hyperexcitable granule cells. These processes involve morphological remodeling of surviving granule cells, selective loss of hilar interneurons, sprouting of new axonal projections, and adaptive changes in neurotransmitter receptor distribution, representing a reorganization of the neural circuits within the dentate gyrus. An attempt to integrate these changes and hypothesize how they may result in granule cell hyperexcitability is given in de Lanerolle and Lee[22] (see Fig. 1 in ref. 22).

Despite the existence of such hyperexcitable granule cells, unanswered is the question of how such hyperexcitability passes from the granule cells to the subiculum to generate behavioral seizures, since the neural pathways connecting the two are interrupted due to extensive neuronal loss in the CA1 area early in pathogenesis. One hypothesis is that in the sclerotic region NG2-like cells, which are depolarizable and excitable, provide a substrate for the spread of waves of depolarization from the dentate gyrus to the subiculum. An alternative, though less likely, possibility is that the neural networks in the stratum oriens, which are very resistant to loss, provide a sufficient substrate for spread of neural excitation from dentate to subiculum.

It is notable that in several surgically removed hippocampi, the dentate gyrus is almost completely devoid of granule cells and Ammon's horn is very gliotic; yet, such hippocampi may take part in seizure generation in these patients. Seizures have been shown to originate from such hippocampi,[14] and in vivo dialysis studies demonstrate elevated extracelluar glutamate ictally in such foci.[105,141] In such hippocampi, the subiculum remains intact; however, the evidence, as discussed above, that the subiculum by itself is responsible for epileptic activity is weak. Alternative explanations must therefore be considered, such as astrocyte and astrocyte-related mechanisms. The unique properties of astrocytes in the hippocampal seizure focus are reviewed above, and an attempt to integrate these properties in the pathophysiology of epilepsy is provided in de Lanerolle et al.[80] Astrocytes may contribute to vascular permeability and increased extracellular glutamate and K^+ levels in the sclerotic hippocampus, which in turn may contribute to the hyperexcitability of the subiculum resulting in epileptic activity.

SPECULATION ON FUTURE CHALLENGES IN THE AREA

Our knowledge of the histopathology of TLE has advanced significantly in the recent past. However, we are still engaged largely in descriptive pathology. One of the major challenges for

the future is to understand the functional significance of the various changes observed. Of primary importance is an understanding of the functional biology of astrocytes and astrocyte-like cells in the sclerotic hippocampus. We have advanced several hypotheses regarding their role, but these need experimental testing. What are the proportions of GluR- and GluT-like astrocytes? How do they contribute to the increased extracellular glutamate and K^+ concentrations observed at the sclerotic seizure focus? Much of our information about their role in these mechanisms has come from the study of astrocytes from embryonic animal tissue and very little from the study of adult astrocytes from human foci. Do these abnormal astrocytes provide an excitable substrate for the spread of neural excitation from the dentate to the subiculum? Understanding the biology of astrocytes in TLE sclerotic foci would be very useful in providing insight into the epileptogenic mechanisms of other seizure foci such as mass lesions, tubers in tuberous sclerosis complex, and many of the neocortical epilepsies in which astrocytes may be a major constituent.

Our understanding of the functional implications of the anatomical changes in the dentate gyrus in the human also remains sketchy. Though much attention has been given to certain aspects, such as the functional significance of granule cell sprouting and glutamatergic and GABAergic mechanisms regulating granule cell function in the sclerotic hippocampus, there is far less experimental analysis of the significance of other changes, such as the new neuropeptidergic inputs to granule cells and the changed distribution of the several receptors. Studies that attempt to correlate the time course of functional changes resulting from dentate gyrus reorganization and abnormal astrocytes in the sclerotic human hippocampus are essential for a better understanding of the causative mechanisms of human TLE.

IMPACT ON FINDING CURES AND REPAIRS FOR EPILEPSIES

The current antiepileptic drugs and other repair strategies have focused attention almost exclusively on neurons as the primary cell type involved in mechanisms underlying epilepsy. The existence of a large group of patients with drug-refractory epilepsy is evidence that more effective therapeutic measures are required. The currently available antiepileptic drugs broadly target glutamatergic and GABAergic mechanisms, though they may have other effects as well. They are mediated through three main mechanisms—blockade of voltage-gated sodium and calcium channels that limit glutamate release; blockade of glutamate receptors; and the potentiation of GABAergic inhibition through increasing GABA availability or modulation of GABA receptors.[142,143] The histopathology of the epileptic hippocampus reviewed above, which shows that there is extensive *loss* of neurons in the hippocampus, suggests why current antiepileptic drugs, which focus on modulating functions of neurons, may be ineffective. New therapeutic agents should target astrocytes and astrocyte-like cells instead of neurons, as these cells are the primary constituents of the sclerotic hippocampus. Cell replacement strategies might consider transplantations of human stem cells that may develop into normal astrocytes that may restore a more normal extracellular glutamate concentration in the seizure focus and compensate for the abnormal astrocytes.

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REFERENCES

1. Kim JH. Pathology of seizure disorders. *Neuroimag Clin North* Am. 1995;5:527–545.
2. Kim JH. Pathology of epilepsy. *Exp Mol Pathol*. 2001;70:345–367.

3. Gloor P. Mesial temporal sclerosis: historical background and an overview from a modern perspective. In: Luders H, ed. *Epilepsy Surgery*. New York: Raven Press; 1991:6889–6903.
4. Sommer W. Erkrankung des Ammonshorns als aetiologisches Moment der Epilepsie. *Arch Psychiatr Nervenkr*. 1880;10:631–675.
5. Bouchet C, Cazauvieilh M. De l'épilepsie considérée dans ses rapports avec l'aliénation mentale. Recherche sur la nature et le siége de ces deux maladies. *Arch Gen Méd*. 1825;9:510–542.
6. Bratz E. Ammonshornbefunde bei Epileptikern. *Arch Psychiatr Nervenkr*. 1899;32:820–835.
7. de Lanerolle NC, Kim JH, Williamson A, Spencer SS, Zaveri HP, Eid T, Spencer DD. A retrospective analysis of hippocampal pathology in human temporal lobe epilepsy: evidence for distinctive patient subcategories. *Epilepsia*. 2003;44:677–687.
8. Kim JH, Guimaraes PO, Shen M-Y, Masukawa LM, Spencer DD. Hippocampal neuronal density in temporal lobe epilepsy with and without gliomas. *Acta Neuropathol*. 1990;80:41–45.
9. Engel J, Brown WJ, Kuhl DE, Phelps ME, Mazziota JC, Crandall PH. Pathological findings underlying focal temporal lobe hypometabolism in partial epilepsy. *Ann Neurol*. 1982;12:518–528.
10. Dlugos DJ, Jaggi J, O'Connor WM, Ding XS, Reivich M, O'Connor MJ, Sperling MR. Hippocampal cell density and subcortical metabolism in temporal lobe epilepsy. *Epilepsia*. 1999;40:408–413.
11. Spencer SS. Neural networks in human epilepsy: evidence of and implications for treatment. *Epilepsia*. 2002;43:219–227.
12. Stauder KH. Epilepsie und schläfenlappen. *Arch Psychiatrie*. 1935;104:181–212.
13. Falconer MA. The significance of mesial temporal sclerosis (Ammon's horn sclerosis) in epilepsy. *Guy's Hosp Rep*. 1968;117:1–12.
14. Babb TL, Lieb JP, Brown WJ, Pretorius J, Crandall PH. Distribution of pyramidal cell density and hyperexcitability in the epileptic human hippocampal formation. *Epilepsia.* 1984;25:721–728.
15. Spencer SS, Berg AT, Vickrey BG, Sperling MR, Bazil CW, Shinnar S, Langfitt JT, Walczak TS, Pacia SV, Surgery TMSoE. Predicting long-term seizure outcome after resective epilepsy surgery: The Multicenter Study. *Neurology*. 2005;65:912–918.
16. Bower SPC, Kilpatrick CJ, Vogrin SJ, Morris K, Cook MJ. Degree of hippocampal atrophy is not related to a history of febrile seizures in patients with proved hippocampal sclerosis. *J Neurol Neurosurg Psychiatry*. 2000;69:733–738.
17. Kothare SV, Sotrel A, Duchowny M, Jayakar P, Marshall PC, Smith TW. Absence of hippocampal sclerosis in children with multiple daily seizures since infancy. *J Child Neurol.* 2001;16:562–564.
18. Thom M, Zhou J, Martinian L, Sisodiya SM. Quantitative post-mortem study of the hippocampus in chronic epilepsy: seizures do not invariably cause neuronal loss. *Brain*. 2005;128(Pt 6):1344–1357.
19. Falconer MA. Surgery of temporal lobe epilepsy. *Proc R Soc Med*. 1952;51:613–616.
20. Spencer DD, Spencer SS. Surgery for epilepsy. *Neurol Clin*. 1985;3:313–330.
21. Blümcke I, Kistner I, Clusmann H, Schramm J, Becker AJ, Elger CF, Bien CG, Merschhemke M, Meencke H-J, Lehmann TN, Buchfelder M, Weigel D, Buslei R, Stefan H, Pauli E, Hildebrandt M. Towards a clinico-pathological classification of granule cell dispersion in human mesial temporal lobe epilepsies. *Acta Neuropathol*. 2009;117:535–544.
22. de Lanerolle NC, Lee TS. New facets of the neuropathology and molecular profile of human temporal lobe epilepsy. *Epilepsy Behav*. 2005;7:190–203.
23. Margerison JH, Corsellis JAN. Epilepsy and the temporal lobes. *Brain*. 1966;89:499–530.
24. Bruton CJ. *The Neuropathology of Temporal Lobe Epilepsy*. Oxford: Oxford University Press; 1988.
25. de Lanerolle NC, Kim JH, Brines ML. Cellular and molecular alterations in partial epilepsy. *Clin Neurosci*. 1994;2:64–81.
26. Zaveri HP, Duckrow RB, de Lanerolle NC, Spencer SS. Distinguishing subtypes of temporal lobe epilepsy with background hippocampal activity. *Epilepsi*a. 2001;42:725–730.
27. Amaral DG, Scharfman HE, Lavenex P. The dentate gyrus: *fundamental neuroanatomical organization (dentate gyrus for dummies). Prog* Brain Res. 2007;163:3–25.
28. Seress L, Mrzljak L. Basal dendrites of granule cells are normal features of the fetal and adult dentate gyrus of both monkey and human hippocampal formations. *Brain Res.* 1987;405:169–174.
29. de Lanerolle NC, Brines ML, Williamson A, Kim JH, Spencer DD. Neurotransmitters and their receptors in human temporal lobe epilepsy. In: Ribak CE, Gall CM, Mody I, eds. *The Dentate Gyrus and Its Role in Seizures*. Amsterdam: Elsevier Science; 1992:235–250.
30. Haas CA, Dudeck O, Kirsch M, Huszka C, Kann G, Pollak S, Zentner J, Frotscher M. Role for reelin in the development of granule cells dispersion in temporal lobe epilepsy. *J Neurosci*. 2002;22:5797–5802.
31. Fahrner A, Kann G, Flubacher A, Heinrich C, Freiman TM, Zentner J, Frotscher M, Haas CA. Granule cell dispersion is not accompanied by enhanced neurogenesis in temporal lobe epilepsy patients. *Exp Neurol*. 2007;203:320–332.
32. Harding B, Thom M. Bilateral hippocampal granule cell dispersion: autopsy study of 3 infants. *Neuropathol Appl Neurobiol*. 2001;27:245–251.
33. Robboli MG, Giulioni M. Role of dentate gyrus alterations in mesial temporal sclerosis. *Clin Neuropathol*. 2010;29:32–35.
34. von Campe G, Rai P, Spencer DD, de Lanerolle NC. Morphology of granular neurons in the human hippocampus. *Soc Neurosci. Abst*. 1994;20:1450.
35. Isokawa M, Levesque MF, Babb TL, Engel JJ. Single mossy fiber axonal systems of human dentate granule cells studied in hippocampal slices from patients with temporal lobe epilepsy. *J Neurosci.* 1993;13:1511–1522.
36. Isokawa M. Preservation of dendrites with the presence of reorganized mossy fiber collaterals in hippocampal dentate granule cells in patients with temporal lobe epilepsy. *Brain Res.* 1997;744:339–343.
37. Pirker S, Gasser E, Czech T, Baumgartner C, Schuh E, Feucht M, Novak K, Zimprich F, Sperk G. Dynamic up-regulation of prodynorphin transcription in temporal lobe epilepsy. *Hippocampus*. 2009;19:1051–1054.
38. Sloviter RS, Sollas AL, Barbaro NM, Laxer KD. Calcium-binding protein (Calbindin-D28K) and

parvalbumin immunocytochemistry in the normal and epileptic human hippocampus. *J Comp Neurol*. 1991;308:381–396.
39. Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ. Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. *J Neurosci*. 2003;30: 6826–6836.
40. de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res*. 1989;495:387–395.
41. Babb TL, Pretorius JK, Crandall PH. Glutamate decarboxylase-immunoreactive neurons are preserved in human epileptic hippocampus. *J Neurosci*. 1989;9: 2562–2574.
42. Sloviter RS. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science*. 1987;235:73–76.
43. Amaral DG. A Golgi study of cell types in the hilar region of the hippocampus in the rat. *J Comp Neurol*. 1978;182:851–914.
44. Soriano E, Frotscher M. Mossy cells of the rat fascia dentata are glutamate-immunoreactive. *Hippocampus*. 1994;4:65–69.
45. Sloviter RS. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: The "dormant basket cell" hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus*. 1991;1:41–66.
46. Seress L. Comparative anatomy of the hippocampal dentate gyrus in adult and developing rodents, non-human primates and humans. *Prog Brain Res*. 2007;163:23–41.
47. de Lanerolle NC, Williamson A, Meredith C, Kim JH, Tabuteau H, Spencer DD, Brines ML. Dynorphin and the kappa 1 ligand [3H]-U69,539 binding in the human epileptogenic hippocampus. *Epilepsy Res*. 1997;28:189–205.
48. Eid T, Meredith C, Kovacs I, de Lanerolle NC. Ultrastructural localization of GluR1 in human temporal lobe epilepsy. *Soc Neurosci Abstr*. 1996;22:1657.
49. Seress L, Abraham H, Horvath Z, Dóczi T, Janszky J, Klemm J, Byrne R, Bakay RAE. Survival of mossy cells of the hippocampal dentate gyrus in humans with mesial temporal lobe epilepsy. *J Neurosurg*. 2009;111: 1237–1247.
50. Babb TL, Kupfer WR, Pretorius JK, Levesque MF. Light and electron microscopy of mossy fiber terminals in human "epileptic" fascia dentata. *Soc Neurosci Abstr*. 1989;14:881.
51. Sutula T, Cascino G, Cavazos J, Pavada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol*. 1989;26: 321–330.
52. Houser CR, Miyashiro JE, Swartz BE, Walsh GO, Rich JR, Delgado-Escueta AV. Altered patterns of dynorphin immunoreactivity suggest mossy fiber reorganization in human hippocampal epilepsy. *J Neurosci*. 1990;10:267–282.
53. Philips MW. *Synaptic and Pathway Remodeling of the Human Hippocampus in Temporal Lobe Epilepsy* [M.D. thesis]: New Haven, CT: Yale University; 1993.
54. Green R, Blume H, Kupperschmid S, Mesulam M-M. Alterations of hippocampal acetylcholinesterase in human temporal lobe epilepsy. *Ann Neurol*. 1989;26:351–367.
55. de Lanerolle NC, Eid T, von Campe G, Kovacs I, Spencer DD, Brines ML. Glutamate receptor subunits GluR1 and GluR2/3 distribution shows reorganization in the human epileptogenic hippocampus. *Eur J Neurosci*. 1998;10:1687–1703.
56. Eid T, Kovacs I, Spencer DD, de Lanerolle NC. Novel expression of AMPA-receptor subunit GluR1 on mossy cells and CA3 pyramidal neurons in the human epileptogenic hippocampus. *Eur J Neurosci*. 2002;15:517–527.
57. Notenboom RGE, Hampson DR, Jansen GH, van Rijen PC, van Veelen CWM, Nieuwenhuizen O, de Grann PN. Up-regulation of hippocampal metabotropic glutamate receptor 5 in temporal lobe epilepsy patients. *Brain*. 2006;129:96–107.
58. Tang F-R, Lee W-L. Expression of the group II and III metabotropic glutamate receptors in the hippocampus of patients with mesial temporal lobe epilepsy. *J Neuroscytol*. 2001;30:137–143.
59. Tang F-R, Lee W-L, Yeo TT. Expression of group 1 metabotropic glutamate receptor in the hippocampus of patients with mesial temporal lobe epilepsy. *J Neurocytol*. 2001;30:403–411.
60. Moldrich RX, Chapman AG, De Sarro G, Meldrum BS. Glutamate metabotropic receptors as targets for drug therapy. *Eur J Pharmacol*. 2003;476:3–16.
61. Loup F, Weiser H-G, Yonekawa Y, Aguzzi A, Fritschy J-M. Selective alterations in GABA-A receptor subtypes in human temporal lobe epilepsy. *J Neurosci*. 2000;20:5401–5419.
62. Billington A, Baird VH, Thom M, Duncan JS, Upton N, Bowery NG. GABA-B receptor autoradiography in hippocampal sclerosis associated with human temporal lobe epilepsy. *Br J Pharmacol*. 2001;132:475–480.
63. Princivalle AP, Duncan JS, Thom M, Bowery NG. GABA-B1a, GABA-B1b and GABA-B2 mRNA variants expression in hippocampus resected from patients with temporal lobe epilepsy. *Neuroscience*. 2003;122: 975–984.
64. Csaba Z, Pirker S, Lelouvier B, Simon A, Videau C, Epelbaum J, Czech T, Baumgartner C, Sperk G, Dournaud P. Somatostatin receptor Type 2 undergoes plastic changes in the human epileptic dentate gyrus. *J Neuropathol Exp Neurol*. 2005;64:956–969.
65. Sundaresan S. *Neurochemical Changes in the Hippocampus in Human Temporal Lobe Epilepsy* [M.D. thesis]. New Haven, CT: Yale University; 1990.
66. Furtinger S, Pirker S, Czech T, Baumgartner C, Ransmayr G, Sperk G. Plasticity of Y1 and Y2 receptors and neuropeptide Y fibers in patients with temporal lobe epilepsy. *J Neurosci*. 2001;21:5804–5812.
67. Tompkins J. *Alterations in D2 Receptor Concentrations in Human Temporal Lobe Epilepsy* [M.D. thesis]. New Haven, CT: Yale University; 1990.
68. de Lanerolle NC, Gunel M, Sundaresan S, Shen MY, Brines ML, Spencer DD. Vasoactive intestinal polypeptide and its receptor changes in human temporal lobe epilepsy. *Brain Res*. 1995;686:182–193.
69. Patrylo PR, Van den Pol AN, Spencer DD, Williamson A. NPY inhibits glutamatergic excitation in the epileptic human dentate gyrus. *J Neurophysiol*. 1999;82:478–483.
70. Dudek FE, Sutula TP. Epileptogenesis in the dentate gyrus: a critical perspective. *Progr Brain Res*. 2007;163:755–773.

71. Williamson A, Patrylo PR, Spencer DD. Decrease in inhibition in dentate granule cells from patients with medial temporal lobe epilepsy. *Ann Neurol*. 1999;45:92–99.
72. Corsellis JAN. The incidence of Ammon's horn sclerosis. *Brain*. 1955;80:193–208.
73. Falconer M. Mesial temporal (Ammon's horn) sclerosis as a common cause of epilepsy. Aetiology, treatment and prevention. *Lancet*. 1974;2:767–770.
74. de Lanerolle NC, Brines ML, Kim JH, Williamson A, Philips MF, Spencer DD. Neurochemical remodelling of the hippocampus in human temporal lobe epilepsy. In: Engel J Jr, Wasterlain C, Cavalheiro EA, Heinemann U, Avanzini G, eds. *Molecular Neurobiology of Epilepsy*. Amsterdam: Elsevier Science; 1992:205–220.
75. de Lanerolle NC, LaMotte CC. The morphological relationships between substance P immunoreactive processes and ventral horn neurons in the human and monkey spinal cord. *J Comp Neurol*. 1982;207: 305–313.
76. Eid T. The use of hydrophobic adhesive tape to produce miniature wells on microscope slides. *Biotech Histochem*. 1993;68:189–192.
77. Ganter P, Szucs P, Paulsen O, Somogyi P. Properties of horizontal axo-axonic cells in the stratum oriens of the hippocampal CA1 area of rats in vitro. *Hippocampus*. 2004;14:232–243.
78. Maccaferri G. Stratum oriens horizontal interneurone diversity and hippocampal network dynamics. *J Physiol*. 2005;562:73–80.
79. Aradi I, Maccaferri G. Cell-type specific synaptic dynamics of synchronized bursting in the juvenile CA3 rat hippocampus. *J Neurosci*. 2004;24:9681–9682.
80. de Lanerolle NC, Lee TS, Spencer DD. Astrocytes and epilepsy. *Neurotherapeutics*. 2010;7:424–438.
81. Steinhäuser C, Haydon PG, de Lanerolle NC. Astroglial mechanisms in epilepsy. In: Engel JJ, Pedley TA, Aicardi J, Dichter M, Moshe S, eds. *Epilepsy: A Comprehensive Textbook*. Philadelphia: Lippincott, Williams & Wilkins; 2008:277–288.
82. Steinhäuser C, Seifert G. Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. *Eur J Pharmacol*. 2002;447:227–237.
83. Lee SH, Magge S, Spencer DD, Sontheimer H, Cornell-Bell A. Human epileptic astrocytes exhibit increased gap junction coupling. *Glia*. 1995;15:195–202.
84. Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci*. 2005;6:626–640.
85. Seifert G, Hüttmann K, Schramm J, Steinhäuser C. Enhanced relative expression of glutamate receptor 1 flip AMPA receptor subunits in hippocampal astrocytes of epilepsy patients with Ammon's horn sclerosis. *J Neurosci*. 2004;24:1996–2003.
86. Seifert G, Schroder W, Hinterkeuser S, Schumacher T, Schramm J, Steinhauser C. Changes in flip/flop splicing of astroglial AMPA receptors in human temporal lobe epilepsy. *Epilepsia*. 2002;43(suppl 5): 162–167.
87. Barres BA, Chun LLY, Corey DP. Ion channel expression by white matter glia: I. Type 2 astrocytes and oligodendrocytes. *Glia*. 1988;1:10–30.
88. Bordey A, Spencer DD. Distinct electrophysiological alterations in dentate gyrus versus CA1 glial cells from epileptic humans with temporal lobe sclerosis. *Epilepsy Res*. 2004;59:107–122.
89. O'Connor ER, Sontheimer H, Spencer DD, de Lanerolle NC. Astrocytes from human hippocampal epileptogenic foci exhibit action potential-like responses. *Epilepsia*. 1998;39:347–354.
90. Djamshidian A, Grassl R, Seltenhammer M, Czech T, Baumgartner C, Schmidbauer M, Ulrich W, Zimprich F. Altered expression of voltage-dependent calcium channel a1 subunits in temporal lobe epilepsy with Ammon's horn sclerosis. *Neuroscience*. 2002;111: 57–69.
91. Hinterkeuser S, Schröder W, Hager G, Seifert G, Blümcke I, Elger CE, Schramm J, Steinhauser C. Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. *Eur J Neurosci*. 2000;12:2087–2096.
92. Schroder W, Hinterkeuser S, Seifert G, Schramm J, Jabs R, Wilkin GP, Steinhauser C. Functional and molecular properties of human astrocytes in acute hippocampal slices obtained from patients with temporal lobe epilepsy. *Epilepsia*. 2000;41:S181–S184.
93. Eid T, Thomas MJ, Spencer DD, Rundén-Pran E, Kim JH, Ottersen OP, de Lanerolle NC. Loss of glutamine synthetase in the human epileptogenic hippocampus: a possible mechanism for elevated extracellular glutamate in mesial temporal lobe epilepsy. *Lancet*. 2004;363:28–37.
94. van der Hel WS, Notenboom RG, Bos IW, van Rijen PC, van Veelen CW, de Grann PN. Reduced glutamine synthetase in hippocampal areas with neuron loss in temporal lobe epilepsy. *Neurology*. 2005;64:326–333.
95. Eid T, Williamson A, Lee T-S, Petroff OA, de Lanerolle NC. Glutamate and astrocytes—key players in human mesial temporal lobe epilepsy. *Epilepsia*. 2008;49:42–52.
96. Petroff OA, Errante LD, Kim JH, Spencer DD. *N*-acetyl-aspartate, total creatine, and myo-inositol in the epileptogenic human hippocampus. *Neurology*. 2003;60:1646–1651.
97. Petroff OA, Errante LD, Rothman DL, Kim JH, Spencer DD. Neuronal and glial metabolite content of the epileptogenic human hippocampus. *Ann Neurol*. 2002;52:635–642.
98. Malthankar-Phatak GH, de Lanerolle NC, Eid T, Spencer DD, Behar KL, Spencer SS, Kim JH, Lai JCK. Differential glutamate dehydrogenase (GDH) activity profile in patients with temporal lobe epilepsy. *Epilepsia*. 2006;47:1292–1299.
99. Eid T, Lee T-SW, Thomas MJ, Amiry-Moghaddam M, Bjornsen LP, Spencer DD, Agre P, Ottersen OP, de Lanerolle NC. Loss of perivascular aquaporin 4 underlies deficient water and K^+ homeostasis in the human epileptogenic hippocampus. *Proc Natl Acad Sci USA*. 2005;102:1193–1198.
100. Mathern GW, Mendoza D, Lozada A, Pretorius JK, Dehnes Y, Danbolt NC, Nelson N, Leite JP, Chimelli L, Born DE, Sakamoto AC, Assirati JA, Fried I, Peacock WJ, Ojemann GA, Adelson PD. Hippocampal GABA and glutamate transporter immunoreactivity in patients with temporal lobe epilepsy. *Neurology*. 1999;52:453–472.
101. Proper EA, Hoogland G, Kappen SM, Jansen GH, Rensen MGA, Schrama LH, van Veelen CWM, van Rijen PC, van Nieuwenhuizen O, Gispen WH, de

Graan PNE. Distribution of glutamate transporters in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. *Brain.* 2002;125:32–43.
102. Tessler S, Danbolt NC, Faull RLM, Storm-Mathisen J, Emson P. Expression of the glutamate transporters in human temporal lobe epilepsy. *Neuroscience.* 1998;88:1083–1091.
103. Bjørnsen LP, Eid T, Holmseth S, Danbolt NC, Spencer DD, de Lanerolle NC. Changes in glial glutamate transporters in human epileptogenic hippocampus: inadequate explanation for high extracellular glutamate during seizures. *Neurobiol Dis.* 2006;25:319–330.
104. Lee T-S, Bjornsen LP, Paz C, Kim JH, Spencer SS, Spencer DD, Eid T, de Lanerolle NC. GAT1 and GAT3 expression are differently localized in the human epileptogenic hippocampus. *Acta Neuropathol*. 2006;111:351–363.
105. During MJ, Spencer DD. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet*. 1993;341:1607–1610.
106. Eid T, Brines M, Cerami A, Spencer DD, Kim JH, Schweitzer JS, Ottersen OP, de Lanerolle NC. Increased expression of erythropoietin receptor on blood vessels in the human epileptogenic hippocampus with sclerosis. *J Neuropathol Exp Neurol*. 2004;63:73–83.
107. Rigau V, Morin M, Rousset M-C, de Bock F, Lebrun A, Coubes P, Picot M-C, Baldy-Moulinier M, Bockaert J, Crespel A, Lerner-Natoli M. Angiogenesis is associated with blood-brain barrier permeability in temporal lobe epilepsy. *Brain*. 2007;130:1942–1956.
108. Van Vliet EA, da Costa Araújo S, Redeker S, van Schaik R, Aronica E. Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. *Brain*. 2007;130:521–534.
109. Cacheaux LP, Ivens S, David Y, Lakhter AJ, Bar-Klein G, Shapira M, Heinemann U, Friedman A, Kaufer D. Transcriptome profiling reveals TGF-β signalling involvement in epileptogenesis. *J Neurosci.* 2009;29:8927–8935.
110. Ivens S, Kaufer D, Flores LP, Bechmann I, Zumsteg D, Tompkins O, Seiffert E, Heinemann U, Friedman A. TGF-β receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. *Brain*. 2007;130:535–547.
111. Tishler DM, Weinberg KI, Hinton DR, Barbaro N, Annett GM, Raffel C. *MDR1* gene expression in brain of patients with medically intractable epilepsy. *Epilepsia*. 1995;36:1–6.
112. Lee T-S, Mane S, Eid T, Zhao H, Lin A, Guan Z, Kim JH, Schweitzer J, King-Stevens D, Weber P, Spencer SS, Spencer DD, de Lanerolle NC. Gene expression in temporal lobe epilepsy is consistent with increased release of glutamate by astrocytes. *Mol Med*. 2007;13:1–13.
113. Matthias K, Kirchhoff F, Seifert G, Hüttmann K, Matyash M, Kettenmann H, Steinhäuser C. Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. *J Neurosci*. 2003;23:1750–1758.
114. Wallraff A, Odermatt B, Willecke K, Steinhauser C. Distinct types of astroglial cells in the hippocampus differ in gap junction coupling. *Glia.* 2004;48: 36–43.
115. Paukert M, Bergles DE. Synaptic communication between neurons and NG2 cells. *Curr Opin Neurobiol*. 2006;16:515–521.
116. Petroff OAC. Metabolic biopsy of the brain. In: Waxman SG, ed. *Molecular Neurology*. New York: Elsevier; 2007:77–100.
117. Malarkey EB, Parpura V. Mechanisms of glutamate release from astrocytes. *Neurochem Int*. 2008;52: 142–154.
118. Kimelberg HK, Mongin AA. Swelling-activated release of excitatory amino acids in the brain: relevance for pathophysiology. *Contrib Nephrol*. 1998;123:240–257.
119. Bordey A, Sontheimer H. Properties of human glial cells associated with epileptic tissue. *Epilepsy Res*. 1998;32:286–303.
120. Williamson A. Electrophysiology of epileptic human neocortical and hippocampal neurons maintained in vitro. *Clin Neurosci*. 1994;2:47–52.
121. Özbas-Gerceker F, Redeker S, Boer K, Özgüc M, Saygi S, Dalkara T, Soylemezoglu F, Akalan N, Baayen JC, Gorter JA, Aronica E. Serial analysis of gene expression in the hippocampus of patients with mesial temporal lobe epilepsy. *Neuroscience.* 2006;138:457–474.
122. van Gassen KLI, de Wit M, Groot Koerkamp MJA, Rensen MGA, van Rijen PC, Holstege FCP, Lindhout D, de Graan PNE. Possible role of the innate immunity in temporal lobe epilepsy. *Epilepsia*. 2008;49:1055–1065.
123. Andjelkovic AV, Pachter JS. Characterization of binding sites for chemokines MCP-1 and MIP-1a on human brain microvessels. *J Neurochem*. 2000;75: 1898–1906.
124. Dong Y, Benveniste EN. Immune function of astrocytes. *Glia.* 2001;36:180–190.
125. John GR, Lee SC, Song X, Rivieccio M, Brosnan CF. IL-1-regulated responses in astrocytes: relevance to injury and recovery. *Glia*. 2005;49:161–176.
126. Ravizza T, Gagliardi B, Noé F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. *Neurobiol Dis*. 2008;29:142–160.
127. Vezzani A, Moneta D, Richichi C, Aliprandi M, Burrows SJ, Ravizza T, Perego C, De Simoni MG. Functional role of inflammatory cytokines and anti-inflammatory molecules in seizures and epileptogenesis. Epilepsia. 2002;43(suppl 5):30–35.
128. Amaral DG, Witter MP. Hippocampal formation. In: Paxinos G, ed. *The Rat Nervous System.* 2nd ed. San Diego, CA: Academic Press; 1995:443–493.
129. Fisher PD, Sperber EF, Moshe SL. Hippocampal sclerosis revisited. *Brain Dev*. 1998;20:563–573.
130. Mathern GW, Babb TL, Armstrong DL. Hippocampal sclerosis. In: Engel J, Pedley TA, eds. *Epilepsy: A Comprehensive Textbook*. Philadelphia: Lippincott-Raven; 1997:133–155.
131. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science*. 2002;298: 1418–1421.
132. Hiuberfield G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, Rivera C. Perturbed chloride homeostasis and GABAergic signalling in human temporal lobe epilepsy. *J Neurosci.* 2007;27:9866–9873.

133. Munoz A, Mendez P, DeFelipe J, Alvarez-Leefmans FJ. Cation-chloride cotransporters and GABAergic innervation in the human epileptic hippocampus. *Epilepsia*. 2007;48:663–673.
134. Palma E, Amici M, Sobrero F, Spinelli G, Di Angelantonio S, Ragozzino D, Mascia A, Scoppetta C, Esposito V, Miledi R, Eusebi F. Anomalous levels of Cl^- transporters in the hippocampal subiculum from temporal lobe epilepsy patients make GABA excitatory. *PNAS*. 2006;103:8465–8468.
135. Wozny C, Kivi A, Lehmann T-N, Dehnicke C, Heinemann U, Behr J. Comment on "On the origin of interictal activity in human temporal lobe epilepsy in vitro." *Science*. 2003;301:463c–464c.
136. Bernasconi N, Andermann F, Arnold DL, Bernasconi A. Entorhinal cortex MRI assessment in temporal, extratemporal and idiopathic generalized epilepsy. *Epilepsia*. 2003;44:1070–1074.
137. Bernasconi N, Bernasconi A, Carmanos Z, Antel SB, Andermann F, Arnold DL. Mesial temporal damage in temporal lobe epilepsy: a volumetric study of the hippocampus and parahippocampal region. *Brain*. 2003;126:462–469.
138. Du F, Whetsell WO Jr, Abou-Khalil B, Blumenkopf B, Lothman EW, Schwarcz R. Preferential neuronal loss in layer III of the entorhinal cortex in patients with temporal lobe epilepsy. *Epilepsy Res*. 1993;16:223–233.
139. Yilmazer-Hanke DM, Wolf HK, Schramm J, Elger CE, Wiestler OD, Blümcke I. Subregional pathology of the amygdala complex and entorhinal region in surgical specimens from patients with pharmacoresistant temporal lobe epilepsy. *J Neuropathol Exp Neurol*. 2000;59:907–920.
140. Dawodu S, Thom M. Quantitative neuropathology of the entorhinal cortex region in patients with hippocampal sclerosis and temporal lobe epilepsy. *Epilepsia*. 2005;46:23–30.
141. Cavus I, Abi-Saab WM, Cassadey M, Jackob R, Sherwin RS, Krysal J, Spencer DD. Basal glutamate, gamma-aminobutyric acid, glucose, and lactate levels in the epileptogenic and non-epileptogenic brain site in neurosuergery patients. *Epilepsia*. 2002; 43(Suppl 7):247.
142. Landmark CJ. Targets for antiepileptic drugs in the synapse. *Med Sci Monit*. 2007;13:RA1–7.
143. White HS, Smith MD, Wilcox KS. Mechanisms of action of antiepileptic drugs. *Int Rev Neurobiol*. 2007;81:85–110.
144. Brines ML, Sundaresan S, Spencer DD, de Lanerolle NC. Quantitative autoradiographic analysis of ionotropic glutamate receptor subtypes in human temporal lobe epilepsy: upregulation in reorganized epileptogenic hippocampus. *Eur J Neurosci*. 1997;9: 2035–2044.

Chapter 31

The Time Course and Circuit Mechanisms of Acquired Epileptogenesis

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In order to develop and test possible therapeutic strategies for preventing or suppressing epileptogenesis, the temporal features of acquired epilepsy and its underlying mechanisms must be understood. These temporal features include the frequency, duration, and cortical extent of spontaneous seizures; this review focuses primarily on seizure frequency. Traumatic brain injury, stroke, status epilepticus, and infection/inflammation are some of the major causes of acquired epilepsy. The spontaneous recurrent epileptic seizures of acquired epilepsy usually occur after a latent period following the injury, and in at least some patients, the epilepsy is progressive (i.e., the seizures become more frequent and severe). Nearly all patients receive antiepileptic drugs (AEDs) after one or a few clinical seizures. Therefore, quantitative analyses of the temporal features of acquired epileptogenesis, independent of the effects of AEDs, cannot be studied in humans. Animal models of acquired epilepsy can circumvent this problem. The research summarized here analyzed the development of spontaneous recurrent seizures (1) in kainate-treated rats,[1] an animal model of temporal lobe epilepsy, and (2) in rats subjected to hypoxic-ischemic brain damage at postnatal day 7, a model of perinatal stroke.[2–4]

Many mechanisms have been hypothesized to account for epileptogenesis after brain injury. Numerous studies have focused on molecular and cellular changes during the latent period (the time from brain injury to the first clinical seizure), which has been thought to mark the duration of epileptogenesis; however, whether the first clinical seizure actually signals the completion of epileptogenesis or whether

epileptogenesis continues well beyond the first seizure is unknown. The frequency and pattern of spontaneous recurrent seizures as a function of time after the brain injury define the time course of epileptogenesis, and this information is fundamental to an understanding of the mechanisms of acquired epilepsy. This time course, which is essentially the natural history of acquired epilepsy, places constraints on the hypothetical mechanisms that contribute to epileptogenesis after brain injury. This chapter uses information on the time course of acquired epileptogenesis to evaluate hypotheses regarding the underlying mechanisms of epilepsy. Our focus is primarily at the level of neuronal circuits and synaptic reorganization: selective loss of specific, vulnerable GABAergic interneurons and the progressive formation of new recurrent excitatory circuits after axonal sprouting. We propose that these two mechanisms may account for—or at least contribute to—the time course of injury-induced epileptogenesis.

TWO MODELS OF ACQUIRED EPILEPSY

Most research using animal models of temporal lobe epilepsy that have spontaneous recurrent seizures is based on status epilepticus; some of the studies described in this chapter used an animal model in which status epilepticus was induced with repeated low-dose injections of kainate.[5–8] In the second animal model of acquired epilepsy, hypoxic-ischemic brain injury was induced in immature rats[2,3,9] as an animal model of perinatal stroke, which is an important cause of intractable epilepsy in children.[10,11] Use of this model allowed determination of the time course of the development of spontaneous recurrent seizures after perinatal brain injury.[4] These two animal models are quite different, but both of them show clear brain damage with neuronal death, and they both unequivocally develop epilepsy, which is manifest as readily documentable electrographic and behavioral seizures. Finally, a reduced in vitro preparation, the organotypic slice preparation, has been used to model posttraumatic epileptogenesis.[12,13] Advantages of this model are the compressed 2-week time scale over which epileptogenesis occurs and the isolation from other brain centers and systems, such as the blood-brain barrier and the immune system, which might influence epileptogenesis.

THE TIME COURSE OF ACQUIRED EPILEPTOGENESIS

The Progressive Nature of Acquired Epilepsy

The question of whether acquired epilepsy is progressive is important and long-standing. One view, as introduced above, is that acquired epilepsy (e.g., temporal lobe epilepsy) starts after a latent period, and subsequent seizure frequency and severity are variable but not necessarily progressive over time (i.e., the step-function hypothesis, Fig. 31–1A1). Another perspective is that acquired epilepsy is progressive; however, technical difficulties in measuring seizure frequency and severity (e.g., long-term, continuous recordings to account for seizure clusters) make it difficult to assess whether acquired epilepsy is progressive (i.e., the continuous-function hypothesis, Fig. 31–1A2). This issue is virtually impossible to evaluate directly in humans, because patient reporting of seizures is inaccurate and human patients are routinely treated with AEDs.[14] Human temporal lobe epilepsy often seems to be progressive, based on clinical[15–18] and magnetic resonance imaging (MRI) data.[19–21] Evidence of ongoing neuronal death in tissue resected for intractable epilepsy also supports this hypothesis.[22,23] In animal models, prolonged and virtually continuous electroencephalographic (EEG) recordings are required to determine whether the frequency and severity of spontaneous recurrent seizures increase in every animal. Bertram and Cornett,[24,25] who obtained extensive chronic recordings from rats with electrically evoked status epilepticus, found that subsequent spontaneous recurrent seizures occurred with variable frequency; however, seizure frequency generally increased over time and appeared to plateau after several months. When averaged across many kainate-treated rats, Hellier and collaborators[6] found that behavioral seizure frequency clearly increased as a function of time for nearly a year.

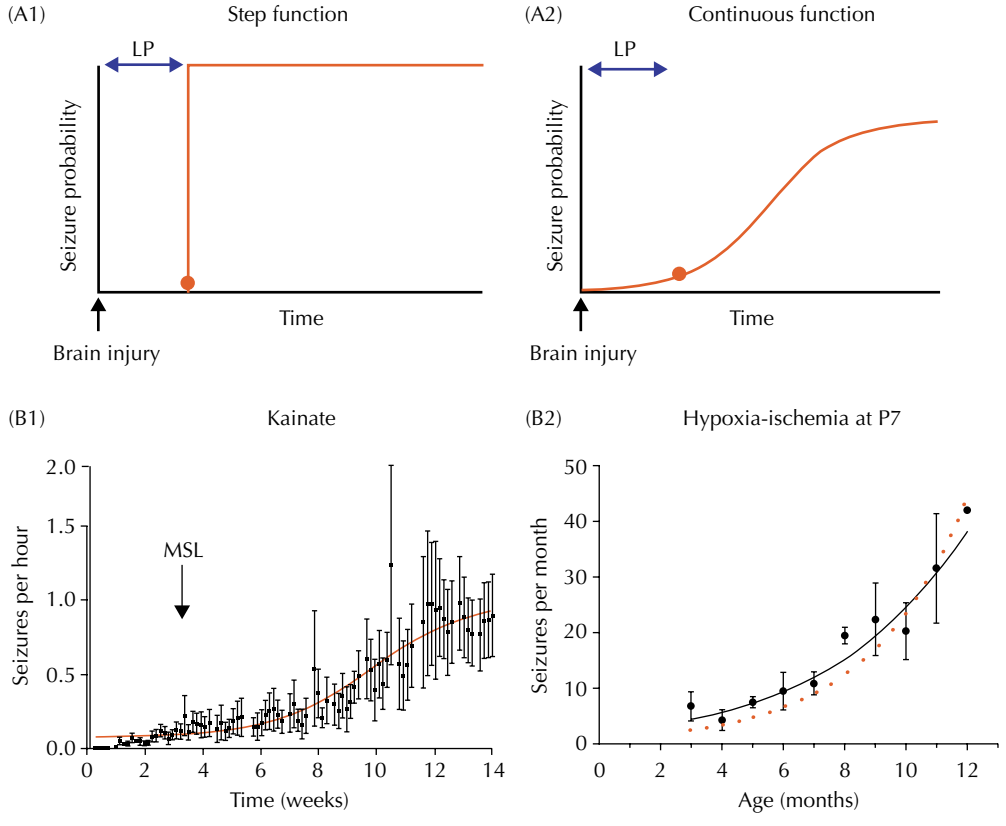

Figure 31–1. Time course of acquired epileptogenesis after brain injury. **A.** Schematic diagrams of the step-function and continuous-function hypotheses of the time course of acquired epileptogenesis. **A1.** The step-function hypothesis proposes a seizure-free period after the initial brain injury (i.e., the latent period [LP], with no epilepsy) and then the occurrence of spontaneous recurrent seizures (i.e., epileptic). The step-function hypothesis indicates that the mechanisms of seizure generation are mature when the first seizure occurs. **A2.** The continuous-function hypothesis proposes that seizure probability increases progressively after a brain injury. The hypothesis predicts an exponential increase in seizure probability or frequency followed by the development of a steady state. In the continuous-function hypothesis, the mechanisms responsible for generation of spontaneous seizures are not completely developed when the first seizure occurs, and the seizure rate continues to increase as epileptogenesis progresses. **B.** Analyses of seizure frequency as a function of time after brain injury. **B1.** Seizure frequency after kainate-induced status epilepticus. Seizure frequency increased as a function of time after kainate treatment (n = 9), which was best described by a sigmoid curve. Previous data obtained with this model suggest that most rats do not reach a steady state in seizure frequency for several months,[6] but some animals ultimately have such a high seizure frequency that they are often in status epilepticus.Note that the motor-seizure latent period (MSL) occurred after the first electrographic seizure. **B2.** Continuous video-EEG monitoring in rats subjected to hypoxia-ischemia at postnatal day 7 (P7) revealed an increase in seizure frequency and severity over time. The rats were implanted at 2 months and at ≥6 months. The data were from 10 hypoxia-ischemia-treated epileptic rats studied over an 11-month period with a best-fit exponential growth curve (dotted line) and a sigmoid curve (solid line). The sigmoid curve showed the best fit for the data. **A** modified from ref. 1. **B1** from ref. 1. **B2** from ref. 4.

After electrically induced status epilepticus (i.e., stimulation of the amygdala), video-EEG recordings every other day showed increased seizure frequency when assessed in 2-week epochs for 6 months.[26] With a similar model, but involving electrical stimulation of the angular bundle, Gorter and colleagues[27] observed a progressive increase in seizure frequency in most rats, although some rats appeared to be nonprogressive. After kainate-induced status epilepticus,[1] nearly continuous radiotelemetric recordings showed a progressive increase in seizure frequency (Fig. 31–1B1). The temporal pattern was highly variable across animals, but all animals (n = 9) showed a progressive increase in seizure frequency over the duration of the study (i.e., about 100 days) and the group data could be fit with a sigmoid curve. Based on

examination of the data from individual rats in the study of Williams and coworkers,[1] the animals from the report by Gorter and coworkers[27] that appeared to be nonprogressive might have had a progressive increase in seizure frequency if more prolonged seizure monitoring had been undertaken; on the other hand, it is possible that some animals may not show progressive worsening of the epilepsy after brain injury. In an animal model of perinatal stroke, however, every animal with a lesion developed progressive epilepsy in which seizure frequency increased over time[3,4] (Fig. 31–1B1). Notably, without virtually continuous recording for many months, seizure clusters and a low overall seizure rate would have obscured the consistent increase in seizure frequency. Thus, rats with epilepsy either after kainate-induced status epilepticus during adulthood or after a hypoxic-ischemic insult at postnatal day 7 showed a progressive increase in seizure frequency when recorded continuously over study durations lasting for at least a few months.

The Latent Period and the Duration of Epileptogenesis

The *latent period*, which is generally defined as the time from a brain insult to the first clinical seizure (Fig. 31–1A), is clearly one of the most intriguing concepts in epilepsy research. In humans, it has been reported to range from a few weeks to many years.[28] Bertram and Cornett[24,25] reported that nonconvulsive seizures always preceded the first convulsive seizure; the data in the report of Williams and coworkers[1] support this conclusion. Although the first convulsive seizures were detected roughly 2 weeks after kainate-induced status epilepticus, nonconvulsive EEG seizures first occurred at about 7 days. Furthermore, several nonconvulsive EEG seizures usually preceded the first convulsive seizure in this study.[1] The studies from these two research groups (and others) raise the possibility that in humans thought to have had latent periods of many years, unrecognized subclinical seizures may have preceded the first convulsive seizures. When examined in more quantitative detail, the longest interseizure intervals for the first 20 seizures in the work of Williams and coworkers[1] were only slightly less than the latent periods for the first electrographic nonconvulsive seizures. This result, and other data, suggest that the latent period can be viewed as the first of many long interseizure intervals (i.e., intercluster intervals), and that epileptogenesis per se involves a smooth and continuous increase in seizure probability over time after the brain injury (Figs. 31–1A2 and 31–1B). Measurements of the latent period are actually quite difficult: first, because the latent period is the time of an asymptotic departure from a baseline; and, second, because determination of the latent period requires continuous recording from the time of the brain insult to the first convulsive seizure. Thus, although the latent period is a genuine phenomenon, it may be best viewed as the initial phase of a continuous process. The continuous nature of epileptogenesis is often obscured by variable seizure frequency and the occurrence of seizure clusters. Therefore, quantitative assessments of acquired epileptogenesis may be accomplished more accurately by long-term measures of seizure frequency than by measurement of the latent period.

CIRCUIT MECHANISMS AND SYNAPTIC REORGANIZATION

Synaptic Reorganization

Acquired epilepsy may involve a wide range of mechanisms, including alterations in neurotransmitter receptors (e.g., gamma-aminobutyric acid A [$GABA_A$] receptors) and/or voltage-dependent currents (e.g., sodium channels). Although other systems may contribute to the time course of epileptogenesis, the progressive nature of this process in reduced preparations[12,13] supports the idea that local circuit alterations are sufficient to induce epilepsy. While local alterations in ligand- and voltage-gated channels may well be important, this chapter focuses on alterations in local synaptic circuits (i.e., synaptic reorganization). Two hypothetical mechanisms (also see the review by Ben-Ari and Dudek[29]) that have been studied extensively are (1) death of GABAergic interneurons and consequent decreases in GABA-mediated inhibition (Fig. 31–2A) and (2) axon sprouting with subsequent increases in recurrent excitation (Fig. 31–2B). The first

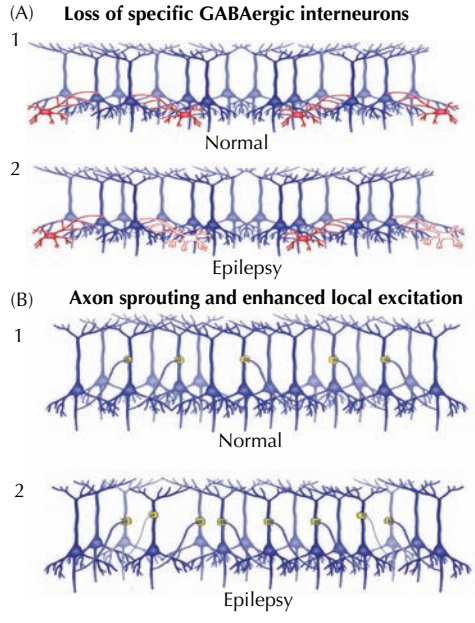

Figure 31–2. Hypothetical changes in local synaptic circuits during epileptogenesis. **A.** Loss of specific interneurons and decreased GABAergic inhibition in the hippocampus during epileptogenesis. Schematic diagram showing hippocampal pyramidal cells and interneurons under normal conditions (**A1**) and after epileptogenesis (**A2**) occurred. The diagrams illustrate the hypothesis that during epileptogenesis, some but not all interneurons are lost. **B.** Axon sprouting and increased recurrent excitation in the hippocampus during epileptogenesis. The diagrams show pyramidal cells under normal conditions (**B1**) and after axon sprouting associated with epileptogenesis (**B2**). They illustrate the hypothesis that although recurrent excitation is normally present among some pyramidal cells before epileptogenesis, it increases during epileptogenesis. From ref. 29.

mechanism involves a rapid loss of GABAergic interneurons after status epilepticus or other injuries, but interneurons may also continue to die during epileptogenesis. A mechanism that has gained considerable attention because it would be expected to require time to occur is axonal sprouting and subsequent formation of new excitatory (and inhibitory?) synaptic circuits; this time dependence could thus account for or contribute to the initial latent period and the subsequent progression of epilepsy.

Loss of GABAergic Interneurons

Experiments based on several independent morphological and physiological techniques in human tissue and animal models of acquired epilepsy support the hypothesis that a modest loss of specific GABAergic interneurons is associated with epileptogenesis (Fig. 31–2A). Immunohistochemical and in situ hybridization techniques have shown that a relatively small number of specific types of GABAergic interneurons are lost in both hippocampal and cortical areas.[5,27,30–32] Several laboratories have used whole-cell recordings of miniature inhibitory postsynaptic currents (mIPSCs) to test more specifically the hypothesis of a loss of interneuron input to granule cells[33,34] and CA1 pyramidal cells[35]; these studies have generally found that the frequency of mIPSCs is reduced (Figs. 31–3 and 31–4), which supports the hypothesis of a reduction of inhibitory GABAergic synaptic terminals (i.e., loss of GABAergic interneurons). Other interpretations of these data, such as reduced release probability, are possible but not congruent with the anatomical findings. Notably, these studies have not found reductions in the amplitude of mIPSCs (Figs. 31–3 and 31–4), thus suggesting that alterations in $GABA_A$ receptors do not contribute substantially to decreases in GABAergic inhibition during epileptogenesis, at least under these conditions. This approach has its limitations, but it uses tetrodotoxin to block action potential-mediated activity and is independent of the problems of extracellular stimulation (e.g., activation of fibers of passage, changes in action potential threshold between control and experimental groups); thus, the number of confounding variables is reduced. Therefore, the evidence from experiments on several animal models from multiple laboratories suggests that epileptogenesis is associated with a modest but significant loss of specific types of vulnerable interneurons in the dentate gyrus and the CA1 area of the hippocampus and in other areas, which in turn is manifest as a reduction of GABAergic inhibition. The reduction in GABAergic inhibition may be more robust early in the course of epileptogenesis and may at least partially resolve by the time of the first seizure.[33] For example, sprouting of GABAergic interneurons may at least partially restore inhibition.[36] Other mechanisms of disinhibition, such as reductions in the transport systems that maintain an inhibitory gradient for GABA-mediated chloride currents, may also contribute to epileptogenesis.[37–39]

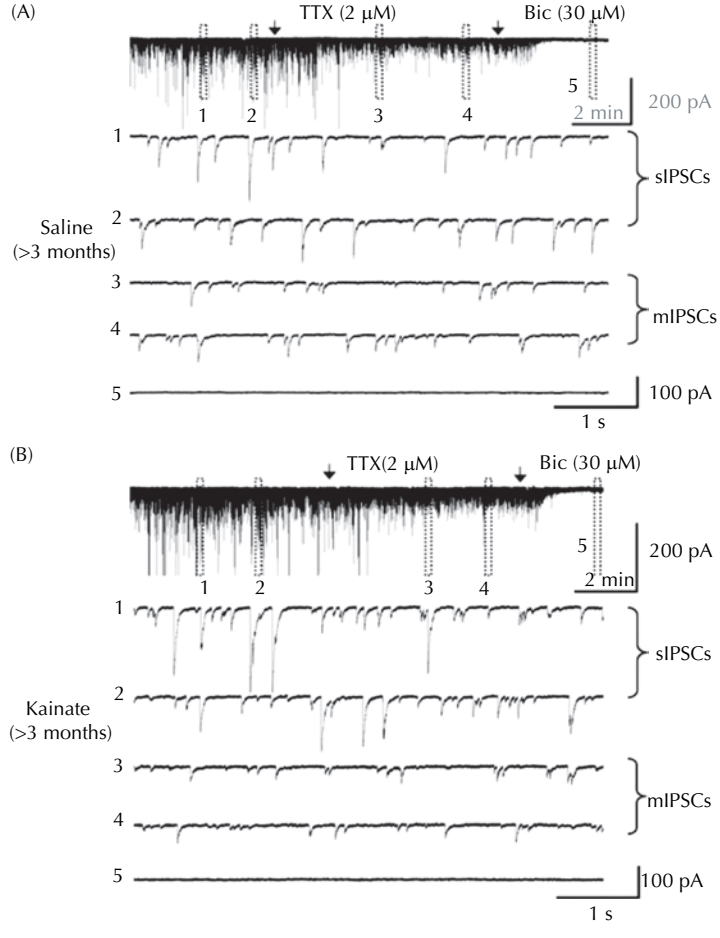

Figure 31–3. Examples of spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs) recorded from granule cells of the dentate gyrus in rats >3 months after saline (**A**) or kainate treatment (**B**). The upper traces are recordings of sIPSCs for 18 min (**A**) and 15 min (**B**). The lower traces are expansions of the boxed parts of the recordings in the upper traces, which show sIPSCs (1 and 2) and mIPSCs (3 and 4). Bicuculline (Bic) blocked these currents, confirming that they were GABAergic. From ref. 34.

Axon Sprouting and Increased Recurrent Excitation

The concept of *synaptic reorganization* was initially based on the observation of mossy fiber sprouting and the associated hypothesis of increased recurrent excitation during acquired epileptogenesis, which has led to many studies aimed at exploring its potential role in temporal lobe epilepsy. One reason for the interest is that several laboratories had reported Timm stain in the inner molecular layer of the dentate gyrus of patients with intractable temporal lobe epilepsy.[40–43] This phenomenon is present in numerous animal models, including pilocarpine- and kainate-treated rats and the kindling model (see refs. 44 and 45 for review). Several types of electrophysiological and ultrastructural data strongly suggest that nearly all of the new mossy fiber connections are excitatory (see refs. 44 and 45 for review). For example, hilar or perforant path stimulation can evoke long-latency excitatory postsynaptic potentials (EPSPs) with prolonged spike bursts several months after status epilepticus, particularly when slices from rats with chronic epilepsy are bathed in $GABA_A$ receptor antagonists and/or high levels of potassium; in similar experiments on slices from control animals, comparable electrical stimulation only evokes an EPSP and/or a single action potential.[46–51] The rationale for performing these experiments

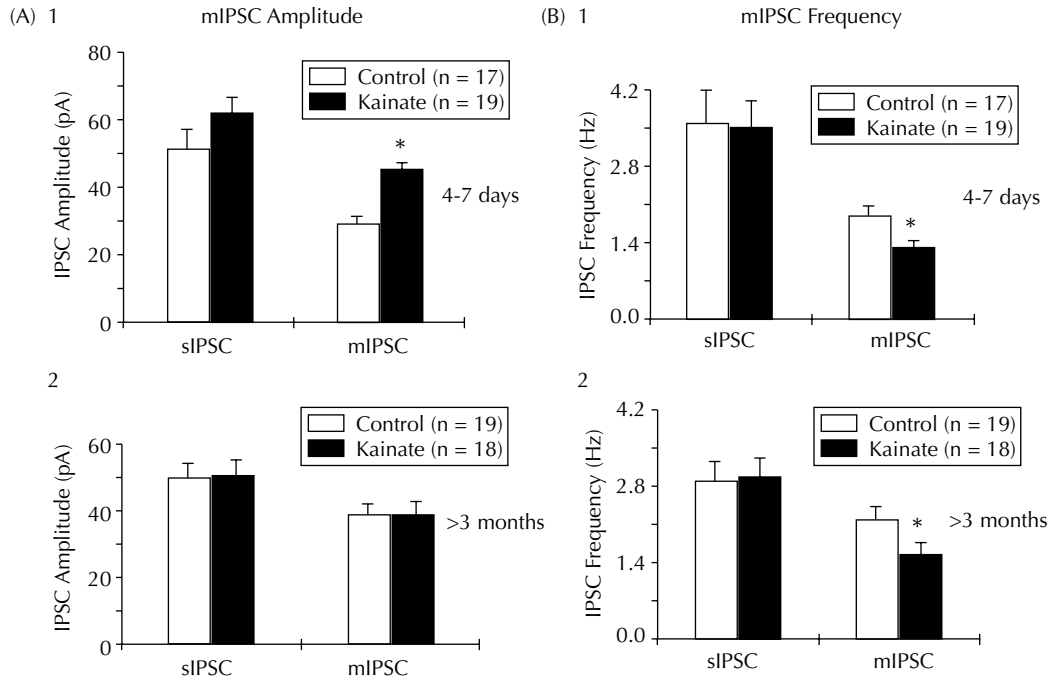

Figure 31–4. Changes in the amplitude and frequency of sIPSCs and mIPSCs during acquired epileptogenesis in kainate-treated rats. **A.** Summary of the data on mean IPSC amplitudes in rats 4–7 days versus >3 months after kainate treatment. **A1.** Although the mean sIPSC amplitude in rats 4–7 days after kainate treatment was not significantly different from that of controls, the mean mIPSC amplitude showed a significant increase. **A2.** Both sIPSC and mIPSC amplitudes in rats >3 months after kainate treatment were similar to those of controls. **B.** Summary of the data on mean mIPSC and sIPSC frequency in rats 4–7 days compared to those >3 months after kainate treatment. **B1.** The mean sIPSC frequency in rats 4–7 days after kainate treatment was similar to that from the saline groups, but the mean mIPSC frequency of the kainate-treated rats was significantly lower than that of the controls. **B2.** At >3 months after saline or kainate treatment, mean sIPSC frequencies were similar, but mean mIPSC frequencies were reduced in the kainate-treated rats. The sIPSC frequency was not significantly different in any of the four groups, and the two control groups in **B1** and **B2** had similar mIPSC frequencies. From ref. 34.

during blockade of $GABA_A$-mediated inhibition is not only that previous work showed that GABA-mediated inhibition has a masking effect on recurrent excitation,[52–55] but also that this procedure controls at least partially for epileptogenesis-associated differences in GABA-mediated inhibition (see above). Electrical stimulation, however, activates axons of passage. Experiments using glutamate microdrops[48,51] or focal photoactivation of caged glutamate,[56,57] which does not stimulate fibers of passage, have provided more direct evidence for the formation of new recurrent excitatory circuits. Dual intracellular recordings have further supported the hypothesis that mossy fiber sprouting in rats with pilocarpine-induced epilepsy is associated with an increase in monosynaptic recurrent excitatory connections among dentate granule cells.[58] Quantitative ultrastructural studies also indicate that new excitatory synapses connect primarily to dentate granule cells versus interneurons.[59,60] Newborn granule cells from seizure-induced neurogenesis in the dentate gyrus may play an important role in synaptic reorganization, at least in the dentate gyrus.[61,62]

Although mossy fiber sprouting and synaptic reorganization may be particularly important in the dentate gyrus, axon sprouting and the formation of new recurrent excitatory circuits is probably a widespread response to brain damage in many areas of the hippocampus and neocortex during epileptogenesis. Numerous studies have reported morphological and electrophysiological data that axonal sprouting (Fig. 31–5A) and enhanced recurrent excitation (Fig. 31–5B) occur in the CA1 area during epileptogenesis.[63–68] The CA1 area of the

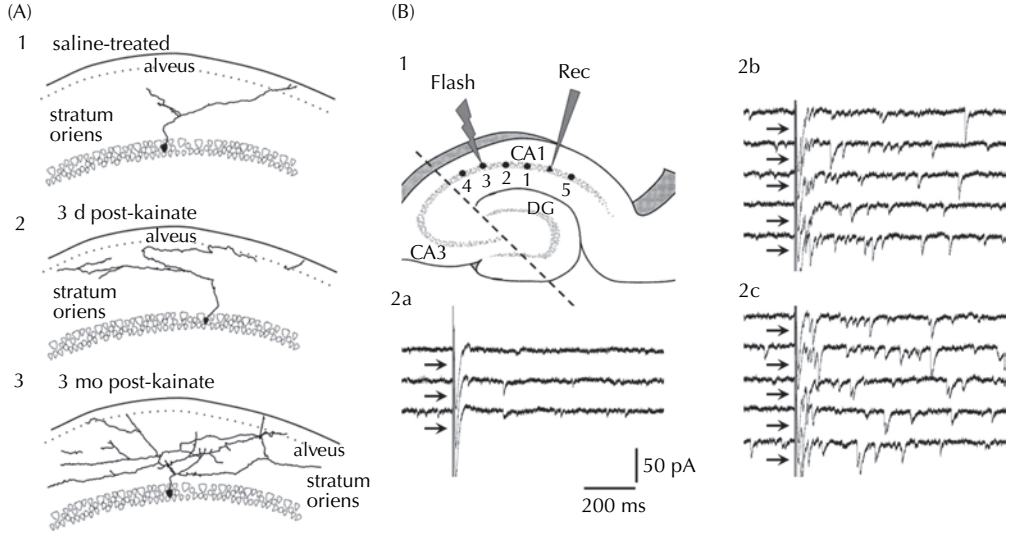

Figure 31–5. Axon sprouting and increased recurrent excitation of CA1 pyramidal cells in kainate-induced epilepsy. **A.** Examples of biocytin-filled axons from CA1 pyramidal cells during whole-cell recordings in isolated CA1 slices. **A1.** Axon from a saline-treated control rat. **A2.** The axons from kainate-treated rats 3 days after treatment were quantitatively similar to those of controls. **A3.** The axons were more highly branched in a rat >3 months after kainate treatment than in control rats or in a rat 3 days after kainate treatment. **B.** Evidence for local excitatory connections of CA1 pyramidal cells in epileptic rats was revealed by flash photolysis of caged glutamate. **B1.** Recordings were made from CA1 pyramidal cells in mini-slices, and flash stimulations were presented at different locations in the slice (circles) at 150 μm to 200 μm intervals along the cell body layer. **B2.** In a slice from a kainate-treated animal, repetitive flash stimulations (arrows, three to five flashes per site at 20 s intervals) consistently evoked a series of EPSCs in a CA1 pyramidal cell at two of five sites. **B2a.** Flashes at three sites (1, 4, and 5) evoked no EPSCs. **B2b, B2c.** Flashes at sites 2 and 3, which were 400 and 600 μm from the recorded neuron, respectively, evoked repetitive EPSCs. **A** from ref. 66. **B** from ref. 68.

hippocampus is highly vulnerable to excitotoxic neuronal death, and loss of neurons in CA1 is a classic marker of mesial temporal sclerosis. Synaptic reorganization in the CA1 area may reflect the types of morphological and functional changes that occur in many areas of the limbic system, possibly early in the epileptogenic process in humans. Many forms of acquired epilepsy in addition to temporal lobe epilepsy, including the epilepsy that occurs after perinatal and adult stroke[2,4,69] and traumatic brain injury,[70] are likely to be a network phenomenon, with at least two reorganizational mechanisms—the loss of vulnerable interneurons along with axon sprouting of principal cells followed by formation of new recurrent excitatory circuits.

POSSIBLE CONCLUSIONS

The spontaneous recurrent seizures after a brain injury, which define acquired epilepsy, typically worsen in terms of frequency and severity. Although this result has not always been seen in previous work, quantitative analyses of long-term continuous recordings are often required to detect progression. Studies of this type in the kainate model[1] and in a model of perinatal stroke[4] have provided clear evidence of progressive epilepsy in virtually all of the animals that were studied. Axonal sprouting and formation of new recurrent excitatory circuits are well known to be progressive processes, and these mechanisms could be the basis for the progressive changes that occur in these and other models. Selective interneuron loss may also be progressive and an important part of the changes that lead to progressive epileptogenesis. Considerable research has targeted the dentate gyrus, but similar mechanisms have been identified in many temporal structures and even in neocortical areas after brain injury (e.g., status epilepticus and perinatal stroke). Acquired epileptogenesis therefore appears to be a slow, time-dependent process (Figs. 31–1A2 and 31–B) rather than

a simple step function (Fig. 31–1A1) with a discrete latent period defining the boundaries of epileptogenesis[1,4]; synaptic reorganization defined by interneuron loss and the formation of sprouting-induced recurrent excitation may be an important component of acquired epilepsy that contributes to its natural history and progressive characteristics.

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REFERENCES

1. Williams PA, White AM, Clark S, Ferraro DJ, Swiercz W, Staley KJ, Dudek FE. Development of spontaneous recurrent seizures after kainate-induced status epilepticus. *J Neurosci*. 2009;29:2103–2112.
2. Williams PA, Dou P, Dudek FE. Epilepsy and synaptic reorganization in a perinatal rat model of hypoxia-ischemia. *Epilepsia*. 2004;45:1210–1218.
3. Kadam S, Dudek FE. Neuropathological features of a rat model for perinatal hypoxic-ischemic encephalopathy with associated epilepsy. *J Comp Neurol*. 2007;505: 716–737.
4. Kadam SD, White AM, Staley KJ, Dudek FE. Continuous electroencephalographic monitoring with radio-telemetry in a rat model of perinatal hypoxia-ischemia reveals progressive post-stroke epilepsy. *J Neurosci*. 2010;30:404–415.
5. Buckmaster PS, Dudek FE. Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol*. 1997;385:385–404.
6. Hellier JL, Patrylo PR, Buckmaster PS, Dudek FE. Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: assessment of a rat model of temporal lobe epilepsy. *Epilepsy Res*. 1998;31:73–84.
7. Dudek FE, Hellier JL, Williams PA, Ferraro DJ, Staley KJ. The course of cellular alterations associated with the development of spontaneous seizures after status epilepticus. *Prog Brain Res*. 2002;135: 53–65.
8. Dudek FE, Clark S, Williams PA, Grabenstatter HL. Kainate-induced status epilepticus: a chronic model of acquired epilepsy. In: Pitkanen A, Schwartzkroin PA, Moshé SL, eds. *Models of Seizures and Epilepsy*. Amsterdam: Elsevier Academic Press; 2006:415–432.
9. Rice JE III, Vannucci RC, Brierley JB. The influence of immaturity on hypoxic-ischemic brain damage in the rat. *Ann Neurol*. 1981;9:131–141.
10. Bergamasco B, Benna P, Ferrero P, Gavinelli R. Neonatal hypoxia and epileptic risk: a clinical prospective study. *Epilepsia*. 1984;25:131–136.
11. Marin-Padilla M. Neuropathologic correlates of perinatal asphyxia. *Int Pediatr*. 2000;15:221–228.
12. McBain CJ, Boden P, Hill RG. Rat hippocampal slices "in vitro" display spontaneous epileptiform activity following long-term organotypic culture. *J Neurosci Methods*. 1989;27:35–49.
13. Dyhrfjeld-Johnsen J, Berdichevsky Y, Swiercz W, Sabolek H, Staley KJ. Interictal spikes precede ictal discharges in an organotypic hippocampal slice culture model of epileptogenesis. *J Clin Neurophysiol*. 2010;27:418–424.
14. Hoppe C, Poepel A, Elger CE. Epilepsy: accuracy of patient seizure counts. *Arch Neurol*. 2007;64: 1595–1599.
15. Engel J Jr. Clinical evidence for the progressive nature of epilepsy. *Epilepsy Res Suppl*. 1996;12:9–20.
16. Engel J. Natural history of mesial temporal lobe epilepsy with hippocampal sclerosis: How does kindling compare with other commonly used animal models? In: Corcoran ME, Moshé SL, eds. Kindling 6. New York: Springer Science + Business Media; 2005:371–384.
17. Engel J Jr. Surgical treatment for epilepsy: too little, too late? *JAMA*. 2008;300:2548–2550.
18. Engel J, Berg AT. From prediction of medical intractability to early surgical treatment. In: Ryvlin P, Beghi E, Camfield P, Hesdorffer D, eds. *From First Unprovoked Seizure to Newly Diagosed Epilepsy: Progress in Epileptic Disorders*. London: John Libbey Eurotext; 2007:209–220.
19. Fuerst D, Shah J, Shah A, Watson C. Hippocampal sclerosis is a progressive disorder: a longitudinal volumetric MRI study. *Ann Neurol*. 2003;53:413–416.
20. Cascino GD. Temporal lobe epilepsy is a progressive neurologic disorder: time means neurons! *Neurology*. 2009;72:1718–1719.
21. Bernhardt BC, Worsley KJ, Kim H, Evans AC, Bernasconi A, Bernasconi N. Longitudinal and cross-sectional analysis of atrophy in pharmacoresistant temporal lobe epilepsy. *Neurology*. 2009;72:1747–1754.
22. Henshall DC, Schindler CK, So NK, Lan JQ, Meller R, Simon RP. Death-associated protein kinase expression in human temporal lobe epilepsy. *Ann Neurol*. 2004;55:485–494.
23. Schindler CK, Pearson EG, Bonner HP, So NK, Simon RP, Prehn JH, Henshall DC. Caspase-3 cleavage and nuclear localization of caspase-activated DNase in human temporal lobe epilepsy. *J Cereb Blood Flow Metab*. 2006;26:583–589.
24. Bertram EH, Cornett JF. The ontogeny of seizures in a rat model of limbic epilepsy: evidence for a kindling process in the development of chronic spontaneous seizures. *Brain Res*. 1993;625:295–300.
25. Bertram EH, Cornett JF. The evolution of a rat model of chronic spontaneous limbic seizures. *Brain Res*. 1994;661:157–162.

26. Nissinen J, Halonen T, Koivisto E, Pitkanen A. A new model of chronic temporal lobe epilepsy induced by electrical stimulation of the amygdala in rat. *Epilepsy Res*. 2000;38:177–205.
27. Gorter JA, van Vliet EA, Aronica E, Lopes da Silva FH. Progression of spontaneous seizures after status epilepticus is associated with mossy fibre sprouting and extensive bilateral loss of hilar parvalbumin and somatostatin-immunoreactive neurons. *Eur J Neurosci*. 2001;13:657–669.
28. Annegers JF, Hauser WA, Coan SP, Rocca WA. A population-based study of seizures after traumatic brain injuries. *N Engl J Med*. 1998;338:20–24.
29. Ben-Ari Y, Dudek FE. Primary and secondary mechanisms of epileptogenesis in the temporal lobe: there is a before and an after. *Epilepsy Curr*. 2010;10: 118–125.
30. Ribak CE, Bradurne RM, Harris AB. A preferential loss of GABAergic, symmetric synapses in epileptic foci: a quantitative ultrastructural analysis of monkey neocortex. *J Neurosci.* 1982;2:1725–1735.
31. Sloviter RS. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science*. 1987;235:73–76.
32. Obenaus A, Esclapez M, Houser CR. Loss of glutamate decarboxylase mRNA-containing neurons in the rat dentate gyrus following pilocarpine-induced seizures. *J Neurosci*. 1993;13:4470–4485.
33. Kobayashi M, Buckmaster PS. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. *J Neurosci*. 2003;23:2440–2452.
34. Shao L-R, Dudek FE. Changes in mIPSCs and sIPSCs after kainate treatment: evidence for loss of inhibitory input to dentate granule cells and possible compensatory responses. *J Neurophysiol*. 2005;94:952–960.
35. Wierenga CJ, Wadman WJ. Miniature inhibitory postsynaptic currents in CA1 pyramidal neurons after kindling epileptogenesis. *J Neurophysiol*. 1999;82: 1352–1362.
36. Zhang W, Yamawaki R, Wen X, Uhl J, Diaz J, Prince DA, Buckmaster PS. Surviving hilar somatostatin interneurons enlarge, sprout axons, and form new synapses with granule cells in a mouse model of temporal lobe epilepsy. *J Neurosci*. 2009;29:14247–14256.
37. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science.* 2002;291:1418–1421.
38. Jin X, Huguenard JR, Prince DA. Impaired Cl^- extrusion in layer V pyramidal neurons of chronically injured epileptogenic neocortex. *J Neurophysiol*. 2005;93: 2117–2126.
39. Pathak HR, Weissinger F, Terunuma M, Carlson GC, Hsu FC, Moss SJ, Coulter DA. Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. *J Neurosci*. 2007;27:14012–14022.
40. Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF. Synaptic reorganization by mossy fibers in human epileptic fascia dentata. *Neuroscience*. 1991;42:351–363.
41. de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res*. 1989;495:387–395.
42. Houser CR, Miyashiro JE, Swartz BE, Walsh GO, Rich JR, Delgado-Escueta AV. Altered patterns of dynorphin immunoreactivity suggest mossy fiber reorganization in human hippocampal epilepsy. *J Neurosci*. 1990;10:267–282.
43. Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol*. 1989;26:321–330.
44. Dudek FE, Sutula TP. Epileptogenesis in the dentate gyrus: a critical perspective. *Prog Brain Res.* 2007;163: 755–773.
45. Sutula TP, Dudek FE. Unmasking recurrent excitation generated by mossy fiber sprouting in the epileptic dentate gyrus: an emergent property of a complex system. *Prog Brain Res*. 2007;163:541–563.
46. Tauck DL, Nadler JV. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. *J Neurosci*. 1985;5:1016–1022.
47. Cronin J, Obenaus A, Houser CR, Dudek FE. Electrophysiology of dentate granule cells after kainate-induced synaptic reorganization of the mossy fibers. *Brain Res*. 1992;573:305–310.
48. Wuarin JP, Dudek FE. Electrographic seizures and new recurrent excitatory circuits in the dentate gyrus of hippocampal slices from kainate-treated epileptic rats. *J Neurosci*. 1996;16:4438–4448.
49. Patrylo PR, Dudek FE. Physiological unmasking of new glutamatergic pathways in the dentate gyrus of hippocampal slices from kainate-induced epileptic rats. *J Neurophysiol*. 1998;79:418–429.
50. Hardison JL, Okazaki MM, Nadler JV. Modest increase in extracellular potassium unmasks effect of recurrent mossy fiber growth. *J Neurophysiol*. 2000;84: 2380–2389.
51. Lynch M, Sutula T. Recurrent excitatory connectivity in the dentate gyrus of kindled and kainic acid-treated rats. *J Neurophysiol.* 2000;83:693–704.
52. Christian EP, Dudek FE. Characteristics of local excitatory circuits studied with glutamate microapplication in the CA3 area of rat hippocampal slices. *J Neurophysiol*. 1988;59:90–109.
53. Traub RD, Wong RK. Cellular mechanism of neuronal synchronization in epilepsy. *Science*. 1982;216: 745–747.
54. Traub RD, Wong RK. Synchronized burst discharge in disinhibited hippocampal slice. II. Model of cellular mechanism. *J Neurophysiol*. 1983;49:459–471.
55. Miles R, Wong RK. Inhibitory control of local excitatory circuits in the guinea-pig hippocampus. *J Physiol*. 1987;388:611–629.
56. Molnar P, Nadler JV. Mossy fiber-granule cell synapses in the normal and epileptic rat dentate gyrus studied with minimal laser photostimulation. *J Neurophysiol.* 1999;82:1883–1894.
57. Wuarin JP, Dudek FE. Excitatory synaptic input to granule cells increases with time after kainate treatment. *J Neurophysiol*. 2001;85:1067–1077.
58. Scharfman HE, Sollas AL, Berger RE, Goodman JH. Electrophysiological evidence of monosynaptic excitatory transmission between granule cells after seizure-induced mossy fiber sprouting. *J Neurophysiol*. 2003;90:2536–2547.
59. Zhang N, Houser CR. Ultrastructural localization of dynorphin in the dentate gyrus in human temporal lobe epilepsy: a study of reorganized mossy fiber synapses. *J Comp Neurol*. 1999;405:472–490.
60. Buckmaster PS, Zhang GF, Yamawaki R. Axon sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. *J Neurosci*. 2002;22:6650–6658.

61. Kron MM, Zhang H, Parent JM. The developmental stage of dentate granule cells dictates their contribution to seizure-induced plasticity. *J Neurosci*. 2010;30:2051–2059.
62. Pierce JP, McCloskey DP, Scharfman HE. Morphometry of hilar ectopic granule cells in the rat. *J Comp Neurol*. 2011;519:1196–1218.
63. Perez Y, Morin F, Beaulieu C, Lacaille JC. Axonal sprouting of CA1 pyramidal cells in hyperexcitable hippocampal slices of kainate-treated rats. *Eur J Neurosci*. 1996;8:736–748.
64. Meier CL, Dudek FE. Spontaneous and stimulation-induced synchronized burst afterdischarges in the isolated CA1 of kainate-treated rats. *J Neurophysiol*. 1996;76:2231–2239.
65. Esclapez M, Hirsch JC, Ben Ari Y, Bernard C. Newly formed excitatory pathways provide a substrate for hyperexcitability in experimental temporal lobe epilepsy. *J Comp Neurol*. 1999;408:449–460.
66. Smith BN, Dudek FE. Short- and long-term changes in CA1 network excitability after kainate treatment in rats. *J Neurophysiol*. 2001;85:1–9.
67. Smith BN, Dudek FE. Network interactions mediated by new excitatory connections between CA1 pyramidal cells in rats with kainate-induced epilepsy. *J Neurophysiol*. 2002;87:1655–1658.
68. Shao LR, Dudek FE. Increased excitatory synaptic activity and local connectivity of hippocampal CA1 pyramidal cells in rats with kainate-induced epilepsy. *J Neurophysiol*. 2004;92:1366–1373.
69. Williams PA, Dudek FE. A chronic histopathological and electrophysiological analysis of a rodent hypoxic-ischemic brain injury model and its use as a model of epilepsy. *Neuroscience*. 2007;149:943–961.
70. Prince DA, Parada I, Scalise, K, Graber K, Jin X, Shen, F. Epilepsy following cortical injury: Cellular and molecular mechanisms as targets for potential prophylaxis. *Epilepsia*. 2009;50(Suppl 2):30–40.

Chapter 32

Mossy Fiber Sprouting in the Dentate Gyrus

Paul S. Buckmaster

UNDER WHAT CIRCUMSTANCES DOES MOSSY FIBER SPROUTING OCCUR?

Granule cell axons (mossy fibers) project into the dentate hilus and stratum lucidum of CA3 in rodents[1] and other species, including humans.[2] Mossy fibers synapse with inhibitory interneurons, hilar mossy cells, and CA3 pyramidal cells[3] but very rarely with other granule cells (see below). Consequently, most granule cells normally do not display functional, monosynaptic, recurrent excitation.[4] A minor mossy fiber projection into the granule cell layer can be visualized with the Timm stain, which generates opaque silver particles specifically within zinc-rich mossy fiber boutons.[5] At all septotemporal levels of the hippocampus, occasional scattered dendrites and cell bodies of interneurons in the granule cell layer are outlined by Timm-positive punctae,[6] which electron microscopy has identified as mossy fiber synaptic boutons.[7] This pattern of Timm staining in the granule cell layer appears to increase with age.[8] At the temporal pole of the hippocampus, Timm staining reveals mossy fiber projections into the inner molecular layer that target granule cell dendrites.[5] This minor, recurrent excitatory pathway also increases with age.[9–12] The more extensive epilepsy-related recurrent mossy fiber pathway is an expansion of the normal minimal circuit already present.[13]

Aberrantly high levels of mossy fiber sprouting were first discovered during experiments investigating lesion-induced changes in neuronal connectivity. After transection of perforant path input to the dentate gyrus in young rats, black Timm staining was detected in the inner molecular layer.[14] More extensive and consistent mossy fiber sprouting developed after lesioning commissural/associational input to the inner molecular layer.[6] Electron microscopy revealed that lesion-induced sprouted mossy fibers synapse with granule cell dendrites.[6,15]

Mossy fiber sprouting was first found in patients with temporal lobe epilepsy by Scheibel et al.,[16] who reported that Golgi-stained mossy fibers project from the hilus, through the granule cell layer, and into the molecular

layer, where their boutons appose granule cell dendrites. Later, Nadler et al.[17] discovered extensive mossy fiber sprouting in rats that had been treated 1 month earlier with the excitotoxant kainic acid. Figure 32–1 shows mossy fiber sprouting in epileptic pilocarpine-treated rats. Perhaps because of the technical challenges of using the Golgi stain to follow individual granule cell axons, mossy fiber sprouting in human epileptic tissue was initially reported as rare (<1% of granule cells).[16] However, later studies used Timm staining and dynorphin immunoreactivity as markers for mossy fibers and found substantial sprouting in patients with mesial temporal lobe epilepsy.[10,18–20] Subsequently, mossy fiber sprouting was discovered in many different epilepsy-related conditions and some nonepileptic conditions (Table 32–1).

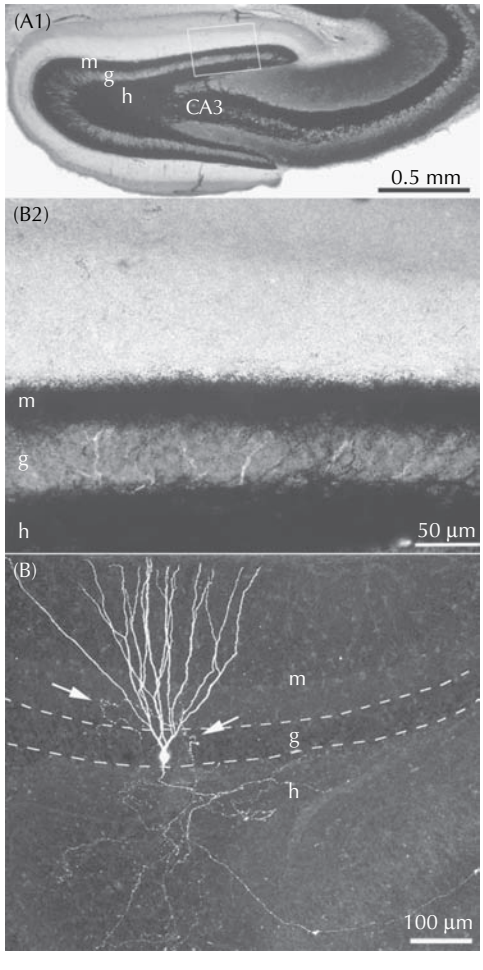

Figure 32–1. Mossy fiber sprouting in epileptic pilocarpine-treated rats. **A1.** Timm staining of the dentate gyrus (h = hilus, g = granule cell layer, m = molecular layer) and the CA3 region. **A2.** Magnified view of the boxed region in **A1** shows a dense band of black Timm staining in the inner molecular layer. **B.** Biocytin-labeled granule cell in a hippocampal slice from another epileptic rat reveals axon collaterals in the hilus and a sprouted mossy fiber (arrows) that projects from the hilus, through the granule cell layer, and into the molecular layer.

Temporal lobe epilepsy, the condition most frequently associated with mossy fiber sprouting, is the most common type of epilepsy in adults.[21] Some patients have a lesion (a hamartoma or glioma, for example) in their temporal lobe, but most have mesial temporal lobe epilepsy, which typically involves extensive neuron loss, especially in the hippocampus and, in at least some cases, a history of a precipitating injury.[22,23] Although robust mossy fiber sprouting is a common pathological finding in patients with mesial temporal lobe epilepsy,[24,25] there are exceptions.[26,27] Lynd-Balta et al.[28] proposed that young children with mesial temporal lobe epilepsy develop mossy fiber sprouting long after other changes, including neuron loss and altered expression of glutamate receptors. However, other investigators found mossy fiber sprouting similar to that of adult patients in children as young as 5.5 months old.[29] Mossy fiber sprouting does not occur exclusively in patients with mesial temporal lobe epilepsy. It also occurs in children secondary to cortical dysplasias without obvious hippocampal damage.[30] In those cases, however, the amount of sprouting is less than in children with hippocampal seizures.[29,31] On average, adult patients with mesial temporal lobe epilepsy have more mossy fiber sprouting than those with lesion-related temporal lobe epilepsy, but there is overlap between groups.[25] Mossy fiber sprouting can occur in epileptic patients without mesial temporal lobe epilepsy.[32] In addition, mild mossy fiber sprouting has been reported in patients with bipolar disorder but not epilepsy.[33] To better understand the circumstances that result in mossy fiber sprouting, it is helpful to consider the molecular and cellular mechanisms underlying its development.

HOW DOES MOSSY FIBER SPROUTING DEVELOP?

The mechanisms underlying mossy fiber sprouting remain unclear, but available evi-

Table 32–1 **Conditions in Which Mossy Fiber Sprouting Occurs in the Dentate Gyrus (Early References Indicated)**

- Patients
  - mesial temporal lobe epilepsy in children[29]
  - mesial temporal lobe epilepsy in adults[10,18]
  - severe epilepsy without hippocampal sclerosis in children[185]
  - nonmesial temporal lobe epilepsy in adults[32]
  - bipolar disorder[33]
- Laboratory animal models
  - chemoconvulsant
    - kainic acid
      - intracerebroventricular[17]
      - systemic[117]
      - intrahippocampal[186]
      - organotypic slices[42]
    - pilocarpine
      - systemic[143]
      - organotypic slices[187]
    - domoic acid
      - repeated subconvulsive systemic in rat pups[188]
  - electrically induced status epilepticus
    - hippocampal stimulation[41]
    - lateral amygdala stimulation[150]
    - angular bundle stimulation[51]
  - nonhuman primate
    - alumina gel into temporal lobe structures[189]
    - intrahippocampal bicuculline[190]
  - kindling
    - electrical[34]
    - pentylenetetrazol[191]
    - audiogenic[192]
    - overkindling[134]
  - genetic
    - tottering mice[193]
    - stargazer mice[11]
    - prion protein null mice[194]
    - ihara rats[195]
    - p35 knockout mice[196]
    - PLC-β1TC –/– mice[197]
  - infectious
    - feline immunodeficiency virus-infected cats[198]
    - herpes simplex virus type 1-injected organotypic slices[199]
  - lesion
    - perforant path transection[14]
    - dentate gyrus commissural/associational pathway transection[6]
    - fimbria/fornix transection[200]
  - organotypic slices[201]
  - experimental electroshock therapy[38]
  - transient forebrain ischemia[202]
  - perinatal hypoxia-ischemia in rats[156]
  - traumatic brain injury[203,204]
  - prolonged experimental febrile seizures[39]
  - intrahippocampal tetanus toxin in infant[205] and adult rats[206]
  - scorpion toxin[207]
  - BDNF infusion into hippocampus[67]

dence suggests likely triggers. One is seizure activity, which appears capable of causing mild mossy fiber sprouting without obvious neuron loss.[34–39] However, seizure activity alone is not always sufficient, because many seizures can propagate through the dentate gyrus without causing mossy fiber sprouting, as in Mongolian gerbils with inherited epilepsy.[7,40] Intense mossy fiber sprouting, like that found in patients with mesial temporal lobe epilepsy, appears to require deafferentation of granule cells either by transecting axons[6] or by killing presynaptic neurons.[17,41,42] Hilar mossy cells give rise to the associational pathway of the dentate gyrus (and, in rodents, the commissural pathway),[43,44] account for ~60% of all hilar neurons,[45–47] and synapse with granule cell dendrites in the inner molecular layer,[48] which also are the primary target of sprouted mossy fibers. The extent of mossy fiber sprouting correlates with hilar neuron loss in patients with mesial temporal lobe epilepsy[19,20,26] and in laboratory animal models.[49–52] More specifically, mossy cell loss correlates with mossy fiber sprouting in epileptic pilocarpine-treated rats.[47] However, fundamental questions persist. Is mossy cell loss alone sufficient to cause mossy fiber sprouting?[53] What is it about mossy cell loss that facilitates mossy fiber sprouting: granule cell deafferentation, removal of a synaptic target of mossy fibers, or both?[54] Complicating the issue, the most common experimental method used to produce mossy fiber sprouting is status epilepticus, which involves many other potential triggers in addition to mossy cell loss.

Whatever the events are that trigger mossy fiber sprouting, presumably they are transduced to granule cells as molecular cues that activate signaling pathways to coordinate mossy fiber growth and synaptogenesis. c-*Fos* expression was proposed as an early step in the process.[35] However, increased expression of c-*fos* and some other immediate early genes does not appear necessary for mossy fiber sprouting.[55,56] Increased expression of GAP-43, a membrane-bound protein concentrated at growth cones and developing presynaptic terminals, was proposed to promote mossy fiber sprouting.[24,57–60] In addition to GAP-43, tubulins[61] and microtubule-associated proteins[62] could be involved in mossy fiber sprouting. However, in regard to the molecular mechanisms underlying mossy fiber sprouting, brain-derived neurotrophic factor (BDNF)

has received the most attention. Expression of BDNF increases in the dentate gyrus after seizures.[63,64] Brain-derived neurotrophic factor promotes granule cell hypertrophy[65] and mossy fiber branching.[66] When infused in control animals, BDNF causes seizure activity and mild mossy fiber sprouting.[67] In addition, electroconvulsive seizure-induced sprouting is diminished in BDNF heterozygote knockout mice.[38] In contrast, other reports challenge the role of BDNF in mossy fiber sprouting. Mild mossy fiber sprouting develops in slice cultures from homozygote BDNF knockout mice[68] and after kindling in heterozygote BDNF knockout mice.[69] Transgenic overexpression of BDNF does not cause mossy fiber sprouting.[70] The timing of BDNF expression relative to development of mossy fiber sprouting has been questioned.[71] Vaidya et al.[38] reported that BDNF infusion does not cause mossy fiber sprouting. Nevertheless, strong evidence comes from organotypic culture experiments that induced mossy fiber sprouting by application of BDNF or a gamma-aminobutyric acid A ($GABA_A$) receptor antagonist.[72] In those experiments, an L-type calcium channel blocker, a sodium channel blocker, a TrkB inhibitor, a function-blocking anti-BDNF antibody, and transfection with dominant-negative TrkB each reduced mossy fiber sprouting. Thus, it seems likely that BDNF plays a role in mossy fiber sprouting, but the specific signaling pathways and molecular targets remain unclear.

The laminar specificity of sprouted mossy fibers is remarkable. For example, in most hippocampal slice cultures, the outer molecular layer is almost completely denervated but is relatively unstained for mossy fibers, which remain confined to the inner molecular layer.[42] Such specificity suggests strong attractant and/or repulsive extracellular signals. Although several molecular candidates have been proposed—including neural cell adhesion molecules (NCAMs),[73,74] tenascin-C,[75] Sema3A,[76] and hyaluronan[77]—none can fully account for precise targeting by sprouted mossy fibers, which remains an important area for research. In summary, there still is much to learn about the molecular mechanisms involved in transducing triggering stimuli, coordinating mossy fiber growth, and directing mossy fibers to their synaptic targets.

At the cellular level, an increasingly detailed picture of mossy fiber sprouting has emerged. After status epilepticus in rats, ~3 months are required for mossy fiber sprouting to fully develop.[12,78,79] Once fully developed, the proportion of granule cells with sprouted mossy fibers appears to be ~60% in patients with mesial temporal lobe epilepsy[80] and laboratory animal models,[81–84] but more precise estimates are needed. Recent findings suggest that only new adult-generated granule cells sprout mossy fibers into the molecular layer. Granule cell neurogenesis normally continues throughout life. For example, in young adult control rats, the number of new granule cells generated each month is 6% of the total population.[85] Granule cell neurogenesis accelerates after status epilepticus[86] or even milder seizure activity.[87] Although earlier studies questioned the role of newborn granule cells in mossy fiber sprouting,[88,89] later work showed that granule cells born up to 4 weeks before status epilepticus[90] and up to 4 days afterward can develop aberrant mossy fiber projections to the inner molecular layer, whereas older granule cells (born ≥7 weeks before status epilepticus) do not.[91] Identification of newborn granule cells as the source of aberrant mossy fibers is an important advance in our understanding of how mossy fiber sprouting develops, but questions persist. Why do newborn granule cells develop aberrant connections? Are the underlying causes attributable to intrinsic, perhaps epigenetic, changes in granule cells, to extrinsic cues in the microenvironment, or to both? Are the underlying causes transient or permanent—and if permanent, can they be reversed? Do all newborn granule cells form aberrant connections or just a subset? And how long following a precipitating injury will newborn granule cells continue to develop aberrant connections?

Human epileptic tissue displays evidence of ongoing synaptic reorganization years after precipitating injuries and the onset of spontaneous seizures.[24,74,80] Although these results might suggest that mossy fiber sprouting continually progresses and becomes increasingly severe with time, in laboratory animal models, which can be evaluated more extensively and with more temporal resolution, levels of mossy fiber sprouting appear to plateau after 3 months.[12] Together, these findings and the neurogenesis data described above suggest that older sprouted mossy fibers might be replaced by new ones. If aberrant circuits continually turn over, there may be opportunities to

interrupt the pathophysiological process even after robust mossy fiber sprouting develops. Consistent with this notion, after a 45 day delay, during which time some sprouting is likely to have developed, grafts of CA3 pyramidal neurons reduce mossy fiber sprouting following kainate infusion.[79] Furthermore, levels of mild mossy fiber sprouting after experimental electroconvulsive treatment peak and then decrease at later time points, suggesting at least partial reversal.[38] Therefore, despite evidence that mossy fiber sprouting is long-lasting or even permanent,[32,78,92] it might be worthwhile to further test its stability.

At the microcircuit level, in vivo spatial features of mossy fiber sprouting have been evaluated using axon tracers[81] and intracellular labeling.[82] Individual granule cells extend mossy fibers into the inner molecular layer over an area with an average radius of 600 μm, which is comparable to the span of hilar collateral projections in control animals. Plotting the corresponding area on a calibrated, flattened map of the rat dentate gyrus,[93] and using an estimate of 1 million granule cells per rat dentate gyrus,[94] one can predict that ~42,000 granule cells are within reach of one granule cell's sprouted mossy fibers. Integrating axon length per cell with synapse density per axon length suggests that each granule cell that sprouts mossy fibers into the molecular layer forms an average of ~500 new synapses with other granule cells.[95] If one assumes that each new synapse is with a different granule cell and that ~60% of granule cells in epileptic rats sprout mossy fibers, then the probability of finding a monosynaptically coupled granule cell within another cell's reach is 0.7%—which is consistent with experimental results.[96] In addition to mossy fiber projections into the inner molecular layer, granule cells in epileptic animals have greater axon length in the hilus,[82] increased branching of hilar collaterals,[83] and more boutons in the hilus.[81] In the hilus of epileptic animals, therefore, mossy fibers might hyperinnervate surviving neurons, consistent with recent findings from recordings of hilar somatostatin interneurons.[97] Epilepsy-related mossy fiber sprouting also occurs in stratum oriens of CA3,[98] but the present review focuses on the dentate gyrus.

In addition to their normal synaptic targets, in epileptic tissue sprouted mossy fibers form asymmetric (excitatory) synapses with ectopic granule cells in the hilus,[99,100] with granule cell basal dendrites in the hilus,[101,102] with granule cell somata in the granule cell layer,[83,103] and with granule cell apical dendrites in the granule cell layer and inner molecular layer[12,20,101,104–106] (including occasional autapses[83,101]). In the inner molecular layer, ~90% of mossy fiber synapses are with dendritic spines, the remainder with dendritic shafts. Mossy fiber synapses with granule cells are abundant, accounting for ~50% of all inner molecular synapses in epileptic pilocarpine-treated rats.[94,100] Compared to other excitatory synapses in the inner molecular layer, mossy fiber synapses appear to be larger and are more likely to be perforated,[94,105] features that suggest greater synaptic strength.[107,108] Overall, there is considerable anatomical evidence that mossy fiber sprouting creates a positive-feedback circuit among granule cells.

Sprouted mossy fibers also synapse with inhibitory interneurons but much less frequently than with granule cells.[83,105] Some have proposed that sprouted mossy fibers hyperinnervate parvalbumin-immunoreactive basket cells.[109,110] Others have countered that even in control tissue, those interneurons receive high levels of mossy fiber input.[111] If interneurons were hyperinnervated by excitatory synapses after mossy fiber sprouting, one would predict the frequency of spontaneous inhibitory postsynaptic currents in granule cells to be more sensitive to glutamatergic receptor antagonists, which was reported.[112] However, one also would expect that the miniature excitatory postsynaptic current frequency in basket cells—a more direct measure of the number of glutamatergic synapses impinging upon basket cells—would increase as mossy fiber sprouting develops; however, it does not.[113] In vivo biocytin-labeling and three-dimensional reconstruction of serial electron micrographs of sprouted mossy fibers revealed that only ~5% of synapses formed by sprouted mossy fibers in the granule cell layer and molecular layer are with GABA-immunoreactive neurons; the other ~95% are with granule cells.[95] Integrating synaptic density, synaptic target frequency, and sprouted mossy fiber length, these findings suggest that the average granule cell with sprouted mossy fiber collaterals forms 20 times more new synapses with granule cells than with interneurons.

WHAT ARE THE FUNCTIONAL CONSEQUENCES OF MOSSY FIBER SPROUTING?

As expected from the anatomical evidence reviewed above, mossy fiber sprouting creates an aberrant, recurrent, excitatory circuit. Functional positive feedback among granule cells has been demonstrated with varying experimental approaches and degrees of confidence. In animals with mossy fiber sprouting, but not in controls, in vivo perforant path stimulation evokes a delayed current sink in the inner molecular layer[114] and reverberating field potential responses, which are consistent with positive feedback among granule cells.[50,82,115] As in hippocampal slice studies,[116] reverberating responses in vivo become most apparent after inhibition is blocked. A limitation of the in vivo approach, however, is that perforant path stimulation activates many circuits in addition to sprouted mossy fibers. In hippocampal slices, especially with $GABA_A$ receptors at least partially blocked or the extracellular potassium ion concentration elevated, antidromic stimulation of mossy fibers evokes depolarizing synaptic responses in granule cells more frequently after sprouting, consistent with the formation of recurrent excitatory connections.[13,26,116–119] However, even with more focal electrical stimulation in hippocampal slice experiments compared to in vivo studies, axons other than sprouted mossy fibers could be activated, including projections from mossy cells and CA3 pyramidal cells. More specific activation of granule cells with glutamate uncaging or glutamate microdrop application evokes synaptic responses in other granule cells more frequently in slices with mossy fiber sprouting compared to controls.[120–123] Although unlikely, the possibility of polysynaptic activation through surviving mossy cells and CA3 pyramidal cells cannot be excluded completely, even with these methods. Scharfman et al.[96] provided the strongest evidence to date that mossy fiber sprouting creates a functional positive feedback circuit among granule cells. In hippocampal slices from epileptic pilocarpine-treated rats with sprouting, but not in controls, granule cells generate monosynaptic excitatory potentials in other granule cells. The probability of monosynaptic coupling between granule cells after sprouting is 0.66%,[96] which is similar to estimates based on anatomical data (see above) and a level approaching that normally found among CA3 pyramidal cells.[124] The average amplitude of granule cell-to-granule cell synaptic responses is ~2 mV, and their failure rate is 60%–70%,[96,122,125] which is not unusual for excitatory synapses in cortical areas. Granule cell-to-granule cell synapses utilize alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate/kainic acid (AMPA/KA) and to a smaller extent *N*-methyl-D-aspartate (NMDA) receptors.[13,122] Kainate receptors account for an unusually large amount of charge transfer at mossy fiber synapses with granule cells.[126] Granule cell-to-granule cell synapses can be presynaptically blocked by Type II metabotropic glutamate receptors and presynaptically facilitated by kainate receptors, and they display frequency-dependent short-term plasticity intermediate between that of normal mossy fiber synapses with interneurons and CA3 pyramidal cells.[125]

Thus, both anatomical and functional evidence confirms that after mossy fiber sprouting, granule cells receive abnormally high levels of synaptic input from other granule cells, which is minimal in control animals. The frequency of spontaneous excitatory postsynaptic currents (EPSCs) in granule cells increases with mossy fiber sprouting,[121,127] which could be attributable to more excitatory synapses. However, another possibility is increased levels of activity in slices from epileptic animals. Consistent with the latter possibility, the frequency of miniature EPSCs—which depends on the number of synapses and the probability of release but not action potentials—is similar in granule cells before and after mossy fiber sprouting,[126] not increased, as would be expected if granule cells were to receive more synapses after sprouting. A stereological electron microscopy study estimated the number of excitatory synapses with proximal dendrites per granule cell in control rats, in rats 5 days after status epilepticus, and in chronically epileptic animals.[94] Shortly after status epilepticus, which kills many hilar mossy cells, the number of synapses is reduced to <40% of control levels, but weeks later recovers to ~85% of control levels. Sprouted mossy fibers are likely to account for much, if not all, of the recovery. Together, these findings suggest that mossy fiber sprouting nearly replaces but does not exceed the original number of inner molecular layer glutamatergic synapses lost by granule cells during precipitating injuries.

The cellular electrophysiological evidence reviewed above demonstrates effects of mossy fiber sprouting at the synaptic level. However, the most important question about mossy fiber sprouting from a clinical standpoint is whether it is epileptogenic, compensatory, or neither. The extensive literature on this topic will be reviewed, beginning with relevant hypotheses. Shortly after it was discovered in kainate-treated rats, Tauck and Nadler[117] proposed that mossy fiber sprouting creates an aberrant positive feedback network among granule cells that synchronizes their activity and facilitates seizure activity. After many years of accumulating data, Nadler restated the hypothesis and added that "the recurrent mossy fiber pathway promotes seizure propagation from the entorhinal cortex to the hippocampus mainly when granule cells are driven at a frequency appropriate to promote synaptic facilitation" (≥1 Hz).[125] Buhl et al.[112] suggested that sprouted mossy fibers contribute to seizures during periods of high activity but through a different mechanism. They proposed that changes in subunit expression of $GABA_A$ receptors on granule cells in epileptic animals make them vulnerable to negative modulation by zinc. Further, they proposed that during periods of intense activity, granule cells synaptically release zinc from sprouted mossy fibers, which diffuses to GABAergic synapses and reduces inhibition when it is critically needed. Thus, two hypotheses (recurrent excitation and zinc-induced collapse of inhibition) contend that mossy fiber sprouting is epileptogenic. In contrast, Sloviter[128] proposed that sprouted mossy fibers preferentially synapse with basket cells and restore powerful recurrent inhibition lost after injuries kill hilar mossy cells. Recently, Sloviter et al.[110] restated the view that "mossy fiber sprouting may play a clinically important role in retarding seizure spread (keeping subclinical seizures subclinical)." Simmons et al.[127] proposed that the effects of mossy fiber sprouting are mixed. Some of their data support the recurrent excitation hypothesis, but they also proposed that sprouted mossy fibers release opioid peptides that have anticonvulsant effects. Other inhibitory transmitters that could be released by sprouted mossy fibers include neuropeptide Y (NPY)[129] and GABA.[130] Finally, Gloor[131] reviewed the literature on neuron loss in temporal lobe epilepsy, considered evidence of synaptic reorganization, and suggested that mossy fiber sprouting might be an epiphenomenon with neither pro- nor antiepileptic effects. Investigators have worked within the context of these diverging hypotheses. For purposes of review, reports on functional consequences of mossy fiber sprouting are summarized below in three categories: kindling studies, experiments that evaluated evoked seizure-like events, and investigations that measured frequencies of spontaneous seizures in patients with temporal lobe epilepsy and laboratory animal models.

In early kindling studies, hyperexcitability and mossy fiber sprouting were shown to develop in parallel, suggesting that sprouting might be epileptogenic.[34,92] Later studies revealed that hyperexcitability and mossy fiber sprouting can be dissociated.[132,133] But in a broader sense, the implications of kindling studies for the role of mossy fiber sprouting in temporal lobe epileptogenesis might be limited. Animals do not display spontaneous seizures unless kindled very extensively—for example, ~100 times in rats.[134] More typical kindling paradigms that involve only 10–20 stimulations generate levels of mossy fiber sprouting far below those found in many patients with mesial temporal lobe epilepsy and in other laboratory animal models.[41] Instead, kindling might more closely model the mild mossy fiber sprouting found with experimental conditions that kill few, if any, neurons and the mild mossy fiber sprouting found in some patients in whom hippocampal neurons are largely spared. This mild form of mossy fiber sprouting might be an effect, not a cause, of seizure activity.

Another set of studies tested whether evoked seizure-like responses correlate with the extent of mossy fiber sprouting. Simulations run on a computer model of dentate circuitry suggested that mossy fiber sprouting has little effect on granule cell activity and attributed the lack of effect to the granule cells' stabilizing intrinsic physiological properties.[135] However, later, more realistic computer models found that mossy fiber sprouting promotes the spread of seizure-like activity.[136] A strength of the in silico approach is the ability to specifically test individual parameters while leaving other conditions unchanged; these studies showed that mossy fiber sprouting alone is sufficient to cause hyperexcitability in the modeled dentate gyrus.[137] Furthermore, incorporating a small number of highly interconnected granule cells greatly increases network activity.[138]

Whether granule cell network hubs actually exist in epileptic tissue and generate seizures is an intriguing prediction to be tested in future experiments. In addition to computer models, actual epileptic tissue has been evaluated. In organotypic slice cultures, kainate treatment causes mossy fiber sprouting but does not affect seizure activity.[139] In contrast, in experiments with acute slices, evoked seizure-like responses by granule cells are more likely after mossy fiber sprouting develops in patients with mesial temporal lobe epilepsy[140] and in rodent models.[120,121,141,142] In summary, much, but not all, computer modeling and experimental slice data are consistent with the hypothesis that mossy fiber sprouting is epileptogenic. Compared to in vivo experiments, these studies reduced confounding influences from outside structures and more specifically evaluated the dentate gyrus region where sprouting occurs. However, caveats include the unclear relevance of computer models and hippocampal slice preparations to in vivo situations, the common requirement for pro-convulsant conditions (for example, $GABA_A$ receptor antagonists and elevated potassium ion concentrations) to unmask seizure-like responses, and dependency on provoking stimuli, which is unlike typically unprovoked seizures in patients.

Spontaneous seizures can be measured in vivo and compared with the extent of mossy fiber sprouting in laboratory animal models and when tissue is surgically resected to treat patients. Some studies found correlations between the extent of mossy fiber sprouting and seizure frequency,[41,83,143–146] but many did not.[26,28,50–52,54,78,128,147–158] Thus, although a loose association between the development of epilepsy and moderate to intense levels of mossy fiber sprouting is commonly reported (in other words, epileptic animals are more likely to display mossy fiber sprouting than nonepileptic individuals), consistently replicable, statistically significant correlations between seizure frequency and mossy fiber sprouting are lacking. Most in vivo evidence indicated above, therefore, supports the hypothesis that mossy fiber sprouting might be an epiphenomenon without major pro- or antiepileptic effects. However, the seizure monitoring methods used in many previous experiments suffered from limited sampling and might have been statistically underpowered, which increases variability in seizure frequency data, making it more difficult to detect subtle correlations. Another potential source of variability are myriad other parameters that might change independently of mossy fiber sprouting and have confounding effects on seizure frequency. Ideally, to more rigorously test its role in epileptogenesis, one would like to specifically block only the development of mossy fiber sprouting after an epileptogenic injury, carefully monitor the frequency and severity of spontaneous seizures, and compare the results to those in a similarly treated group in which mossy fiber sprouting developed.

Most efforts to block mossy fiber sprouting have been unsuccessful, despite the testing of reasonable candidate mechanisms. One prior attempt neutralized nerve growth factor with antibodies, which suppressed sprouting by cholinergic axons but not mossy fibers.[159] Treatment with the anticonvulsant vigabatrin did not block mossy fiber sprouting when administered to rats after kainate-induced status epilepticus.[160] Blocking neural activity by continuously infusing tetrodotoxin into the dentate gyrus for 1 month after status epilepticus did not suppress mossy fiber sprouting and might have made it worse.[161] It was reported that blocking protein synthesis with cycloheximide at around the time of epileptogenic injury reduced mossy fiber sprouting.[53,162,163] However, cycloheximide pretreatment reduces excitotoxic damage during status epilepticus;[87,89,164] therefore, mossy fiber sprouting may have been reduced indirectly by reducing hilar neuron loss.[165] Furthermore, when administered systemically, as in the original experiments,[166] or infused directly into the dentate gyrus,[167] cycloheximide's effect on mossy fiber sprouting could not be reproduced by other investigators. Therefore, it is doubtful that transiently blocking protein synthesis directly prevents mossy fiber sprouting.

Mossy fiber sprouting presumably begins with formation of growth cones, and previous attempts targeted growth cone function by blocking L-type calcium channels and inhibiting the calcium-activated phosphatase, calcineurin. Systemic administration of the L-type calcium channel blocker nicardipine was reported to suppress mossy fiber sprouting after pilocarpine-induced status epilepticus.[168] Also, the calcineurin inhibitor FK506 was reported to inhibit kindling[169] and block mossy fiber sprouting.[170] However, after 1 month of continuous, direct infusion into the dentate gyrus of nicardipine, FK506, or cyclosporin A

(another calcineurin inhibitor), the extent of mossy fiber sprouting was similar in infused versus noninfused hippocampi of rats that had experienced status epilepticus.[171]

The ketogenic diet was reported to reduce mossy fiber sprouting after kainate-induced status epilepticus,[172] but sample sizes in that study were small and results should be verified. Chronic treatment with oral lithium at therapeutically relevant concentrations was reported to suppress mossy fiber sprouting after pilocarpine-induced status epilepticus.[173] However, in that study, status epilepticus was curtailed early, and the level of excitotoxicity appeared insufficient to produce an adequate baseline level of mossy fiber sprouting for comparison. The NR2B-selective NMDA antagonist (Ro 25,6981) suppressed mossy fiber sprouting in organotypic cultures,[174] but it is unclear whether it would be effective in vivo. Grafting embryonic CA3 pyramidal cells into the hippocampus reduces mossy fiber sprouting after kainate-induced status epilepticus,[79] but grafted neurons sprout axons into the inner molecular layer of the dentate gyrus, suggesting that they might suppress development of one recurrent excitatory circuit (among granule cells) by establishing another aberrant positive feedback circuit in its place (a disynaptic circuit between CA3 pyramidal cells and granule cells).

Recently, a new treatment was discovered that suppresses mossy fiber sprouting. Rapamycin administered systemically[175] or directly infused into the dentate gyrus[176] reduces mossy fiber sprouting after chemoconvulsant-induced status epilepticus in rats. Rapamycin inhibits the mTOR signaling pathway that transduces extracellular signals, including BDNF, to control protein synthesis and cell growth.[177,178] Systemic treatment with rapamycin was reported to suppress both mossy fiber sprouting and seizure frequency in rats.[175] In pilocarpine-treated mice, however, systemic rapamycin suppressed mossy fiber sprouting but did not affect seizure frequency.[179] Possible explanations for the apparently contradictory results include a confounding anticonvulsant effect of rapamycin specifically in rats.[180] In conclusion, the role of mossy fiber sprouting in epileptogenesis remains unclear. As additional methods are discovered to block its development selectively, more opportunities will arise to test its functional effects. In addition to granule cells, epilepsy-related axon reorganization occurs among other excitatory neurons, including CA3 pyramidal cells,[181,182] CA1 pyramidal cells,[183] subicular neurons,[152] and neocortical neurons.[184] Therefore, future lessons learned from continued study of mossy fiber sprouting might have more general relevance for a broad range of patients with epilepsy and other brain disorders that involve synaptic reorganization.

DISCLOSURE STATEMENT

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REFERENCES

1. Ramón y Cajal S. Histology of the nervous system of man and vertebrates. Vol. 2. Swanson N, Swanson LW, trans. New York: Oxford University Press; 1995: 614–625.
2. Lim C, Blume HW, Madsen JR, Saper CB. Connections of the hippocampal formation in humans: I. The mossy fiber pathway. *J Comp Neurol*. 1997;385:325–351.
3. Acsády L, Kamondi A, Sik A, Freund T, Buzsáki G. GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J Neurosci*. 1998;18:3386–3403.
4. Fricke RA, Prince DA. Electrophysiology of dentate gyrus granule cells. *J Neurophysiol*. 1984;51: 195–209.
5. Haug, F-M Š. Light microscopical mapping of the hippocampal region, the piriform cortex and the corticomedial amygdaloid nuclei of the rat with Timm's sulphide silver method. *Z Anat Entwickl-Gesch*. 1974;145:1–27.
6. Laurberg S, Zimmer J. Lesion-induced sprouting of hippocampal mossy fiber collaterals to the fascia dentata in developing and adult rats. *J Comp Neurol*. 1981;200:433–459.
7. Ribak CE, Peterson GM. Intragranular mossy fibers in rats and gerbils form synapses with the somata and proximal dendrites of basket cells in the dentate gyrus. *Hippocampus*. 1991;1:355–364.
8. Wolfer DP, Lipp H-P. Evidence for physiological growth of hippocampal mossy fiber collaterals in the guinea pig during puberty and adulthood. *Hippocampus*. 1995;5:329–340.
9. Cassell MD, Brown MW. The distribution of Timm's stain in the nonsulphide-perfused human hippocampal formation. *J Comp Neurol*. 1984;222:461–471.
10. Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol*. 1989;26: 321–330.
11. Qiao X, Noebels JL. Developmental analysis of hippocampal mossy fiber outgrowth in a mutant mouse with inherited spike-wave seizures. *J Neurosci*. 1993;12:4622–4635.

12. Okazaki MM, Evenson DA, Nadler JV. Hippocampal mossy fiber sprouting and synapse formation after status epilepticus in rats: visualization after retrograde transport of biocytin. *J Comp Neurol*. 1995;352:515–534.
13. Okazaki MM, Molnár P, Nadler JV. Recurrent mossy fiber pathway in rat dentate gyrus: synaptic currents evoked in presence and absence of seizure-induced growth. *J Neurophysiol*. 1999;81:1645–1660.
14. Zimmer J. Changes in the Timm sulfide silver staining pattern of the rat hippocampus and fascia dentata following early postnatal deafferentation. *Brain Res.* 1973;64:313–326.
15. Frotscher M, Zimmer J. Lesion-induced mossy fibers to the molecular layer of the rat fascia dentata: identification of postsynaptic granule cells by the Golgi-EM technique. *J Comp Neurol*. 1983;215:299–311.
16. Scheibel ME, Crandall PH, Scheibel AB. The hippocampal-dentate complex in temporal lobe epilepsy. *Epilepsia*. 1974;15:55–80.
17. Nadler JV, Perry BW, Cotman CW. Selective reinnervation of hippocampal area CA1 and the fascia dentata after destruction of CA3–CA4 afferents with kainic acid. *Brain Res*. 1980;182:1–9.
18. de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res.* 1989;495:387–395.
19. Houser CR, Miyashiro JE, Swartz BE, Walsh GO, Rich JR, Delgado-Escueta AV. Altered patterns of dynorphin immunoreactivity suggest mossy fiber reorganization in human hippocampal epilepsy. *J Neurosci*. 1990;10:267–282.
20. Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF. Synaptic reorganization by mossy fibers in human epileptic fascia dentata. *Neuroscience*. 1991;42:351–363.
21. Engel J Jr, Williamson PD, Wieser HG. Mesial temporal lobe epilepsy. In: Engel J Jr, Pedley TA, eds. *Epilepsy: A Comprehensive Textbook*. Philadelphia: Lippincott-Raven; 1997:2417–2426.
22. Babb TL, Brown WJ. Pathological findings in epilepsy. In: Engel J Jr, ed. *Surgical Treatment of the Epilepsies.* New York: Raven Press; 1987:511–540.
23. Mathern GW, Babb TL, Vickrey BG, Melendez M, Pretorius JK. The clinical-pathogenic mechanisms of hippocampal neuron loss and surgical outcomes in temporal lobe epilepsy. *Brain.* 1995;118:105–118.
24. Proper EA, Oestreicher AB, Jansen GH, Veelen CW, van Rijen PC, Gispen WH, de Graan PNE. Immunohistochemical characterization of mossy fibre sprouting in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. *Brain.* 2000;123:19–30.
25. Mathern GW, Pretorius JK, Babb TL. Quantified patterns of mossy fiber sprouting and neuron densities in hippocampal and lesional seizures. *J Neurosurg*. 1995;82:211–219.
26. Masukawa LM, Uruno K, Sperling M, O'Connor MJ, Burdette LJ. The functional relationship between antidromically evoked field responses of the dentate gyrus and mossy fiber reorganization in temporal lobe epileptic patients. *Brain Res.* 1992;579:119–127.
27. de Lanerolle NC, Kim JH, Williamson A, Spencer SS, Zaveri HP, Eid T, Spencer DD. A retrospective analysis of hippocampal pathology in human temporal lobe epilepsy: evidence for distinctive patient subcategories. *Epilepsia*. 2003;44:677–687.
28. Lynd-Balta E, Pilcher WH, Joseph SA. AMPA receptor alterations precede mossy fiber sprouting in young children with temporal lobe epilepsy. *Neuroscience*. 2004;126:105–114.
29. Mathern GW, Babb TL, Mischel PS, Vinters HV, Pretorius JK, Leite JP, Peacock WJ. Childhood generalized and mesial temporal epilepsies demonstrate different amounts and patterns of hippocampal neuron loss and mossy fibre synaptic reorganization. *Brain*. 1996;119:965–987.
30. Mathern GW, Leite JP, Pretorius JK, Quinn B, Peacock WJ, Babb TL. Children with severe epilepsy: evidence of hippocampal neuron losses and aberrant mossy fiber sprouting during postnatal granule cell migration and differentiation. *Dev Brain Res*. 1994;78:70–80.
31. Ying Z, Babb TL, Hilbig A, Wylie E, Mohamed A, Bingaman W, Prayson R, Staugaitis S, Najm I, Lüders HO. Hippocampal chemical anatomy in pediatric and adolescent patients with hippocampal or extrahippocampal epilepsy. *Dev Neurosci*. 1999;21:236–247.
32. Thom M, Martinian L, Catarino C, Yogarajah M, Koepp MJ, Caboclo L, Sisodiya SM. Bilateral reorganization of the dentate gyrus in hippocampal sclerosis: a postmortem study. *Neurology*. 2009;73:1033–1040.
33. Dowlatshahi D, MacQueen G, Wang J-F, Chen B, Young LF. Increased hippocampal supragranular Timm staining in subjects with bipolar disorder. *NeuroReport.* 2000;11:3775–3778.
34. Sutula T, Xiao-Xian H, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science.* 1988;239:1147–1150.
35. Watanabe Y, Johnson RS, Butler LS, Binder DK, Spiegelman BM, Papaioannou VE, McNamara JO. Null mutation of c-*fos* impairs structural and functional plasticities in the kindling model of epilepsy. *J Neurosci*. 1996;16:3827–3836.
36. Stringer JL, Agarwal KS, Dure LS. Is cell death necessary for hippocampal mossy fiber sprouting? *Epilepsy Res*. 1997;27:67–76.
37. Holmes GL, Gairsa J-L, Chevassus-Au-Lois N, Ben-Ari Y. Consequences of neonatal seizures in the rat: morphological and behavioral effects. *Ann Neurol*. 1998;44:845–857.
38. Vaidya VA, Siuciak JA, Du F, Duman RS. Hippocampal mossy fiber sprouting induced by chronic electroconvulsive seizures. *Neuroscience.* 1999;89:157–166.
39. Bender RA, Dubé C, Gonzalez-Vega R, Mina EW, Baram TZ. Mossy fiber plasticity and enhanced hippocampal excitability, without hippocampal cell loss or altered neurogenesis, in an animal model of prolonged febrile seizures. *Hippocampus.* 2003;13:399–412.
40. Peterson GM, Ribak CE. Hippocampus of the seizure-sensitive gerbil is a specific site for anatomical changes in the GABAergic system. *J Comp Neurol*. 1987;261:405–422.
41. Mathern GW, Bertram EH III, Babb TL, Pretorius JK, Kuhlman PA, Spradlin S, Mendoza D. In contrast to kindled seizures, the frequency of spontaneous epilepsy in the limbic status model correlates with greater aberrant fascia dentate excitatory and inhibitory axon sprouting, and increased staining for *N*-methyl-D-aspartate, AMPA and $GABA_A$ receptors. *Neuroscience*. 1997;77:1003–1019.
42. Routbort MJ, Bausch SB, McNamara JO. Seizures, cell death, and mossy fiber sprouting in kainic acid-treated

organotypic hippocampal cultures. *Neuroscience*. 1999;94:755–765.
43. Zimmer J. Ipsilateral afferents to the commissural zone of the fascia dentate, demonstrated in decommissurated rats by silver impregnation. *J Comp Neurol*. 1971;142:393–416.
44. Berger TW, Semple-Rowland S, Basset JL. Hippocampal polymorph neurons are the cells of origin for ipsilateral association and commissural afferents to the dentate gyrus. *Brain Res.* 1980;215:329–336.
45. Buckmaster PS, Jongen-Rêlo AL. Highly specific neuron loss preserves lateral inhibitory circuits in the dentate gyrus of kainate-induced epileptic rats. *J Neurosci*. 1999;19:9519–9529.
46. Austin JE, Buckmaster PS. Recurrent excitation of granule cells with basal dendrites and low interneuron density and inhibitory postsynaptic current frequency in the dentate gyrus of macaque monkeys. *J Comp Neurol*. 2004;476:205–218.
47. Jiao Y, Nadler JV. Stereological analysis of GluR2-immunoreactive hilar neurons in the pilocarpine model of temporal lobe epilepsy: correlation of cell loss with mossy fiber sprouting. *Exp Neurol*. 2007;205:569–582.
48. Buckmaster PS, Wenzel HJ, Kunkel DD, Schwartzkroin PA. Axon arbors and synaptic connections of hippocampal mossy cells in the rat in vivo. *J Comp Neurol*. 1996;366:270–292.
49. Cavazos JE, Sutula TP. Progressive neuronal loss induced by kindling: a possible mechanism for mossy fiber synaptic reorganization and hippocampal sclerosis. *Brain Res.* 1990;527:1–6.
50. Buckmaster PS, Dudek FE. Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol.* 1997;385:385–404.
51. Gorter JA, van Vliet EA, Aronica E, Lopes da Silva FH. Progression of spontaneous seizures after status epilepticus is associated with mossy fibre sprouting and extensive bilateral loss of hilar parvalbumin and somatostatin-immunoreactive neurons. *Eur J Neurosci*. 2001;13:657–669.
52. Nissinen J, Lukasiuk K, Pitkänen A. Is mossy fiber sprouting present at the time of the first spontaneous seizures in rat experimental temporal lobe epilepsy? *Hippocampus.* 2001;11:299–310.
53. Silva JG, Mello LEAM. The role of mossy cell death and activation of protein synthesis in the sprouting of dentate mossy fibers: evidence from calretinin and neo-Timm staining in pilocarpine-epileptic mice. *Epilepsia.* 2000;41(suppl 6):S18–S23.
54. Rao MS, Hattiangady B, Reddy DS, Shetty AK. Hippocampal neurodegeneration, spontaneous seizures, and mossy fiber sprouting in the F344 rat model of temporal lobe epilepsy. *J Neurosci Res*. 2006;83:1088–1105.
55. Nahm WK, Noebels JL. Nonobligate role of early or sustained expression of immediate-early gene proteins c-*fos*, c-*jun*, and *zif*/268 in hippocampal mossy fiber sprouting. *J Neurosci*. 1998;18:9245–9255.
56. Zheng D, Butler LS, McNamara JO. Kindling and associated mossy fibre sprouting are not affected in mice deficient of *NGFI-A/NGFI-B* genes. *Neuroscience.* 1998;83:251–258.
57. Meberg PJ, Gall CM, Routtenberg A. Induction of F1/GAP-43 gene: expression in hippocampal granule cells after seizures. *Mol Brain Res*. 1993;17:295–297.
58. Bendotti C, Pende M, Sarmanin R. Expression of GAP-43 in the granule cells of rat hippocampus after seizure-induced sprouting of mossy fibres: in situ hybridization and immunocytochemical studies. *Eur J Neurosci*. 1994;6:509–515.
59. Bendotti C, Pende M, Guglielmetti F, Sarmanin R. Cycloheximide inhibits kainic acid-induced GAP-43 mRNA in dentate granule cells in rats. *NeuroReport*. 1996;7:2539–2542.
60. Cantallops I, Routtenberg A. Rapid induction by kainic acid of both axonal growth and F1/GAP-43 protein in the adult rat hippocampal granule cells. *J Comp Neurol*. 1996;366:303–319.
61. Represa A, Pollard H, Moreau J, Ghilini G, Khrestchatisky M, Ben-Ari Y. Mossy fiber sprouting in epileptic rats is associated with a transient increased expression of α-tubulin. *Neurosci Lett*. 1993;156:149–152.
62. Pollard H, Khrestchatisky M, Moreau J, Ben-Ari Y, Represa A. Correlation between reactive sprouting and microtubule protein expression in epileptic hippocampus. *Neuroscience.* 1994;61:773–787.
63. Gall CM. Seizure-induced changes in neurotrophin expression: implications for epilepsy. *Exp Neurol*. 1993;124:150–166.
64. Suzuki F, Junier M-P, Guilhem D, Sørensen J-C, Onteniente B. Morphogenetic effect of kainate on adult hippocampal neurons associated with a prolonged expression of brain-derived neurotrophic factor. *Neuroscience.* 1995;64:665–674.
65. Guilhem D, Dreyfus PA, Makiura Y, Suzuki F, Onteniente B. Short increase of BDNF messenger RNA triggers kainic acid-induced neuronal hypertrophy in adult mice. *Neuroscience.* 1996;72:923–931.
66. Danzer SC, Crooks KRC, Lo DC, McNamara JO. Increased expression of brain-derived neurotrophic factor induces formation of basal dendrites and axonal branching in dentate granule cells in hippocampal explant cultures. *J Neurosci*. 2002;22:9754–9763.
67. Scharfman HE, Goodman JH, Sollas AL, Croll SD. Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. *Exp Neurol.* 2002;174:201–214.
68. Bender R, Heimrich B, Meyer M, Frotscher M. Hippocampal mossy fiber sprouting is not impaired in brain-derived neurotrophic factor-deficient mice. *Exp Brain Res*. 1998;120:399–402.
69. Kokaia M, Ernfors P, Kokaia Z, Elmér E, Jaenisch R, Lindvall O. Suppressed epileptogenesis in BDNF mutant mice. *Exp Neurol*. 1995;133:215–224.
70. Qiao X, Suri C, Knusel B, Noebels JL. Absence of hippocampal mossy fiber sprouting in transgenic mice overexpressing brain-derived neurotrophic factor. *J Neurosci Res*. 2001;64:268–276.
71. Shetty AK, Zaman V, Shetty GA. Hippocampal neurotrophin levels in a kainate model of temporal lobe epilepsy: lack of correlation between brain-derived neurotrophic factor content and progression of aberrant dentate mossy fiber sprouting. *J Neurochem*. 2003;87:147–159.
72. Koyama R, Yamada MK, Fujisawa S, Katoh-Semba R, Matsuki N, Ikegaya Y. Brain-derived neurotrophic factor induces hyperexcitable reentrant circuits in the dentate gyrus. *J Neurosci*. 2004;24:7215–7224.
73. Niquet J, Jorquera I, Ben-Ari Y, Represa A. NCAM immunoreactivity on mossy fibers and reactive astro-

cytes in the hippocampus of epileptic rats. *Brain Res.* 1993;626:106–116.
74. Mikkonen M, Soininen H, Kälviäinen R, Tapiola T, Ylinen A, Vapalahti M, Paljärvi L, Pitkänen A. Remodeling of neuronal circuitries in human temporal lobe epilepsy: increased expression of highly polysialylated neural cell adhesion molecular in the hippocampus and entorhinal cortex. *Ann Neurol*. 1998;44: 923–934.
75. Niquet J, Jorquera I, Faissner A, Ben-Ari Y, Represa A. Gliosis and axonal sprouting in the hippocampus of epileptic rats are associated with an increase of tenascin-C immunoreactivity. *J Neurocytol.* 1995;24:611–624.
76. Holtmaat AJGD, Gorter JA, De Wit J, Tolner EA, Spijker S, Giger RJ, Lopes da Silva FH, Verhaagen J. Transient downregulation of Sema3A mRNA in a rat model for temporal lobe epilepsy. A novel molecular event potentially contributing to mossy fiber sprouting. *Exp Neurol*. 2003;182:142–150.
77. Bausch SZ. Potential roles for hyaluronan and CD44 in kainic acid-induced mossy fiber sprouting in organotypic hippocampal slice cultures. *Neuroscience.* 2006;143:339–350.
78. Mello LEAM, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, Finch DM. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia*. 1993;34:985–995.
79. Shetty AK, Zaman V, Hattiangady B. Repair of the injured adult hippocampus through graft-mediated modulation of the plasticity of the dentate gyrus in a rat model of temporal lobe epilepsy. *J Neurosci*. 2005;25:8391–8401.
80. Isokawa M, Levesque MF, Babb TL, Engel JE Jr. Single mossy fiber axonal systems of human dentate granule cells studied in hippocampal slices from patients with temporal lobe epilepsy. *J Neurosci*. 1993;13:1511–1522.
81. Sutula T, Zhang Z, Lynch M, Sayin U, Golarai G, Rod R. Synaptic and axonal remodeling of mossy fibers in the hilus and supragranular region of the dentate gyrus in kainate-treated rats. *J Comp Neurol*. 1998;390:578–594.
82. Buckmaster PS, Dudek FE. In vivo intracellular analysis of granule cell axon reorganization in epileptic rats. *J Neurophysiol*. 1999;81:712–721.
83. Wenzel HJ, Woolley CS, Robbins CA, Schwartzkroin PA. Kainic acid-induced mossy fiber sprouting and synapse formation in the dentate gyrus of rats. *Hippocampus*. 2000;10:244–260.
84. Kobayashi M, Buckmaster PS. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. *J Neurosci*. 2003;23:2440–2452.
85. Cameron HA, McKay RDG. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J Comp Neurol*. 2001;435:406–417.
86. Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J Neurosci*. 1997;17:3727–3738.
87. Bengzon J, Kokaia Z, Elmér E, Nanobashvili A, Kokaia M, Lindvall O. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *Proc Natl Acad Sci USA*. 1997;94:10432–10437.
88. Parent JM, Tada E, Fike JR, Lowenstein DH. Inhibition of dentate granule cell neurogenesis with brain irradiation does not prevent seizure-induced mossy fiber synaptic reorganization in the rat. *J Neurosci*. 1999;19:4508–4519.
89. Covolan L, Ribeiro LTC, Longo BM, Mello LEAM. Cell damage and neurogenesis in the dentate granule cell layer of adult rats after pilocarpine- or kainate-induced status epilepticus. *Hippocampus*. 2000;10:169–180.
90. Jessberger S, Zhao C, Toni N, Clemenson GD Jr, Li Y, Gage FH. Seizure-associated, aberrant neurogenesis in adult rats characterized with retrovirus-mediated cell labeling. *J Neurosci.* 2007;27:9400–9407.
91. Kron MM, Zhang H, Parent JM. The developmental stage of dentate granule cells dictates their contribution to seizure-induced plasticity. *J Neurosci*. 2010;30:2051–2059.
92. Cavazos JE, Golarai G, Sutula TP. Mossy fiber synaptic reorganization induced by kindling: time course of development, progression, and permanence. *J Neurosci*. 1991;11:2795–2803.
93. Swanson LW, Wyss JM, Cowan WM. An autoradiographic study of the organization of intrahippocampal association pathways in the rat. *J Comp Neurol*. 1978;181:681–716.
94. Thind KK, Yamawaki R, Phanwar I, Zhang G, Wen X, Buckmaster PS. Initial loss but later excess of GABAergic synapses with dentate granule cells in a rat model of temporal lobe epilepsy. *J Comp Neurol*. 2010;518:647–667.
95. Buckmaster PS, Zhang GF, Yamawaki R. Axon sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. *J Neurosci*. 2002;22:6650–6658.
96. Scharfman HE, Sollas AL, Berger RE, Goodman JH. Electrophysiological evidence of monosynaptic excitatory transmission between granule cells after seizure-induced mossy fiber sprouting. *J Neurophysiol*. 2003;90:2536–2547.
97. Halabisky B, Parada I, Buckmaster PS, Prince DA. Excitatory input onto hilar somatostatin interneurons is increased in a chronic model of epilepsy. *J Neurophysiol*. 2010;104:2214–2223.
98. Represa A, Le Gal La Salle G, Ben-Ari Y. Hippocampal plasticity in the kindling model of epilepsy in rats. *Neurosci Lett.* 1989;99:345–350.
99. Dashtipour K, Tran PH, Okazaki MM, Nadler JV, Ribak CE. Ultrastructural features and synaptic connections of hilar ectopic granule cells in the rat dentate gyrus are different from those of granule cells in the granule cell layer. *Brain Res*. 2001;890:261–271.
100. Pierce JP, Melton J, Punsoni M, McCloskey DP, Scharfman HE. Mossy fibers are the primary source of afferent input to ectopic granule cells that are born after pilocarpine-induced seizures. *Exp Neurol.* 2005;196:316–331.
101. Franck JE, Pokorny J, Kunkel DD, Schwartzkroin PA. Physiologic and morphologic characteristics of granule cell circuitry in human epileptic hippocampus. *Epilepsia*. 1995;36:543–558.
102. Ribak CE, Tran PH, Spigelman I, Okazaki MM, Nadler JV. Status epilepticus-induced hilar basal dendrites on rodent granule cells contribute to recurrent excitatory circuitry. *J Comp Neurol*. 2000;428: 240–253.

103. Cavazos JE, Zhang P, Qazi R, Sutula TP. Ultrastructural features of sprouted mossy fiber synapses in kindled and kainic acid-treated rats. *J Comp Neurol*. 2003;458:272–292.
104. Represa A, Jorquera I, Le Gal La Salle G, Ben-Ari Y. Epilepsy induced collateral sprouting of hippocampal mossy fibers: does it induce the development of ectopic synapses with granule cell dendrites? *Hippocampus*. 1993;3:257–268.
105. Zhang N, Houser CR. Ultrastructural localization of dynorphin in the dentate gyrus in human temporal lobe epilepsy: a study of reorganized mossy fiber synapses. *J Comp Neurol*. 1999;405:472–490.
106. Patel LS, Wenzel HJ, Schwartzkroin PA. Physiological and morphological characterization of dentate granule cells in the p35 knock-out mouse hippocampus: evidence for an epileptic circuit. *J Neurosci*. 2004;34: 9005–9014.
107. Nusser Z, Lujan R, Laube G, Roberts JDB, Molnar E, Somogyi P. Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron*. 1998;21:545–559.
108. Ganeshina O, Berry RW, Petralia RS, Nicholson DA, Geinesman Y. Differences in the expression of AMPA and NMDA receptors between axospinous perforated and nonperforated synapses are related to the configuration and size of postsynaptic densities. *J Comp Neurol*. 2004;468:86–95.
109. Kotti T, Riekkinen PJ, Miettinen R. Characterization of target cells for aberrant mossy fiber collaterals in the dentate gyrus of epileptic rat. *Exp Neurol*. 1997;146:323–330.
110. Sloviter RS, Zappone CA, Harvey BD, Frotscher M. Kainic acid-induced recurrent mossy fiber innervation of dentate gyrus inhibitory interneurons: possible anatomical substrate of granule cell hyperinhibition in chronically epileptic rats. *J Comp Neurol*. 2006;494:944–960.
111. Blasco-Ibáñez JM, Martinez-Guijarro FJ, Freund TF. Recurrent mossy fibers preferentially innervate parvalbumin-immunoreactive interneurons in the granule cell layer of the rat dentate gyrus. *NeuroReport*. 2000;11:3219–3225.
112. Buhl EH, Otis TS, Mody I. Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. *Science*. 1996;271:369–373.
113. Zhang W, Buckmaster PS. Dysfunction of the dentate basket cell circuit in a rat model of temporal lobe epilepsy. *J Neurosci*. 2009;29:7846–7856.
114. Golarai G, Sutula TP. Functional alterations in the dentate gyrus after induction of long-term potentiation, kindling, and mossy fiber sprouting. *J Neurophysiol*. 1996;75:343–353.
115. Buckmaster PS, Dudek FE. Network properties of the dentate gyrus in epileptic rats with hilar neuron loss and granule cell axon reorganization. *J Neurophysiol*. 1997;77:2685–2696.
116. Cronin J, Obenaus A, Houser CR, Dudek FE. Electrophysiology of dentate granule cells after kainate-induced synaptic reorganization of the mossy fibers. *Brain Res*. 1992;573:305–310.
117. Tauck DL, Nadler JV. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. *J Neurosci*. 1985;5:1016–1022.
118. Hardison JL, Okazaki MM, Nadler JV. Modest increase in extracellular potassium unmasks effect of recurrent mossy fiber growth. *J Neurophysiol*. 2000;84:2380–2389.
119. Otsu Y, Maru E, Ohata H, Takashima I, Kajiwara R, Iijima T. Optical recording study of granule cell activities in the hippocampal dentate gyrus of kainate-treated rats. *J Neurophysiol*. 2000;83:2421–2430.
120. Wuarin J-P, Dudek FE. Electrographic seizures and new recurrent excitatory circuits in the dentate gyrus of hippocampal slices from kainate-treated epileptic rats. *J Neurosci*. 1996;16:4438–4448.
121. Wuarin J-P, Dudek FE. Excitatory synaptic input to granule cells increases with time after kainate treatment. *J Neurophysiol*. 2001;85:1067–1077.
122. Molnár P, Nadler JV. Mossy fiber-granule cell synapses in the normal and epileptic rat dentate gyrus studied with minimal laser photostimulation. *J Neurophysiol*. 1999;82:1883–1894.
123. Lynch M, Sutula T. Recurrent excitatory connectivity in the dentate gyrus of kindled and kainic acid-treated rats. *J Neurophysiol*. 2000;83:693–704.
124. Miles R, Wong RKS. Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus. *J Physiol*. 1986;373:397–418.
125. Feng L, Molnár P, Nadler JV. Short-term frequency-dependent plasticity at recurrent mossy fiber synapses of the epileptic brain. *J Neurosci*. 2003;23: 5381–5390.
126. Epsztein J, Represa A, Jorquera I, Ben-Ari Y, Crépel V. Recurrent mossy fibers establish aberrant kainate receptor-operated synapses on granule cells from epileptic rats. *J Neurosci*. 2005;25:8229–8239.
127. Simmons ML, Terman GW, Chavkin C. Spontaneous excitatory currents and κ-opioid receptor inhibition in dentate gyrus are increased in the rat pilocarpine model of temporal lobe epilepsy. *J Neurophysiol*. 1997;7:1860–1868.
128. Sloviter RS. Possible functional consequences of synaptic reorganization in the dentate gyrus of kainate-treated rats. *Neurosci Lett*. 1992;137:91–96.
129. Tu B, Timofeeva O, Jiao Y, Nadler JV. Spontaneous release of neuropeptide Y tonically inhibits recurrent mossy fiber synaptic transmission in epileptic brain. *J Neurosci*. 2005;25:1718–1729.
130. Schwarzer C, Sperk G. Hippocampal granule cells express glutamic acid decarboxylase-67 after limbic seizures in the rat. *Neuroscience*. 1995;69:705–709.
131. Gloor P. *The Temporal Lobe and Limbic System*. New York: Oxford University Press; 1997:677–691.
132. Elmér E, Kokaia Z, Kokaia M, Lindvall O, McIntyre DC. Mossy fibre sprouting: evidence against a facilitatory role in epileptogenesis. *NeuroReport*. 1997;8:1193–1196.
133. Armitage LL, Mohapel P, Jenkins EM, Hannesson DK, Corcoran ME. Dissociation between mossy fiber sprouting and rapid kindling with low-frequency stimulation of the amygdala. *Brain Res*. 1998;781:37–44.
134. Sayin U, Osting S, Hagen J, Rutecki P, Sutula T. Spontaneous seizures and loss of axo-axonic and axosomatic inhibition induced by repeated brief seizures in kindled rats. *J Neurosci*. 2003;23:2759–2768.
135. Lytton WW, Hellman KM, Sutula TP. Computer models of hippocampal circuit changes of the kindling model of epilepsy. *Artif Intell Med*. 1998;13:81–97.
136. Santhakumar V, Aradi I, Soltesz I. Role of mossy fiber sprouting and mossy cell loss in hyperexcitability: a network model of the dentate gyrus incorporating

cell types and axonal topography. *J Neurophysiol*. 2005;93:437–453.
137. Dyhrfjeld-Johnsen J, Santhakumar V, Morgan RJ, Huerta R, Tsimring L, Soltesz I. Topological determinants of epileptogenesis in large-scale structural and functional models of the dentate gyrus derived from experimental data. *J Neurophysiol*. 2007;97: 1566–1587.
138. Morgan RJ, Soltesz I. Nonrandom connectivity of the epileptic dentate gyrus predicts a major role for neuronal hubs in seizures. *Proc Natl Acad Sci USA*. 2008;105:6179–6184.
139. Bausch SZ, McNamara JO. Contributions of mossy fiber and CA1 pyramidal cell sprouting to dentate granule cell hyperexcitability in kainic acid-treated hippocampal slice cultures. *J Neurophysiol*. 2004;92: 3582–3595.
140. Gabriel S, Njunting M, Pomper JK, Merschhemke M, Sanabria ERG, Eilers A, Kivi A, Zeller M, Meencke H-J, Cavalheiro EA, Heinemann U, Lehmann T-N. Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. *J Neurosci*. 2004;24:10416–10430.
141. Patrylo PR, Dudek FE. Physiological unmasking of new glutamatergic pathways in the dentate gyrus of hippocampal slices from kainate-induced epileptic rats. *J Neurophysiol*. 1998;79:418–429.
142. Winokur RS, Kubal T, Liu D, Davis SF, Smith BN. Recurrent excitation in the dentate gyrus of a murine model of temporal lobe epilepsy. *Epilepsy Res*. 2004;48:93–105.
143. Lemos T, Cavalheiro EA. Suppression of pilocarpine-induced status epilepticus and the late development of epilepsy in rats. *Exp Brain Res*. 1995;102:423–428.
144. Mathern GW, Cifuentes F, Leite JP, Pretorius JK, Babb TL. Hippocampal EEG excitability and chronic spontaneous seizures are associated with aberrant synaptic reorganization in the rat intrahippocampal kainate model. *EEG Clin Neurophysiol*. 1993;87:326–339.
145. Pitkänen A, Kharatishvili I, Narkilahti S, Lukasiuk K, Nissinen J. Administration of diazepam during status epilepticus reduces development and severity of epilepsy in rat. *Epilepsy Res*. 2005;63:27–42.
146. Kharatishvili I, Nissinen JP, McIntosh TK, Pitkänen A. A model of posttraumatic epilepsy induced by lateral fluid-percussion brain injury in rats. *Neuroscience*. 2006;140:685–697.
147. Cronin J, Dudek FE. Chronic seizures and collateral sprouting of dentate mossy fibers after kainic acid treatment in rats. *Brain Res*. 1988;474:181–184.
148. Timofeeva OA, Peterson GM. Dissociation of mossy fiber sprouting and electrically-induced seizure sensitivity: rapid kindling versus adaptation. *Epilepsy Res*. 1999;33:99–115.
149. Spencer SS, Kim J, de Lanerolle N, Spencer DD. Differential neuronal and glial relations with parameters of ictal discharge in mesial temporal lobe epilepsy. *Epilepsia*. 1999;40:708–712.
150. Pitkänen A, Nissinen J, Lukasiuk K, Jutila L, Paljärvi L, Salmenperä T, Karkola K, Vapalahti M, Ylinen A. Association between the density of mossy fiber sprouting and seizure frequency in experimental and human temporal lobe epilepsy. *Epilepsia*. 2000;41 (suppl 6):S24–S29.
151. Wenzel HJ, Born DE, Dubach MF, Gundersen VM, Maravilla KR, Robbins CA, Szot P, Zierath D, Schwartzkroin PA. Morphological plasticity in an infant monkey model of temporal lobe epilepsy. *Epilepsia*. 2000;41(suppl 6):S70–S75.
152. Lehmann T-N, Gabriel S, Eilers A, Njunting M, Kovacs R, Schulze K, Lanksch WR, Heinemann U. Fluorescent tracer in pilocarpine-treated rats shows widespread aberrant hippocampal neuronal connectivity. *Eur J Neurosci*. 2001;14:83–95.
153. Zhang X, Cui S-S, Wallace AE, Hannesson DK, Schmued LC, Saucier DM, Honer WG, Corcoran ME. Relations between brain pathology and temporal lobe epilepsy. *J Neurosci*. 2002;22:6052–6061.
154. Raol VSH, Budreck EC, Brooks-Kayal AR. Epilepsy after early-life seizures can be independent of hippocampal injury. *Ann Neurol*. 2003;53:503–511.
155. Jung K-H, Chu K, Kim M, Jeong S-W, Song Y-M, Lee S-T, Kim J-Y, Lee SK, Roh J-K. Continuous cytosine-b-D-arabinofuranoside infusion reduces ectopic granule cells in adult rat hippocampus with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. *Eur J Neurosci*. 2004;9:3219–3226.
156. Williams PA, Dou P, Dudek FE. Epilepsy and synaptic reorganization in a perinatal rat model of hypoxia-ischemia. *Epilepsia*. 2004;45:1210–1218.
157. Harvey BD, Sloviter RS. Hippocampal granule cell activity and c-*fos* expression during spontaneous seizures in awake, chronically epileptic, pilocarpine-treated rats: implications for hippocampal epileptogenesis. *J Comp Neurol*. 2005;488:442–463.
158. Kadam SD, Dudek FE. Neuropathological features of a rat model for perinatal hypoxic-ischemic encephalopathy with associated epilepsy. *J Comp Neurol*. 2007;505:716–737.
159. Holtzman DM, Lowenstein DH. Selective inhibition of axon outgrowth by antibodies to NGF in a model of temporal lobe epilepsy. *J Neurosci*. 1995;15: 7062–7070.
160. Pitkänen A, Nissinen J, Jolkkonen E, Tuunanen J, Halonen T. Effects of vigabatrin treatment on status epilepticus-induced neuronal damage and mossy fiber sprouting in the rat hippocampus. *Epilepsy Res*. 1999;33:67–85.
161. Buckmaster PS. Chronic infusion of tetrodotoxin does not block mossy fiber sprouting in pilocarpine-treated rats. *Epilepsia*. 2004;45:452–458.
162. Longo BM, Mello LEAM. Blockade of pilocarpine- or kainate-induced mossy fiber sprouting by cycloheximide does not prevent subsequent epileptogenesis in rats. *Neurosci Lett*. 1997;226:163–166.
163. Longo BM, Mello LEAM. Supragranular mossy fiber sprouting is not necessary for spontaneous seizures in the intrahippocampal kainate model of epilepsy in the rat. *Epilepsy Res*. 1998;32:172–182.
164. Schreiber SS, Tocco G, Najm I, Thompson RF, Baudry M. Cycloheximide prevents kainate-induced neuronal death and c-*fos* expression in adult rat brain. *J Mol Neurosci*. 1993;4:149–159.
165. Longo BM, Covolan L, Chadi G, Mello LEAM. Sprouting of mossy fibers and the vacating of post-synaptic targets in the inner molecular layer of the dentate gyrus. *Exp Neurol*. 2003;181:57–67.
166. Williams PA, Wuarin J-P, Dou P, Ferraro DJ, Dudek FE. Reassessment of the effects of

cycloheximide on mossy fiber sprouting and epileptogenesis in the pilocarpine model of temporal lobe epilepsy. *J Neurophysiol*. 2002;88:2075–2087.
167. Toyoda I, Buckmaster PS. Prolonged infusion of cycloheximide does not block mossy fiber sprouting in a model of temporal lobe epilepsy. *Epilepsia*. 2005;46:1017–1020.
168. Ikegaya Y, Nishiyama N, Matsuki N. L-type Ca^{2+} channel blocker inhibits mossy fiber sprouting and cognitive deficits following pilocarpine seizures in immature mice. *Neuroscience*. 2000;98:647–659.
169. Moia LJMP, Matsui H, de Barros GAM, Tomizawa K, Miyamoto K, Kuwata Y, Tokuda M, Itano T, Hatase O. Immunosuppressants and calcineurin inhibitors, cyclosporin A and FK506, reversibly inhibit epileptogenesis in amygdaloid kindled rat. *Brain Res*. 1994;648:337–341.
170. Moriwaki A, Lu Y-F, Hayashi Y, Tomizawa K, Tokuda M, Itano T, Hatase O, Matsui H. Immunosuppressant FK506 prevents mossy fiber sprouting induced by kindling stimulation. *Neurosci Res.* 1996;25:191–194.
171. Ingram EA, Toyoda I, Wen X, Buckmaster PS. Prolonged infusion of inhibitors of calcineurin or L-type calcium channels does not block mossy fiber sprouting in a model of temporal lobe epilepsy. *Epilepsia*. 2009;50:56–64.
172. Muller-Schwarze AB, Tandon P, Liu Z, Yang Y, Stafstrom CE. Ketogenic diet reduces spontaneous seizures and mossy fiber sprouting in the kainic acid model. *NeuroReport.* 1999;10:1517–1522.
173. Cadotte DW, Xu B, Racine RJ, MacQueen GM, Wang JF, McEwen B, Young LT. Chronic lithium treatment inhibits pilocarpine-induced mossy fiber sprouting in rat hippocampus. *Neuropsychopharmacology.* 2003;28:1448–1453.
174. Wang X-M, Bausch SB. Effects of distinct classes of *N*-methyl-D-aspartate receptor antagonists on seizures, axonal sprouting and neuronal loss in vitro: suppression by NR2B-selective antagonists. *Neuropharmacology.* 2004;47:1008–1020.
175. Zeng L-H, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J Neurosci.* 2009;29:6964–6972.
176. Buckmaster PS, Ingram EA, Wen X. Inhibition of the mammalian target of rapamycin signaling pathway suppresses dentate granule cell axon sprouting in a rodent model of temporal lobe epilepsy. *J Neurosci*. 2009;29:8259–8269.
177. Harris TE, Lawrence JC Jr. TOR signaling. *Sci STKE*. 2003:re15.
178. Swiech L, Perycz M, Malik A, Jaworski J. Role of mTOR in physiology and pathology of the nervous system. *Biochem Biophys Acta*. 2008;1784:116–132.
179. Buckmaster PS, Lew FH. Rapamycin suppresses mossy fiber sprouting but not seizure frequency in a mouse model of temporal lobe epilepsy. *J Neurosci*. 2011;31:2337–2347.
180. Huang X, Zhang H, Yang J, Wu J, McMahon J, Lin Y, Cao Z, Gruenthal M, Huang Y. Pharmacological inhibition of the mammalian target of rapamycin pathway suppresses acquired epilepsy. *Neurobiol Dis.* 2010;40:193–199.
181. McKinney RA, Debanne D, Gähwiler BH, Thompson SM. Lesion-induced axonal sprouting and hyperexcitability in the hippocampus in vitro: implications for the genesis of posttraumatic epilepsy. *Nat Med.* 1997;3:990–996.
182. Siddiqui AH, Joseph SA. CA3 axonal sprouting in kainate-induced chronic epilepsy. *Brain Res.* 2005;1066:129–146.
183. Perez Y, Morin F, Beaulieu C, Lacaille J-C. Axonal sprouting of CA1 pyramidal cells in hyperexcitable hippocampal slices of kainate-treated rats. *Eur J Neurosci*. 1996;8:736–748.
184. Salin P, Tseng G-F, Hoffman S, Parada I, Prince DA. Axonal sprouting in layer V pyramidal neurons of chronically injured cerebral cortex. *J Neurosci*. 1995;15:8234–8245.
185. Represa A, Robain O, Tremblay E, Ben-Ari Y. Hippocampal plasticity in childhood epilepsy. *Neurosci Lett*. 1989;99:351–355.
186. Suzuki F, Makiura Y, Guilhem D, Sørensen J-C, Onteniente B. Correlated axonal sprouting and dendritic spine formation during kainate-induced neuronal morphogenesis in the dentate gyrus of adult mice. *Exp Neurol*. 1997;145:203–213.
187. Thomas AM, Corona-Morales AA, Ferraguti F, Capogna M. Sprouting of mossy fibers and presynaptic inhibition by group II metabotropic glutamate receptors in pilocarpine-treated rat hippocampal slice cultures. *Neuroscience*. 2005;131:303–320.
188. Bernard PB, MacDonald DS, Gill DA, Ryan CL, Tasker RA. Hippocampal mossy fiber sprouting and elevated trkB receptor expression following systemic administration of low dose domoic acid during neonatal development. *Hippocampus*. 2007;17:1121–1133.
189. Ribak CE, Seress L, Weber P, Epstein CM, Henry TR, Bakay RAE. Alumina gel injections into the temporal lobe of rhesus monkeys cause complex partial seizures and morphological changes found in human temporal lobe epilepsy. *J Comp Neurol*. 1998;401:266–290.
190. Gunderson VM, Dubach M, Szot P, Born DE, Wenzel HJ, Maravilla KR, Zierath DK, Robbins CA, Schwartzkroin PA. Development of a model of status epilepticus in pigtailed macaque infant monkeys. *Dev Neurosci*. 1999;21:352–364.
191. Golarai G, Cavazos JE, Sutula TP. Activation of the dentate gyrus by pentylenetetrazol evoked seizures induces mossy fiber synaptic reorganization. *Brain Res*. 1992;593:257–264.
192. Garcia-Cairasco N, Wakamatsu H, Oliveira JAC, Gomes ELT, Del Bel EA, Mello LEAM. Neuroethological and morphological (neo-Timm staining) correlates of limbic recruitment during the development of audiogenic kindling in seizure susceptible Wistar rats. *Epilepsy Res*. 1996;26:177–192.
193. Stanfield BB. Excessive intra- and supragranular mossy fibers in the dentate gyrus of tottering (*tg/tg*) mice. *Brain Res*. 1989;480:294–299.
194. Colling SB, Khana M, Collinge J, Jefferys JG. Mossy fibre reorganization in the hippocampus of prion protein null mice. *Brain Res*. 1997;755:28–35.
195. Amano S, Ikeda M, Uemura S, Fukuoka J, Tsuji A, Sasahara M, Hayase Y, Hazama F. Mossy fiber sprouting in the dentate gyrus in a newly developed epileptic mutant, Ihara epileptic rat. *Brain Res.* 1999;834:214–218.
196. Wenzel HJ, Robbins CA, Tsai L-H, Schwartzkroin PA. Abnormal morphological and functional organization of the hippocampus in a p35 mutant model of cortical

dysplasia associated with spontaneous seizures. *J Neurosci*. 2001;21:983–998.
197. Böhm D, Schwegler H, Kotthaus L, Nayernia K, Rickmann M, Köhler M, Rosenbusch J, Engel W, Flügge G, Burfeind P. Disruption of PLC-β1-mediated signal transduction in mutant mice causes age-dependent hippocampal mossy fiber sprouting and neurodegeneration. *Mol Cell Neurosci*. 2002;21: 584–601.
198. Mitchell TW, Buckmaster PS, Hoover EA, Whalen LR, Dudek FE. Neuron loss and axon reorganization in the dentate gyrus of cats infected with feline immunodeficiency virus. *J Comp Neurol*. 1999;411:563–577.
199. Chen S-F, Huang C-C, Wu H-M, Chen S-H, Liang Y-C, Ksu K-S. Seizure, neuron loss, and mossy fiber sprouting in herpes simplex virus type 1-infected organotypic hippocampal cultures. *Epilepsia*. 2004;45:322–332.
200. Hannesson DK, Armitage LL, Mohapel P, Corcoran ME. Time course of mossy fiber sprouting following bilateral transection of the fimbria/fornix. *NeuroReport*. 1997;8:2299–2303.
201. Coltman BW, Earley EM, Shahar A, Dudek FE, Ide CF. Factors influencing mossy fiber collateral sprouting in organotypic slice cultures of neonatal mouse hippocampus. *J Comp Neurol.* 1995;362:209–222.
202. Onodera H, Aoki H, Yae T, Kogure K. Post-ischemic synaptic plasticity in the rat hippocampus after long-term survival: histochemical and autoradiographic study. *Neuroscience.* 1990;38:125–136.
203. Golarai G, Greenwood AC, Feeney DM, Connor JA. Physiological and structural evidence for hippocampal involvement in persistent seizure susceptibility after traumatic brain injury. *J Neurosci*. 2001;21: 8523–8537.
204. Santhakumar V, Ratzliff ADH, Jeng J, Toth Z, Soltesz I. Long-term hyperexcitability in the hippocampus after experimental head trauma. *Ann Neurol*. 2001;50:708–717.
205. Anderson AE, Hrachoby RA, Antalffy BA, Armstrong DL, Swann JW. A chronic focal epilepsy with mossy fiber sprouting follows recurrent seizures induced by intrahippocampal tetanus toxin injection in infant rats. *Neuroscience*. 1999;92: 73–82.
206. Mitchell J, Gatherer M, Sundstrom LE. Aberrant Timm-stained fibres in the dentate gyrus following tetanus toxin-induced seizures in the rat. *Neuropathol Appl Neurobiol*. 1996;22:129–135.
207. Sandoval MRL, Lebrun I. TsTx toxin isolated from *Tityus serrulatus* scorpion venom induces spontaneous recurrent seizures and mossy fiber sprouting. *Epilepsia*. 2003;44:904–911.

Chapter 33

Kainate and Temporal Lobe Epilepsies

Three Decades of Progress

Yehezkel Ben-Ari

INTRODUCTION

At the end of July 2010, there were 10,168 references under "kainic acid" and 5149 references under "kainic acid AND neurons" in *PubMed*. The first reference to kainate[1] reported the structures of kainate and allokainate. In the next 20 years, attention to kainate was focused on its strong excitatory actions on crayfish muscles and other systems. The first references by the Curtis-Watkins group described the excitatory actions of this new agent that had just been purified from a seaweed known to exert an antiascaris action.[2,3] Kainate—a glutamate analogue—is a stronger excitant than other amino acids, and pharmacological

observations pointed to its unique structure/actions features that suggested the presence of a specific receptor, an observation that was rapidly confirmed. The identification of kainate-specific receptors stimulated a flurry of studies on the excitatory actions of kainate that were soon to be viewed as excitotoxic when Olney, Nadler, and colleagues discovered that kainate in fact selectively destroys various neuronal populations.[4–7] Their finding was the starting point for two parallel lines of research focused on the following questions: Why does kainate kill neurons selectively? How can this action be used as a tool to reproduce animal models of neurological disorders?

During the late 1970s and early 1980s, extensive investigation showed that kainate excites a large number of neuronal populations where receptors are present; this excitation is followed by selective neuronal loss at the site of injection, while the axons en passant are spared. Given this finding, McGeers, Schwartz and Coyle, and other investigators produced several animal models of neurological disorders, relying on the axon-sparing excitotoxic actions of kainate to model Parkinson's disease and Huntington's disease.[8–12] The technique was also used to selectively destroy cell bodies (i.e., spare axons), thus providing a method to trace neuronal connections in the brain and to determine the selective sequelae produced by neuron-specific lesions of a brain structure without altering en passant fibers. Thus, the first phase of kainate research exploited the lesioning capabilities of kainate to delete specific neuronal populations and determine the consequences of this loss on brain operation.

In 1978, during an investigation aimed at using the axon-sparing effects of kainate in the amygdala, Ben-Ari and Lagowska discovered the epileptogenic actions of amygdaloid injections of kainate.[13] Injections of low concentrations of kainic acid in the amygdala generated a status epilepticus that persisted for several hours, often until the animal died. Injections of benzodiazepine were used to interrupt the seizures, providing an animal model of limbic status epilepticus in which the mechanisms underlying the generation of a status and its effects on brain operation could be investigated.[14] This publication, in turn, led to a flurry of studies; in three decades, over 2495 references to "kainate AND epilepsies" appeared in *PubMed* (July 2010). Extensive investigations used injections of kainate parenterally and intracerebrally to test the properties of epileptogenic neurons and networks. These studies have provided a wide range of advances in our understanding of the mechanisms of seizure generation, propagation, and their sequelae, with the emergence of concepts extending from apoptotic cell death to reactive plasticity and sprouting of mossy fibres in temporal lobe epilepsy (TLE) to the selective loss of GABAergic neurons[15] (see Fig. 33–1). These concepts were subsequently confirmed with other animal models of TLE, notably pilocarpine[16] and electrical brain stimulation.[17] Parallel studies led to the discovery of kainate high-affinity receptors in the brain having a specific distribution—notably in regions that are known to play a central role in seizures, particularly in the hippocampus.[18–20] With the development of molecular biology and genetic tools, kainate receptors were cloned, and shown to belong to the glutamate receptor family, with several subunits conferring unique properties; these characterizations provided clear examples of the intricate links between biologically active natural molecules and recognition signals in the brain for these molecules.[21] This insight was further expanded by the development of relatively specific antagonists that block alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) but not kainate receptor-mediated currents (see below). Investigators were then able to identify centrally active kainatergic synapses that, when activated by glutamate, generate synaptic currents. Compelling evidence that kainate plays a role in central transmission was obtained in studies showing spontaneous kainatergic excitatory postsynaptic currents (EPSCs) and miniature kainatergic PSCs with electrical properties that differ from those of the predominant fast-acting AMPA receptor-mediated PSCs generated by glutamate. Glutamate activates a series of receptors, with kainate receptors playing a specific role different from those of *N*-methyl-D-aspartate (NMDA) and AMPA receptors. This finding, in turn, raised formidable questions: Why do certain neuronal populations use these long-lasting PSCs mediated through kainatergic receptors? Why are these receptors enriched in regions known to be highly vulnerable to seizures and to be destroyed in patients suffering from TLE? The evolution of an en passant discovery of the destructive properties

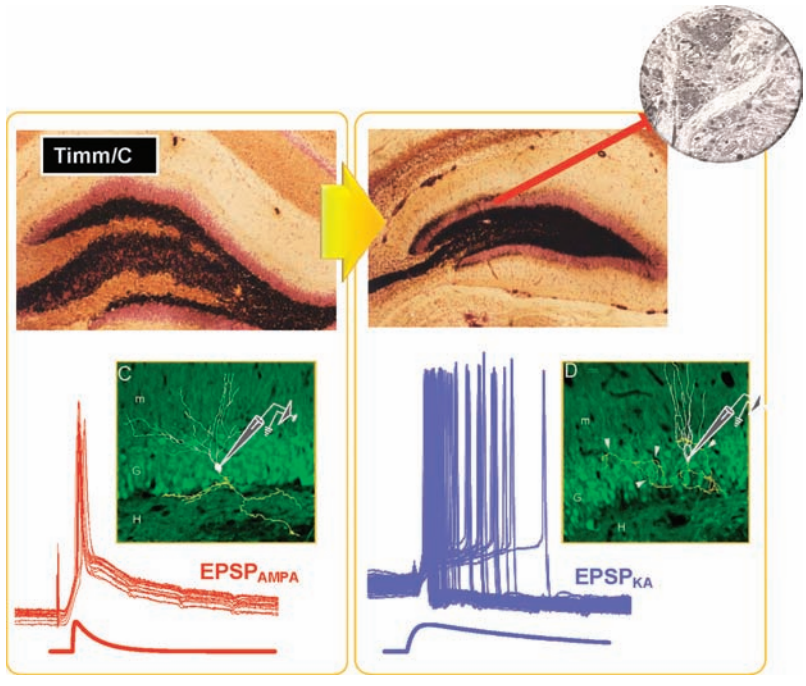

Figure 33–1. Sprouting of mossy fibers and formation of novel synapses that alter the operation of evoked synaptic currents. Sprouting of mossy fibers depicted by the typical Timm stain in the supragranular layer and the thorny excrescences of the mossy fibers in an electron micrograph. Bottom left: In a naive granule cell reconstructed to show its restricted arbor outside the granule layer, electrical stimuli evoke an EPSP mediated exclusively by AMPA receptors ($EPSP_{AMPA}$) associated with a time-locked response. Bottom right: In contrast, in a granule cell recorded from an epileptic hippocampus—induced several weeks earlier by pilocarpine—the same protocol evokes an EPSP mediated by a kainate receptor ($EPSP_{KA}$) associated with a variable-latency jitter response. Arrowhead depict the differences between naive (left) and epileptic (right) hippocampi. The microelectrodes illustrate granule cells recorded, filed with dyes and reconstructed to show the extensive sprouting of axons within the granule layer in epileptic but not in naive hippocampi. From refs. 178 and 186.

of a biologically active molecule to an endogenous selective set of synapses employing these receptors to carry out physiological functions stands at the core of present kainate research. This discovery has provided important insights into brain operation and will undoubtedly also lead to novel therapeutic strategies for TLE.

SEIZURES AND BRAIN DAMAGE PRODUCED BY KAINATE IN THE ADULT BRAIN

The Kainate Model of TLE

From the late 1970s, studies revealed that intra-amygdaloid, intrahippocampal, intracerebroventricular, or systemic injections of kainate generates a syndrome of seizures and brain damage that mimics human TLE (reviewed in refs. 15 and 22–28). Injections of kainic acid, either peripherally or centrally in various brain structures, generate seizures and brain damage syndromes in which limbic structures play key roles. Clinically, after a variable delay, animals display wet dog shakes, facial motor signs and chewing, paw tremor associated with rearing and falling, and other complex motor manifestations. Electroencephalographic (EEG) and depth recordings reveal recurrent tonic-clonic severe ictal discharges and a long-lasting status epilepticus that are reminiscent of those observed in TLE. Electroencephalographic recordings established that the seizures are initiated in regions that are highly excitable and central to TLE, notably the hippocampal CA3 region and the amygdala. The description of the propagation of seizures discharges has been repeatedly shown to include triggering zones—notably within the hippocampal CA3 region and various amygdaloid and adjacent pyriform

and entorhinal cortex regions—that appear to have among the lowest thresholds for seizure generation in the rat brain. The anatomical pathways that enable seizures to express motor manifestations are consistent with classical axonal pathways that interconnect limbic structures and their projection targets. Thus, facial signs are activated when the seizure activity in amygdala propagates to its brainstem targets, as defined by extensive human and animal investigations. Studies using 2-deoxyglucose metabolic methods, as well as a wide range of other imaging and recording techniques, have confirmed the general pattern of structures involved and the crucial role of limbic structures. In addition, following kainate-induced status epilepticus, there is a silent period followed by ongoing chronic seizure activity; although the initial status is not often observed in humans, this pattern of seizure development in animal models has been useful for dissecting out the mechanisms underlying cell death and chronic epilepsies. The kainate and pilocarpine models of TLE have been extensively used for such investigations. Other closely related models include kindling, in which daily electrical stimuli (usually of the amygdala) transforms a naive structure into one that seizes upon stimulation.[29,30] The pros and cons of these models have been reviewed and discussed extensively. The goal here is to stress that they provide, collectively, useful contexts in which to study mechanisms of epileptogenesis in limbic structures.[31–33] The clinical and pathological effects of kainate are reminiscent of those observed in partial TLEs.[15,26,30,32]

Seizure-Specific and Nonspecific Cell Loss Produced by Kainate

Administration of kainate produces a seizure and brain damage syndrome. Following recurrent seizures, there is a pattern of cell loss primarily—but not solely—in limbic structures including the hippocampus, amygdala, and pyriform cortex,[15,34] Within the hippocampus, there is a gradient of vulnerability, with CA3 pyramidal neurons being particularly vulnerable, as cell loss can be produced with low doses (that may be truly homeopathic concentrations) of kainate applied locally or at distant sites,[15,23] These observations raised considerable interest in determining the mechanisms underlying cell loss. One question was whether the damage is due to the direct actions of kainate (i.e., directly exciting the cell, increasing calcium influx, and initiating a cascade of events that have been extensively investigated in relation to neurotoxicity[4,7]). Two types of cell loss have been identified on the basis of morphological and chemical studies: apoptotic—or programmed—cell loss and necrosis (i.e., swelling followed by cell death[35,36]). It is most likely that the mechanisms underlying cell death in the kainate model (as in other models, such as ischemic cell loss[35]) depend on the severity of seizures and the concentrations of kainate injected; small doses appear to produce preferentially apoptosis, and higher doses produce necrotic cell damage. In keeping with this scheme, the regional susceptibility of hippocampal neurons in seizure models parallels the pattern of vulnerability to ischemic insults.

These observations raise yet another important question: Does kainate induce cell loss because of its direct actions or because of the seizures it generates? The following observations suggest that seizures generated by kainate can induce selective secondary damage due to seizures per se: (1) following intra-amygdaloid injections of kainate, injections of valium block seizures and prevent distal hippocampal damage but not local cell loss in the amygdala[14,37]; (2) quantitative measures of the severity of intrahippocampal electrographic seizures revealed a strong relation between the severity of paroxysmal events (including the duration of ictal events and postictal depression) and subsequent damage[38]; (3) damage in the hippocampus is prevented by lesions of mossy fiber synapses on CA3 neurons, confirming the essential role of propagated activity in these synapses in the high degree of vulnerability of target neurons to seizures[39–42]; (4) other types of limbic seizures, produced by pilocarpine or by long-lasting recurrent electrical stimulation of the fascia dentate, also trigger cell death with a similar pattern of vulnerability[16,43]; (5) direct local determination of blood flow, oxygen consumption, and partial pressure of carbon dioxide (PCO_2) showed that during recurrent seizures, the increase in blood flow overcompensates for the enhanced oxygen consumption, suggesting that the damage is not due to a metabolic failure.[44] Therefore, recurrent seizures can directly produce cell loss in vulnerable neurons.

Loss of GABAergic Interneurons and Failure of Inhibition

Because gamma-aminobutyric acid (GABA)-mediated inhibition controls the excitability of principal neurons and networks, failure of inhibition has long been favored to explain seizure generation and propagation. In keeping with this hypothesis, GABA receptor antagonists generate seizures and agents thought to specifically reinforce the GABAergic drive often reduce seizures and are used extensively as antiepileptic agents. However, there is a large variety of GABAergic populations, each of which innervates specific targets controlling highly specific cellular and network features.[45–47] By means of their cell- or domain-specific targets, activation of GABAergic synapses will differently alter neuronal and network excitability.[45–47] For example, some GABAergic neurons innervate the axon hillock of projection cells where spikes are generated; the release of GABA at these synapses alters the generation of action potentials by a large population of pyramidal neurons.[48–50] Other GABAergic neurons innervate the dendrites of pyramidal neurons; oriens lacunosum moleculare (O-LM) interneurons release GABA that would be expected to reduces the excitatory drive that impinges on pyramidal neurons[48] (see Fig. 33–2). The former synapses will entrain large ensembles of principal neurons in coordinated patterns, whereas the latter will more locally alter the responses of these neurones to inputs. GABAergic neurons also exert their inhibitory actions by a variety of different mechanisms, including a shunting action and an intracellular accumulation of chloride that tends to shift the GABA reversal potential (E_{GABA}), which results in an excitatory action of GABA (see below).

Experiments using kainate and/or pilocarpine illustrate the multiple facets of the suggested failure of inhibition and its implications in seizures and epilepsies. A decrease in the number of glutamic acid decarboxylase (GAD) mRNA-containing neurons has been observed following pilocarpine seizures primarily in interneurons labeled for somatostatin only (O-LM and bistratified cells).[17,51] Electron microscopic observations suggested that the loss of somatostatin-containing neurons

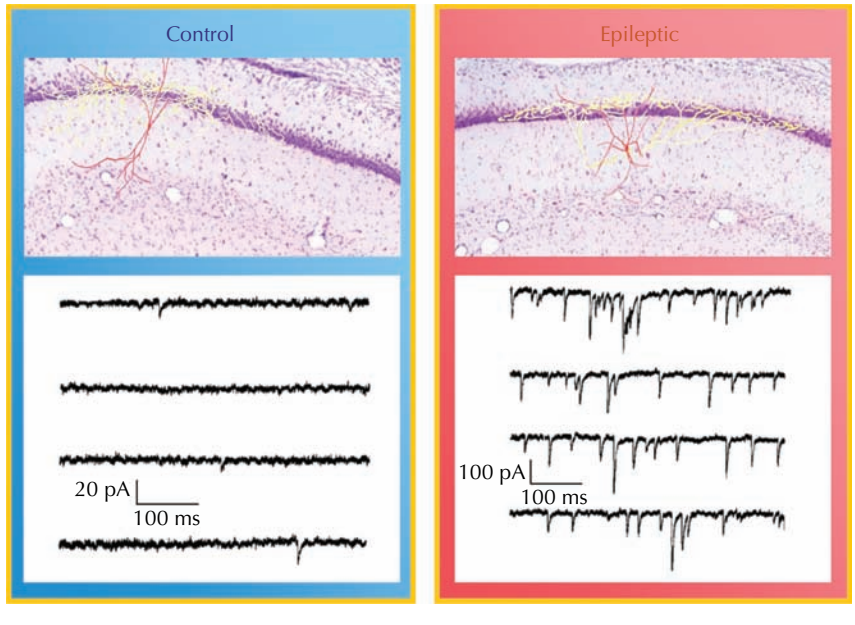

Figure 33–2. Somatic projecting GABAergic interneurons are more active in the hippocampus of epileptic animals. Recurrent seizures were generated by pilocarpine injections and weeks later, once the reactive reaction had taken place, slices were recorded from the sclerotic hippocampus. Many somatostatin-positive GABAergic neurons were lost. Surviving interneurons were patch-clamp recorded and morphologically reconstructed for identification. Interneurons in TLE had a higher frequency of ongoing synaptic currents suggesting enhanced activity in synaptic inputs. This, in turn, compensated for the loss and preserved some somatic inhibition. From ref. 186.

corresponds preferentially to the degeneration of interneurons with an axon projecting to stratum lacunosum-moleculare (O-LM cells).[51] In contrast, stratum oriens interneurons labeled for parvalbumin were preserved, suggesting that somatic inhibition is maintained, whereas dendritic inhibition is not. In keeping with this, direct recording from the somata and dendrites of principal neurons using the same model[52] revealed a marked reduction of GABAergic inhibition in the dendrites, whereas somatic inhibition was preserved.[52,53] Also, the activity of basket cells that were not destroyed by the seizures[52–54] was enhanced, thereby compensating for the loss of other interneurons. These observations collectively suggest that the glutamatergic inputs to the dendrites of principal cells will be less inhibited in epileptic neurons, whereas somatic inhibition will not. Other mechanisms affecting the strength of inhibition have been suggested, including notably (1) a reduction of miniature GABAergic postsynaptic currents (mIPSCs), suggesting decreased release of GABA from presynaptic elements,[53] and (2) postsynaptic reduction of the efficacy of zinc,[55–57] NMDA receptors, and Ca^{2+} signals,[58] and the density of GABAergic synapse subunit composition.[59,60]

The suggestion that inhibition is not reduced per se, but rather that there is a loss of the excitatory drive onto basket cells following a seizure (an induced denervation of interneurons—the so-called *dormant basket cell*[61]), has been confirmed by pair recordings from interneurons and their target pyramidal neurons in TLE hippocampi. These studies showed that GABAergic inhibition is operative.[54] It bears stressing that alterations of GABAergic inhibition are area and cell specific (but also see refs. 62 and 63). Therefore, some (but not all) facets of GABAergic actions are reduced in TLE, most likely explaining why seizures are not continuously generated by the sclerotic hippocampus.

Physiological Actions of Kainate on Hippocampal Neurons

One important question is whether kainate exerts it actions through specific kainate receptors. Direct recordings from limbic neurons have revealed that the hippocampus, and particularly CA3 neurons, are strongly excited by kainate. Submicromolar applications of kainate excite CA3 pyramidal neurons,[64–66] generating large currents and triggering action potentials. Kainate-induced currents are also observed in the presence of tetrodotoxin (TTX; refs. 64–68). At higher concentrations, kainate also activates AMPA receptors.[25] In the presence of specific AMPA and NMDA receptor blockers, kainate depolarizes rat and mouse hippocampal interneurons.[69–71]

How is this excitatory drive generated? A large repertoire of actions underlies the excitatory actions of kainate. Kainate strongly reduces evoked GABAergic PSCs,[71–74] an effect that would augment excitability. However, small submicromolar concentrations of kainate augment spontaneous inhibitory postsynaptic currents (IPSCs) in interneurons[75] as well as GABAergic mIPSPs in CAI interneurons (also see ref. 74), suggesting presynaptic regulation by kainate receptors of GABA release that increases the efficacy of GABAergic transmission between interneurons. In keeping with this, in paired recordings, low concentrations of kainate (300 nM) reduced the failures and increase the occurrence of postsynaptic responses[76] but higher concentrations (5 μM) depressed IPSCs, confirming the dose dependence of the dual actions of kainate.

Kainate also acts on voltage-gated currents. It reduces the slow hyperpolarizing K^+ current,[77,78] effects that are mediated by intracellular second messengers.[79–81] These effects can be induced with nanomolar concentrations of kainate, suggesting that they are mediated by the high-affinity receptors observed on these neurons (see below). This strong excitatory action mediated by high-affinity kainate receptors plays an important role in the epileptogenic actions of kainate (also see ref. 82).

The Yin and the Yang of Kainate Receptors: Molecular Considerations

The advent of novel genetic tools has enabled investigators to distinguish between different subtypes of glutamate receptors, including kainate receptors. Kainate receptors are

composed of various combinations of five subunits: GluR5, GluR6, GluR7, KA1, and KA2.[21,83–85] In studies of hyperexcitability and epilepsy, the GluR6 and GluR5 subtypes of kainate receptors have been of particular interest. The former has been thought to be related to limbic epilepsies because of its distribution, especially in the vulnerable CA3 pyramidal neurons.[86–88] In addition, GluR6 knockouts have greatly reduced vulnerability to seizures and to kainite-induced injury.[39,68,89] In contrast, GluR5 subunits are mostly expressed by certain types of interneurons, notably somatostatin interneurons that are enriched within stratum oriens; in these cells, the activation of kainate receptors leads to a dramatic increase in cell activity and the consequent inhibitory drive onto target pyramidal cells[69,70,90] (see Fig. 33–3). Given these data, it appears that kainate may exert a dual action: it may have pro-convulsive effects in principal neurons due to the activation of GluR6 receptor subtypes and anticonvulsive effects due to the activation of GluR5 subunits in interneurons. The strong excitation by GluR5 receptor agonists could explain the vulnerability of interneurons to seizures, as this action will dramatically excite these neurons. Additional subunit-specific actions have been reported and reviewed extensively elsewhere.[25,91,92]

SEIZURES BUT NO BRAIN DAMAGE PRODUCED BY KAINATE IN PUPS

Maturation of the Kainate System

The syndrome of seizures and brain damage produced by kainate is age dependent. Injections of kainate into immature pups generate seizures, but these seizures are not followed by cell loss.[93–97] Conspicuous damage is first seen at the end of the second postnatal week; before then, even high doses of systemic kainate (that generate seizures) fail to produce cell damage.[93–97] The clinical reflection of kainite-induced seizures is quite restricted initially—little or no facial movement or other manifestations that implicate the activation of the amygdaloid complex and its projections to the brainstem—suggesting that the critical network is not yet mature. This assumption was confirmed by direct recordings and by 2-deoxyglucose studies showing that kainate-induced seizures are initially local, with no sign of propagation to

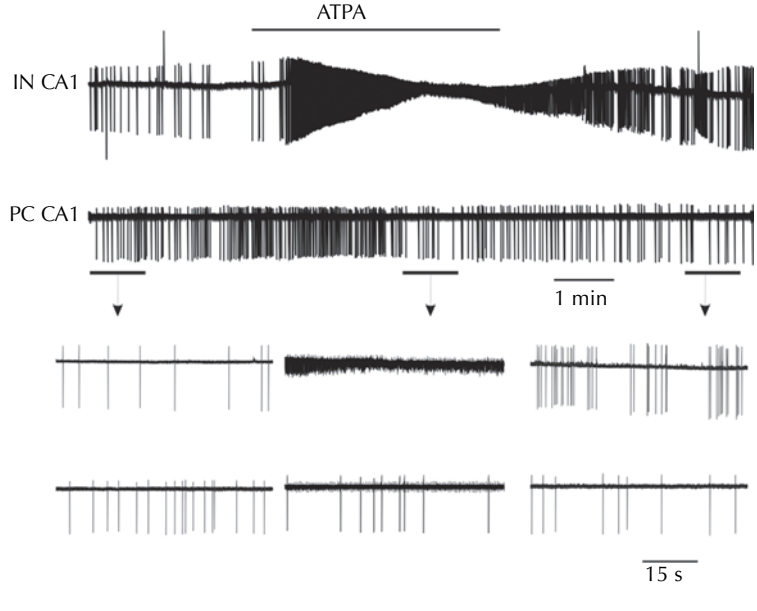

Figure 33–3. Dual actions of kainate. The GluR5 agonist (ATPA) excited interneurons, thereby inhibiting the pyramidal neurons. A pyramidal neuron and an interneuron were recorded simultaneously, and the GluR5-selective agonist was applied. Note the dramatically increased activity of the CA1 interneuron (top) and the reduced activity of the pyramidal neuron (bottom). From ref. 90.

the entire limbic system.[93–96] This time frame correlates with the progressive and protracted maturation of the fascia dentate-mossy fiber system that extends to the third week of age at least in rodents.[98] Consistent with this finding, lesions of the mossy fibers (produced by irradiation or other means) also protect CA3 pyramidal neurons from kainate-induced damage (see below). In addition to reduced hippocampal cell loss following status epilepticus, there is less mossy fiber sprouting in young animals than in adult animals, suggesting that the neuronal damage and subsequent reactive plasticity are correlated.[99,100] Interestingly, cell loss is also less readily produced in neonatal neurons in other experimental models of TLE, suggesting that the full maturation of networks and connections is indeed required for the cell loss to be produced.[101]

We do not fully comprehend why immature neurons are more resistant than those of adults to insults and epilepsies. This is likely a general phenomenon, as suggested by the observation that immature neurons are also much more resistant to anoxic insults[102,103] and have reduced pro-inflammatory cytokines associated with seizures.[104] The reduced sensitivity to glutamate[105–108] and reduced oxidative stress compared to adult seizures[109] may be pertinent in this context. Also, GABA synthesis is better preserved during status epilepticus in neonatal neurons than in those of adults.[110] Therefore, although this reduced vulnerability is not restricted to the maturation of the kainate system, it illustrates its general pertinence to epilepsies of the developing brain.

Recurrent Seizures in the Immature Brain Produce Long-Term Effects

Although seizures in pups produce a less severe syndrome and no cell loss until the end of the second week, they are not harmless. Extensive human and experimental data suggest that seizures early in life can lead to lifelong severe, intractable neurological disorders, a result of particular concern since infants and children are at high risk for seizures compared with adults.[111,112] Although most seizures in children are benign and result in no long-term consequences, experimental animal data strongly suggest that frequent or prolonged seizures in the developing brain result in long-lasting sequelae.[113–115] Behavioral effects of acute seizures or status epilepticus are also associated with the age of the animal, adult animals having substantial deficits in learning, memory, and behavior.[113–115]

Recurrent seizures in infants and children can be harmful, causing long-lasting sequel.[116,117] Similarly, recurrent seizures in pups can cause long-term behavioral and physiological alterations. Neonatal seizures induced by the inhalant fluothyl produce impairment of visual memory without any discernible cell loss.[118] This study also shows that after recurrent seizures, the number of newly formed granule cells is reduced in neonates but increased in adults. Since recurrent seizures do not readily cause cell loss and extensive damage, the long-term deficits must be due to other mechanisms.

What do these observations suggest, and how can they be reconciled and unified in a coherent theory? Can these seemingly diverse observations be related to a similar feature of developing neurons? If immature neurons are more resistant to insults in terms of cell loss but are still affected by early insults, as reflected by the long-term consequences these produce, then it is reasonable to suggest that the insults are programmatic rather than lesional and are due to alterations of developmental programs. I have recently introduced the *neuroarcheology* concept of presymptomatic electrical or morphological signatures of the developing brain that has experienced seizures or other insults.[119] The suggestion is that recurrent seizures—or other insults—produce early malformations that lead to an ensemble of misplaced and/or misconnected neurons but also alter the properties of adjacent neurons that have already reached their assigned region and developed as programmed their synaptic connections. The former remain with immature features in the adult brain, including high input resistance and a strong tendency to oscillate and generate intrinsic activities that can entrain other neurons to seizures. The latter, notably in the neocortex, are also affected and show a reexpression of immature features—notably excitatory actions of GABA.[120–127] This postinsult recapitulation of ontogenesis[119–128] has important implications, since the determination of the properties of neurons in the sclerotic hippocampus will help guide the development of suitable therapeutic agents. Although this issue deserves a great deal of description and debate,

it is important to keep in mind the major differences between immature and adult mechanisms that connect an insult and the subsequent expression of epilepsies.

ROLE OF KAINATERGIC SYNAPSES IN SEIZURE-INDUCED EPILEPTOGENESIS

One of the most salient and specific properties of neurons and synapses is their plasticity. In fact, almost all the procedures and mechanisms that take place during the operation of brain networks are altered by incoming information and depend on the magnitude of neuronal activity that impinges on its synapses. Activity-dependent alterations of synapse operation are classically observed with recurrent stimuli such as in long term potentiation (LTP), considered a cellular model of memory processes (at least with respect to cellular mechanisms and signaling pathways). Activity-dependent alterations of almost all of the steps in brain maturation have been reported, including neuronal proliferation and migration, synapse formation, network construction, and sequential development of voltage- and synapse-mediated currents.

This plasticity is not restricted to development and integration of activity in the adult brain. Following a wide range of insults—including traumatic brain injury, sensory deprivation or brain lesions, and cell loss—there is considerable synaptic reorganization, with formation of novel synapses and changes in the properties of the involved cortical regions. This plasticity has been demonstrated after visual impairments, limb deafferentation, and central insults; cortical regions are invaded by elements from adjacent structures, with neuronal sprouting and formation of novel aberrant synaptic connections in sites where they are not present normally. There is little doubt that recurrent severe seizures trigger a similar cascade of events, and the history and recurrence of seizures cannot be obliterated as if the environment is not affected. In keeping with this, seizures—notably TLE—are associated with cell loss and degeneration of a substantial part of the hippocampus and other limbic structures that are known to engage in various forms of reactive plasticity.[129,130] For over two decades, there has been direct evidence that at least in TLE in humans and in animal models, there is considerable sprouting of fibers and establishment of novel synapses that may, in turn, lead to enhanced excitability and thus contribute to the generation of further seizures. In this domain, studies using kainate have provided major breakthroughs in understanding the alterations produced by insults.

The Mossy Fibers: An Ideal Location to Look for Changes

The story starts with the mossy fibers, which are a helpful target in studies to determine the long-term changes produced by seizures. The mossy fibers constitute the main and sole output of the granule neurons of the fascia dentate—a major gate to the hippocampus through which an inflow of information that originates in the entorhinal cortex reaches Ammon's horn and modulates its operation[98,131,132] (see Fig. 33–1). Mossy fibers innervate a wide range of interneurons, but their most visible and most widely investigated targets are the giant CA3 pyramidal neurons. Mossy fibers establish their synapses with the proximal apical dendrites of pyramidal neurons—within the stratum lucidum, which, in most rodents, is located immediately above the pyramidal layer. In primates and humans, the region innervated is interspersed within the pyramidal layer, in part because the pyramidal neurons are not aligned as tightly as in rodents. Yet, in all species, the mossy fiber terminals and innervation zones are readily visible because mossy fiber terminals are very large and enriched in zinc; this property enables investigators to visualize the terminals with simple histological techniques.[133,134] Mossy fibers accumulate and release zinc in very large amounts, although the role of zinc is not fully understood.[135]

Mossy Fibers Have an Intimate Relation with Kainate Signaling

In the early period of receptor identification, autoradiographic investigations were used to determine the distribution of subtypes of receptors. Several observations have shown

that KAR-mediated synaptic transmission in hippocampus is strongly linked to the presence of mossy fiber terminals: (1) the stratum lucidum (the target zone of mossy fibers on CA3 pyramidal neurons) contains among the highest density of KARs in the brain[18]; (2) the stimulation of mossy fibers selectively generates $EPSC_{KA}$ in CA3 pyramidal cells[67,68,136,137]; (3) lesions of the mossy fibers both reduce the density of KARs[19,137] and suppress KAR-mediated synaptic transmission in CA3 pyramidal cells[137]; (4) the expression of $EPSC_{KA}$ in CA3 pyramidal cells is correlated with the postnatal development of mossy fiber synapses[138]; (5) the severity of kainate-induced seizures and brain damage is reduced in GluR6 Kos.[39,68] The $EPSC_{KA}$ evoked by mossy fiber stimulation in CA3 pyramidal neurons[67,69,136,139] has several interesting features. Stimulation of a single mossy fiber generates three types of EPSCs: (1) fast EPSCs that are mediated by AMPA receptors—the traditional ionotropic receptor-mediated currents; (2) slow EPSCs that are selectively mediated by kainate receptors; and (3) mixed EPSCs composed of both AMPA and kainate EPSCs.[137] Blocking ongoing activity with TTX revealed the presence of three comparable types of mEPSCs, indicating that kainate receptors are located at the core of synaptic terminals and act as a conventional transmitter-mediated signaling device (i.e., they are not located on extrasynaptic sites, as was initially thought),[137] $EPSC_{KA}$ are selectively restricted to some neuronal populations (i.e., they are not found on every neuron, in contrast to AMPA receptors that mediate the general fast glutamatergic ionotropic synaptic current). In particular, in the hippocampus, $EPSC_{KA}$ are enriched on CA3 pyramidal neurons and certain interneurons but not on CA1 pyramidal neurons or on granule cells. Neurons enriched with *kainatergic synapses* are also the ones that degenerate most readily in human and animal TLE—stratum oriens somatostatin-containing interneurons, CA3 pyramidal neurons, and so on—suggesting that the presence of a high density of kainatergic synapses triggers a cascade of events that is deleterious to neurons. In addition, $EPSC_{KA}$ have long-lasting slow kinetics with important implications.

A multitude of effects of kainate on synaptic transmission have been reported. Kainate inhibits mossy fiber synaptic inputs,[140] Low and high concentrations of kainite facilitate and inhibit synaptic transmission, respectively,[89,141,142] Presynaptic KARs (with GluR 7 subunits) are localized in the presynaptic active zone, close to release sites of mossy fibers, where they facilitate the release of glutamate.[143,144] Therefore, at both pre- and postsynaptic sites, kainate modulates the effects of mossy fiber synapses in a dose-dependent manner.

Mossy Fibers Sprout after Seizures: Anatomical Observations

That mossy fibers sprout after kainate injections (and consequent seizure generation) was reported over two decades ago.[19,145,146] These studies identified a novel band of mossy fiber terminals below the CA3 pyramidal neuron layer after recurrent seizures caused by the convulsive agent. This sprouting was also associated with the formation of a band of mossy fibers immediately above the granule layer, suggesting that an additional sprouting of fibers—and formation of synapses—had also occurred within the granule cells. Investigators speculated that in epileptic tissue, sprouting mossy fibers may innervate other granule cells (in contrast to the situation in the seizure-naive granule cells layer)[19,41,147–150] (see Fig. 33–1). Interestingly, this new band of mossy fiber terminals was characterized by both a high concentration of zinc (Timm stain) and high-affinity kainate receptors, suggesting that the innervations of neurons by mossy fibers entrain the postsynaptic expression of the features that normally characterize these synapses.[19,145,147,151,152] Direct demonstration that the newly formed mossy fiber terminals indeed have all the features and constituents of conventional mossy fibers was then performed using electron microscopy and kainate binding.[19,150,151] As stressed above, mossy fiber terminals have unique features (very large diameters with multiple invaginations and large numbers of vesicles). After recurrent seizures, typical mossy fiber terminals were observed in the aberrant regions above the granule layers and the infrapyramidal (CA3) zone, where they are not present in naive animals. Whether mossy fibers also sprout and increase their innervation of GABAergic interneurons has not been firmly established.

Epileptic hippocampus exhibits an aberrant mossy fiber terminal zone that is not present in naive animals. Similar observations

have been repeatedly made in a variety of animal models of TLE, including kindling and pilocarpine, providing a potential substrate for seizure-induced epileptogenesis (see below).[41,148,149,153–166] Sprouting has also been observed in human epileptic patients.[148,154,167–171] Parallel studies performed in postmortem hippocampi, first in non-TLE types of infantile seizures and then on many different types of epilepsies, suggest that this plasticity is not restricted to rodents.[148,149] If functional, this sprouting could lead to profound rearrangements in the operation of the hippocampal circuit, with increased excitation of CA3 pyramidal neurons and granule cells.

Recurrent Mossy Fiber Synapses in TLE Include Kainatergic Synapses that Are Not Observed in Naive Neurons

In spite of overwhelming anatomical evidence that mossy fibers sprout, it remained to be shown that they also are functional in their aberrant localization and that sprouting therefore contributes to enhancement of the excitability of their targets. Experimental evidence suggests that changes in voltage-gated conductances—in addition to mossy fiber sprouting—could promote the generation of epileptiform activity.[172–177] Since mossy fiber synapses are closely associated with kainate signaling, one can hypothesize that the formation of aberrant mossy fiber synapses on dentate granule cells (DGCs) would trigger the formation of functional KAR-operated synapses in chronic epileptic rats. Indeed, recordings from granule cells in an animal model of TLE—selected because they have no mossy fiber terminals in naive conditions—revealed major differences from control cells. In control granule cells, stimulation of the perforant pathway generated exclusively fast AMPA receptor-mediated EPSCs ($EPSC_{AMPA}$).[137] In contrast, in TLE DGCs, a similar stimulation paradigm generated long-lasting $EPSC_{KA}$ originating from recurrent mossy fiber synapses[178] (see Figs. 33–11 and 33–4). Therefore, epileptic DG neurons operate by means of aberrant glutamatergic synaptic currents that are not observed in naïve neurons.

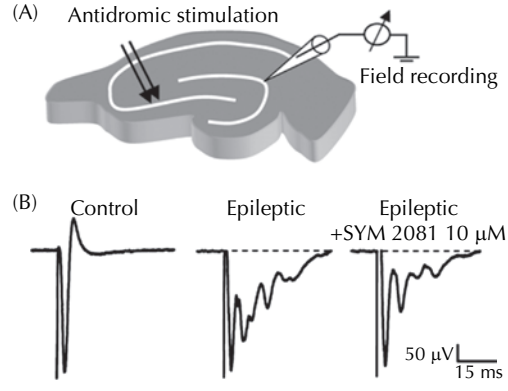

Figure 33–4. A: experimental protocol: field recordings in the granule layer and antidromic stimulation in CA3. **B**: illustration of responses evoked by the stimulation in naive and epileptic hippocampi. A kainate receptor antagonist blocked the epileptiform events generated by electrical stimulation of the perforant pathway. In a naive slice, this generated an all-or-none field EPSP. In an epileptic slice—prepared weeks after in vivo treatment with pilocarpine—the same stimulation generated an epileptiform event that was partly blocked by an antagonist of kainate receptors. From ref.178.

How do aberrant KAR-operated synapses, with their slow kinetics, impact the temporal precision of EPSP-spike coupling in DGCs of epileptic rats and generate seizures? There are indications that the loss of time-locked EPSC-evoked action potentials, and a large degree of jitter, facilitate the generation of paroxysmal synchronized activities.[179] Many studies have shown that the shape of an excitatory synaptic event, and its modulation by voltage-gated conductance, are important determinants of the temporal precision of hippocampal and neocortical cell operation.[180–185] In naive DCGs, the generation of an $EPSP_{AMPA}$ leads to a time-locked spike with a fixed latency and very little jitter. This precision is instrumental in the operation of the entorhinal cortex/perforant pathway/Ammon's horn and most likely underlies the behaviorally relevant patterns that this pathway entrains. In contrast, in TLE DGCs, there is a dramatic decrease in spike timing precision.[186] Jittery spikes are selectively evoked by $EPSP_{KA}$ (not by $EPSP_{AMPA}$, which only generate highly time-locked spikes in both control and TLE conditions).[178,186] Direct proof of the link between kainatergic synapses and reduced time-locked responses was presented in an experiment in which a simulated electrical pulse, with kainate-like kinetics, was

injected intracellularly into control DGCs—which are endowed only with fast kinetic $EPSP_{AMPA}$. The injection of a kainate-like EPSC converted the time-locked spikes to jittery responses. In other words, it is not the amplitude of the EPSC but its kinetics that is here determinant: $EPSP_{KA}$ are endowed with a long-lasting decay time constant that is ideal for the loss of these time-locked responses. Indeed, the activation by EPSPs of voltage-gated conductances near threshold is an important parameter in the modulation of the EPSP time course and of EPSP-spike coupling temporal precision.[182,187–190] We showed that $EPSP_{KA}$ but not $EPSP_{AMPA}$ activate voltage-gated currents and specifically the persistent Na^+ current (I_{NaP}), which is activated below firing threshold and amplifies EPSPs in hippocampal and neocortical neurons.[188,191,192] Using two blockers of I_{NaP}, phenytoin[180,188,193,194] and a low dose of TTX,[195,196] we found that blockade of I_{NaP} restores the temporal precision of EPSP-spike coupling in DGCs of epileptic rats. Therefore, DG epileptic but not naive neurons generate an $EPSP_{KA}$ that triggers selectively the activation of I_{NaP}, which, in turn, facilitates the generation of bursts of action potentials in a dispersed jittery pattern instead of the time-locked $EPSP_{AMPA}$ response. These observations suggest that selective interplay between an aberrant $EPSP_{KA}$ and I_{NaP} alters the temporal precision of EPSP-spike coupling in epileptic but not naive DGCs. This action is not due to an enhancement of I_{NaP} but to the unique long-lasting kinetics of kainatergic EPSCs that are needed to activate I_{NaP}. Interestingly, I_{NaP} is enhanced in neurons in both animal models and patients with temporal lobe epilepsy.[196a] These aberrant KAR-operated synapses exert a strong influence on the operation of hippocampal circuitry, given the high frequency of ongoing excitatory synaptic events in DGCs from epileptic rats.[197] It is likely that these mechanisms underlie the alterations of place cell and phase/precession patterns and the temporal organization of firing among pairs of neurons in TLE.[198,199]

The importance of sprouting and neosynapse formation in chronic epileptogenesis has been challenged by Mello and colleagues on the basis of experiments using the blocker of protein synthesis cycloheximide. In their experiments, pretreatment with cycloheximide following pilocarpine-induced status epilepticus allowed epileptogenesis but prevented aberrant mossy fiber sprouting as assessed by Timm staining. This result suggested that mossy fiber sprouting is not required for seizures to beget seizures in this model.[200,201] However, Dudek and colleagues[197,202] showed that pretreatment with cycloheximide neither altered the spontaneous motor seizure rate posttreatment (compared to that of untreated TLE animals) nor changed the pattern of Timm staining. Cycloheximide also did not prevent hilar, CA1, or CA3 neuronal loss compared to that of the untreated TLE rats. Direct evidence for functional aberrant mossy fiber synapses was obtained, suggesting that pretreatment with cycloheximide does not affect aberrant mossy fiber sprouting in epileptic rats and does not prevent the formation of recurrent excitatory circuits (also see ref. 168).

Implications of Kainatergic Pathways for the Development of Efficient Antiepileptic Agents

Several lessons can be drawn from the use of kainate and other animal models of TLE. First, to understand and eventually cure TLE, reactive plasticity must be incorporated and taken into account in the animal models used for basic and applied research. This conclusion may be valid in general for other insults and neurological disorders, since although mossy fiber synapses offer a unique opportunity to demonstrate postlesional plasticity, this is by no means restricted to mossy fibers. Axonal sprouting and innervation of aberrant targets have been observed in CA1 pyramidal neurons that are deprived of an important input by seizures,[203,204] and a wide range of lesions and insults produce fiber sprouting and formation of novel connections that impact the operation of brain networks.[205–208] These observations imply that the animal models used to mimic human neurological disorders must be chronic and must incorporate alterations. After seizures, sprouting and functional aberrant synapse operation require several weeks to take place and to express the aberrant features (including the novel aberrant kainatergic synapses); then and only then—weeks after the inaugurating status—can one investigate genuine epileptic

networks and develop suitable antiepileptic drugs. In keeping with this important point, in epileptic animals, activation of granule cell synaptic inputs generate all-or-none epileptiform bursts (instead of the normal field population spike evoked in naive neurons) that are blocked by SYM2081, a relatively specific kainate receptor antagonist,[178] This observation suggests that this aberrant synapse plays an important role in the generation of seizures by epileptic neurons, and that suitable kainate receptor antagonists could provide novel therapeutic avenues. This conclusion could not have been reached had the experimental procedures relied only on naive acutely treated animals.

SEIZURES BEGET SEIZURES IN VITRO IN THE DEVELOPING HIPPOCAMPUS

The observations that seizures induced by kainate (or other convulsive agents or procedures) do not produce cell loss and mossy fiber sprouting in immature tissue reflect an important difference between adult and infantile brain. Yet, recurrent seizures do produce long-term, often severe, neurological and behavioral sequelae. To address this puzzle, we have developed an in vitro preparation that has allowed several important observations regarding how recurrent seizures generated by kainate could lead directly to long-lasting alterations in neuronal activity.

An Experimental Protocol to Generate an Epileptogenic Mirror Focus

In spite of their essential contributions, in vivo studies do not readily provide access to the full mechanisms involved in seizure generation. Acute slice preparations also have some limitations, including the difficulties in generating ictal events similar to those observed in vivo. To circumvent these limitations, Khalilov and colleagues developed a technique for studying an intact ex vivo hippocampus preparation; the neonatal hippocampus is dissected, and the entire repertoire of recording techniques applied in vitro can be utilized,[209] This intact preparation can be used for long-lasting recordings, with excellent preservation of essential variables and physiological parameters. It is, however, limited to neonatal preparations and requires suitable relatively fast perfusion rates. Recordings from this preparation revealed larger and better network-driven signals than those observed in slices, allowing the generation and propagation of giant depolarizing potentials (GDPs)—the dominant synaptic pattern of developing cortical networks[210,211] along the entire hippocampal axis—to be described. Combined whole-cell and extracellular field recordings from the CA3 hippocampal region and the septum indicated that spontaneous GDPs are most often initiated in the septal poles of the hippocampus and propagate to the medial septum and temporal poles of both hippocampi simultaneously.[212]

This preparation was subsequently extended to a triple chamber that accommodates the two interconnected hippocampi and their connecting commissures; each hippocampus is placed in an independent chamber so that a convulsive agent can be applied exclusively to one chamber (whereas the naive contralateral hippocampus and/or the associative/commissural connections are perfused with a different solution).[213] This approach provides a unique opportunity to separate the network submitted to a convulsive agent from the naive network that experiences only recurrent seizures that propagate from the other side. Indeed, one can allow the propagation of a predetermined number of seizures before interrupting the flow of activity. Further, putative anti-epileptic drugs can be applied to either the treated or the naive network.

Using this preparation, several important observations have been made concerning the mechanisms underlying the effects of kainate-induced seizures. First, gradual developmentally dependent actions of kainate were observed. Kainate did not generate ictal seizures in postnatal day 2 (P2) tissue, but it did trigger ictal seizures at P7. The propagation of seizures is also developmentally regulated, with interictal seizures propagating to the contralateral hippocampus and septum at P2 and also to the entorhinal cortex; the latter pattern is seen only starting from P4, confirming the crucial role of the hippocampus at an early age in acting as the pacemaker of kainite-induced seizures.[214] Applications of kainate (300 nM)

to one hippocampus generate a tonic-clonic seizure pattern with ictal high-frequency oscillations (HFOs ≥ 40 Hz) similar to those observed in vivo in experimental animals and in epileptic patients.[215–219] The seizures propagate to the other hippocampus, leading to the generation of a similar electrographic event. If the connections between the two hippocampi are interrupted after one seizure, the contralateral hippocampus does not generate seizures spontaneously; that is, it does not become epileptic. In contrast, after 10–15 kainate applications to the stimulated hippocampus, the contralateral hippocampus—which has never been perfused with kainite—becomes epileptic; after disconnection from the stimulated side, it generates ongoing seizures. The networks are "chronically" epileptic; even after 2 days in vitro, ongoing seizures are generated by slices prepared from the mirror foci and artificially maintained. This preparation therefore enables investigators to directly assess the consequences of seizures on a naive network and how seizures beget seizures.

Conditions Required for Recurrent Seizures to Generate a Mirror Focus

Using this preparation, we first determined the conditions required for seizures to beget seizures and to form a mirror focus. Recurrent seizures must include HFOs (>40 Hz) to transform a naive network into an epileptic one. Seizures without HFOs do not generate a mirror focus (see Fig. 33–5). To test this, we repetitively applied kainate to one hippocampus—referred to as the *treated hippocampus*—and NMDA or GABA receptor antagonists to the other hippocampus—referred to as the *naive hippocampus*, as it did not receive kainate. The GABA or NMDA receptor antagonists did not block the propagated seizures but eliminated only their HFO components and completely prevented the formation by seizures of a mirror focus.[122,123,220] Clearly, GABA and NMDA receptors are necessary and sufficient for both the generation of HFOs *and* the formation by seizures of a mirror focus. An interesting implication of these observations is that seizures

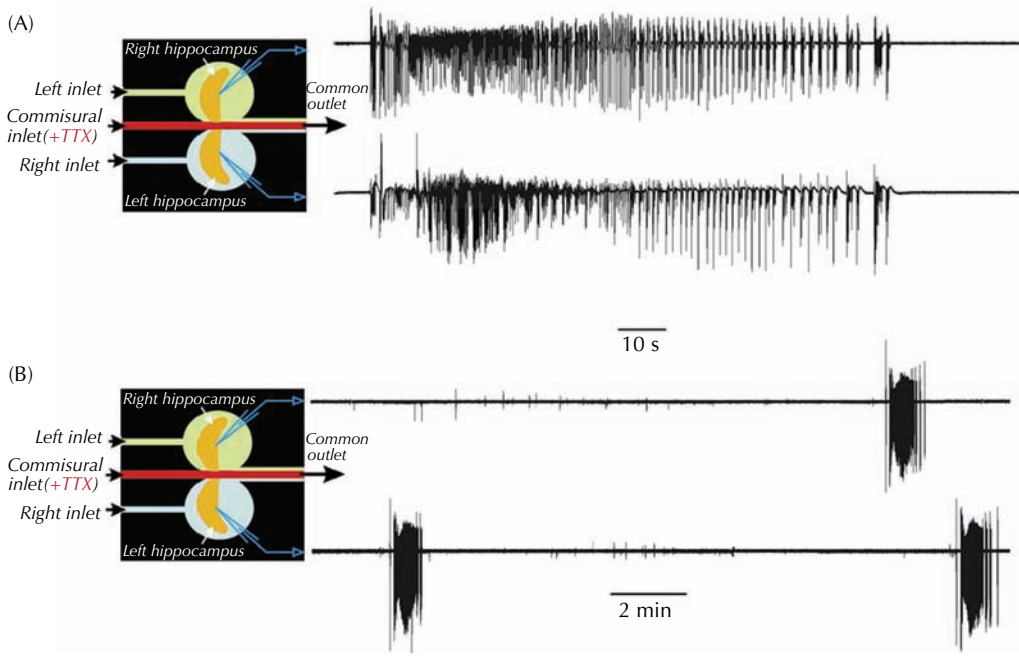

Figure 33–5. **A**: triple chamber with recordings from both hippocampi: seizures propagate from one hippocampus to the other. Recurrent seizures generated in one intact hippocampus by kainate propagated to the other hippocampus and formed an epileptogenic mirror focus. Triple chamber with the two interconnected hippocampi and their connecting commissures. **B**: formation of an epileptogenic mirror focus. After 15 kainate applications and seizures, the naive hippocampus was disconnected from the kainate-treated one by applications of TTX to the commissural chamber, thereby interrupting the flow of activity. The disconnected hippocampus generated ictal seizures for the duration of the preparation. From ref.123.

generated by GABA receptor blockers are *not* epileptogenic, as they do not lead to long-term consequences. This was directly demonstrated by applications of GABA receptor antagonists to both hippocampi that produced seizures but not epileptogenic mirror foci, as the hippocampi did not generate ongoing seizures when the drugs were washed out. Therefore, at least in the neonatal hippocampus, GABA and NMDA receptors must be operative for seizures to include HFOs, and this determines whether seizures will beget seizures. Interestingly, observations in human and animal TLE also reflect the importance of HFOs,[216–218]

This preparation was then used to determine the persistent alterations occurring in an epileptogenic mirror focus formed by the propagation or recurrent seizures from the other hippocampus. Several alterations have been reported. Gamma-aminobutyric acid strongly depolarizes and excites epileptic neurons because of a permanent shift of E_{GABA}; indeed, single-channel recordings of GABA channels showed a highly significant alteration of $[Cl^-]_I$ and the driving force of GABA (DF_{GABA}). Gamma-aminobutyric acid excites neurons in the epileptic tissue, generating action potentials.[122] The accumulation of chloride is most likely mediated by a loss of the chloride exporter KCC2, perturbing the capacity of neurons to remove chloride that accumulates during recurrent seizures[221] (but also see ref. 124). This excitatory-to-inhibitory shift of GABA actions (I to E) has been reported in several other preparations, including human TLE neurons[121]and animal models—although other underlying mechanisms (notably, increased efficacy of the chloride importer NKCC1) have been suggested.[222] This preparation also has been quite useful in testing the actions of known and novel antiepileptic drugs. For example, the NKCC1 chloride cotransporter antagonist bumetanide (which has been used for decades as a diuretic agent) also reduces seizures.[124,223,224] In the triple chamber, bumetanide applied to the naive hippocampus while kainate was repeatedly applied to the other hippocampus failed to prevent the formation by seizures of a mirror focus but efficiently reduced ongoing seizures generated by the mirror focus.[223] These actions are mediated by its potent reduction of intracellular chloride that counteracts the depolarizing and excitatory actions of GABA in the mirror focus and thereby reinforces the inhibitory actions of GABA. This modulation of GABA effects is also illustrated in a recent study in which the dynamic regulation of chloride (DCR) was determined using a perforated patch-clamp recording with focal applications of GABA and V rest selected so as to have a nil DF_{GABA}—that is, no net current at that voltage.[224] A large depolarizing step led to a chloride influx, and then the time required for chloride to return to control values was determined. In mirror focus neurons this time course was significantly augmented, and this was mimicked by specific antagonists of NKCC1/KCC2. As seizures also beget seizures in NKCC1 knockout mice and lead to a permanent rise of DF_{GABA}, it appears that NKCC1 is neither necessary nor sufficient (see a contrasting conclusion in ref. 225). We also showed that in this preparation, KCC2 is internalized—and thus is not operational—in epileptic neurons. Therefore, recurrent seizures reduce DCR, leading to more excitatory actions of GABA, at least in part because of a downregulation of KCC2 (now known to be heavily controlled by tyrosine phosphorylation and dimerization).[226] An important consequence of these effects is that phenobarbital will efficiently block seizure inauguration but aggravate established seizures.[225] Therefore, the history of seizures prior to phenobarbital administration is instrumental in determining its effects.

GENERAL CONCLUSIONS

Understanding the role of kainate signals in modulating ongoing neuronal and network-driven patterns of activity has enormously benefited from the parallel investigation of pathological and normal tissue. The past three decades have shown how kainate generates seizures and how neurons susceptible to kainate are also the ones that use it for ongoing biological functions. Clearly, the system relies on the unique capacity of kainate signals to generate biologically relevant patterns by activating a wide range of cellular mechanisms that converge to enhance neuronal excitability. Theses systems, however, "live dangerously," since insults can transform these activities into severe life-long neurological disorders. It is this link that we must better understand if we want to efficiently block pharmacoresistant TLE.

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REFERENCES

1. Nitta I, Watase H, Tomie Y. Structure of kainic acid and its isomer, allokainic acid. *Nature*. 1958;181:761–762.
2. Curtis DR, Duggan AW, Felix D, Johnston GA, Tebecis AK, Watkins JC. Excitation of mammalian central neurones by acidic amino acids. *Brain Res*. 1972;41:283–301.
3. Curtis DR, Watkins JC. Acidic amino acids with strong excitatory actions on mammalian neurones. *J Physiol*. 1963;166:1–14.
4. Coyle JT, Bird SJ, Evans RH, et al. Excitatory amino acid neurotoxins: selectivity, specificity, and mechanisms of action. Based on an NRP one-day conference held June 30, 1980. *Neurosci Res Program Bull*. 1981;19:1–427.
5. Nadler JV. Kainic acid: neurophysiological and neurotoxic actions. *Life Sci*. 1979;24:289–299.
6. Olney JW, Fuller T, De Gubareff T. Acute dendrotoxic changes in the hippocampus of kainate treated rats. *Brain Res*. 1979;176:91–100.
7. Olney JW. Excitotoxicity: an overview. *Can Dis Wkly Rep*. 1990;16(suppl 1E):47–57.
8. McGeer EG, McGeer PL. Neurotoxins as tools in neurobiology. *Int Rev Neurobiol*. 1981;22:173–204.
9. McGeer PL, McGeer EG. Kainic acid: the neurotoxic breakthrough. *Crit Rev Toxicol*. 1982;10:1–26.
10. Schwarcz R, Coyle JT. Striatal lesions with kainic acid: neurochemical characteristics. *Brain Res*. 1977;127:235–249.
11. Coyle JT, Molliver ME, Kuhar MJ. In situ injection of kainic acid: a new method for selectively lesioning neural cell bodies while sparing axons of passage. *J Comp Neurol*. 1978;180:301–323.
12. Schwarcz R, Zaczek R, Coyle JT. Microinjection of kainic acid into the rat hippocampus. *Eur J Pharmacol*. 1978;50:209–220.
13. Ben-Ari Y, Lagowska J. Epileptogenic action of intra-amygdaloid injection of kainic acid. *CR Acad Sci Hebd Seances Acad Sci D*. 1978;287:813–816.
14. Ben-Ari Y, Lagowska Y, Le Gal La SG, Tremblay E, Ottersen OP, Naquet R. Diazepam pretreatment reduces distant hippocampal damage induced by intra-amygdaloid injections of kainic acid. *Eur J Pharmacol*. 1978;52:419–420.
15. Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience*. 1985;14:375–403.
16. Cavalheiro EA, Santos NF, Priel MR. The pilocarpine model of epilepsy in mice. *Epilepsia*. 1996;37:1015–1019.
17. Sloviter RS. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science*. 1987;235:73–76.
18. Monaghan DT, Cotman CW. The distribution of [3H] kainate acid binding sites in rat CNS as determined by autoradiography. *Brain Res*. 1982;252:91–100.
19. Represa A, Tremblay E, Ben-Ari Y. Kainate binding sites in the hippocampal mossy fibers: localization and plasticity. *Neuroscience*. 1987;20:739–748.
20. Berger M, Ben-Ari Y. Autoradiographic visualization of [3H]kainic acid receptor subtypes in the rat hippocampus. *Neurosci Lett*. 1983;39:237–242.
21. Hollmann M, O'Shea-Greenfield A, Rogers SW, Heinemann S. Cloning by functional expression of a member of the glutamate receptor family. *Nature*. 1989;342:643–648.
22. Lothman EW, Collins RC. Kainic acid induced limbic seizures: metabolic, behavioral, electroencephalographic and neuropathological correlates. *Brain Res*. 1981;218:299–318.
23. Sperk G, Lassmann H, Baran H, Kish SJ, Seitelberger F, Hornykiewicz O. Kainic acid induced seizures: neurochemical and histopathological changes. *Neuroscience*. 1983;10:1301–1315.
24. Sperk G. Kainic acid seizures in the rat. *Prog Neurobiol*. 1994;42:1–32.
25. Vincent P, Mulle C. Kainate receptors in epilepsy and excitotoxicity. *Neuroscience*. 2009;158:309–323.
26. Ben-Ari Y, Cossart R. Kainate, a double agent that generates seizures: two decades of progress. *Trends Neurosci*. 2000;23:580–587.
27. Lothman EW. Seizure circuits in the hippocampus and associated structures. *Hippocampus*. 1994;4:286–290.
28. Lothman EW, Bertram EH 3rd, Stringer JL. Functional anatomy of hippocampal seizures. *Prog Neurobiol*. 1991;37:1–82.
29. McIntyre DC, Racine RJ. Kindling mechanisms: current progress on an experimental epilepsy model. *Prog Neurobiol*. 1986;27:1–12.
30. Sato M, Racine RJ, McIntyre DC. Kindling: basic mechanisms and clinical validity. *Electroencephalogr Clin Neurophysiol*. 1990;76:459–472.
31. Loscher W, Leppik IE. Critical re-evaluation of previous preclinical strategies for the discovery and the development of new antiepileptic drugs. *Epilepsy Res*. 2002;50:17–20.
32. Loscher W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Res*. 2002;50:105–123.
33. Schwartzkroin PA, Scharfman HE, Sloviter RS. Similarities in circuitry between Ammon's horn and dentate gyrus: local interactions and parallel processing. *Prog Brain Res*. 1990;83:269–286.
34. Nadler JV, Okazaki MM, Gruenthal M, Ault B, Armstrong DR. Kainic acid seizures and neuronal cell death: insights from studies of selective lesions and drugs. *Adv Exp Med Biol*. 1986;203:673–686.

35. Charriaut-Marlangue C, Aggoun-Zouaoui D, Represa A, Ben-Ari Y. Apoptotic features of selective neuronal death in ischemia, epilepsy and gp 120 toxicity. *Trends Neurosci*. 1996;19:109–114.
36. Timsit S, Rivera S, Ouaghi P, et al. Increased cyclin D1 in vulnerable neurons in the hippocampus after ischaemia and epilepsy: a modulator of in vivo programmed cell death? *Eur J Neurosci*. 1999;11:263–278.
37. Ben-Ari Y, Represa A. Brief seizure episodes induce long-term potentiation and mossy fibre sprouting in the hippocampus. *Trends Neurosci*. 1990;13:312–318.
38. Ben-Ari Y, Tremblay E, Ottersen OP, Meldrum BS. The role of epileptic activity in hippocampal and "remote" cerebral lesions induced by kainic acid. *Brain Res*. 1980;191:79–97.
39. Gaiarsa JL, Zagrean L, Ben-Ari Y. Neonatal irradiation prevents the formation of hippocampal mossy fibers and the epileptic action of kainate on rat CA3 pyramidal neurons. *J Neurophysiol*. 1994;71:204–215.
40. Nadler JV, Evenson DA, Smith EM. Evidence from lesion studies for epileptogenic and non-epileptogenic neurotoxic interactions between kainic acid and excitatory innervation. *Brain Res*. 1981;205:405–410.
41. Buckmaster PS, Dudek FE. In vivo intracellular analysis of granule cell axon reorganization in epileptic rats. *J Neurophysiol*. 1999;81:712–721.
42. Okazaki MM, Nadler JV. Protective effects of mossy fiber lesions against kainic acid-induced seizures and neuronal degeneration. *Neuroscience.* 1988;26:763–781.
43. Sloviter RS. Hippocampal pathology and pathophysiology in temporal lobe epilepsy. *Neurologia*. 1996; 11(suppl 4):29–32.
44. Pinard E, Tremblay E, Ben-Ari Y, Seylaz J. Blood flow compensates oxygen demand in the vulnerable CA3 region of the hippocampus during kainate-induced seizures. *Neuroscience.* 1984;13:1039–1049.
45. Freund TF, Buzsáki G. Interneurons of the hippocampus. *Hippocampus*. 1996;6:347–470.
46. Klausberger T, Roberts JD, Somogyi P. Cell type- and input-specific differences in the number and subtypes of synaptic GABA(A) receptors in the hippocampus. *J Neurosci*. 2002;22:2513–2521.
47. Klausberger T, Magill PJ, Marton LF, et al. Brain-state- and cell-type-specific firing of hippocampal interneurons in vivo. *Nature*. 2003;421:844–848.
48. Miles R, Toth K, Gulyas AI, Hajos N, Freund TF. Differences between somatic and dendritic inhibition in the hippocampus. *Neuron*. 1996;16:815–823.
49. Szabadics J, Varga C, Molnar G, Olah S, Barzo P, Tamas G. Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. *Science*. 2006;311:233–235.
50. Khirug S, Yamada J, Afzalov R, Voipio J, Khiroug L, Kaila K. GABAergic depolarization of the axon initial segment in cortical principal neurons is caused by the Na-K-2Cl cotransporter NKCC1. *J Neurosci*. 2008;28:4635–4639.
51. Dinocourt C, Petanjek Z, Freund TF, Ben-Ari Y, Esclapez M. Loss of interneurons innervating pyramidal cell dendrites and axon initial segments in the CA1 region of the hippocampus following pilocarpine-induced seizures. *J Comp Neurol*. 2003;459: 407–425.
52. Cossart R, Denocourt C, Hirsch J, et al. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. *Nat Neurosci*. 2001;4:52–62.
53. Hirsch JC, Agassandian C, Merchan-Perez A, et al. Deficit of quantal release of GABA in experimental models of temporal lobe epilepsy. *Nat Neurosci*. 1999;2:499–500.
54. Esclapez M, Hirsch JC, Khazipov R, Ben-Ari Y, Bernard C. Operative GABAergic inhibition in hippocampal CA1 pyramidal neurons in experimental epilepsy. *Proc Natl Acad Sci USA.* 1997;94:12151–12156.
55. Shumate MD, Lin DD, Gibbs JW, Holloway KL, Coulter DA. GABA(A) receptor function in epileptic human dentate granule cells: comparison to epileptic and control rat. *Epilepsy Res*. 1998;32:114–128.
56. Mody I, Miller JJ. Levels of hippocampal calcium and zinc following kindling-induced epilepsy. *Can J Physiol Pharmacol*. 1985;63:159–161.
57. Buhl EH, Otis TS, Mody I. Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. *Science.* 1996;271:369–373.
58. Staley KJ, Soldo BL, Proctor WR. Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors. *Science.* 1995;269:977–981.
59. Houser CR, Esclapez M. Vulnerability and plasticity of the GABA system in the pilocarpine model of spontaneous recurrent seizures. *Epilepsy Res*. 1996;26: 207–218.
60. Esclapez M, Houser CR. Up-regulation of GAD65 and GAD67 in remaining hippocampal GABA neurons in a model of temporal lobe epilepsy. *J Comp Neurol*. 1999;412:488–505.
61. Sloviter RS. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the "dormant basket cell" hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus.* 1991;1:41–66.
62. Bekenstein JW, Lothman EW. Dormancy of inhibitory interneurons in a model of temporal lobe epilepsy. *Science.* 1993;259:97–100.
63. Gibbs JW, Sombati S, DeLorenzo RJ, Coulter DA. Physiological and pharmacological alterations in postsynaptic GABA(A) receptor function in a hippocampal culture model of chronic spontaneous seizures. *J Neurophysiol*. 1997;77:2139–2152.
64. Robinson JH, Deadwyler SA. Kainic acid produces depolarization of CA3 pyramidal cells in the in vitro hippocampal slice. *Brain Res*. 1981;221:117–127.
65. Ben-Ari Y, Gho M. Long-lasting modification of the synaptic properties of rat CA3 hippocampal neurones induced by kainaic acid. *J Physiol* (*Lond*). 1988;404: 365–384.
66. Westbrook GL, Lothaman EW. Cellular and synaptic basis of kainic acid-induced hippocampal epileptiform activity. *Brain Res*. 1983;273:97–109.
67. Castillo PE, Malenka RC, Nicoll RA. Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. *Nature*. 1997;388:182–186.
68. Mulle C, Sailer A, Perez-Otano I, et al. Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. *Nature*. 1998;392:601–605.
69. Frerking M, Malenka RC, Nicoll RA. Synaptic activation of kainate receptors on hippocampal interneurons. *Nat Neurosci*. 1998;1:479–486.
70. Cossart R, Esclapez M, Hirsch JC, Bernard C, Ben-Ari Y. Activation of GluR5 receptors in interneurons increases tonic GABAergic inhibition of pyramidal neurons. *Nat Neurosci*. 1998;1:470–478.

71. Cossart R, Esclapez M, Hirsch JC, Bernard C, Ben Ari Y. GluR5 kainate receptor activation in interneurons increases tonic inhibition of pyramidal cells. *Nat Neurosci*. 1998;1:470–478.
72. Fisher RS, Alger BE. Electrophysiological mechanisms of kainic acid-induced epileptiform activity in the rat hippocampal slice. *J Neurosci*. 1984;4:1312–1323.
73. Rodriguez-Moreno A, Herreras O, Lerma J. Kainate receptors presynaptically downregulate GABAergic inhibition in the rat hippocampus. *Neuron*. 1997;19: 893–901.
74. Mulle C, Sailer A, Swanson GT, et al. Subunit composition of kainate receptors in hippocampal interneurons. *Neuron*. 2000;28:475–484.
75. Cossart R, Tyzio R, Dinocourt C, et al. Presynaptic kainate receptors that enhance the release of GABA on CA1 hippocampal interneurons. *Neuron.* 2001;29: 497–508.
76. Jiang L, Xu J, Nedergaard M, Kang J. A kainate receptor increases the efficacy of GABAergic synapses. *Neuron*. 2001;30:503–513.
77. Gho M, King AE, Ben-Ari Y, Enrico E. kainate reduce two voltage-dependent potassium conductances in rat hippocampal neurons in vitro. *Brain Res*. 1986;385: 411–414.
78. Ashwood TJ, Lancaster B, Wheal HV. Intracellular electrophysiology of CA1 pyramidal neurones in slices of kainic acid lesioned hippocampus of the rat. *Exp Brain Res*. 1986;62:189–198.
79. Rodriguez-Moreno A, Lerma J. Kainate receptor modulation of GABA release involves a metabotropic function. *Neuron*. 1998;20:1211–1218.
80. Ruiz A, Sachidhanandam S, Utvik JK, Coussen F, Mulle C. Distinct subunits in heteromeric kainate receptors mediate ionotropic and metabotropic function at hippocampal mossy fiber synapses. *J Neurosci*. 2005;25:11710–11718.
81. Fisahn A, Heinemann SF, McBain CJ. The kainate receptor subunit GluR6 mediates metabotropic regulation of the slow and medium AHP currents in mouse hippocampal neurones. *J Physiol.* 2005;562: 199–203.
82. Le DC, Bouilleret V, Miles R. Epileptiform activities in slices of hippocampus from mice after intra-hippocampal injection of kainic acid. *J Physiol*. 2008;586: 4891–4904.
83. Hollmann M, Heinemann S. Cloned glutamate receptors. *Annu Rev Neurosci*. 1993;17:31–108.
84. Coussen F, Mulle C. Kainate receptor-interacting proteins and membrane trafficking. *Biochem Soc Trans*. 2006;34:927–930.
85. Keinanen K, Wisden W, Sommer B, et al. A family of AMPA-selective glutamate receptors. *Science*. 1990;249:556–560.
86. Werner P, Voigt M, Keinämen K, Widsen W, Seeburg PH. Cloning of a putative high-affinity kainate receptor expressed predominantly in hippocampal CA3 cells. *Nature*. 1991;351:742–744.
87. Herb A, Burnashev N, Werner P, Sakmann B, Widsen W, Seeburg PH. The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly subunits. *Neuron.* 1992;8:775–785.
88. Wisden W, Seeburg PH. A complex mosaic of high-affinity kainate receptors in rat brain. *J Neurosci*. 1993;13:3582–3598.
89. Fisahn A, Contractor A, Traub RD, Buhl EH, Heinemann SF, McBain CJ. Distinct roles for the kainate receptor subunits GluR5 and GluR6 in kainate-induced hippocampal gamma oscillations. *J Neurosci*. 2004;24:9658–9668.
90. Khalilov I, Hirsch J, Cossart R, Ben Ari Y. Paradoxical anti-epileptic effects of a GluR5 agonist of kainate receptors. *J Neurophysiol*. 2002;88:523–527.
91. Ruano D, Lambolez B, Rossier J, Paternain AV, Lerma J. Kainate receptor subunits expressed in single cultured hippocampal neurons: molecular and functional variants by RNA editing. *Neuron*. 1995;14:1009–1017.
92. Lerma J. Roles and rules of kainate receptors in synaptic transmission. *Nat Rev Neurosci*. 2003;4:481–495.
93. Tremblay E, Nitecka L, Berger ML, Ben-Ari Y. Maturation of kainic acid seizure-brain damage syndrome in the rat. I. Clinical, electrographic and metabolic observations. *Neuroscience*. 1984;13: 1051–1072.
94. Berger ML, Tremblay E, Nitecka L, Ben-Ari Y. Maturation of kainic acid seizure-brain damage syndrome in the rat. III. Postnatal development of kainic acid binding sites in the limbic system. *Neuroscience*. 1984;13:1095–1104.
95. Nitecka L, Tremblay E, Charton G, Bouillot JP, Berger ML, Ben-Ari Y. Maturation of kainic acid seizure-brain damage syndrome in the rat. II. Histopathological sequelae. *Neuroscience.* 1984;13: 1073–1094.
96. Albala BJ, Moshe SL, Okada R. Kainic-acid-induced seizures—a developmental study. *Dev Brain Res*. 1984;13:139–148.
97. Sankar R, Shin D, Liu H, Wasterlain C, Mazarati A. Epileptogenesis during development: injury, circuit recruitment, and plasticity. Epilepsia. 2002; 43(suppl 5): 47–53.
98. Amaral DG, Dent JA. Development of the mossy fibers of the dentate gyrus: I. A. light and electron microscopic study of the mossy fibers and their expansions. *J Comp Neurol*. 1981;195:51–86.
99. Yang YL, Tandon P, Liu Z, Sarkisian MR, Stafstrom CE, Holmes GL. Synaptic reorganization following kainic acid-induced seizures during development. *Dev Brain Res.* 1998;107:169–177.
100. Sperber EF, Haas KZ, Stanton PK, Moshe SL. Resistance of the immature hippocampus to seizure-induced synaptic reorganization. *Brain Res Dev Brain Res.* 1991;60:88–93.
101. Cavalheiro EA, Silva DF, Turski WA, Calderazzo-Filho LS, Bortolotto ZA, Turski L. The susceptibility of rats to pilocarpine-induced seizures is age-dependent. *Brain Res*. 1987;465:43–58.
102. Cherubini E, Ben-Ari Y, Krnjevic K. Anoxia produces smaller changes in synaptic transmission, membrane potential, and input resistance in immature rat hippocampus. *J Neurophysiol*. 1989;62:882–895.
103. Haut SR, Veliskova J, Moshe SL. Susceptibility of immature and adult brains to seizure effects. *Lancet Neurol*. 2004;3:608–617.
104. Rizzi M, Perego C, Aliprandi M, et al. Glia activation and cytokine increase in rat hippocampus by kainic acid-induced status epilepticus during postnatal development. *Neurobiol Dis*. 2003;14:494–503.
105. Bickler PE, Hansen BM. Hypoxia-tolerant neonatal CA1 neurons: relationship of survival to evoked

glutamate release and glutamate receptor-mediated calcium changes in hippocampal slices. *Brain Res Dev Brain Res.* 1998;106:57–69.
106. Bickler PE, Gallego SM, Hansen BM. Developmental changes in intracellular calcium regulation in rat cerebral cortex during hypoxia. *J Cereb Blood Flow Metab*. 1993;13:811–819.
107. Liu Z, Stafstrom CE, Sarkisian M. et al. Age-dependent effects of glutamate toxicity in the hippocampus. *Brain Res Dev Brain Res*. 1996;97:178–184.
108. Marks JD, Friedman JE, Haddad GG. Vulnerability of CA1 neurons to glutamate is developmentally regulated. *Brain Res Dev Brain Res.* 1996;97:194–206.
109. Patel M, Li QY. Age dependence of seizure-induced oxidative stress. *Neuroscience.* 2003;118:431–437.
110. Sankar R, Shin DH, Wasterlain CG. GABA metabolism during status epilepticus in the developing rat brain. *Brain Res Dev Brain Res*. 1997;98:60–64.
111. Hauser WA. Epidemiology of epilepsy in children. *Neurosurg Clin North Am.* 1995;6:419–429.
112. Cowan LD. The epidemiology of the epilepsies in children. *Ment Retard Dev Disabil Res Rev.* 2002;8: 171–181.
113. Villeneuve N, Ben-Ari Y, Holmes GL, Gaïarsa J-L. Neonatal seizures induced persistent changes in intrinsic properties of CA1 rat hippocampal cells. *Ann Neurol*. 2000;47:729–738.
114. Ben Ari Y, Holmes GL. Effects of seizures on developmental processes in the immature brain. *Lancet Neurol*. 2006;5:1055–1063.
115. Holmes GL. Effects of seizures on brain development: lessons from the laboratory. *Pediatr Neurol*. 2005;33:1–11.
116. Brunquell PJ, Glennon CM, DiMario FJ Jr, Lerer T, Eisenfeld L. Prediction of outcome based on clinical seizure type in newborn infants. *J Pediatr*. 2002;140: 707–712.
117. Miller SP, Weiss J, Barnwell A. et al. Seizure-associated brain injury in term newborns with perinatal asphyxia. *Neurology*. 2002;58:542–548.
118. Riviello P, de RL I, Holmes GL. Lack of cell loss following recurrent neonatal seizures. *Brain Res Dev Brain Res*. 2002;135:101–104.
119. Ben-Ari Y. Neuro-archaeology: pre-symptomatic architecture and signature of neurological disorders. *Trends Neurosci*. 2008;31:626–636.
120. Huberfeld G, Wittner L, Clemenceau S, et al. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. *J Neurosci*. 2007;27:9866–9873.
121. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science*. 2002;298:1418–1421.
122. Khalilov I, Le Van QM, Gozlan H, Ben Ari Y. Epileptogenic actions of GABA and fast oscillations in the developing hippocampus. *Neuron.* 2005;48:787–796.
123. Khalilov I, Holmes GL, Ben Ari Y. In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. *Nat Neurosci*. 2003;6:1079–1085.
124. Dzhala VI, Talos DM, Sdrulla DA. et al. NKCC1 transporter facilitates seizures in the developing brain. *Nat Med*. 2005;11:1205–1213.
125. Dzhala VI, Staley KJ. Excitatory actions of endogenously released GABA contribute to initiation of ictal epileptiform activity in the developing hippocampus. *J Neurosci*. 2003;23:1840–1846.
126. Glykys J, Dzhala VI, Kuchibhotla KV. et al. Differences in cortical versus subcortical GABAergic signaling: a candidate mechanism of electroclinical uncoupling of neonatal seizures. *Neuron*. 2009;63:657–672.
127. Payne JA, Rivera C, Voipio J, Kaila K. Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci*. 2003;26: 199–206.
128. Cohen I, Navarro V, Le DC, Miles R. Mesial temporal lobe epilepsy: a pathological replay of developmental mechanisms? *Biol Cell*. 2003;95:329–333.
129. Nadler JV, Perry BW, Gentry C, Cotman CW. Loss and reacquisition of hippocampal synapses after selective destruction of CA3-CA4 afferents with kainic acid. *Brain Res*. 1980;191:387–403.
130. Parnavelas JG, Lynch G, Brecha N, Cotman CW, Globus A. Spine loss and regrowth in hippocampus following deafferentation. *Nature.* 1974;248:71–73.
131. Lothman EW, Stringer JL, Bertram EH. The dentate gyrus as a control point for seizures in the hippocampus and beyond. *Epilepsy Res Suppl*. 1992;7: 301–313.
132. Freund T, Buzsaki G. Interneurons of the hippocampus. *Hippocampus*. 1996;6:345–470.
133. Haug FM. Electron microscopical localization of the zinc in hippocampal mossy fibre synapses by a modified sulfide silver procedure. *Histochemie*. 1967;8: 355–368.
134. Haug FM, Blackstad TW, Simonsen AH, Zimmer J. Timm's sulfide silver reaction for zinc during experimental anterograde degeneration of hippocampal mossy fibers. *J Comp Neurol*. 1971;142:23–31.
135. Aniksztejn L, Charton G, Ben-Ari Y. Selective release of endogenous zinc from the hippocampal mossy fibers in situ. *Brain Res*. 1987;404:58–64.
136. Vignes M, Collingridge GL. The synaptic activation of kainate receptors. *Nature*. 1997;388:179–182.
137. Cossart R, Epsztein J, Tyzio R, et al. Quantal release of glutamate generates pure kainate and mixed AMPA/kainate EPSCs in hippocampal neurons. *Neuron.* 2002;35:147–159.
138. Marchal C, Mulle C. Postnatal maturation of mossy fibre excitatory transmission in mouse CA3 pyramidal cells: a potential role for kainate receptors. *J Physiol.* 2004;561:27–37.
139. Frerking M, Nicoll RA. Synaptic kainate receptors. *Curr Opin Neurobiol*. 2000;10:342–351.
140. Kamiya H, Ozawa S. Kainate receptor-mediated presynaptic inhibition at the mouse hippocampal mossy fibre synapse. *J Physiol*. 2000;523(pt 3):653–665..
141. Schmitz D, Mellor J, Frerking M, Nicoll RA. Presynaptic kainate receptors at hippocampal mossy fiber synapses. *Proc Natl Acad Sci USA*. 2001;98: 11003–11008.
142. Lauri SE, Delany C, VR JC, et al. Synaptic activation of a presynaptic kainate receptor facilitates AMPA receptor-mediated synaptic transmission at hippocampal mossy fibre synapses. *Neuropharmacology*. 2001;41:907–915.
143. Pinheiro PS, Mulle C. Kainate receptors. *Cell Tissue Res*. 2006;326:457–482.
144. Pinheiro PS, Mulle C. Presynaptic glutamate receptors: physiological functions and mechanisms of action. *Nat Rev Neurosci*. 2008;9:423–436.

145. Sutula T, Xio-Xia H, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science*. 1988;239:1147–1150.
146. Okazaki MM, Evenson DA, Nadler JV. Hippocampal mossy fiber sprouting and synapse formation after status epilepticus in rats: visualization after retrograde transport of biocytin. *J Comp Neurol*. 1995;352: 515–534.
147. Tauck DL, Nadler JV. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. *J Neurosci*. 1985;5:1016–1022.
148. Isokawa M, Levesque MF, Babb TL, Engel J Jr. Single mossy fiber axonal systems of human dentate granule cells studied in hippocampal slices from patients with temporal lobe epilepsy. *J Neurosci*. 1993;13:1511–1522.
149. Franck JE, Pokorny J, Kunkel DD, Schwartzkroin PA. Physiologic and morphologic characteristics of granule cell circuitry in human epileptic hippocampus. *Epilepsia.* 1995;36:543–558.
150. Represa A, Jorquera I, Le Gal La Salle G, Ben-Ari Y. Epilepsy induced collateral sprouting of hippocampal mossy fibers: does it induce the development of ectopic synapses with granule cell dendrites? *Hippocampus*. 1993;3:257–268.
151. Cavazos JE, Zhang P, Qazi R, Sutula TP. Ultrastructural features of sprouted mossy fiber synapses in kindled and kainic acid-treated rats. *J Comp Neurol*. 2003;458:272–292.
152. Sutula TP, Dudek FE. Unmasking recurrent excitation generated by mossy fiber sprouting in the epileptic dentate gyrus: an emergent property of a complex system. *Prog Brain Res*. 2007;163:541–563.
153. Represa A, Le Gall L, Ben-Ari Y. Hippocampal plasticity in the kindling model of epilepsy in rats. *Neurosci Lett*. 1989;99:345–350.
154. Mello LE, Cavalheiro EA, Tan AM, et al. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia.* 1993;34:985–995.
155. Morimoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog Neurobiol*. 2004;73:1–60.
156. Dudek FE, Hellier JL, Williams PA, Ferraro DJ, Staley KJ. The course of cellular alterations associated with the development of spontaneous seizures after status epilepticus. *Prog Brain Res*. 2002;135: 53–65.
157. Williams PA, White AM, Clark S, et al. Development of spontaneous recurrent seizures after kainate-induced status epilepticus. *J Neurosci*. 2009;29:2103–2112.
158. Sutula TP. Secondary epileptogenesis, kindling, and intractable epilepsy: a reappraisal from the perspective of neural plasticity. *Int Rev Neurobiol*. 2001;45: 355–386.
159. Sutula TP. Seizure-induced plasticity and adverse long-term effects of early-life seizures. *Ann Neurol*. 2004;56:164–165.
160. Okazaki MM, Evenson DA, Nadler JV. Hippocampal mossy fiber sprouting and synapse formation after status epilepticus in rats: visualization after retrograde transport of biocytin. *J Comp Neurol*. 1995;352: 515–534.
161. Scharfman HE, Sollas AL, Berger RE, Goodman JH. Electrophysiological evidence of monosynaptic excitatory transmission between granule cells after seizure-induced mossy fiber sprouting. *J Neurophysiol*. 2003;90:2536–2547.
162. Wuarin JP, Dudek FE. Electrographic seizures and new recurrent excitatory circuits in the dentate gyrus of hippocampal slices from kainate-treated epileptic rats. *J Neurosci.* 1996;16:4438–4448.
163. Represa A, Niquet J, Pollard H, Khrestchatisky M, Ben-Ari Y. From seizures to neo-synaptogenesis: intrinsic and extrinsic determinants of mossy fiber sprouting in the adult hippocampus. *Hippocampus*. 1994;4:270–274.
164. Represa A, Tremblay E, Ben-Ari Y. Sprouting of mossy fibers in the hippocampus of epileptic human and rat. *Adv Exp Med Biol*. 1990;268:419–424.
165. Represa A, Ben-Ari Y. Kindling is associated with the formation of novel mossy fibre synapses in the CA3 region. *Exp Brain Res*. 1992;92:69–78.
166. Represa A, Tremblay E, Ben-Ari Y. Aberrant growth of mossy fibers and enhanced kainic acid binding sites induced in rats by early hyperthyroidism. *Brain Res*. 1987;423:325–328.
167. Represa A, Robain O, Tremblay E, Ben-Ari Y. Hippocampal plasticity in childhood epilepsy. *Neurosci Lett*. 1989;99:351–355.
168. Dudek FE, Sutula TP. Epileptogenesis in the dentate gyrus: a critical perspective. *Prog Brain Res*. 2007;163:755–773.
169. Ben-Ari Y, Crepel V, Represa A. Seizures beget seizures in temporal lobe epilepsies: the boomerang effects of newly formed aberrant kainatergic synapses. *Epilepsy Curr*. 2008;8:68–72.
170. Blaabjerg M, Zimmer J. The dentate mossy fibers: structural organization, development and plasticity. *Prog Brain Res.* 2007;163:85–107.
171. Nadler JV. The recurrent mossy fiber pathway of the epileptic brain. *Neurochem Res*. 2003;28:1649–1658.
172. Bernard C, Anderson A, Becker A, Poolos NP, Beck H, Johnston D. Acquired dendritic channelopathy in temporal lobe epilepsy. *Science.* 2004;305:532–535.
173. Beck H, Steffens R, Elger CE, Heinemann U. Voltage-dependent Ca^{2+} currents in epilepsy. *Epilepsy Res.* 1998;32:321–332.
174. Yaari Y, Yue C, Su H. Recruitment of apical dendritic T-type Ca^{2+} channels by backpropagating spikes underlies de novo intrinsic bursting in hippocampal epileptogenesis. *J Physiol*. 2007;580:435–450.
175. Jensen MS, Yaari Y. Role of intrinsic burst firing, potassium accumulation, and electrical coupling in the elevated potassium model of hippocampal epilepsy. *J Neurophysiol*. 1997;77:1224–1233.
176. Heinemann U, Franceschetti S, Hamon B, Konnerth A, Yaari Y. Effects of anticonvulsants on spontaneous epileptiform activity which develops in the absence of chemical synaptic transmission in hippocampal slices. *Brain Res*. 1985;325:349–352.
177. Jensen MS, Azouz R, Yaari Y. Spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. *J Physiol* (*Lond*). 1996;492: 199–210.
178. Epsztein J, Represa A, Jorquera I, Ben Ari Y, Crepel V. Recurrent mossy fibers establish aberrant kainate receptor-operated synapses on granule cells from epileptic rats. *J Neurosci.* 2005;25:8229–8239.
179. Foffani G, Uzcategui YG, Gal B, Menendez de la Prida L. Reduced spike-timing reliability correlates with the emergence of fast ripples in the rat epileptic hippocampus. *Neuron*. 2007;55:930–941.

180. Fricker D, Miles R. EPSP amplification and the precision of spike timing in hippocampal neurons. *Neuron*. 2000;28:559–569.
181. Maccaferri G, Dingledine R. Complex effects of CNQX on CA1 interneurons of the developing rat hippocampus. *Neuropharmacology*. 2002;43:523–529.
182. Axmacher N, Miles R. Intrinsic cellular currents and the temporal precision of EPSP-action potential coupling in CA1 pyramidal cells. *J Physiol*. 2004;555: 713–725.
183. Rodriguez-Molina VM, Aertsen A, Heck DH. Spike timing and reliability in cortical pyramidal neurons: effects of EPSC kinetics, input synchronization and background noise on spike timing. *PLoS One*. 2007;2:e319.
184. Daoudal G, Hanada Y, Debanne D. Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. *Proc Natl Acad Sci USA*. 2002;99:14512–14517.
185. Campanac E, Debanne D. Spike timing-dependent plasticity: a learning rule for dendritic integration in rat CA1 pyramidal neurons. *J Physiol*. 2008;586:779–793.
186. Epsztein J, Sola E, Represa A, Ben-Ari Y, Crepel V. A Selective interplay between aberrant EPSPKA and INaP reduces spike timing precision in dentate granule cells of epileptic rats. *Cereb Cortex*. 2009;20: 898–911.
187. Stafstrom CE, Schwindt PC, Chubb MC, Crill WE. Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex *in vitro*. *J Neurophysiol*. 1985;53: 153–170.
188. Stuart G, Sakmann B. Amplification of EPSPs by axosomatic sodium channels in neocortical pyramidal neurons. *Neuron*. 1995;15:1065–1076.
189. Fricker D, Miles R. EPSP amplification and the precision of spike timing in hippocampal neurons. *Neuron*. 2002;28:559–569.
190. Vervaeke K, Hu H, Graham LJ, Storm JF. Contrasting effects of the persistent Na^+ current on neuronal excitability and spike timing. *Neuron*. 2006;49:257–270.
191. Schwindt PC, Crill WE. Amplification of synaptic current by persistent sodium conductance in apical dendrite of neocortical neurons. *J Neurophysiol*. 1995;74:2220–2224.
192. Andreasen M, Lambert JD. Somatic amplification of distally generated subthreshold EPSPs in rat hippocampal pyramidal neurones. *J Physiol*. 1999; 519(pt 1):85–100.
193. Kuo CC, Bean BP. Slow binding of phenytoin to inactivated sodium channels in rat hippocampal neurons. *Mol Pharmacol*. 1994;46:716–725.
194. Segal MM, Douglas AF. Late sodium channel openings underlying epileptiform activity are preferentially diminished by the anticonvulsant phenytoin. *J Neurophysiol*. 1997;77:3021–3034.
195. Hammarstrom AK, Gage PW. Oxygen-sensing persistent sodium channels in rat hippocampus. *J Physiol*. 2000;529(pt 1):107–118.
196. Yue C, Remy S, Su H, Beck H, Yaari Y. Proximal persistent Na^+ channels drive spike afterdepolarizations and associated bursting in adult CA1 pyramidal cells. *J Neurosci*. 2005;25:9704–9720.
196a. Vreugdenhil M, Hoogland G, van Veelen CW, Wadman WJ. Persistent sodium current in subicular neurons isolated from patients with temporal lobe epilepsy. *Eur J Neurosci*. 2004;19:2769–2778.
197. Wuarin JP, Dudek FE. Excitatory synaptic input to granule cells increases with time after kainate treatment. *J Neurophysiol*. 2001;85:1067–1077.
198. Liu X, Muller RU, Huang LT, et al. Seizure-induced changes in place cell physiology: relationship to spatial memory. *J Neurosci*. 2003;23:11505–11515.
199. Lenck-Santini PP, Holmes GL. Altered phase precession and compression of temporal sequences by place cells in epileptic rats. *J Neurosci*. 2008;28: 5053–5062.
200. Longo BM, Mello LE. Supragranular mossy fiber sprouting is not necessary for spontaneous seizures in the intrahippocampal kainate model of epilepsy in the rat. *Epilepsy Res*. 1998;32:172–182.
201. Dos SJ Jr, Longo BM, Blanco MM, Menezes de Oliveira MG, Mello LE. Behavioral changes resulting from the administration of cycloheximide in the pilocarpine model of epilepsy. *Brain Res*. 2005;1066: 37–48.
202. Williams PA, Wuarin JP, Dou P, Ferraro DJ, Dudek FE. Reassessment of the effects of cycloheximide on mossy fiber sprouting and epileptogenesis in the pilocarpine model of temporal lobe epilepsy. *J Neurophysiol*. 2002;88:2075–2087.
203. Esclapez M, Hirsch J, Ben-Ari Y, Bernard C. Newly formed excitatory pathways provide a substrate for hyperexcitability in experimental temporal lobe epilepsy. *J Comp Neurol*. 1999;408:449–460.
204. Esclapez M, Hirsch JC, Ben-Ari Y, Bernard C. Newly formed excitatory pathways provide a substrate for hyperexcitability in experimental temporal lobe epilepsy. *J Comp Neurol*. 1999;408:449–460.
205. Wolpaw JR, Carp JS. Plasticity from muscle to brain. *Prog Neurobiol*. 2006;78:233–263.
206. Paz JT, Christian CA, Parada I, Prince DA, Huguenard JR. Focal cortical infarcts alter intrinsic excitability and synaptic excitation in the reticular thalamic nucleus. *J Neurosci*. 2010;30:5465–5479.
207. Green JB. Brain reorganization after stroke. *Top Stroke Rehabil*. 2003;10:1–20.
208. Keck T, Mrsic-Flogel TD, Vaz AM, Eysel UT, Bonhoeffer T, Hubener M. Massive restructuring of neuronal circuits during functional reorganization of adult visual cortex. *Nat Neurosci*. 2008;11: 1162–1167.
209. Khalilov I, Esclapez M, Medina I, et al. A novel in vitro preparation: the intact hippocampal formation. *Neuron*. 1997;19:743–749.
210. Ben-Ari Y, Cherubini E, Corradetti R, Gaïarsa J-L. Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiol* (*Lond*). 1989;416:303–325.
211. Ben-Ari Y. Developing networks play similar melody. *Trends Neurosci*. 2001;24:354–360.
212. Leinekugel X, Khalilov I, Ben-Ari Y, Khazipov R. Giant depolarizing potentials: the septal pole of the hippocampus paces the activity of the developing intact septohippocampal complex in vitro. *J Neurosci*. 1998;18:6349–6357.
213. Khazipov R, Desfreres L, Khalilov I, Ben-Ari Y. Three-independent-compartment chamber to study in vitro commissural synapses. *J Neurophysiol*. 1999;81:921–924.
214. Khalilov I, Dzhala V, Medina I, et al. Maturation of kainate-induced epileptiform activities in

interconnected intact neonatal limbic structures in vitro. *Eur J Neurosci*. 1999;11:3468–3480.
215. Bragin A, Jando G, Nadasdy Z, Hetke J, Wise K, Buzsaki G. Gamma (40–100 Hz) oscillation in the hippocampus of the behaving rat. *J Neurosci*. 1995;15: 47–60.
216. Bragin A, Engel J Jr, Wilson CL, Vizentin E, Mathern GW. Electrophysiologic analysis of a chronic seizure model after unilateral hippocampal KA injection. *Epilepsia*. 1999;40:1210–1221.
217. Bragin A, Engel J Jr, Wilson CL, Fried I, Buzsaki G. High-frequency oscillations in human brain. *Hippocampus*. 1999;9:137–142.
218. Bragin A, Wilson CL, Staba RJ, Reddick M, Fried I, Engel J Jr. Interictal high-frequency oscillations (80–500 Hz) in the human epileptic brain: entorhinal cortex. *Ann Neurol*. 2002;52:407–415.
219. Staba RJ, Wilson CL, Bragin A, Fried I, Engel J Jr. Quantitative analysis of high-frequency oscillations (80–500 Hz) recorded in human epileptic hippocampus and entorhinal cortex. *J Neurophysiol*. 2002;88:1743–1752.
220. Le Van QM, Khalilov I, Ben Ari Y. The dark side of high-frequency oscillations in the developing brain. *Trends Neurosci*. 2006;29:419–427.
221. Rivera C, Voipio J, Thomas-Crusells J, et al. Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. *J Neurosci*. 2004;24:4683–4691.
222. Dzhala VI, Brumback AC, Staley KJ. Bumetanide enhances phenobarbital efficacy in a neonatal seizure model. *Ann Neurol*. 2008;63:222–235.
223. Nardou R, Ben-Ari Y, Khalilov I. Bumetanide, an NKCC1 antagonist, does not prevent formation of epileptogenic focus but blocks epileptic focus seizures in immature rat hippocampus. *J Neurophysiol*. 2009;101:2878–2888.
224. Nardou R, Yamamoto S, Chazal G, Bhar A, Ferrand N, Dulac O, Ben-Ari Y, Khalilov I. Neuronal chloride accumulation and excitatory GABA underlie aggravation of neonatal epileptiform activities by phenobarbital. *Brain*. 2011;134:987–1002.
225. Dzhala VI, Kuchibhotla KV, Glykys JC, et al. Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. *J Neurosci*. 2010;30:11745–11761.
226. Lee HH, Jurd R, Moss SJ. Tyrosine phosphorylation regulates the membrane trafficking of the potassium chloride co-transporter KCC2. *Mol Cell Neurosci*. 2010;45:173–179.

Chapter 34

Abnormal Dentate Gyrus Network Circuitry in Temporal Lobe Epilepsy

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DEFINING EPILEPTOGENESIS

Significant progress has been made in understanding the causes of cryptogenic temporal lobe epilepsy when aberrant genes[1] and developmental malformations[2] are involved, but the cause of acquired temporal lobe epilepsy, in which presumably normal individuals develop epilepsy following brain injury, remains poorly understood.[3,4] Epileptogenic injuries include head trauma, infection, ischemia, and prolonged febrile seizures, although the latter insult apparently develops as a result of pre-existing abnormalities.[5] Regardless, injuries that occur in normal brains often cause both temporal lobe pathology and temporal lobe epilepsy, and therefore the possible relationship between pathology and epileptogenesis is a compelling one. However, it is not entirely clear what the term "epileptogenesis" signifies exactly, when the process of hippocampal epileptogenesis is first complete, and which mechanisms develop with a time-course that permits an inference of epileptogenic causality. For definitional purposes, we use the term "epileptogenesis" (literally the birth of epilepsy) to encompass the primary process that causes principal neurons to generate their first spontaneous, multiple

population spike-containing epileptiform discharges, whether or not they produce a clinically detected "aura" or any other behavioral manifestation. We regard the process by which spontaneous and subclinical focal seizure discharges propagate to other cell populations to produce clinically detectable behavioral signs, and changes in seizure phenotype and frequency, to be part of a separate, and often prolonged, secondary process ("epileptic maturation") that follows epileptogenesis.

WHY FOCUS ON TEMPORAL LOBE EPILEPSY AND THE DENTATE GYRUS?

There are many forms of epilepsy and many brain regions that can generate spontaneous seizures, so why focus specifically on acquired temporal lobe epilepsy and the dentate gyrus in particular? There are several reasons. Of all the epilepsies, temporal lobe epilepsy is perhaps the most amenable to study because seizures often arise from the hippocampal formation, a brain region with a highly organized structure that facilitates the assessment of often subtle structural changes. In addition, the temporal lobe is removed surgically to treat refractory epilepsy and is therefore often available for study.[6] From an experimental perspective, the similarity of hippocampal structure in rodents and humans, and the fact that epilepsy can be produced in rodents by the same insults that cause epilepsy in humans, make it feasible to relate the results of experimental studies to the human neurological disorder.

The early discovery that spontaneous epileptic seizures appeared to arise from a damaged and shrunken temporal lobe[7] focused attention on surviving hippocampal neurons as likely sources of seizure activity. The likely role of the dentate gyrus as a primary source of seizures was supported by the observation that dentate granule cells are consistently among the few surviving hippocampal neuron populations[8,9] and by the fact that the loss of neurons in the hippocampal endfolium was the single common pathology shared by all epilepsy patient brains that exhibited any detectable hippocampal cell loss.[9] The anatomical realization that the neurons of the vulnerable endfolium, previously thought to be part of the pyramidal cell layer,[10] were instead synaptically connected with the dentate gyrus[11–13] raised the possibility that the loss of dentate hilar neurons[9] might be associated with granule cell pathophysiology.[14]

Additional reasons for focusing on the dentate gyrus relate to the observation that several conspicuous secondary structural abnormalities develop after injury. In this chapter, we address selective neuron loss,[14] synaptic reorganization,[15] granule cell dispersion,[16] and the timing of their development in relation to the time of onset of spontaneous granule cell epileptiform discharges and behavioral seizures. Clearly, whether a particular pathology precedes or follows the onset of spontaneous seizures is directly relevant to whether that pathology can be causally related to the epileptogenic process.

EPILEPTOGENIC NEURON LOSS AND IMMEDIATE GRANULE CELL HYPEREXCITABILITY: IS NEURON LOSS SUFFICIENT TO CAUSE EPILEPSY?

Do dentate granule cells become hyperexcitable and also spontaneously epileptic after hippocampal injury? And if so, precisely how soon after hippocampal injury do granule cells begin to generate spontaneous epileptiform discharges? A series of experimental studies in vivo has consistently demonstrated that granule cell hyperexcitability is caused immediately by prolonged seizures or head trauma and is closely associated with extensive hilar neuron loss.[14,17–20] In our most detailed study, we found that whenever unilateral perforant pathway stimulation produced bilateral hilar neuron loss, then and only then was bilateral granule cell hyperexcitability evident.[18] Conversely, whenever granule cell hyperexcitability was restricted to the ipsilateral dentate gyrus, only extensive ipsilateral hilar neuron loss was evident. These different responses to identical stimulation controlled for the effects of seizure activity per se because although unilateral stimulation always produced bilateral seizure discharges, contralateral granule cell hyperexcitability was uniquely associated with contralateral hilar neuron loss.[18]

We attributed the immediate granule cell hyperexcitability that we observed after

prolonged perforant pathway stimulation to the extensive loss of the two main hilar neuron populations. These are the peptide-containing inhibitory interneurons that innervate the dentate outer molecular layer and the excitatory hilar mossy cells that innervate the dentate inner molecular layer.[14,18] Loss of hilar inhibitory interneurons that project to the distal dendritic region innervated by the entorhinal cortex was hypothesized to produce a direct disinhibitory effect on granule cells.[14,18] The loss of excitatory hilar mossy cells was also hypothesized to cause granule cell hyperexcitability, but to do so indirectly as a result of decreased excitation of surviving inhibitory basket cells,[14,18] which mossy cells normally innervate and excite.[21–24]

The hypothesized direct disinhibitory effect of hilar inhibitory interneuron loss[14,18] was supported by several subsequent studies.[25,26] However, the observation that extensive loss of hilar peptide-containing interneurons following ischemic insult[27] has minimal effect on granule cell excitability[28] argues against a prominent role for hilar interneuron loss in the specific injury-associated granule cell hyperexcitability that reliably follows prolonged perforant pathway stimulation.[14,18] Conversely, selective and extensive (~90%) mossy cell loss in a conditional mossy cell-knockout mouse caused immediate granule cell hyperexcitability,[29] supporting the view that extensive mossy cell loss is the primary cause of the granule cell hyperexcitability observed following kainate-, pilocarpine-, or stimulation-induced status epilepticus.[14,18,30,31]

The main obstacle to our hypothesis—that seizure-induced neuron loss alone is a sufficient and primary epileptogenic mechanism[18,32]—was stated succinctly by Wasterlain and colleagues, who noted that "this hypothesis does not tackle the problem of the 'silent period' between the initial injury and the development of spontaneous seizures. Because mossy cells are injured at the time of the original seizures and disappear within hours to days, it is hard to understand why spontaneous seizures are delayed by weeks to months, unless the loss of inhibition is permissive but not sufficient for epileptogenesis, and its role is only to permit further changes which, in turn, produce chronic epilepsy."[33]

The widely accepted notions that status epilepticus-induced brain damage in animals is reliably followed by a silent, seizure-free preepileptic state lasting for weeks to months,[33–35] and that spontaneous epileptic seizures only begin because synaptic reorganization or other secondary processes that develop during the silent period have finally matured,[15,33,34] are anecdotal and based mainly on occasional behavioral observation,[36] which inevitably risks missing early behavioral seizures and all subclinical seizures.[37] The belief in a seizure-free postinjury latent period[35] has nonetheless formed the logical basis for focusing on delayed secondary processes as likely epileptogenic mechanisms, rather than on the effects of initial neuron loss and other immediate events. However, recent studies that involved continuous monitoring of chemoconvulsant-treated rats have now consistently shown that spontaneous behavioral seizures begin almost immediately after status epilepticus-induced injury.[31,38–41] Continuous video and electrographic monitoring of electrically stimulated rats, in which residual chemoconvulsants can play no role in any early seizures that occur, have now also shown that spontaneous granule cell-onset population spikes and epileptiform discharges, as well as behavioral seizures, also begin within the first days post-status epilepticus.[41] Thus, epileptogenesis after status epilepticus is coincident with initial neuron loss (Fig. 34–1), which occurs over a period of several days,[42] indicating that neuron loss could well be the primary epileptogenic mechanism.[41]

Redefining the Term *Epileptogenesis*

Genesis literally means "birth," "coming into being," or the "beginning of something." The genesis phase in the life of a human being ends in the process of birth, not in reaching maturity. The growth and maturation of the individual is a separate and secondary process distinct from genesis. By analogy, it seems logical to define the completion of epileptogenesis as the moment when the machinery and mechanisms necessary for spontaneous abnormal epileptiform behaviors first exist. The subsequent chronic epileptic state (epileptic maturation) undoubtedly evolves and progresses, but we regard this process to be distinct from epileptogenesis. For example, a seizure focus may expand, or it may transition from a focal to a generalized process, or the frequency of seizure events may change. However, these changes would seem to be an

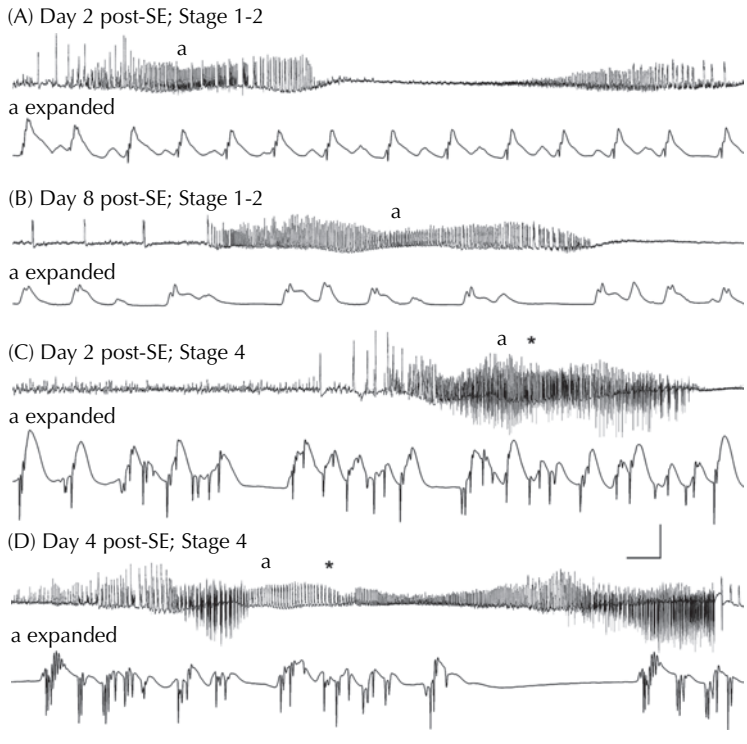

Figure 34–1. Correlation between spontaneous granule cell layer activity and behavioral seizure expression in an epileptic rat 2–8 days after 3 h of perforant pathway stimulation-induced convulsive status epilepticus (SE). Two focal (subclinical) seizures (**A,B**) on days 2 and 8 post-SE and two stage 4 behavioral seizures (**C,D**) on days 2 and 4 post-SE. **A.** On day 2 post-SE, spontaneous high-amplitude activity, consisting of field excitatory postsynaptic potentials (EPSPs) and small-amplitude population spikes, was recorded from the granule cell layer electrode. During this spontaneous event, the animal exhibited only a frozen stare, followed by stereotyped chewing movements. **B.** A similar spontaneous granule cell layer event was recorded on day 8 post-SE, which was only associated with staring and stereotyped head movements. **C,D.** On days 2 and 4 post-SE in the same animal, spontaneous granule cell layer events included larger-amplitude and downwardly deflected population spikes. These events were invariably followed by stage 3–5 behavioral seizures. Note that high-frequency spiking began prior to the first behavioral manifestation (asterisks in **C,D**) of each behavioral seizure. These events from a single rat are representative of all 72 stage 3–5 behavioral seizures recorded in 10 chronically implanted, continuously monitored rats. Calibration bars equal 1.4 s and 9 mV in **A**; 56 ms and 9 mV in **A** (expanded), **B** (expanded), **C** (expanded), **D** (expanded); 3.2 s and 9 mV in **B** and **C**; 4.5 s and 9 mV in **D**. From ref. 41.

evolving process of growth and maturation, not part of the birth process. Clearly, the conundrum quoted above, regarding "the problem of the 'silent period' between the initial injury and the development of spontaneous seizures,"[33] is the product of using the appearance of the first spontaneous generalized behavioral seizures in rats as the definitive marker of the end of epileptogenesis.[34]

Based on the results of studies in which both behavior and granule cell layer activity have been simultaneously and continuously monitored in awake animals after a highly controlled and reproducible injury,[31,41,43] we suggest that epileptogenesis is not a unitary process that ends when the first spontaneous generalized behavioral seizure is visually observed.[33–36] Rather, we regard epileptogenesis to be a process that culminates in the first spontaneous epileptiform discharge that causes a focal seizure or, at minimum, a process that ends when the brain is changed in such a way that the first spontaneous epileptiform discharge and focal seizure are imminent. We suggest that this is the process that needs to be aborted if an antiepileptogenic strategy is to be effective. However, if identified principal cell populations are not directly monitored in awake animals after injury, and if true epileptiform discharges are not clearly differentiated from high-amplitude depolarizations that contain no population spikes, but only superficially appear

to be epileptiform discharges,[31] it is doubtful that accurate inferences can be made regarding the nature, duration, or location of the process called *epileptogenesis*. Even then, important events in unmonitored cell populations may be missed, placing limits on what we should think we know about where seizures originate or where and when epileptogenesis has actually occurred.

Car manufacturing provides a useful analogy for defining epileptogenesis. Every car is assigned a "build date," which marks completion of the process that culminates in the ability of the car to operate as designed, with no additional physical changes required. It is not a date that indicates its first test drive, its first maximum speed attempt, or its first assignment of title, which are all defining characteristics of car ownership and operation. A fully built car that has not yet been driven is still a car, and giving it a build date does not preclude making additional modifications that change the car's attributes and capabilities. Thus, we regard the end of epileptogenesis as the brain's unfortunate build date when all cellular and network changes needed for the spontaneous generation of focal epileptiform discharges are first present. This altered brain state is most likely complete sometime before the first clinically detectable seizure event occurs. The chronic state that follows the initial epileptogenesis phase, which might best be called *epileptic maturation*, undoubtedly involves continuing modification and progression, but we regard epileptogenesis as the process that describes completion of the initially altered brain state, that is, the earliest time when a brain can be said to be epileptic.

Hyperexcitable Is Not Synonymous with *Spontaneously Epileptic*: Does Hippocampal Epileptogenesis Require a Second Pathology?

Although extensive and specific mossy cell loss causes immediate granule cell hyperexcitability, this hyperexcitability alone is apparently not enough to cause spontaneous granule cell epileptiform discharges or spontaneous behavioral seizures.[29] This is not surprising because granule cell hyperexcitability to afferent excitation does not require that granule cells become a spontaneously discharging population. It is therefore notable that there is a second temporal lobe pathology common to all status epilepticus models in the entorhinal cortex[41,43,44] (Fig. 34–2).

One possible explanation for the immediate development in awake animals of both granule cell hyperexcitability to afferent excitation and spontaneous granule cell-onset seizures comes from the analysis of an animal model in which extensive hilar neuron loss, entorhinal cortex damage, and extratemporal injury are consistently produced by electrical stimulation-induced status epilepticus.[41] In this model, as in animals subjected to kainate-[20,30] or pilocarpine-induced status epilepticus[31] or experimental head trauma,[19] extensive hilar neuron loss is reliably associated with immediate granule cell hyperexcitability to afferent

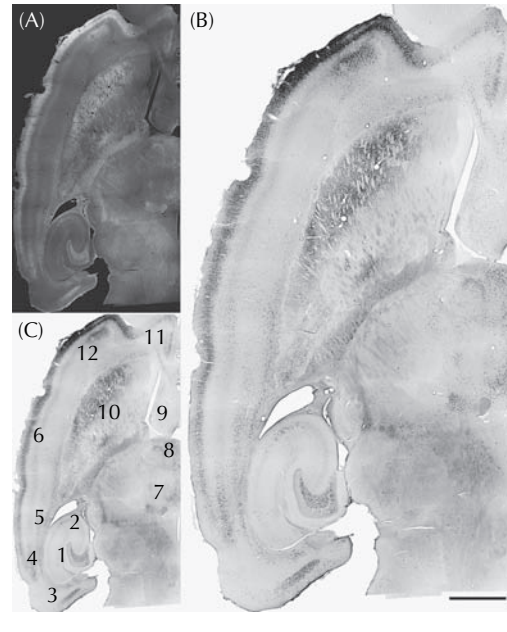

Figure 34–2. Acute Fluoro-Jade B (FJB) staining showing neurodegeneration 4 days after 3 h of perforant pathway stimulation-induced SE. **A.** Fluoro-Jade B fluorescence. **B,C.** Gray-scale inverted image of the same horizontal brain section. Note the selective degeneration of neurons in the dentate hilar region (**C1**); hippocampal area CA3a (**C2**); entorhinal cortex layer III (**C3**); perirhinal cortex (**C4,5**); layer II throughout the neocortex (**C6**); parafascicular thalamic nucleus (**C7**); intermediodorsal-, mediodorsal, paratenial, paraventricular, and central medial thalamic nuclei (**C8**); lateral septum (**C9**); lateral caudate/putamen (**C10**); infralimbic cortex (**C11**); and deep pyramidal cells of the agranular insular cortex (**C12**). Scale bar: 1 mm in **B**; 2 mm in **A** and **C**. From ref. 41.

excitation.[41] In the case of perforant pathway-stimulated rats, in which granule cell activity and behavior were monitored continuously, spontaneous granule cell epileptiform discharges began almost immediately and preceded each spontaneous behavioral seizure[41] (Fig. 34–1). Importantly, clinical behavioral seizures always and only occurred when granule cells generated prolonged negative-going population spikes (Fig. 34–1). Clearly, these spontaneous granule cell discharges cannot be the result of recurrent excitatory connections among granule cells[15] because the hyperexcitability, spontaneous epileptiform discharges, and behavioral seizures preceded the formation of recurrent axonal connections.[41] We suggest that although granule cells become immediately hyperexcitable[14,18–20,30] as a direct result of extensive hilar mossy cell loss,[18,29] granule cells do not generate synchronous epileptiform discharges until they receive abnormal excitatory input from the disinhibited layer II neurons of the injured entorhinal cortex.[43,45] The appearance in the granule cell layers of spontaneous large-amplitude dendritic field depolarizations virtually identical to those evoked by perforant pathway stimulation[41] (Fig. 34–1) suggests that spontaneous granule cell discharges may involve at least two principal pathologies: (1) mossy cell loss that disinhibits granule cells[29,32] and (2) entorhinal cortex damage (Fig. 34–2), which causes abnormal entorhinal layer II neuron activity that invades the dentate gyrus.[41] Future experiments will need to determine whether synchronous epileptiform activity in the entorhinal cortex precedes granule cell epileptiform discharges or whether asynchronous entorhinal activity precedes synchronous granule cell discharges. Regardless, whether spontaneous granule cell seizure discharges cause behavioral seizures immediately (Fig. 34–1) or result in a latent period before clinical seizures first occur may be a measure of the extent of injury in the entorhinal cortex, in the hilus, and in the downstream barriers to seizure spread (hippocampal pyramidal cells and synaptically linked cell populations farther down the chain). We suggest that the only network change needed for spontaneous granule cell epileptiform discharges to develop is extensive neuron loss in the hilus of the dentate gyrus and in the entorhinal cortex. We do not suggest that minor neuron loss is necessarily epileptogenic; only extensive hilar neuron loss is predicted to have a significant epileptogenic effect.[20]

MOSSY FIBER SPROUTING

Few ideas are more conceptually appealing than the hypothesis of epileptogenic mossy fiber sprouting, as formulated originally by Tauck and Nadler.[15,46] According to this scenario, normal granule cells have no recurrent excitatory connections, and therefore granule cells do not normally generate spontaneous population spikes or epileptiform discharges. Hilar mossy cell axotomy[47,48] or injury-induced mossy cell degeneration[15,49] denervates the granule cell dendrite segment normally innervated by mossy cells, and this loss of mossy cell input apparently triggers the reinnervation of granule cells by newly formed granule cell axons (mossy fibers).[50] The *mossy fiber sprouting* hypothesis posits that it is the formation of aberrant excitatory connections among normally unconnected granule cells that causes granule cells to become spontaneously epileptic.[15] Many studies have replicated and extended the original findings of Tauck and Nadler,[15,46] but several critical issues were not considered in the many studies that were designed to support the hypothesis, and other observations suggest an alternative hypothesis.

First, the mossy fiber sprouting hypothesis ignores the network effects of the initial injury-induced neuron loss and regards mossy cell loss as only a trigger for mossy fiber sprouting. However, the effects of neuron loss and reactive synaptic reorganization cannot be separated since these effects coexist, with the former apparently causing the latter.[50] Therefore, any parameters that have been correlated with mossy fiber sprouting in a multitude of studies could have been similarly correlated with hilar neuron loss, although the role of hilar neuron loss has rarely been discussed in studies that have sought to establish a causal link between mossy fiber sprouting and granule cell hyperexcitability. Second, we have found that the granule cell hyperexcitability observed in hippocampal slices from kainate-treated rats, and attributed to mossy fiber sprouting that takes weeks to develop,[15,30,31] is present in vivo immediately after kainate-induced injury, before mossy fiber sprouting develops.[30,51] Third, in

rats in which immediate postinjury granule cell hyperexcitability was confirmed in our in vivo experiments, we have shown that the growth of mossy fiber sprouting was temporally associated with gradually increasing granule cell paired-pulse inhibition and an inability to evoke granule cell epileptiform discharges rather than hyperexcitability.[30,51] Importantly, the judgment that granule cells were hyperexcitable shortly after injury, and later were powerfully inhibited, was based not only on the assessment of responses to paired-pulse stimulation,[30,51] but also on the immediate appearance of multiple population spikes in response to single-afferent stimuli and the inability to evoke granule cell epileptiform discharges during the later synaptic reorganization phase, even by high-frequency afferent excitation.[31] Fourth, direct recording from the granule cell layers in awake pilocarpine- and kainate-treated animals revealed that the granule cells do not generate epileptiform discharges before the spontaneous behavioral seizures that develop in chemoconvulsant-treated rats.[31,52] This result is in contrast to the result of identical recordings made in perforant pathway-stimulated rats, in which all spontaneous behavioral seizures were preceded by granule cell layer epileptiform discharges.[41,43] Fifth, and perhaps most definitively, in all studies in rats subjected to convulsive status epilepticus and then monitored continuously, spontaneous behavioral seizures precede mossy fiber sprouting.[38–41]

Discussion of the mossy fiber sprouting hypothesis in the literature has focused almost exclusively on the aberrant autoinnervation of granule cells, and has largely ignored the possible impact of mossy cell death and mossy fiber sprouting on inhibitory basket cells.[30,51] In fact, mossy cell loss denervates all target cell dendrites in the dentate inner molecular layer, not just granule cells, and the reinnervation of vacated synaptic sites by newly formed granule cell axons would be predicted to target both inhibitory neurons and granule cells.[30,31,51] The consistent finding that synaptic reorganization does, in fact, reinnervate both granule cells and inhibitory neurons[30,31,51,53] (Figs. 34–3 and 34–4) challenges the assumption that mossy fiber sprouting must be exclusively excitatory in nature.[15] To the contrary, we interpret the available data as indicating that mossy fiber sprouting may play a mainly compensatory or restorative role.[30,51]

Inhibitory Circuitry, Mossy Cells, and Mossy Fiber Sprouting

Elegant in vivo studies first revealed the paradoxical net inhibitory effects of the excitatory commissural projections of the dentate gyrus,[21–24] which originate from hilar mossy cells.[11,12] These studies clearly showed that although the commissural fibers were excitatory, and might be predicted to excite granule cells, activation of this pathway in vivo had a predominantly inhibitory effect on granule cells because the mossy cell-derived commissural pathway directly excited inhibitory basket cells.[21–24] Thus, although numerically superior, the excitatory input to granule cells is apparently dominated in vivo by the influence of the mossy cell-basket cell innervation. Consistent with the idea that the seizure-induced loss of mossy cells should denervate basket cells, as well as granule cells, and that mossy fiber sprouting should reinnervate both basket cells and granule cells, granule cells were found to be disinhibited and hyperexcitable immediately after hilar neuron loss, prior to mossy fiber sprouting.[30,51] Then as mossy fiber sprouting developed, the granule cells were found to become powerfully hyperinhibited in the same animals.[30,51] These and additional results are consistent with the view that (1) excitatory mossy cells normally and paradoxically produce predominantly inhibitory, rather than excitatory, effects on granule cells in vivo,[20–24] (2) mossy cell loss denervates inhibitory basket cells, possibly causing immediate granule cell hyperexcitability,[14,18,20,29] and (3) mossy fiber sprouting reinnervates basket cells as well as granule cells (Figs. 34–3 and 34–4), which correlates temporally with an apparent partial restoration of granule cell inhibition.[30,31,51,53]

Must Mossy Fiber Sprouting Be Either Entirely Excitatory or Entirely Inhibitory?

The question of whether mossy fiber sprouting is epileptogenic or restorative in nature may be simplistic regardless of how the electrophysiological and anatomical data are weighted.[51] Mossy cells and mossy fiber sprouting innervate both granule cells and inhibitory neurons and might play distinct roles in different behavioral

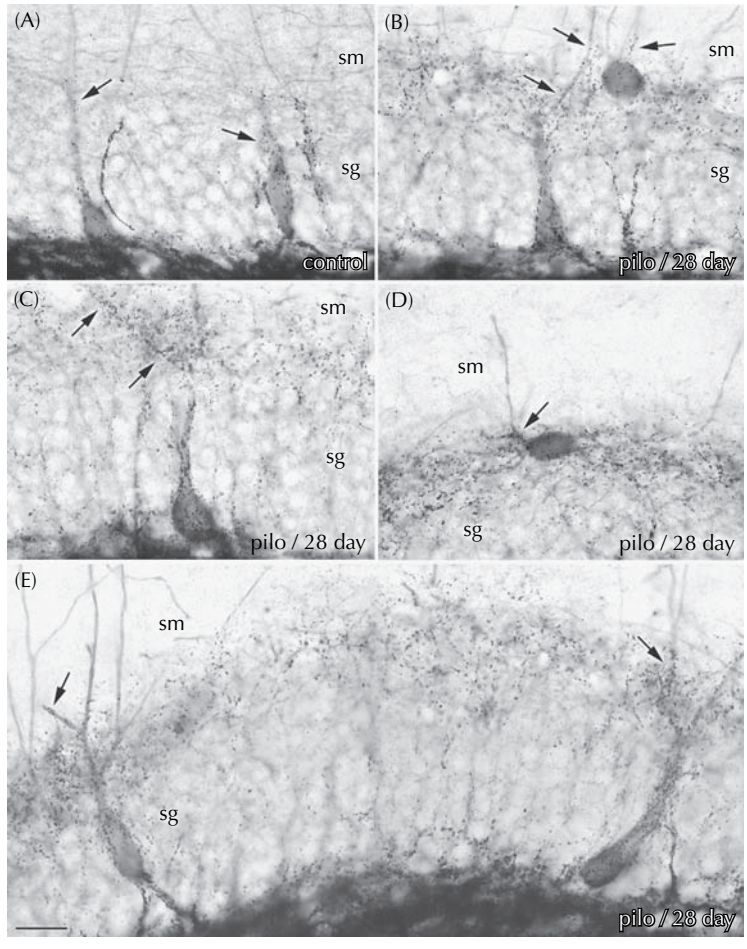

Figure 34–3. Parvalbumin-positive inhibitory interneurons are targets of aberrant mossy fiber sprouting at the time of early recovery of granule cell paired-pulse inhibition. **A.** Timm staining in a control rat. Note that Timm-positive terminals surround and outline the somata and proximal dendrites of normal granule cell layer interneurons (arrows). **B–E.** Timm-stained sections from an animal perfusion-fixed 28 days post-SE, the recovery period when 1 Hz afferent stimulation first failed to evoke epileptiform discharges (i.e., the epileptiform discharge threshold was increased). Note that aberrant mossy fiber terminals targeted the somata and dendrites of identified inhibitory interneurons of the granule cell and inner molecular layers (arrows). sg, stratum granulosum; sm, stratum moleculare. Scale bar: 26 μm in **E**; 25 μm in **A–D**. From ref. 31.

states. It is conceivable that mossy fiber sprouting could be predominantly inhibitory interictally, regulating seizure frequency, but could then play an excitatory role when inhibition is overcome and seizures occur. Although we do not know the net effect of mossy fiber sprouting under all conditions, we contend that the notion that mossy fiber sprouting must be purely epileptogenic in nature because granule cell interconnections are formed is not supported by either the time course of postinjury granule cell excitability in vivo or the relationship between mossy fiber sprouting and the latency to the first spontaneous epileptic seizures.[30,54] Understanding the role of mossy cell loss and reactive mossy fiber sprouting clearly requires (1) an unbiased consideration of the effects of both neuron loss and synaptic reorganization on dentate gyrus function and (2) testing the mossy fiber sprouting hypothesis in animals that exhibit confirmed granule cell-onset epilepsy.[41,43] The latter consideration probably excludes testing in chemoconvulsant-treated rats, which exhibit frequent generalized seizures that appear to minimally involve the hippocampus.[31,52]

The subject of cell loss and synaptic reorganization is made even more complex by the

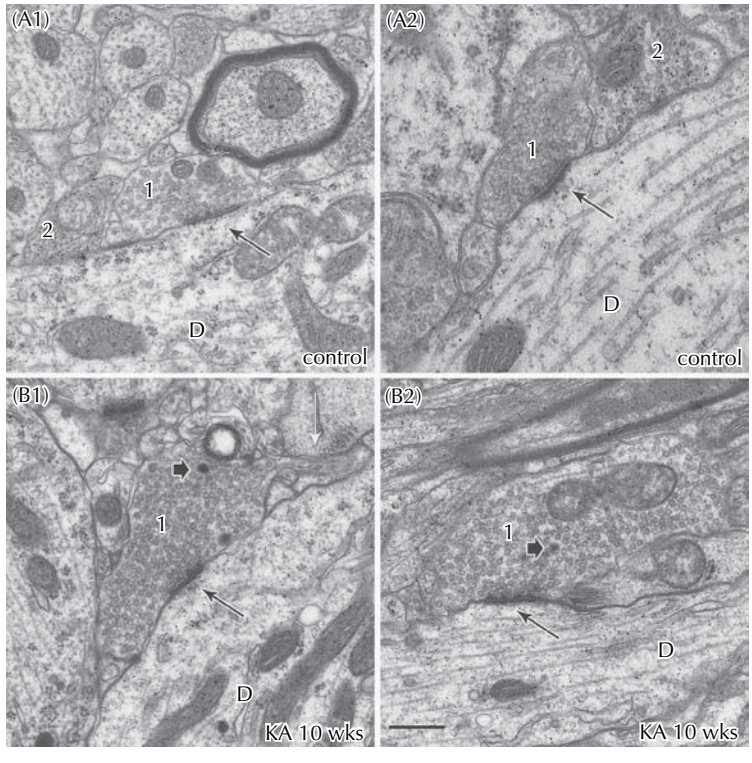

Figure 34–4. Gamma-aminobutyric acid immunocytochemical electron microscopy of the dentate inner molecular layer 10 weeks after saline or kainate (KA)-induced status epilepticus (SE). **A1,2.** In control sections, proximal dendrites (D) of GABA-positive interneurons are contacted by small immunonegative boutons (terminal 1 in both panels) establishing asymmetric synaptic contacts, and by GABA-positive terminals (terminal 2 in both panels) forming symmetric synapses. Arrows point to asymmetric synaptic contacts. **B1,2.** Ten weeks after KA injection, numerous large terminals, densely filled with clear synaptic vesicles and containing dense-core vesicles (thick black arrows), emerge from thin, unmyelinated preterminal axons (white arrow in **B1**). These mossy fiber boutons establish asymmetric synaptic contacts (thin black arrows) with GABA-positive dendritic shafts (D) in the inner molecular layer. Scale bar: 0.5 μm in **B2** (applies to **A1-B2**). From ref. 51.

fact that hilar mossy cells normally constitute a long-distance, longitudinally projecting axonal system,[20,55] whereas aberrant mossy fiber sprouting is more localized[56] and presumably cannot fully restore the translamellar influences that are lost when mossy cells die.[20] In addition, clustering of spontaneous seizures[39] and the duration of the interictal period, that is, seizure frequency, could be powerfully influenced by both the net inhibitory effects of mossy fiber sprouting and the upregulation of glutamic acid decarboxylase 67 (GAD67) and gamma-aminobutyric acid (GABA) that seizure activity produces specifically in granule cells.[57] Clearly, the roles of cell loss, synaptic reorganization, glial abnormalities, and altered expression of transmitters, receptors, and channels remain to be addressed in animal models that reliably exhibit confirmed hippocampal-onset seizures.

GRANULE CELL DISPERSION

Granule cell dispersion in temporal lobe epilepsy, first described by Houser,[58] is another example of a frequently observed structural abnormality that may or may not play a role in altered dentate gyrus excitability.[16] In the normal dentate gyrus, granule cells are tightly packed, forming a clearly delineated and relatively uniform cell layer. Although there is some structural variability among these neurons, particularly in the primate brain,[59] granule cells send their cone-shaped dendrites to

the molecular layer, with virtually all dendrites reaching the hippocampal fissure. Mossy fiber axons emerge from the granule cell somata and enter the hilus. This bipolarity of dentate granule cells clearly separates the input region of the cell from its output region, resulting in minimal granule cell-granule cell connectivity under normal conditions. In granule cell dispersion, the compact lamination of granule cell bodies is lost. As a result, the axons of granule cells dispersed into the molecular layer traverse the molecular layer for some distance and could contact the dendrites of more deeply located neurons, possibly increasing granule cell interconnectivity. Since granule cell dispersion is found in tissues from epileptic patients, the following scenario appears plausible: migration defects of granule cells during development, or dispersion of granule cells following hippocampal injury, might result in increased granule cell interconnectivity and hyperexcitability.

The results of studies conducted over the last 10 years suggest a different scenario. In tissue samples from epileptic patients, the expression of the extracellular matrix protein Reelin was found to be significantly decreased.[60] Moreover, it was noticed that the extent of granule cell dispersion correlated with the extent of decreased Reelin expression. Reelin is known for its role in layer formation in the cerebral cortex, cerebellum, and hippocampus,[61–67] and Reelin-deficient "reeler" mutants show a severe loss of granule cell lamination,[68–70] which is at least structurally reminiscent of granule cell dispersion in epilepsy. Thus, Reelin apparently stabilizes dentate gyrus architecture, and an injury-induced decrease in Reelin expression might cause granule cell dispersion. To test this hypothesis, Heinrich and colleagues[71] infused a Reelin-neutralizing antibody into the dentate gyrus of mature mice. They unexpectedly observed granule cell dispersion at sites of Reelin antibody infusion, and this effect could not be induced when the Reelin antibody was replaced by a nonspecific immunoglobulin G (IgG). The interpretation of these findings is that Reelin establishes or maintains the laminated organization of the dentate gyrus, and that decreased Reelin expression or antibody blockade of Reelin results in granule cell dispersion.

Unilateral granule cell dispersion is reliably produced in normal mice during the first month following unilateral intrahippocampal injections of the glutamate receptor agonist kainate,[72] and it has been shown that Reelin expression was dramatically decreased on the kainate-injected side, but not contralaterally.[71] These results suggest that granule cell dispersion results from decreased Reelin expression after cell loss or hypermethylation of the Reelin gene.[73] Although Reelin appears to play an important role in stabilizing cortical architecture in the mature brain,[67] it remains to be determined whether granule cell dispersion directly influences granule cell interconnectivity and excitability. In this regard, it is notable that reeler mice, which are deficient in Reelin and exhibit granule cell dispersion, do not show spontaneous seizures. In addition, recent recordings from dispersed granule cells in kainate-treated epileptic mice provided evidence of reduced, rather than increased, granule cell excitability.[74] Importantly, many patient hippocampi do not exhibit granule cell dispersion, and other animal models that exhibit confirmed granule cell-onset epilepsy do not show granule cell dispersion.[41,43] Although intrahippocampal kainate injection causes both granule cell dispersion and epilepsy in mice, perforant pathway stimulation-induced hippocampal injury in mice produces epilepsy without producing granule cell dispersion.[75] Thus, the pathophysiological implications of granule cell dispersion, if any, remain to be clarified.

THE LATENT PERIOD AND EPILEPTOGENESIS

The belief that all status epilepticus models exhibit a silent postinjury latent period during which seizures do not occur[33–36] is no longer tenable for the reasons cited above.[31,37–41,76] Although there is no delay between injury and epilepsy after prolonged status epilepticus in animals, and although a similar lack of any detectable latent period has been reported after prolonged status epilepticus in humans,[77] delays in the appearance of clinical seizures after injury usually exist.[34,76,78] Why do seizures sometimes develop in humans immediately after injury but, in other cases, only after years or decades? Unfortunately, estimates of the latency to the appearance of clinical epilepsy

are inherently unreliable because they are almost always based on the time that elapses between a presumed injury and the observation of a significant behavioral event.[78] This is understandable, as the proper metric, that is, the time of onset of the first focal epileptiform discharges, cannot be determined. For example, should a patient who has experienced a clinically unrecognized aura for 30 years, prior to a first clinical seizure, be regarded as having had epilepsy ever since the first aura, or only after the first clinical seizure has been observed and recognized? That is, does epileptogenesis really take 30 years to mature in this case, with the 30-year-long period of subclinical auras considered to be a preepileptic state during which a single epileptogenic process was slowly maturing? From a neurobiological perspective, the important event for the concept of epileptogenesis would seem to be the first onset of epileptiform discharges that produced the first aura (a focal seizure), regardless of whether the first discharges spread sufficiently to disrupt behavior or consciousness and become clinically obvious. We contend that delays in the appearance of clinical seizures following brain injuries have been inferred incorrectly to indicate that epileptogenesis is a specific and progressive unitary process that is as long in duration as the time it takes for generalized or clinically obvious seizures to appear and be recognized. Clearly, the calculated latent period cannot reflect the length of epileptogenesis if the time to the appearance of the first epileptiform discharges cannot be accurately assessed. Regardless, recent results indicate that highly experimenter-controlled hippocampal injuries consistently produce immediate hippocampal epileptogenesis that is coincident with initial neuron loss.[41]

Based on our most recent studies,[41,43] we suggest that acquired temporal lobe epilepsy involves a relatively straightforward two-stage process. First, an injury causes changes that result in spontaneous principal cell epileptiform discharges, which are presumably subclinical in most cases. This stage (*epileptogenesis*) might be most effectively impeded in the immediate postinjury period by a neuroprotective treatment that minimizes the extent of initial neuron loss, some of which is delayed,[42] and may be initially susceptible to treatment. Second, subclinical discharges increase gradually in duration, and invade and recruit other neuronal populations that act initially as barriers to seizure spread, ultimately causing clinical epilepsy, which includes subtle focal seizures.[41] This distinct second phase (*epileptic maturation*) might be most effectively targeted by treatments that retard the kindling process or that interfere with pro-epileptic secondary processes such as glial abnormalities, neurogenesis, synaptic reorganization, altered receptor expression, and so on.

A GRID CELL HYPOTHESIS OF TEMPORAL LOBE EPILEPTOGENESIS

The data discussed above regarding the possible epileptogenicity of damage in the entorhinal cortex and dentate gyrus permit a conceptual synthesis that may have implications for thinking about temporal lobe epileptogenesis and devising strategies to prevent it. If all animals subjected to a uniform insult exhibit pathological changes that closely resemble the human neurological condition, and if all of these animals exhibit spontaneous hippocampal-onset seizures that develop without delay,[41] then epilepsy in these animals is the likely result of the immediate effects of injury, rather than being the result of delayed secondary processes that develop after the animal is already epileptic.[41,76]

If hippocampal injury causes immediate granule cell hyperexcitability, and if no recurrent excitatory connections are necessary for epileptiform discharges to occur, why do granule cells spontaneously generate only brief, intermittent seizure discharges and behavioral seizures[41] rather than continuous epileptiform discharges and status epilepticus? We hypothesize that some of the answers may lie in the nature of incomplete hilar and entorhinal cortex neuron loss, and the behavior of layer II entorhinal neurons, which form the main excitatory input to the dentate granule cells.[79] Layer II entorhinal cortical neurons (EC2) are hyperexcitable following status epilepticus,[45] presumably as a consequence of cell loss in the adjacent layers III (EC3) and V (EC5)[44,80,81] or other closely related nuclei. Recent studies in normal animals have shown that EC2 neurons constitute a system of *grid cells* in which individual EC2 neurons form an

environment-independent spatial coordinate system.[82,83] These grid cells normally discharge strictly independently in a spatial environment and exhibit discrete inhibitory surrounds.[82] Apparently, the independent firing patterns of EC2 grid cells constitute a universal map of the spatial environment, and these grid cells feed this information to their target cells in the dentate gyrus.[84] We predict that the pathology reliably produced in the EC3 and EC5 layers by prolonged convulsive or nonconvulsive status epilepticus[41,43,44] reduces the size of the grid cell inhibitory surround and decreases the location-based specificity of EC2 neuron discharges. EC2 neuron hyperexcitability may occur as a result of the loss of EC3 and EC5 neurons, which normally influence EC2 neurons.[85] Loss of EC2 neuronal inhibition would cause EC2 pyramidal cells to coalesce functionally, to disrupt the grid function that establishes normal spatial memory, and to generate abnormal synchronous discharges that propagate directly to the granule cell layers.[86] Therefore, we predict that after extensive stimulation-induced injury of hilar neurons and EC3 and EC5 cells,[41,43] EC2 grid cells should lose their inhibitory surrounds and their spatial separation immediately, and begin to generate synchronous discharges that cause the spontaneous granule cell layer depolarizations, population spikes (apparent biomarkers of imminent epileptiform discharges), and epileptiform discharges that we have consistently recorded in awake rats prior to each granule cell-onset seizure[41,43] (Fig. 34–1). Changes in the GABAergic projection from entorhinal cortex to hippocampus[87] might also affect hippocampal excitability.

CONCLUSION

In summary, we suggest that acquired temporal lobe epileptogenesis involves two causal pathologies: (1) extensive hilar neuron (mossy cell) loss, which reduces translamellar granule cell inhibition,[20] causing granule cell hyperexcitability to afferent input,[29,32] and (2) entorhinal cortex damage, which causes a loss of functional separation in layer II grid cells, resulting in abnormal and synchronous excitation of disinhibited granule cells, which generates spontaneous granule cell-onset seizures.[32,41,43] We hypothesize that widespread brain damage, such as that caused by prolonged convulsive status epilepticus, can result in immediate clinical epilepsy in both rats and humans[41,77] because all cortical and subcortical barriers to the spread of focal seizures are damaged or functionally altered by the initial insult. Conversely, after more limited injury in the entorhinal cortex and hilus, it may take time for EC2 neurons to begin to generate abnormal discharges. Once begun, it may require additional time for entorhinal discharges to overcome the granule cell inhibition that remains as a result of incomplete hilar neuron loss. Thus, granule cell seizures would not start until the entorhinal cortex generates abnormal discharges capable of overcoming granule cell inhibition and evoking granule cell epileptiform activity. From this perspective, the inhibitory effects of mossy fiber sprouting may establish or extend the latency to the first granule cell-onset seizures by making granule cells more resistant to generating their first epileptiform discharges.[30,31,51]

A postinjury delay of the first spontaneous granule cell discharges, plus additional time needed to recruit hippocampal pyramidal cells and to overcome downstream barriers to seizure spread, could explain the prolonged period of subclinical focal discharges that precedes the appearance of clinical life-disrupting seizures. The hypothesis that the progression from subclinical to clinical epilepsy involves a time-consuming kindling process[88] that should be targeted in the immediate postinjury period[41,43] is not original, but it is wholly consistent with the ideas of Graham Goddard, which were cogently summarized in Goddard's obituary by Frank Morrell.[89] Thus, we suggest that two mechanisms should be primary targets for pharmacological treatment: (1) initial neuron loss, which has a delayed component[42] that may constitute a therapeutic window that remains open for several days, and (2) a kindling process,[88] interruption of which could, at least theoretically, extend the latent period to clinical seizures indefinitely, even if epileptogenic neuron loss cannot be substantially reduced.

In addition to the need for experimental resolution of a variety of issues using animal models that reliably involve hippocampal epileptogenesis, it may be worthwhile to consider that the terms *epileptogenesis*, *latent period*, and *kindling* are names of concepts created by the human mind, rather than being real and readily definable or identifiable neurobiological

entities.[90] Giving the name *epileptogenesis* to a probably multifactorial process of unknown nature has implications for how we think about that process, and implies that it is something singular that can be prevented or aborted if only "it" can be identified. Similarly, the term *latent period* is a conception that confers significance on an ill-defined time period with an uncertain beginning and an unknown maturation date.[34,76] The use of the term *latent period* has significant implications for the importance we ascribe to a perceived interval during which nothing is observed. Clearly, an inability to hear a distant conversation is not evidence that nothing has been said. Given these considerations, the clarity of the discussion of the process that causes the brain to generate abnormal synchronized discharges for the first time (epileptogenesis), and of the secondary processes that enable focal epileptiform discharges to spread and become clinically detectable (epileptic maturation), might benefit from referring to the neurobiological processes themselves, rather than using created names applied to difficult-to-define subjective conceptions of those processes.[90]

Finally, given that many endogenous homeostatic mechanisms normally limit excitation, epilepsy may be viewed as an unusually powerful network defect that, once established, cannot be completely suppressed by any combination of homeostatic mechanisms and cannot be easily controlled pharmacologically. If any pharmacological intervention can successfully interfere with the processes of epileptogenesis and epileptic maturation, the combination of a neuroprotective compound and an antikindling compound in the immediate postinjury period, followed by long-term treatment with an antikindling compound alone, might be the most logical treatment approach.

SYNOPSIS

The mechanisms that cause acquired temporal lobe epilepsy are unknown. Suspected mechanisms include neuron loss, synaptic reorganization, and granule cell dispersion, but determining which abnormalities mediate epileptogenesis has been problematic because the most frequently used chemoconvulsant-based animal models exhibit extreme variability and minimal evidence of hippocampal epileptogenesis. Continuous monitoring of behavior and granule cell layer activity in awake rats after hippocampal injury caused by stimulation-induced status epilepticus has now shown that granule cells generate spontaneous field depolarizations, population spikes, and epileptiform discharges in the first days postinjury, prior to each generalized behavioral seizure. Thus, injury-associated hippocampal epileptogenesis is coincident with initial neuron loss, not delayed secondary processes. We hypothesize that neuron loss in the entorhinal cortex disrupts the functional separation of Layer II "grid cells," causing abnormal synchronous discharges that invade the dentate gyrus. This, in turn, produces population spikes and epileptiform discharges in granule cells disinhibited by injury-induced hilar neuron loss. Long delays between injury and generalized behavioral seizures, when they occur, may primarily involve a "kindling" process in which initially focal (subclinical) discharges gradually increase in duration and cause clinical seizures. Neuroprotection in the immediate post-injury period, and prolonged anti-kindling therapy, might be the most effective anti-epileptic strategy.

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DISCLOSURE STATEMENT

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REFERENCES

1. Reid CA, Berkovic SF, Petrou S. Mechanisms of human inherited epilepsies. *Prog Neurobiol*. 2009;87:41–57.

2. Gaitanis JN, Walsh CA. Genetics of disorders of cortical development. *Neuroimag Clin North Am.* 2004;14: 219–229.
3. McNamara JO. Cellular and molecular basis of epilepsy. *J Neurosci*. 1994;14:3413–3425.
4. Chang BS, Lowenstein DH. Mechanisms of disease; epilepsy. *N Engl J Med*. 2003;349:1257–1266.
5. Fernández G, Effenberger O, Vinz B, Steinlein O, Elger CE, Döhring W, Heinze HJ. Hippocampal malformation as a cause of familial febrile convulsions and subsequent hippocampal sclerosis. *Neurology*. 1998;50:909–917.
6. Falconer MA, Taylor DC. Surgical treatment of drug-resistant epilepsy due to mesial temporal lobe sclerosis; etiology and significance. *Arch Neurol*. 1968;19:353–361.
7. Jasper HH, Pertuiset B, Flanigin H. EEG and cortical electrograms in patients with temporal lobe seizures. *Arch Neurol Psychiatry*. 1951;65:272–290.
8. Meldrum BS, Bruton CJ. Epilepsy. In Adams JH, Duchen LW, eds. *Greenfield's Neuropathology.* New York: Oxford University Press; 1992:1246–1283.
9. Margerison JH, Corsellis JA. Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain.* 1966;89:499–530.
10. Lorente de Nó R. Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *J Psychol Neurol*. 1934;46:113–177.
11. Amaral DG. A Golgi study of cell types in the hilar region of the hippocampus in the rat. *J Comp Neurol.* 1978;182:851–914.
12. Berger TW, Semple-Rowland S, Bassett JL. Hippocampal polymorph neurons are the cells of origin for ipsilateral association and commissural afferents to the dentate gyrus. *Brain Res*. 1981;224:329–336.
13. Bakst I, Avendano C, Morrison JH, Amaral DG. An experimental analysis of the origins of somatostatin-like immunoreactivity in the dentate gyrus of the rat. *J Neurosci.* 1986;6:1452–1462.
14. Sloviter RS. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science*. 1987;235:73–76.
15. Tauck DL, Nadler JV. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid treated rats. *J Neurosci.* 1985;5:1016–1022.
16. Haas CA, Frotscher M. Reelin deficiency causes granule cell dispersion in epilepsy. *Exp Brain Res*. 2010;200:141–149.
17. Sloviter RS. "Epileptic" brain damage in rats induced by sustained electrical stimulation of the perforant path. I. Acute electrophysiological and light microscopic studies. *Brain Res Bull*. 1983;10:675–697.
18. Sloviter RS. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the dormant basket cell hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus*. 1991;1:41–66.
19. Lowenstein DH, Thomas MJ, Smith DH, McIntosh TK. Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. *J Neurosci*. 1992;12:4846–4853.
20. Zappone CA, Sloviter RS. Translamellar disinhibition in the rat hippocampal dentate gyrus after seizure-induced degeneration of vulnerable hilar neurons. *J Neurosci.* 2004;24:853–864.
21. Buzsáki G, Eidelberg E. Commissural projection to the dentate gyrus of the rat: evidence for feed-forward inhibition. *Brain Res*. 1981;230:346–350.
22. Buzsáki G, Eidelberg E. Direct afferent excitation and long-term potentiation of hippocampal interneurons. *J Neurophysiol*. 1982;48:597–607.
23. Douglas RM, McNaughton BL, Goddard GV. Commissural inhibition and facilitation of granule cell discharge in fascia dentata. *J Comp Neurol*. 1983;219: 285–294.
24. Bilkey DK, Goddard GV. Septohippocampal and commissural pathways antagonistically control inhibitory interneurons in the dentate gyrus. *Brain Res*. 1987;405:320–325.
25. Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, De Felipe J, Ben-Ari Y, Esclapez M, Bernard C. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. *Nat Neurosci*. 2001;4:52–62.
26. Sun C, Mtchedlishvili Z, Bertram EH, Erisir A, Kapur J. Selective loss of dentate hilar interneurons contributes to reduced synaptic inhibition of granule cells in an electrical stimulation-based animal model of temporal lobe epilepsy. *J Comp Neurol*. 2007;500:876–893.
27. Johansen FF, Zimmer J, Diemer NH. Early loss of somatostatin neurons in dentate hilus after cerebral ischemia in the rat precedes CA1 pyramidal cell loss. *Acta Neuropathol*. 1987;73:110–114.
28. Mody I, Otis TS, Bragin A, Hsu M, Buzsáki G. GABAergic inhibition of granule cells and hilar neuronal synchrony following ischemia-induced hilar neuronal loss. *Neuroscience.* 1995;69:139–150.
29. Jinde S, Zsiros V, Kohno K, Nakazawa K. Generation and characterization of inducible-dentate mossy cell ablation mice. Program number 645.11. 2008 Neuroscience Meeting Planner, Chicago, IL: Society for Neuroscience, 2008. the URL is: http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=c9f36363-a10a-479-b-b930–57f8fdfc81f0&cKey=e4cbc77e-83bf-4-ca5-b871-df97324d0fbc&mKey={AFEA068D-D012–4520–8E42–10E4D1AF7944}
30. Sloviter RS. Possible functional consequences of synaptic reorganization in the dentate gyrus of kainate-treated rats. *Neurosci Lett*. 1992;137:91–96.
31. Harvey BD, Sloviter RS. Hippocampal granule cell activity and c-Fos expression during spontaneous seizures in awake, chronically epileptic, pilocarpine-treated rats; implications for hippocampal epileptogenesis. *J Comp Neurol*. 2005;488:441–462.
32. Sloviter RS. The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy. *Ann Neurol*. 1994;35:640–654.
33. Wasterlain CG, Mazarati AM, Shirasaka Y, Thompson KW, Penix L, Liu H, Katsumori H. Seizure-induced hippocampal damage and chronic epilepsy: a Hebbian theory of epileptogenesis. *Adv Neurol*. 1999;79: 829–843.
34. Bragin A, Wilson CL, Engel J Jr. Chronic epileptogenesis requires development of a network of pathologically interconnected neuron clusters: a hypothesis. Epilepsia. 2000;41(suppl 6):S144–S152.
35. Stables JP, Bertram E, Dudek FE, Holmes G, Mathern G, Pitkänen A, White HS. Therapy discovery

for pharmacoresistant epilepsy and for disease-modifying therapeutics: summary of the NIH/NINDS/AES models II workshop. *Epilepsia*. 2003;44:1472–1478.
36. Kobayashi M, Buckmaster PS. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. *J Neurosci*. 2003;23:2440–2452.
37. Mazarati A, Bragin A, Baldwin R, Shin D, Wilson C, Sankar R, Naylor D, Engel J, Wasterlain CG. Epileptogenesis after self-sustaining status epilepticus. Epilepsia. 2002;43(suppl 5):74–80.
38. Raol YH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, Russek SJ, Brooks-Kayal AR. Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. *J Neurosci*. 2006;26:11342–11346.
39. Goffin K, Nissinen J, Van Laere K, Pitkänen A. Cyclicity of spontaneous recurrent seizures in pilocarpine model of temporal lobe epilepsy in rat. *Exp Neurol*. 2007;205:501–505.
40. Jung S, Jones TD, Lugo JN, Sheerin JH, Miller JW, D'Ambrosio R, Anderson AE, Poolos NP. Progressive dendritic HCN channelopathy during epileptogenesis in the rat pilocarpine model of epilepsy. *J Neurosci*. 2007;27:13012–13021.
41. Bumanglag AV, Sloviter RS. Minimal latency to hippocampal epileptogenesis and clinical epilepsy after perforant pathway stimulation-induced status epilepticus in awake rats. *J Comp Neurol*. 2008;510:561–580.
42. Sloviter RS, Dean E, Sollas AL, Goodman JH. Apoptosis and necrosis induced in different hipocampal neuron populations by repetitive perforant path stimulation in the rat. *J Comp Neurol*. 1996;366:516–533.
43. Norwood BA, Bumanglag AV, Osculati F, Sbarbati A, Marzola P, Nicolato E, Fabene PF, Sloviter RS. Classic hippocampal sclerosis and hippocampal-onset epilepsy produced by a single "cryptic" episode of focal hippocampal excitation in awake rats. *J Comp Neurol*. 2010;518:3381–3407.
44. Du F, Eid T, Lothman EW, Köhler C, Schwarcz R. Preferential neuronal loss in layer III of the medial entorhinal cortex in rat models of temporal lobe epilepsy. *J Neurosci*. 1995;15:6301–6313.
45. Kobayashi M, Wen X, Buckmaster PS. Reduced inhibition and increased output of layer II neurons in the medial entorhinal cortex in a model of temporal lobe epilepsy. *J Neurosci*. 2003;23:8471–8479.
46. Nadler JV. The recurrent mossy fiber pathway of the epileptic brain. *Neurochem Res*. 2003;28:1649–1658.
47. Laurberg S, Zimmer J. Lesion-induced sprouting of hippocampal mossy fiber collaterals to the fascia dentata in developing and adult rats. *J Comp Neurol*. 1981;200:433–459.
48. Frotscher M, Zimmer J. Lesion-induced mossy fibers to the molecular layer of the rat fascia dentata: identification of postsynaptic granule cells by the Golgi-EM technique. *J Comp Neurol*. 1983;215:299–311.
49. Nadler JV, Perry BW, Gentry C, Cotman CW. Loss and reacquisition of hippocampal synapses after selective destruction of CA3-CA4 afferents with kainic acid. *Brain Res*. 1980;191:387–403.
50. Jiao Y, Nadler JV. Stereological analysis of GluR2-immunoreactive hilar neurons in the pilocarpine model of temporal lobe epilepsy: correlation of cell loss with mossy fiber sprouting. *Exp Neurol*. 2007;205:569–582.
51. Sloviter RS, Zappone CA, Harvey BD, Frotscher M. Kainic acid-induced recurrent mossy fiber innervation of dentate gyrus inhibitory interneurons: possible anatomical substrate of granule cell hyperinhibition in chronically epileptic rats. *J Comp Neurol*. 2006;494:944–960.
52. Queiroz CM, Gorter JA, Lopes da Silva FH, Wadman WJ. Dynamics of evoked local field potentials in the hippocampus of epileptic rats with spontaneous seizures. *J Neurophysiol*. 2009;101:1588–1597.
53. Kotti T, Riekkinen PJ Sr, Miettinen R. Characterization of target cells for aberrant mossy fiber collaterals in the dentate gyrus of epileptic rat. *Exp Neurol*. 1997;146:323–330.
54. Sloviter RS, Zappone CA, Harvey BD, Bumanglag AV, Bender RA, Frotscher M. "Dormant basket cell" hypothesis revisited; relative vulnerabilities of dentate gyrus mossy cells and inhibitory interneurons after hippocampal status epilepticus in the rat. *J Comp Neurol*. 2003;459:44–76.
55. Amaral DG, Witter MP. The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience*. 1989;31:571–591.
56. Sutula T, Zhang P, Lynch M, Sayin U, Golarai G, Rod R. Synaptic and axonal remodeling of mossy fibers in the hilus and supragranular region of the dentate gyrus in kainate-treated rats. *J Comp Neurol*. 1998;390:578–594.
57. Sloviter RS, Dichter MA, Rachinsky TL, Dean E, Goodman JH, Sollas AL, Martin DL. Basal expression and induction of glutamate decarboxylase and GABA in excitatory granule cells of the rat and monkey hippocampal dentate gyrus. *J Comp Neurol*. 1996;373:593–618.
58. Houser CR. Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. *Brain Res*. 1990;535:195–204.
59. Seress L, Frotscher M. Morphological variability is a characteristic feature of granule cells in the primate fascia dentata: a combined Golgi/electron microscope study. *J Comp Neurol*. 1990;293:253–267.
60. Haas CA, Dudeck O, Kirsch M, Huszka C, Kann G, Pollak S, Zentner J, Frotscher M. Role for reelin in the development of granule cell dispersion in temporal lobe epilepsy. *J Neurosci*. 2002;22:5797–5802.
61. Rakic P, Caviness VS Jr. Cortical development: view from neurological mutants two decades later. *Neuron*. 1995;14:1101–1104.
62. Frotscher M. Cajal-Retzius cells, Reelin, and the formation of layers. *Curr Opin Neurobiol*. 1998;8:570–575.
63. Rice DS, Curran T. Role of the reelin signaling pathway in central nervous system development. *Annu Rev Neurosci*. 2001;24:1005–1039.
64. Tissir, F, Goffinet AM. Reelin and brain development. *Nat Rev Neurosci*. 2003;4:496–505.
65. Förster E, Zhao S, Frotscher M. Laminating the hippocampus. *Nat Rev Neurosci*. 2006;7:259–267.
66. Cooper JA. A mechanism for inside-out lamination in the neocortex. *Trends Neurosci*. 2008;31:113–119.
67. Frotscher M. Role for Reelin in stabilizing cortical architecture. *Trends Neurosci*. 2010;33:407–414.
68. Stanfield BB, Cowan WM. The morphology of the hippocampus and dentate gyrus in normal and reeler mice. *J Comp Neurol*. 1979;185:393–422.

69. Stanfield BB, Cowan WM. The development of the hippocampus and dentate gyrus in normal and reeler mice. *J Comp Neurol*. 1979;185:423–460.
70. Drakew A, Deller T, Heimrich B, Gebhardt C, Del Turco D, Tielsch A, Förster E, Herz J, Frotscher M. Dentate granule cells in reeler mutants and VLDLR and ApoER2 knockout mice. *Exp Neurol*. 2002;176:12–24.
71. Heinrich C, Nitta N, Flubacher A, Müller M, Fahrner A, Kirsch M, Freiman T, Suzuki F, Depaulis A, Frotscher M, Haas CA. Reelin deficiency and displacement of mature neurons, but not neurogenesis, underlie the formation of granule cell dispersion in the epileptic hippocampus. *J Neurosci*. 2006;264701–4713.
72. Bouilleret V, Ridoux V, Depaulis A, Marescaux C, Nehlig A, Le Gal La Salle G. Recurrent seizures and hippocampal sclerosis following intrahippocampal kainate injection in adult mice: electroencephalography, histopathology and synaptic reorganization similar to mesial temporal lobe epilepsy. *Neuroscience*. 1999;89:717–729.
73. Kobow K, Jeske I, Hildebrandt M, Hauke J, Hahnen E, Buslei R, Buchfelder M, Weigel D, Stefan H, Kasper B, Pauli E, Blümcke I. Increased Reelin promoter methylation is associated with granule cell dispersion in human temporal lobe epilepsy. *J Neuropathol Exp Neurol*. 2009;68:356–364.
74. Young CC, Stegen M, Bernard R, Müller M, Bischofberger J, Veh RW, Haas CA, Wolfart J. Upregulation of inward rectifier K^+ (Kir2) channels in dentate gyrus granule cells in temporal lobe epilepsy. *J Physiol*. 2009;587:4213–4233.
75. Kienzler F, Norwood BA, Sloviter RS. Hippocampal injury, atrophy, synaptic reorganization, and epileptogenesis after perforant pathway stimulation-induced status epilepticus in the mouse. *J Comp Neurol*. 2009;515:181–196.
76. Sloviter RS. Hippocampal epileptogenesis in animal models of mesial temporal lobe epilepsy with hippocampal sclerosis; the importance of the "latent period" and other concepts. *Epilepsia*. 2008; 49(suppl 9):85–92.
77. Mikaeloff Y, Jambaque I, Hertz-Pannier L, Zamfirescu A, Adamsbaum C, Plouin P, Dulac O, Chiron C. Devastating epileptic encephalopathy in school-aged children (DESC): a pseudo encephalitis. *Epilepsy Res*. 2006;69:67–79.
78. French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, Spencer DD. Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. *Ann Neurol.* 1993;34: 774–780.
79. Ruth RE, Collier TJ, Routtenberg A. Topography between the entorhinal cortex and the dentate septotemporal axis in rats: I. Medial and intermediate entorhinal projecting cells. *J Comp Neurol*. 1982;209: 69–78.
80. Schwarcz R, Eid T, Du F. Neurons in layer III of the entorhinal cortex. A role in epileptogenesis and epilepsy? *Ann NY Acad Sci*. 2000;911:328–342.
81. Kumar SS, Buckmaster PS. Hyperexcitability, interneurons, and loss of GABAergic synapses in entorhinal cortex in a model of temporal lobe epilepsy. *J Neurosci*. 2006;26:4613–4623.
82. Hafting T, Fyhn M, Molden S, Moser MB, Moser EI. Microstructure of a spatial map in the entorhinal cortex. *Nature*. 2005;436:801–806.
83. Sargolini F, Fyhn M, Hafting T, McNaughton BL, Witter MP, Moser MB, Moser EI. Conjunctive representation of position, direction, and velocity in entorhinal cortex. *Science.* 2006;312:758–762.
84. van Strien NM, Cappaert NL, Witter MP. The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. *Nat Rev Neurosci*. 2009;10:272–282.
85. Quilichini P, Sirota A, Buzsáki G. Intrinsic circuit organization and theta-gamma oscillation dynamics in the entorhinal cortex of the rat. *J Neurosci.* 2010;30: 11128–11142.
86. Scimemi A, Schorge S, Kullmann DM, Walker MC. Epileptogenesis is associated with enhanced glutamatergic transmission in the perforant path. *J Neurophysiol*. 2006;95:1213–1220.
87. Germroth P, Schwerdtfeger WK, Buhl EH. GABAergic neurons in the entorhinal cortex project to the hippocampus. *Brain Res*. 1989;494:187–192.
88. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol*. 1969;25:295–330.
89. Morrell F. In memoriam Graham Goddard: an appreciation. *Epilepsia*. 1987;28:717–719.
90. Sloviter RS. Apoptosis: a guide for the perplexed. *Trends Pharmacol Sci*. 2002;23:19–24.

Chapter 35

Alterations in Synaptic Function in Epilepsy

Christophe Bernard

Epilepsy is characterized by the occurrence of seizures and by the presence of comorbidities such as cognitive deficits. Their underlying mechanisms remain unknown. Since seizures and cognition usually involve large networks of millions of neurons, it is difficult to formulate working hypotheses regarding potential mechanisms. The activity of these networks is controlled by numerous parameters. Hence, why should a given parameter (or a set of parameters) be considered to play a more important role than any other? Further, some parameter changes may be crucial for epilepsy, while others may be central for the associated comorbidities. Which parameters are common to both issues, and which ones are specific to the various aspects of epilepsy? Since we do not have a comprehensive understanding of how the brain works, it is very difficult to generate a sound hypothesis-driven research strategy to uncover the mechanisms of epileptogenesis. Our strategy must rely simply upon correlations and comparisons with normal circuits. Since one major locus for the transfer of information/activity is the synapse, it seems reasonable to propose that synaptic modifications may be involved in epilepsy and associated comorbidities.

What do we mean by the phrase *synaptic modifications*? A synapse includes three compartments: the presynaptic terminal, the postsynaptic site, and the glial cell processes surrounding them. The extrasynaptic space will not be considered, although it is known to play a central role in controlling synaptic transmission. A simplified scheme of the transfer of information between two neurons involves the release of neurotransmitters by a presynaptic terminal, the activation of postsynaptic receptors, and the uptake of the neurotransmitters (Fig. 35–1). Many features of the synapse can be modified. The number of synapses established

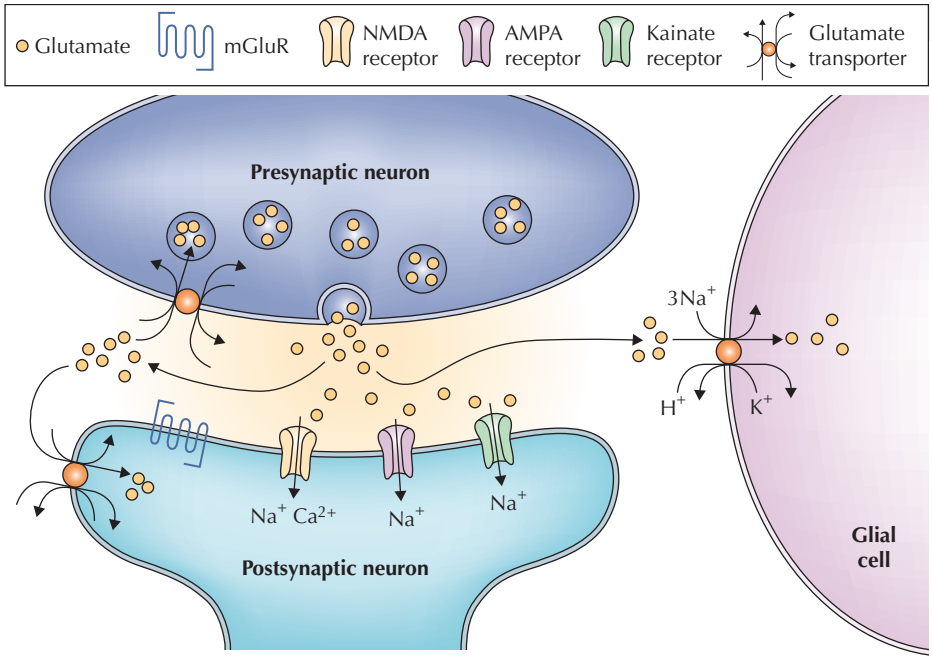

Figure 35–1. Schematic drawing of a glutamatergic synapse, with postsynaptic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), *N*-methyl-D-aspartate (NMDA), kainate, and metabotropic receptors. Glutamate transporters are present on the presynaptic and postsynaptic sites, as well as on the nearby glial cell process. From Attwell D, Gibb A. Neuroenergetics and the kinetic design of excitatory synapses. *Nat Rev Neurosci*. 2005;6:841–849.

by a given neuron on its targets can decrease (*pruning*, or death of the presynaptic neuron) or increase (sprouting, neosynaptogenesis). The properties of the presynaptic terminal can be changed (release probability, neurotransmitter concentration in vesicles, control by presynaptic receptors). On the postsynaptic site, the number, subunit composition, and function (e.g., phosphorylation, anchoring) of the receptors can be changed. Finally, alterations at the glial cell level may affect the environment of the synapse and its function (neurotransmitter uptake, energy supply to neurons, etc.).

How do alterations in synaptic function relate to seizures and their comorbidities? This question has been particularly difficult to address since epilepsy is often a time-dependent disorder, involving (for example) an initial insult (which may involve genetic alterations, meningitis, brain trauma, etc.) and the subsequent trigger of a number of network modifications. Ultimately, some of these modifications may be directly linked to seizure generation and/or comorbidities. It is therefore important to understand the time course of these changes. This issue has been extensively investigated in experimental models of temporal lobe epilepsy, which are characterized by a latent seizure-free period of about 2 weeks following the initial insult (usually a period of status epilepticus). We will use these models to describe synaptic remodeling and its possible functional consequences in the adult brain, focusing on the hippocampus.

PRESYNAPTIC MODIFICATIONS

Neurons receive information from different sources; information from other neurons is generally transmitted at the level of the synapse. The transfer of information starts with the release of neurotransmitter from the presynaptic terminal. The transfer of information ends with the transformation of this chemical signal into an electrochemical one (the flux of ions via the opening of ionotropic receptors and/or the activation of second messengers via metabotropic receptors).

There are multiple ways to change the transfer of information at the presynaptic level between two neurons. These changes can involve the disappearance of the presynaptic terminals themselves, a modification of the neurotransmitter content in the vesicles, and an alteration in control of the release machinery. Several of these modifications have been described in epilepsy. They are presented below, starting with the most drastic one, the loss of the presynaptic terminals.

Cell Death

The loss of the presynaptic terminals can result from the death of the source neuron. Early work demonstrated the rapid loss of glutamatergic synapses on CA1 pyramidal cells due to the death of CA3 pyramidal cells following kainic acid injection in adult animals.[1–3] Similarly, GABAergic (GABA, gamma-aminobutyric acid) synapses disappear due to the death of interneurons soon after status epilepticus.[4–6] Interestingly, interneuron loss is cell-type specific and an early event. For example, it affects axo-axonic cells and oriens lacunosum moleculare (O-LM) interneurons, which project to initial segment and the distal part of the dendritic tree of pyramidal cells, respectively[5,6] (Fig. 35–2).

How does neuron loss affect the network/system? Certainly, neuronal death should change the way these networks process information. For example, the loss of O-LM interneurons is associated with a decreased GABAergic drive in the distal dendrites of CA1 pyramidal cells and with a large facilitation of entorhinal inputs (which O-LM cells control under normal conditions).[6] This reorganization occurs soon after the initial status epilepticus, during the latent period, days before the first spontaneous seizure.[7] Clearly, such a loss of neurons and synapses is not sufficient, in itself, to facilitate/trigger seizures. Using a theoretical approach, we have proposed that the decreased GABAergic drive provides sufficient conditions for the emergence of interictal activity,[7] which

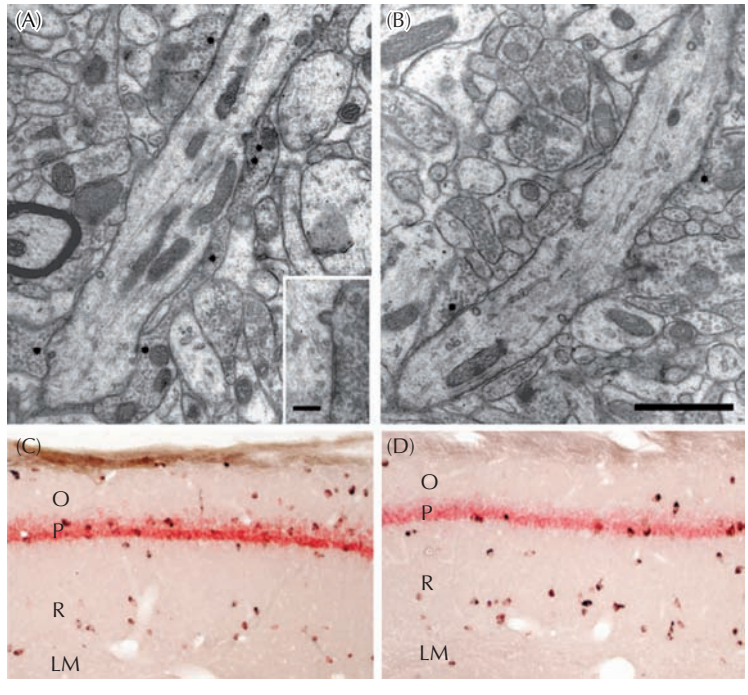

Figure 35–2. Loss of GABAergic synapses. Photomicrograph showing the loss of coverage by symmetrical (GABAergic) synapses (*) of the initial segment of the axon of CA1 pyramidal cells in pilocarpine-treated rats (**B**) compared to controls (**A**). Scale bar, 0.5 µm. **C.** In a control animal, many NeuN- (red) and GAD65 mRNA- (dark) containing neurons can be seen in CA1 stratum oriens (O). Many of these neurons are lost in pilocarpine-treated animals (**D**). They include O-LM interneurons. Adapted from ref. 5.

appears soon after the status epilepticus and which is predictive of epileptogenesis.[7–10] We have also proposed that this reorganization would be causally linked to the degradation of theta rhythm and spatial memory found soon after status epilepticus in these models.[11]

Another example is provided by the loss of cholecystokinin (CCK) basket cells.[12] Basket cells target the soma and proximal dendrites of CA1 pyramidal cells. Two major classes of basket cells can be distinguished based on their neurochemical content: CCK and parvalbumin (PV).[13] Cholecystokinin basket cells appear to degenerate soon after status epilepticus occurs,[12] further supporting the idea that the loss of GABAergic cells is not sufficient to trigger seizures. Cholecystokinin basket cells carry type1 cannabinoid (CB1) receptors on their presynaptic terminals.[14] The activation of CB1 receptors decreases the release of neurotransmitter, in particular GABA, from CCK basket cells.[14] The functional consequence of the loss of such a regulatory pathway remains to be investigated. One might hypothesize that the disappearance of CCK basket cells would increase the functional weight of PV basket cells. Since the latter appear to play a key role in synchronizing large sets of neurons to produce oscillations, an increased PV basket cell contribution may increase the ability of the network to synchronize in a pathological manner.[12,13] It is important to note that the number of CB1-containing GABAergic terminals is not modified in the dentate gyrus from epileptic patients,[15] so it is unclear if this subpopulation of GABAergic neurons is selectively lost in human brain. A change in the CCK/PV basket cell ratio may be species- and/or brain region-dependent.

Finally, the loss of interneurons affects not only GABAergic cells that target principal cells, but also calretinin GABAergic interneurons that contact other interneurons.[16] Such reorganization would remove an inhibitory drive onto interneurons, which may, in turn, contribute to their hyperexcitability in epileptic animals.[6] Since calretinin interneurons may play a central role in synchronizing large ensembles of interneurons, their loss may hamper the capacity of the networks to generate/propagate physiological oscillations[16]—which may help explain the observed decrease in theta rhythm and associated cognitive deficits in epilepsy.[11] Whether the loss of calretinin interneurons increases the synchronization associated with seizures remains to be investigated.

This functional scheme, as intuitively simple as it seems, is based upon what we think we know about brain function. It is in fact simplistic. Losing GABAergic function is generally considered to be bad for the system. But is it? Mutations in the $\alpha 7$ and $\beta 2$ neuronal nicotinic acetylcholine receptor subunit genes result in epilepsy (i.e., GEFS+) . Yet, mouse models of such mutations are characterized by *increased* GABAergic activity, and spontaneous seizures are abolished by the injection of low doses of the $GABA_A$ receptor antagonist picrotoxin.[17]

Similarly, we know that the activation of GABAergic axo-axonic cells can directly excite their targets; GABA has an excitatory action in the initial segment due to the low level of expression of the KCC2 transporter at this site.[18] It could therefore be argued that losing axo-axonic synapses in epilepsy[5,19] would in fact be protective by removing a powerful excitatory and synchronization mechanism. These two examples clearly show that it is very difficult to interpret the observations made in a pathological tissue, if only because we lack a conceptual framework that accounts for the various physiological components of the system.

The loss of neurons can be seen as a drastic modification of the architecture of the network. However, new synapses can be generated, perhaps as a direct consequence of the loss of neurons.

Neosynaptogenesis

Axonal sprouting of glutamatergic neurons has been identified in all hippocampal subfields, including dentate gyrus (mossy fibers), Schaffer collaterals, and CA1 pyramidal cell collaterals.[1,2,20–23] In the CA1 region, the sprouting of CA1 pyramidal cell axons can clearly be identified during the chronic period (i.e., when animals have spontaneous seizures),[22,23] although its time course after the initial insult remains to be established. Interestingly, in the chronically epileptic animal, CA1 axon collaterals innervate the stratum radiatum (Fig. 35–3; a region rarely contacted by CA1 collaterals in normal control animals[23]) and extend farther into the subiculum.[24]

The targeting of new regions has also been established for mossy fibers.[25] The

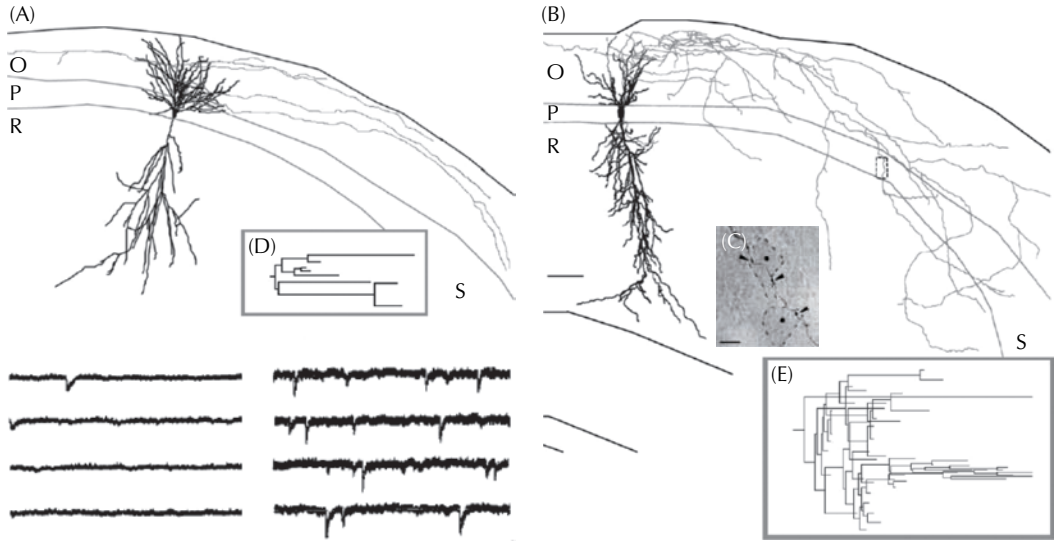

Figure 35–3. Sprouting of CA1 pyramidal cell axon in epileptic animals and increased glutamatergic activity received by pyramidal cells. **A.** Tridimensional reconstruction of a CA1 pyramidal cell in a control animal. Note that the axon (light gray) emits few branches in stratum oriens (O), as shown on the axogram (**D**). **B.** Tridimensional reconstruction of a CA1 pyramidal cell in an epileptic animal. Note that the axon (light gray) displays profuse branching, as shown on the axogram (**E**). Axonal branches cross the pyramidal cell layer (P; panel **C**) and enter stratum radiatum (R), which rarely occurs in control animals. **F.** Patch-clamp recording of the control cell. The downward deflections represent spontaneous AMPA receptor-mediated currents. These glutamatergic events are rare. In contrast, in an epileptic animal, the frequency of these events is considerably increased as a consequence of sprouting (**G**). P: stratum pyramidale, R: stratum radiatum, LM: stratum lacunosum moleculare, S: septum. Adapted from ref. 23.

collateralization of this system appears to depend upon the activation of a serine/threonine kinase (mTOR) that controls protein synthesis linked to cell growth.[26,27] Sprouting is associated with the formation of new synapses[1, 2, 21, 25] that appear to be functional.[23,28] The functional consequence of this neosynaptogenesis include increased connectivity between principal cells and increased glutamatergic drive.[23,28] Although glutamatergic neosynaptogenesis onto GABAergic cells remains to be established, indirect evidence based upon the measure of the frequency of glutamatergic currents received by the surviving interneurons suggests that the sprouting of excitatory axons also targets interneurons.[6]

Early work on the Schaffer collateral pathway led to the hypothesis that the loss of synapses due to presynaptic neuronal death would trigger sprouting and reactive synaptogenesis.[1,2] Whether the death of interneurons following the initial insult also triggers a similar mechanism remains to be clearly established. If it does, it does not fully compensate for the initial loss, since the number of GABAergic synapses on principal cells remains decreased in epileptic animals compared to controls.[5] However, a class of GABAergic cells located in the hilus, and containing somatostatin, displays axonal sprouting and establishes more synaptic contacts with granule cells in epileptic animals.[29] It has been argued that such sprouting may act as a mechanism to compensate for some of the GABAergic neurotransmission that is lost after the death of vulnerable populations of GABAergic interneurons.[29] It will be important to determine the time course of this sprouting after the initial insult in order to relate it to epileptogenesis.

Available information indicates that sprouting of glutamatergic and GABAergic fibers is not an early event. It is tempting to propose that it is causally related to the occurrence of seizures, that is, that increased connectivity is necessary for seizure genesis and propagation. According to this hypothesis, sprouting in the dentate gyrus would create hub cells, which could favor seizure genesis.[30,31] At present, we can only propose a correlation, not causality.

In addition to a morphological reorganization (loss of terminals and neosynaptogenesis),

presynaptic terminals function is modified in epileptic tissue.

Presynaptic Terminal

One key function of presynaptic terminals is the release of neurotransmitter, a process that involves complex machinery regulated by numerous proteins, including presynaptic receptors. There are numerous ways to affect neurotransmitter release, many of which could play a role in epileptogenesis and seizure genesis/propagation. The end product of such modifications is the modulation of the amount of neurotransmitter that is released.

The filling of vesicles can be dynamically modulated. For example, the filling of glutamate vesicles requires allosteric activation by Cl^-.[32] Ketone bodies compete with Cl^- for this allosteric activation and reduce glutamate content in the vesicle, which may contribute to the antiepileptic effect of the ketogenic diet.[32] The filling of GABAergic vesicles can also be modulated. Reactive astrocytosis induces downregulation of glutamine synthetase, which results in decreased GABA synthesis and GABA content in synaptic vesicles (cf. the section "Glial Modifications" later in this chapter).[33] Inflammation, reactive astrocytosis, and decreased glutamine synthetase activity occur soon after the initial insult and are maintained during epileptogenesis.[34,35] These changes should result in decreased GABA content in vesicles. Since there is currently no direct way to measure precisely the neurotransmitter content in vesicles, one must rely on the amplitude distribution of miniature inhibitory postsynaptic currents (mIPSCs) for an indication. In CA1 pyramidal cells, the amplitude of mIPSCs is decreased in epileptic animals, lending support to this hypothesis.[36] In contrast, the amplitude of mIPSCs is increased in dentate granule cells[37] (but see ref. 38). Since the amplitude of mIPSCs depends upon many factors (including the subunit composition, the number of receptors, their phosphorylation state, etc.), it is difficult to draw strong conclusions regarding synaptic filling based on mIPSC analysis (it only provides indirect arguments).

In addition to altered vesicle filling, defects in neurotransmitter release may alter synaptic transmission. A decrease in the reserve pool of GABA-containing vesicles in presynaptic terminals contacting CA1 pyramidal cells may explain a decrease in mIPSC frequency,[36] since miniature events appear to reflect release of the reserve pool rather than release from the immediate releasable pool, at least in glutamatergic terminals.[39] Paired recordings between basket cells and dentate granule cells revealed a decreased probability of release despite a larger size of the immediate releasable pool.[40] Changes in the release machinery (e.g., in presynaptic Ca^{2+} channels) or a different tonic control by presynaptic receptors (e.g., by $GABA_B$, mGluRs, CB1) may explain these modifications.

For example, there is a downregulation of CB1 receptors and of the endocannabinoid machinery in human epileptic tissue.[41] Interestingly, CB1 downregulation affects glutamatergic but not GABAergic terminals, which would remove an important regulatory component of glutamate (excitatory neurotransmitter) release and act as a pro-epileptic factor.[14,42]

The presynaptic terminal is a complex unit of signal integration with multiple control pathways. There are thus countless ways to alter synaptic transmission, many/all of which may be brain state-dependent, since the transmission of information between pre- and postsynaptic elements depends upon the frequency and duration of presynaptic signals. At present, it is not possible to provide a clear picture of the real functional consequences of these reorganizations.

Once neurotransmitters are released, they activate postsynaptic receptors. As is the case for the presynaptic terminal, there are countless ways to change the transfer of information by altering components of the postsynaptic site.

POSTSYNAPTIC MODIFICATIONS

The mechanisms that are known to control $GABA_A$ receptors will be used below as typical examples of how postsynaptic receptors might be modulated; similar considerations could be developed for ionotropic and metabotropic glutamate receptors. These mechanisms are described in the caption of Fig. 35–4.

One key point about this synapse is that $GABA_A$ receptor trafficking is very fast (5 min for subunit assembly, 20 min for cycling).

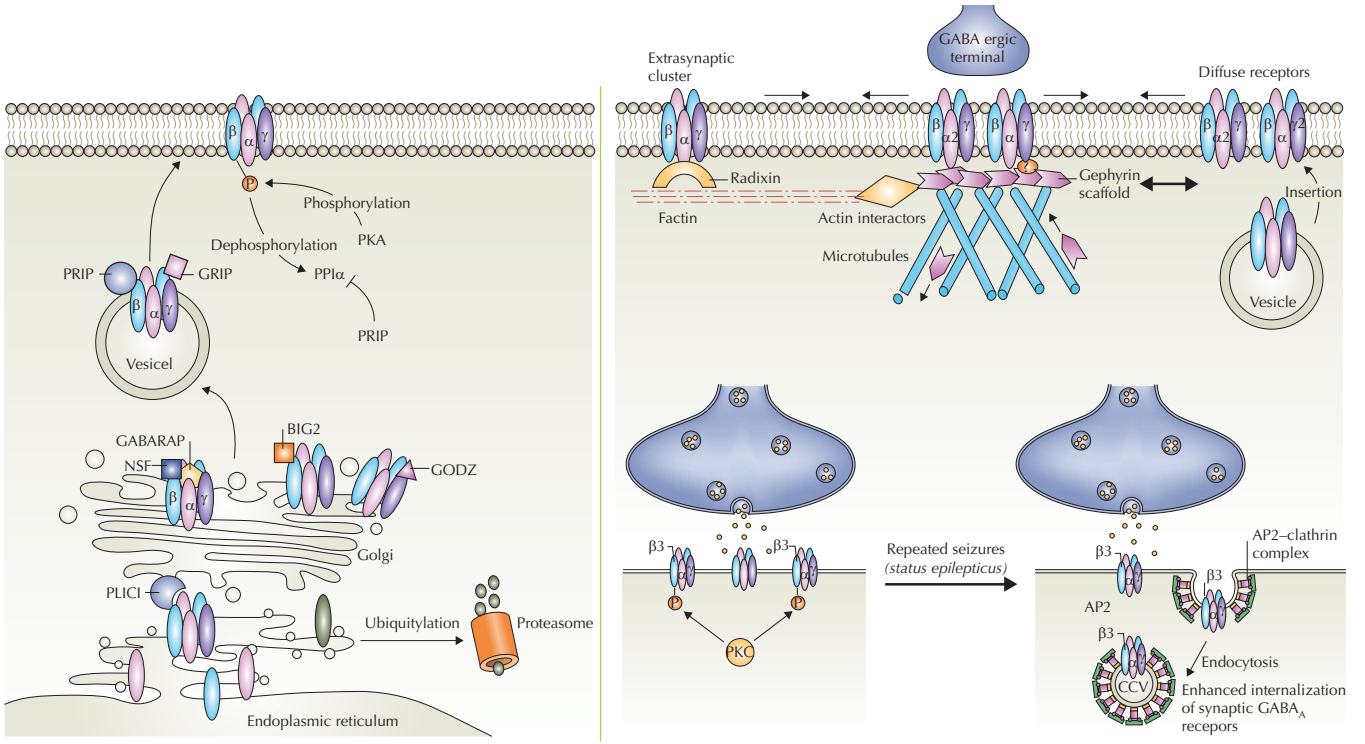

Figure 35–4. Gamma-aminobutyric acid A receptor trafficking. Left panel: Gamma-aminobutyric acid A receptors have a pentameric structure usually made of two α, two β, and one γ subunit. These subunits are assembled in the endoplasmic reticulum to form the receptor. Some of these are ubiquinated to be degraded by the ubiquitin-proteasome system. The ubiquitin-like protein PLIC1 prevents this degradation. Exit from the Golgi apparatus is facilitated by a number of proteins, which can associate with β subunits including GABARAP ($GABA_A$ receptor-associated protein), NSF (*N*-ethylmaleimide-sensitive factor), BIG2 (brefeldin-A-inhibited guanosine diphosphate/guanosine triphosphate exchange factor 2), and γ2 subunits such as GODZ (palmitoyltransferase Golgi-specific DHHC zinc-finger-domain protein). The receptors are then trafficked to the membrane in vesicles, a process that depends upon other proteins such as PRIP (phospholipase-C-related catalytically inactive protein) and GRIF ($GABA_A$ receptor-interacting factor protein). Once inserted, $GABA_A$ receptor properties can be modulated by phosphorylation and dephosphorylation processes. Right top panel: Mobility of $GABA_A$ receptors. Postsynaptic receptors are anchored to microtubules via the interaction between α2 subunits and gephyrin. Gephyrin is also associated with γ2 subunits via an unknown interacting protein. Gamma-aminobutyric acid A receptors can diffuse into and out of the synapse (lateral diffusion). In addition to postsynaptic clusters of receptors, extrasynaptic $GABA_A$ receptors can also be identified. They contain the α5 subunit, which links them to radixin, which is bound to F-actin. These receptors participate in the tonic current. Right bottom panel: Internalization of $GABA_A$ receptors during status epilepticus. Status epilepticus leads to the dephosphorylation of β3 subunits (usually phosphorylated by protein kinase C [PKC]). As a consequence, AP2 (clathrin-adaptor protein 2) can associate with $GABA_A$ receptors, leading to clathrin-dependent endocytosis. Adapted from Jacob TC, Moss SJ, Jurd R. GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci*. 2008;9:331–343.

Although many mechanisms and interacting proteins remain to be identified, the schemes presented in Fig. 35–4 clearly show the diversity of the postsynaptic control systems of GABAergic neurotransmission. These various mechanisms reflect the many parameters that can be altered in epilepsy.

Trafficking of $GABA_A$ Receptors in Epilepsy

After a status epilepticus, $GABA_A$ receptors are internalized.[43,44] The resulting loss of postsynaptic $GABA_A$ receptors may explain why status epilepticus may become pharmacoresistant, at least in response to drugs targeting $GABA_A$ receptors such as benzodiazepines.[43,44] The underlying mechanism involves the dephosphorylation of the β3 subunit, which enables the association between clathrin adaptor 2 (AP2) and β3, and internalization[45] (Fig. 35–4). As might be predicted, blocking this pathway restores GABAergic activity.[45] In addition to internalization, network hyperexcitability increases the lateral diffusion of receptors, which naturally occurs in physiological conditions (Fig. 35–4). As a result, fewer $GABA_A$ receptors are present postsynaptically in hyperexcitable brain, decreasing the amplitude of mIPSCs.[46] These two mechanisms—receptor internalization and receptor diffusion—constitute two examples of fast removal of $GABA_A$ receptors from the postsynaptic site. Whether increased lateral diffusion and endocytosis occur after a spontaneous seizure, and whether they constitute a stable feature during epileptogenesis and the chronic period, remain to be investigated.

Changes in Subunit Composition

A straightforward way to change GABAergic function (in a permanent fashion) is to alter the subunit composition of the receptors.[47] Such modifications constitute a hallmark of human epileptic tissue.[48,49] The modifications observed in human tissue are very similar to those reported in experimental animal models.[50] These changes are area-specific; for example, $GABA_A$ receptors appear to be upregulated in the dentate gyrus but downregulated in CA1.[50] A detailed analysis of the dentate gyrus revealed a change in subunit composition, which appears early during epileptogenesis and persists during the chronic phase.[51] This reorganization is associated with increased GABAergic currents, decreased sensitivity to zolpidem (a nonbenzodiazepine that can potentiate $GABA_A$ receptors), and increased inhibition by Zn^{2+}.[51] Since network function may depend upon the kinetics of receptors, alterations in $GABA_A$ receptor kinetics may potentially lead to altered network dynamics. The functional impact of these changes remains to be addressed.

As displayed in Fig. 35–4, $GABA_A$ receptor clusters can also be formed extrasynaptically. They are responsible for the presence of a tonic GABAergic current, which represents more than 75% of the total (transient and tonic) GABAergic current received by principal cells.[52] The subunit composition of extrasynaptic receptors is also modified after the initial insult.[53,54] In particular, there is decreased expression of the δ subunit, which results in decreased sensitivity of the tonic current to neurosteroids.[55] Despite the loss of δ subunit-containing receptors, the magnitude of the tonic current is not modified in epileptic animals, suggesting the presence of compensatory mechanisms.[54] A redistribution of the γ2 subunit to the perisynaptic domain may also participate in the decrease of the amplitude of synaptic GABAergic currents in epileptic tissue.[54]

Alterations in Chloride Homeostasis

Two main ions can transit via the channel opened by $GABA_A$ receptors: Cl^- and bicarbonate. Bicarbonate leaves the cell, but the direction of Cl^- flux critically depends upon the intracellular concentration of Cl^-.[56] If it is low, Cl^- enters the cell, which hyperpolarizes the membrane. If it is high, Cl^- leaves the cell, which depolarizes the membrane. The internal concentration of Cl^- depends upon various transporters (e.g., KCC2, which extrudes Cl^-, and NKCC1, which pumps Cl^- into the cell) and chloride channels (e.g., ClC2). These systems can be dynamically regulated. For example, GABA appears to have an inhibitory action in physiological conditions in the immature intact hippocampus in vitro,[57] and also in vivo in the cerebellum,[58] but shifts to a depolarizing

action after several seizures due to hyperactivity of NKCC1.[57] This result may explain why seizures become resistant to drugs potentiating $GABA_A$ receptors at early developmental stages in humans. Counteracting NKCC1 with the diuretic bumetanide restores the inhibitory action of GABA and renders seizures sensitive to drugs potentiating $GABA_A$ receptors.[57]

In adult human epilepsy, 20% of subicular cells are depolarized by GABA[59] due to the downregulation of the KCC2 transporter in these cells.[60] A shift to a depolarizing action in a minority of cells may be sufficient to favor the occurrence of interictal spikes.[59]

GLIAL MODIFICATIONS

Astrocytes play crucial roles in regulating numerous functions, from blood flow to neuronal activity. In addition to controlling extracellular levels of K^+ and neurotransmitters (e.g., glutamate), they can respond to various neurotransmitters and hormones and release several factors, such as glutamate, adenosine triphosphate (ATP), and others.[61,62] Alterations in astrocytic function in epilepsy may directly alter synaptic function. Several important modifications have been reported.[62] Only alterations that could change synaptic function are described below.

The first obvious change is morphological. Epileptic tissue is characterized by the presence of reactive astrocytes, as assessed by the overexpression of glial fibrillary acidic protein (GFAP). What could be the functional consequences of this reaction? Astrocytes possess an extensive network of processes. One astrocytic domain can cover approximately 150,000 synapses, a volume that does not overlap with that of neighboring astrocytes in normal conditions. This organization is lost in experimental epilepsy, where there is a 10-fold increase in overlap.[63] The functional consequence of such reorganization is not known, but it has been suggested to be linked to the parallel increase in dendritic spines.[63]

Increased overlap of astrocytic domains could facilitate neuronal synchronization. Astrocytes can release glutamate (or D-serine), producing *N*-methyl-D-aspartate (NMDA) receptor-dependent slow inward currents in neurons, which, in turn, may facilitate neuronal synchronization.[64] Such a scheme has been proposed to play a central role in seizure genesis,[65] although the issue remains controversial.[66] Furthermore, the release of glutamate appears to be increased in astrocytes in epilepsy.[62,67–69] Reactive astrocytes and microglia release tumor necrosis factor α (TNFα), a cytokine that can act on TNFR1 receptors. The activation of TNFR1 increases the production of prostaglandin 2, which, in turn, can amplify glutamate release. If such a mechanism is at play in epilepsy, there should be an increase of slow inward currents in neurons, which remains to be tested experimentally. Another important cytokine released by reactive astrocytes is interleukin 1β (IL-1β). Interleukin 1β can boost NMDA receptor responses via the activation of Src tyrosine kinases and subsequent NR2A/B subunit phosphorylation, which could favor hyperexcitability.[70]

Under physiological conditions, NMDA receptors are blocked most of the time by Mg^{2+}, that is, they do not conduct any current when activated by glutamate. In contrast, in the epileptic hippocampus, NMDA receptors contribute directly to glutamatergic neurotransmission and produce a long-lasting depolarization of neurons.[71] The origin of such an increase in the NMDA contribution has remained elusive, although a change in the redox state of NMDA receptors has been proposed as an important contributing factor.[72]

Other changes in astrocytic function may increase the likelihood of neuronal synchronization. Astrocytes play an active role in regulating the extracellular concentration of glutamate, K^+ and water content.[62] Epileptogenesis is associated with changes in gene expression in astrocytes.[73] A marked downregulation of two genes coding for two astrocytic glutamate transporters results in reduced astrocytic glutamate uptake. Current data suggest that the functional consequence of this change is marginal and is seen only during high-frequency (>100 Hz) stimulation.[73] In addition to glutamate transporters, there is a marked reduction in the expression of the inwardly rectifying K^+ channel, Kir4.1, in astrocytes, resulting in decreased K^+ buffering capacity and facilitation of glutamatergic synaptic responses.[73]

It is important to note that Kir1.4 acts in concert with aquaporin 4 (AQP4) to regulate K^+ and water levels in the extracellular space. Seizure genesis leads to cell swelling, reducing

the extracellular space and favoring hyperexcitability.[74] The downregulation of APQ4 and its redistribution away from the astrocytic endfeet in epilepsy[75,76] may further impair K^+ buffering and favor hyperexcitability.

In addition to postsynaptic/extrasynaptic consequences, as described above, astrocytic dysfunction may have pro-epileptic effects at presynaptic sites. Synaptic function critically depends upon the glutamate-glutamine cycle. Glutamate transported into astrocytes is transformed into glutamine by glutamine synthetase. Glutamine is then exported to neurons, where it is transformed into glutamate by the mitochondrial glutaminase. Glutamate is then reused as such or transformed into GABA. Inhibition of glutamine synthesis results in specific downregulation of GABAergic neurotransmission involving a presynaptic mechanism (decreased vesicular GABA content).[77] Reactive astrocytosis reduces GABAergic neurotransmission,[33] perhaps since there is a downregulation of glutamine synthetase in epileptic tissue.[73] Depleted GABAergic terminals may favor the occurrence of paroxysmal discharges.

FUNCTIONAL CONSEQUENCES OF THESE MODIFICATIONS

The reorganizations occurring around the synapse are extremely diverse and complex. What could be the functional consequences? The hypotheses are admittedly very speculative, since we do not know the role of each parameter under physiological conditions. Very importantly, a drastic alteration of one parameter (e.g., the loss of GABAergic inhibition or its transformation into excitation) may have no functional impact. This is a key concept derived from Eve Marder's work,[78] summarized below. Simply put, this work, performed in the stomatogastric system of the lobster, has led to the concept that there are multiple solutions to a given biological problem. The stomatogastric system, which generates a rhythm vital for the animal, is composed of three nuclei connected to each other via different neurotransmitter systems. Knowing the types of channels expressed by the neurons in each nucleus and the types of connections, the researchers built a computer model in which each parameter (e.g., amplitude of the ionic current, strength of the connection) could take any biologically realistic value. They varied all the parameters and selected the sets of parameters that produced the same rhythm recorded in vivo. They found that there are countless possible solutions, which produce the same behavior at the network level.[78] Importantly, they also found that the system is resistant even if one type of channel is not expressed or if a connection between two nuclei is missing. Further, the values taken by a given parameter (among the sets of solutions) match the biological variability.[79,80] That is, the variability of a given parameter measured in a biological system (e.g., amplitude of $GABA_A$ receptor-mediated currents) may just reflect the different solutions that enable networks to function adequately. One might therefore consider that all the modifications occurring in epileptic networks (including those described above) may simply constitute the expression of another set of solutions to perform normal physiological function. Seizures are, after all, very infrequent events—which suggests that most of the time the system can cope with various parameters' permutations without engaging in abnormal activity.

Nevertheless, important functional changes in epilepsy appear to stem from some of the synaptic modifications identified so far. We'll consider interictal activity and cognitive deficits.

Interictal Activity

Interictal-like activity appears very early after the initial insult in experimental models, and precedes (by days) and even predicts the appearance of the chronic phase of epilepsy defined by recurrent seizures.[7,8,81] Using a crude model of hippocampal circuitry, we have tried to determine the conditions sufficient for the genesis of interictal spikes.[7] Many different solutions exist, which include decreased dendritic GABAergic inhibition and increased glutamatergic excitation,[7] in a range of values found experimentally.[6,7] This model does not explain why interictal activity is not permanent in vivo, but it suggests clues regarding its underlying mechanisms. Since interictal activity is rarely encountered in nonepileptic individuals, it has been proposed that it is pathological. Studies performed in vitro suggest that interictal-like activity can produce long-term potentiation of

synapses, thus contributing to the construction of hyperexcitable networks.[82] The presence of interictal-like activity during the earliest stages of epileptogenesis may not only constitute biomarkers for at-risk patients, but also one core mechanism of epileptogenesis.[7,8,81] One study performed in patients with temporal lobe epilepsy suggests that the size of the epileptogenic zone increases with the duration of epilepsy.[83] The brain regions outside the epileptogenic zone (i.e., the irritative zone) are often characterized by the presence of interictal spikes. Some of these regions become part of the epileptogenic zone as epilepsy evolves over time.[83] It is therefore tempting to propose that interictal spikes participate in the transformation of the irritative regions into epileptic ones.

Cognitive Deficits

Interictal spikes may have other deleterious consequences, in particular for learning and memory processes. Interictal spikes correspond to a more or less synchronous firing of large populations of cells.[84,85] In physiological conditions, groups of hippocampal cells fire in a coordinated and precisely timed fashion, in particular during replay for the storage of information or its transfer.[86] In keeping with this scheme, interictal spikes have deleterious cognitive consequences during memory retrieval in experimental epilepsy.[87]

Cognitive deficits are consistently found in epileptic patients, but their underlying basis is yet to be determined. Since hippocampal circuitry is drastically modified after the initial insult in experimental animals, it is tempting to propose that the normal physiological functions performed by these circuits are altered. Consistent with this prediction, spatial memory performance is decreased soon after the initial insult.[11] This type of memory depends upon the hippocampus (which is considerably reorganized); in contrast, nonspatial memory, which is hippocampus-independent, is preserved during epileptogenesis and the chronic period.[11] The same study showed that the theta rhythm (4–12 Hz), which plays a key role in numerous cognitive processes,[86] is altered soon after the initial insult, and that spatial performance is proportional to the power of the theta rhythm.[11] These results are consistent with the proposal that altered circuitry should result in dysfunctions. However, they are only correlative; causality has not been demonstrated.

REPAIR STRATEGIES

Several biomarkers have been proposed to predict the construction of an epileptic brain, including inflammatory factors, interictal spikes, and cognitive deficits.[7,11,81,88] Validation of these markers in prospective studies of at-risk patients would open the way to the development of preventive treatments. Since the circuitry is modified soon after the initial insult, it is important to determine the mechanisms responsible for these reorganizations. Alternatively, one could simply try to repair the circuits (without knowledge of the pathological mechanisms). As mentioned above, cell death is a hallmark of epilepsy. It is also an early event. Interestingly, the delivery of viral vectors to supply fibroblast growth factor-2 (FGF-2) and brain-derived neurotrophic factor (BDNF) after the initial insult increased neurogenesis, limited cell damage, and reduced the severity of epilepsy in an experimental model.[89] These findings suggest that the circuitry may be repaired, even partially, after the damage has been done.

Is it possible to act upstream to prevent these changes? To do so would require an understanding of the mechanisms responsible for the reorganization of the circuitry. Key aspects of the early processes triggered by the initial insult are now under investigation and include the rupture of the brain-blood barrier, oxidative stress, and inflammation.[34,35,90,91] Experimental studies have shown that acting on these parameters can be disease-modifying (i.e., may decrease the damage and/or the severity of epilepsy), although none of these attempts have managed to stop the occurrence of spontaneous seizures.[34,35,90,91]

CONCLUSION

The previous considerations clearly demonstrate that many types of reorganization take place at the synaptic level soon after an initial epileptogenic insult. Whether these reorganizations play a role in epileptogenesis, seizure genesis/propagation, or cognitive

deficits remains an open question. All of our studies are correlative. None can show causality since we do not know the role of each parameter under physiological conditions.

DISCLOSURE STATEMENT

The authors have not disclosed any conflicts of interest.

REFERENCES

1. Nadler J, Perry B, Cotman C. Selective reinnervation of hippocampal area CA1 and fascia dentate after destruction of CA3-CA4 afferents with kainic acid. *Brain Res.* 1980;182: 1–9.
2. Nadler J, Perry B, Gentry C , Cotman C. Loss and reacquisition of hippocampal synapses after selective destruction of hippocampal synapses after selective destruction of CA3-CA4 afferents with kainic acid. *Brain Res.* 1980;191:387–403.
3. Nadler J, Perry B, Cotman C. Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells. *Nature.* 1978;271:676–677.
4. Houser CR, Esclapez M. Vulnerability and plasticity of the GABA system in the pilocarpine model of spontaneous recurrent seizures. *Epilepsy Res.* 1996;26: 207–218.
5. Dinocourt C, Petanjek Z, Freund TF, Ben Ari Y, Esclapez M. Loss of interneurons innervating pyramidal cell dendrites and axon initial segments in the CA1 region of the hippocampus following pilocarpine-induced seizures. *J Comp Neurol.* 2003;459:407–425.
6. Cossart R, Dinocourt C, Hirsch JC, et al. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. *Nat Neurosci.* 2001;4:52–62.
7. El-Hassar L, Milh M, Wendling F, et al. Cell domain-dependent changes in the glutamatergic and GABAergic drives during epileptogenesis in the rat CA1 region. *J Physiol.* 2007;578:193–211.
8. Williams PA, White AM, Clark S, et al. Development of spontaneous recurrent seizures after kainate-induced status epilepticus. *J Neurosci.* 2009;29:2103–2112.
9. White A, Williams PA, Hellier JL, et al. EEG spike activity precedes epilepsy after kainate-induced status epilepticus. *Epilepsia.* 2010;51:371–383.
10. Staley KJ, Dudek FE. Interictal spikes and epileptogenesis. *Epilepsy Curr.* 2006;6:199–202.
11. Chauvière L, Rafrafi N, Thinus-Blanc C, et al. Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy. *J Neurosci.* 2009;29:5402–5410.
12. Wyeth MS, Zhang N, Mody I, Houser CR. Selective reduction of cholecystokinin-positive basket cell innervation in a model of temporal lobe epilepsy. *J Neurosci.* 2010;30:8993–9006.
13. Freund TF, Katona I. Perisomatic inhibition. *Neuron.* 2007;56:33–42.
14. Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med.* 2008;14:923–930.
15. Ludányi A, Hu SS, Yamazaki M, Tanimura A, et al. Complementary synaptic distribution of enzymes responsible for synthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in the human hippocampus. *Neuroscience.* 2011;174:50–63.
16. Tóth K, Eross L, Vajda J, et al. Loss and reorganization of calretinin-containing interneurons in the epileptic human hippocampus. *Brain.* 2010;133:2763–2777.
17. Klaassen A, Glykys J, Maguire J, et al. Seizures and enhanced cortical GABAergic inhibition in two mouse models of human autosomal dominant nocturnal frontal lobe epilepsy. *Proc Natl Acad Sci USA.* 2006;103: 19152–19157.
18. Szabadics J, Varga C, Molnár G, et al. Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. *Science.* 2006;311:233–235.
19. Arellano JI, Munoz A, Ballesteros-Yanez I, Sola RG, DeFelipe J. Histopathology and reorganization of chandelier cells in the human epileptic sclerotic hippocampus. *Brain.* 2004;127:45–64.
20. Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol.* 1989;26:321–330.
21. Tauck D, Nadler J. Evidence for functional mossy fiber sprouting in hippocampal formation of kainic acid treated rats. *J Neurosci.* 1985;5:1016–1022.
22. Perez Y, Morin F, Jutras I, Beaulieu C, Lacaille J-C. CA1 pyramidal cells in hyperexcitable slices of kainate-treated rats show sprouting of local axon collaterals. *Eur J Neurosci.* 1996;8:736–748.
23. Esclapez M, Hirsch JC, Ben-Ari Y, Bernard C. Newly formed excitatory pathways provide a substrate for hyperexcitability in experimental temporal lobe epilepsy. *J Comp Neurol.* 1999;408:449–460.
24. Cavazos JE, Jones SM, Cross DJ. Sprouting and synaptic reorganization in the subiculum and CA1 region of the hippocampus in acute and chronic models of partial-onset epilepsy. *Neuroscience.* 2004;126: 677–688.
25. Ribak CE, Tran PH, Spigelman I, Okazaki MM, Nadler JV. Status epilepticus-induced hilar basal dendrites on rodent granule cells contribute to recurrent excitatory circuitry. *J Comp Neurol.* 2000;428: 240–253.
26. Zeng LH, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J Neurosci.* 2009;29:6964–6972.
27. Buckmaster PS, Ingram EA, Wen X. Inhibition of the mammalian target of rapamycin signaling pathway suppresses dentate granule cell axon sprouting in a rodent model of temporal lobe epilepsy. *J Neurosci.* 2009;29:8259–8269.
28. Wuarin JP, Dudek FE. Excitatory synaptic input to granule cells increases with time after kainate treatment. *J Neurophysiol.* 2001;85:1067–1077.
29. Zhang W, Yamawaki R, Wen X, et al. Surviving hilar somatostatin interneurons enlarge, sprout axons, and form new synapses with granule cells in a mouse model of temporal lobe epilepsy. *J Neurosci.* 2009;29: 14247–14256.
30. Dyhrfjeld-Johnsen J, Santhakumar V, Morgan RJ, et al. Topological determinants of epileptogenesis in large-scale structural and functional models of the dentate gyrus derived from experimental data. *J Neurophysiol.* 2007;97:1566–1587.
31. Morgan RJ, Soltesz I. Nonrandom connectivity of the epileptic dentate gyrus predicts a major role for

neuronal hubs in seizures. *Proc Natl Acad Sci USA.* 2008;105:6179–6184.
32. Juge N, Gray JA, Omote H, et al. Metabolic control of vesicular glutamate transport and release. *Neuron.* 2010;68:99–112.
33. Ortinski PI, Dong J, Mungenast A, et al. Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nat Neurosci.* 2010;13:584–591.
34. Vezzani A, Balosso S, Aronica E, Ravizza T. Basic mechanisms of status epilepticus due to infection and inflammation. *Epilepsia.* 2009;50(Suppl 12):56–57.
35. Shlosberg D, Benifla M, Kaufer D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nat Rev Neurol.* 2010;6: 393–403.
36. Hirsch JC, Agassandian C, Merchán-Pérez A, et al. Deficit of quantal release of GABA in experimental temporal lobe epilepsy. *Nat Neurosci*. 1999;2:499–500.
37. Kobayashi M, Buckmaster PS. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. *J Neurosci.* 2003;23:2440–2452.
38. Shao LR, Dudek FE. Changes in mIPSCs and sIPSCs after kainate treatment: evidence for loss of inhibitory input to dentate granule cells and possible compensatory responses. *J Neurophysiol.* 2006;96:961–962.
39. Fremeau RT Jr, Kam K, Qureshi T, et al. Vesicular glutamate transporters 1 and 2 target to functionally distinct synaptic release sites. *Science.* 2004;304:1815–1819.
40. Zhang W, Buckmaster PS. Dysfunction of the dentate basket cell circuit in a rat model of temporal lobe epilepsy. *J Neurosci.* 2009;29:7846–7856.
41. Ludányi A, Eross L, Czirják S, et al. Downregulation of the CB1 cannabinoid receptor and related molecular elements of the endocannabinoid system in epileptic human hippocampus. *J Neurosci.* 2008;28:2976–2990.
42. Bernard C, Milh M, Morozov YM, et al. Altering cannabinoid signaling during development disrupts neuronal activity. *Proc Natl Acad Sci USA.* 2005;102: 9388–9393.
43. Goodkin HP, Yeh JL, Kapur J. Status epilepticus increases the intracellular accumulation of $GABA_A$ receptors. *J Neurosci.* 2005;25:5511–5520.
44. Naylor DE, Liu H, Wasterlain CG. Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci.* 2005;25:7724–7733.
45. Terunuma M, Xu J, Vithlani M, et al. Deficits in phosphorylation of GABA(A) receptors by intimately associated protein kinase C activity underlie compromised synaptic inhibition during status epilepticus. *J Neurosci.* 2008;28:376–384.
46. Bannai H, Lévi S, Schweizer C, et al. Activity-dependent tuning of inhibitory neurotransmission based on $GABA_A$R diffusion dynamics. *Neuron.* 2009;62:670–682.
47. Sperk G, Drexel M, Pirker S. Neuronal plasticity in animal models and the epileptic human hippocampus. *Epilepsia.* 2009;50(Suppl 12):29–31.
48. Loup F, Wieser HG, Yonekawa Y, Aguzzi A, Fritschy JM. Selective alterations in $GABA_A$ receptor subtypes in human temporal lobe epilepsy. *J Neurosci.* 2000;20:5401–5419.
49. Pirker S, Schwarzer C, Czech T, et al. Increased expression of GABA(A) receptor beta-subunits in the hippocampus of patients with temporal lobe epilepsy. *J Neuropathol Exp Neurol.* 2003;62:820–834.
50. Bouilleret V, Loup F, Kiener T, Marescaux C, Fritschy JM. Early loss of interneurons and delayed subunit-specific changes in GABA(A)-receptor expression in a mouse model of mesial temporal lobe epilepsy. *Hippocampus*. 2000;10:305–324.
51. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat Med.* 1998;4:1166–1172.
52. Glykys J, Mody I. Activation of $GABA_A$ receptors: views from outside the synaptic cleft. *Neuron.* 2007;56: 763–770.
53. Coulter DA, Carlson GC. Functional regulation of the dentate gyrus by GABA-mediated inhibition. *Prog Brain Res.* 2007;163:235–243.
54. Zhang N, Wei W, Mody I, Houser CR. Altered localization of GABA(A) receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *J Neurosci.* 2007;27: 7520–7531.
55. Peng Z, Huang CS, Stell BM, Mody I, Houser CR. Altered expression of the delta subunit of the $GABA_A$ receptor in a mouse model of temporal lobe epilepsy. *J Neurosci.* 2004;24:8629–8639.
56. Ben Ari Y. Excitatory actions of gaba during development: the nature of the nurture. *Nat Rev Neurosci.* 2002;3:728–739.
57. Dzhala VI, Kuchibhotla KV, Glykys JC, et al. Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. *J Neurosci.* 2010;30:11745–11761.
58. Bernard C, Axelrad H. Effects of recurrent collateral inhibition on Purkinje cell activity in the immature rat cerebellar cortex—an in vivo electrophysiological study. *Brain Res.* 1993;626:234–258.
59. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science.* 2002;298: 1418–1421.
60. Huberfeld G, Wittner L, Clemenceau S, et al. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. *J Neurosci.* 2007;27:9866–9873.
61. Hamilton NB, Attwell D. Do astrocytes really exocytose neurotransmitters? *Nat Rev Neurosci.* 2010;11: 227–238.
62. Wetherington J, Serrano G, Dingledine R. Astrocytes in the epileptic brain. *Neuron.* 2008;58:168–178.
63. Oberheim NA, Tian GF, Han X, et al. Loss of astrocytic domain organization in the epileptic brain. *J Neurosci.* 2008;28:3264–3276.
64. Angulo MC, Kozlov AS, Charpak S, Audinat E. Glutamate released from glial cells synchronizes neuronal activity in the hippocampus. *J Neurosci.* 2004;24: 6920–6927.
65. Tian GF, Azmi H, Takano T, et al. An astrocytic basis of epilepsy. *Nat Med.* 2005;11:973–981.
66. Fellin T, Gomez-Gonzalo M, Gobbo S, Carmignoto G, Haydon, PG. Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices. *J Neurosci.* 2006;26:9312–9322.
67. Bezzi P, Volterra A. A neuron-glia signalling network in the active brain. *Curr Opin Neurobiol.* 2001;11: 387–394.
68. Bezzi P, Carmignoto G, Pasti L, et al. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature.* 1998;391:281–285.

69. Domercq M, Brambilla L, Pilati E, et al. P2Y1 receptor-evoked glutamate exocytosis from astrocytes: Control by tumor necrosis factor-alpha and prostaglandins. *J Biol Chem.* 2006;281:30684–30696.
70. Viviani B, Bartesaghi S, Gardoni F, et al. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J Neurosci.* 2003;23:8692–8700.
71. Turner D, Wheal H. Excitatory synaptic potentials in kainic acid-denervated rat CA1 pyramidal neurons. *J Neurosci.* 1991;11:2786–2794.
72. Quesada O, Hirsch J, Ben-Ari Y, Bernard C. Redox sites of NMDA receptors can modulate epileptiform activity in hippocampal slices from kainic acid-treated rats. *Neurosci. Letters.* 1996;212:171–174.
73. David Y, Cacheaux LP, Ivens S, et al. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *J Neurosci.* 2009;29:10588–10599.
74. Dudek FE, Obenaus A, Tasker JG. Osmolality-induced changes in extracellular volume alter epileptiform bursts independent of chemical synapses in the rat: importance of non-synaptic mechanisms in hippocampal epileptogenesis. *Neurosci Lett.* 1990;120:267–270.
75. Eid T, Lee TS, Thomas MJ, et al. Loss of perivascular aquaporin 4 may underlie deficient water and K^+ homeostasis in the human epileptogenic hippocampus. *Proc Natl Acad Sci USA.* 2005;102:1193–1198.
76. Lee TS, Eid T, Mane S, et al. Aquaporin-4 is increased in the sclerotic hippocampus in human temporal lobe epilepsy. *Acta Neuropathol.* 2004;108:493–502.
77. Liang SL, Carlson GC, Coulter DA. Dynamic regulation of synaptic GABA release by the glutamate-glutamine cycle in hippocampal area CA1. *J Neurosci.* 2006;26:8537–8548.
78. Prinz AA, Bucher D, Marder E. Similar network activity from disparate circuit parameters. *Nat Neurosci.* 2004;7:1345–1352.
79. Schulz DJ, Goaillard JM, Marder EE. Quantitative expression profiling of identified neurons reveals cell-specific constraints on highly variable levels of gene expression. *Proc Natl Acad Sci USA.* 2007;104:13187–13191.
80. Marder E, Goaillard JM. Variability, compensation and homeostasis in neuron and network function. *Nat Rev Neurosci.* 2006;7:563–574.
81. White A, Williams PA, Hellier JL, et al. EEG spike activity precedes epilepsy after kainate-induced status epilepticus. *Epilepsia.* 2010;51:371–383.
82. Dzhala VI, Staley KJ. Transition from interictal to ictal activity in limbic networks in vitro. *J Neurosci.* 2003;23:7873–7880.
83. Bartolomei F, Chauvel P, Wendling F. Epileptogenicity of brain structures in human temporal lobe epilepsy: a quantified study from intracerebral EEG. *Brain.* 2008;131:1818–1830.
84. de la Prida LM, Huberfeld G, Cohen I, Miles R. Threshold behavior in the initiation of hippocampal population bursts. *Neuron.* 2006;49:131–142.
85. Cohen I, Huberfeld G, Miles R. Emergence of disinhibition-induced synchrony in the CA3 region of the guinea pig hippocampus in vitro. *J Physiol.* 2006;570:583–594.
86. Buzsaki G. *Rhythms of the Brain*. New York: Oxford University Press; 2006.
87. Kleen JK, Scott RC, Holmes GL, Lenck-Santini PP. Hippocampal interictal spikes disrupt cognition in rats. *Ann Neurol.* 2010;67:250–257.
88. Dubé CM, Ravizza T, Hamamura M, et al. Epileptogenesis provoked by prolonged experimental febrile seizures: mechanisms and biomarkers. *J Neurosci.* 2010;30:7484–7494.
89. Paradiso B, Marconi P, Zucchini S, et al. Localized delivery of fibroblast growth factor-2 and brain-derived neurotrophic factor reduces spontaneous seizures in an epilepsy model. *Proc Natl Acad Sci USA.* 2009;106:7191–7196.
90. Fabene PF, Navarro Mora G, et al. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. *Nat Med.* 2008;14:1377–1383.
91. Maroso M, Balosso S, Ravizza T, et al. Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. *Nat Med.* 2010;16:413–419.

Chapter 36

Seizure-Induced Formation of Basal Dendrites on Granule Cells of the Rodent Dentate Gyrus

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Granule cells of the normal adult rodent dentate gyrus generally have the typical morphology of bipolar cells. Their apical dendrites arise from one pole and arborize into the molecular layer, while the axon originates from the base of the granule cell body and extends into the hilus subjacent to the granule cell layer.[1] Two exceptions to this rule have been observed. Sometimes recurrent basal dendrites arise from the base of granule cell bodies and then curve back through the granule cell layer in the direction of the molecular layer, where they join apical dendrites.[2–4] Despite this unusual origination, dendrites of dentate granule cells in rodents arborize exclusively in the molecular layer. The other exception is the rare instance of an axon originating from the granule cell's apical dendrite or the apical pole of its cell body.[4] In this instance, the axon descends into the hilus without giving rise to collaterals. Both of these morphologies suggest that rat granule cells are more heterogeneous than was previously indicated.

Dentate granule cells from humans and nonhuman primates differ from granule cells from rodents; primate granule cells commonly have

basal dendrites. Seress and Mrzljak[5] were the first to show that primate granule cells display basal dendrites in normal brain. Other studies confirmed this observation and showed that many granule cells in monkey have basal dendrites that enter the hilus.[6] These basal dendrites have large, complex spines and smaller "stubby" spines. About 10% of granule cells in the monkey dentate gyrus exhibit basal dendrites. Pertinent to this review is the finding that greater numbers of granule cells with hilar basal dendrites are found in the temporal lobes of epileptic humans compared to normal human control tissues.[7,8] The remainder of this chapter will focus on the seizure-induced formation of hilar basal dendrites in rodents and the potential significance of hilar basal dendrites in epileptogenesis.

SEIZURE-INDUCED HILAR BASAL DENDRITES IN RODENTS

Seizure-induced hilar basal dendrites on dentate granule cells have been observed in the brains of epileptic animals (Fig. 36–1). Using rats in which the perforant path was stimulated to cause seizures, Spigelman et al.[9] showed that 6%–15% of Golgi-impregnated granule cells had basal dendrites that extended into the hilus. In contrast, basal dendrites were not observed in control animals. The presence of basal dendrites on dentate granule cells in the kainic acid model of temporal lobe epilepsy was described shortly afterward by Buckmaster and Dudek.[10] A subsequent study of rats with pilocarpine-induced seizures provided evidence for basal dendrites on dentate granule cells in yet another model of epilepsy.[3] Therefore, in three unique models of temporal lobe epilepsy, anatomical studies demonstrate the formation of basal dendrites on granule cells of the dentate gyrus. In addition, rats with amygdala kindling also have granule cells with hilar basal dendrites.[11] Therefore, it is reasonable to assume that hilar basal dendrites in rats form after both seizures and excessive neuronal activity within the limbic system.

The basal dendrites observed on granule cells from epileptic rats were densely packed with spines along their length, and these spines were similar in morphology to those found on the apical dendrites of granule cells.[9] These

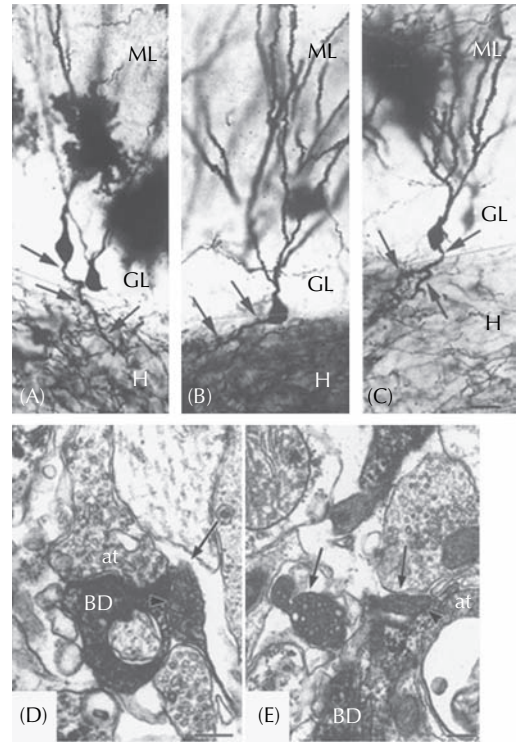

Figure 36–1. Light (**A–C**) and electron (**D,E**) micrographs of hilar basal dendrites. As can be seen in the light micrograph (**A–C**), the basal side of the neuron (arrows) has a dendritic process that extends into the deep hilus. In **D**, there is a labeled basal dendrite (BD) that is postsynaptic (arrowhead) to a labeled axon terminal (arrow). **E.** A hilar basal dendrite with a spine that is postsynaptic (arrowhead) to a labeled axon (arrows). Scale bar: 10 μm in **A–C**, 0.5 μm in **D** and **E** ML, molecular layer; GL, granule cell layer; H, hilus.

dendrites commonly originated from the base of the granule cell body (on the hilar side) and could be clearly distinguished from the axon initial segment.[3] It was rare for the basal dendrite to arise from the lateral side of the granule cell body or the apical dendrite.[9] These basal dendrites run horizontally beneath the granule cell layer or extend relatively straight into the hilus, perpendicular to the granule cell layer. The basal dendrites either branch or remain unbranched. Their lengths vary between 200 and 500 μm, and the basal dendrites populate the subgranular region of the hilus (previously defined as the first 50 μm subjacent to the granule cell layer). Most of the granule cells with basal dendrites have cell bodies located at the hilar border of the granule cell layer or within one or two cell bodies away from this border.

Using electron microscopy, hilar basal dendrites were analyzed in hippocampal slices following biocytin injections into the stratum lucidum of CA3 to retrogradely label the projecting granule cells.[3] Granule cells at the border of the hilus had spiny dendrites projecting into the hilus. Both the spines and dendritic shafts of these biocytin-labeled basal dendrites were postsynaptic to axon terminals (Fig. 36–1). The fact that some of the labeled spines and dendrites were postsynaptic to small biocytin-labeled axon terminals[3] suggested that the granule cells with basal dendrites participated in synaptic circuitry with mossy fiber synapses derived from other dentate granule cells. Subsequent electron microscopic analysis revealed that less than 10% of the synapses on seizure-induced basal dendrites were GABAergic (GABA, gamma-aminobutyric acid); the great majority appeared to be excitatory.[12] These electron microscopic data support the view that the synapses on basal dendrites of granule cells from epileptic animals are likely involved in additional excitatory feedback circuits that could play a role in seizure propensity.

SYNAPTIC CONNECTIONS OF HILAR BASAL DENDRITES

Granule cell basal dendrites are observed in temporal lobe epilepsy,[3,9,10] in other pathophysiological conditions,[13] and normally (to a variable extent) in primates.[6] The functional consequences of this special subpopulation of granule cells depend largely on the synaptic inputs received by basal dendrites and how those inputs differ from those of granule cells with only apical dendrites. Available evidence indicates that basal dendrites receive excitatory input primarily from neighboring granule cells, and this input establishes a recurrent excitatory positive-feedback circuit.[3,12] Basal dendrite involvement in additional circuits has been shown or is suspected, but many questions remain.

Excitatory Synapses

Ultrastructural evidence has been used to characterize excitatory synapses onto basal dendrites.[3,12,14–16] Ribak et al.[3] were the first to demonstrate that granule cell axons (mossy fibers) are at least one source of excitatory synaptic input onto basal dendrites. Mossy fiber synapses with basal dendrites were identified later in epileptic p35 knockout mice.[17] Mossy fiber synapses with basal dendrites create a monosynaptic and local positive-feedback circuit among granule cells. The aberrant circuit is local (and constrained along the septotemporal axis) because mossy fibers remain close to their granule cell of origin, even in epileptic animals with mossy fiber sprouting.[10] Since mossy fibers are concentrated in the hilus, they probably account for a substantial fraction of excitatory synaptic input to basal dendrites. In addition, mossy fibers from epileptic pilocarpine-treated rats are the major source of excitatory synapses to apical dendrites and somata of hilar ectopic granule cells.[18,19]

Widespread convergence of excitatory connections onto hilar basal dendrites might contribute to seizure activity in epileptic animals. For example, other glutamatergic neurons, including mossy cells and CA3 pyramidal cells, extend their axons into the hilus,[20] where they may synapse with basal dendrites. Normally in control animals, mossy cells and CA3 pyramidal cells are directly and strongly excited by granule cells. Mossy cells, in turn, project their axons into the inner molecular layer, where they synapse with granule cell apical dendrites.[21,22] To a lesser extent, some CA3 pyramidal cells also extend axons into the inner molecular layer[23,24] and likely synapse with granule cell apical dendrites. Disynaptic recurrent excitation to granule cells might be exaggerated in epileptic animals because of axonal sprouting by the surviving mossy cells and CA3 pyramidal cells[25] and the formation of new synapses between these aberrant axon collaterals with basal dendrites.

Computational models can provide additional confirmation of the significance of the increased excitatory drive due to basal dendrite formation. Several studies in recent years have demonstrated the role of reduced numbers of hilar mossy cells in subsequent hippocampal excitability.[26–29] Soltesz and colleagues[27,28] have used large-scale modeling of hippocampal excitability, in particular the dentate gyrus, to evaluate network architectural changes and their contributions to

epileptogenesis and hyperexcitability. They constructed a functional model of the dentate gyrus containing several of the major glutamatergic and GABAergic cellular subtypes. Simulations using this model demonstrated that decreasing the number of hilar cells resulted in significant decreases in global connectivity, but the sprouting of granule cell axons resulted in increased local connectivity.[27] The net effect of hilar cell loss and mossy fiber sprouting was increased hyperexcitability within the dentate gyrus. A recent extension of these computational studies suggested that granule cells with hilar basal dendrites could play an important role in generating seizure activity.[28] Specifically, using the functional model containing hilar cell loss and mossy fiber sprouting, the inclusion of various nonrandom granule cell microcircuits in the dentate gyrus was explored. It was shown that a small number of highly interconnected granule cells, or *hubs*, in the network were sufficient to enhance hyperexcitability. The establishment of new synapses on basal dendrites[12] is consistent with the predictions generated from this model. Even though the estimates for such hub cells range from 5% to 20% of the total granule cell population, the relatively low number of hubs was apparently large enough to promote hyperexcitability. In these computational models, it is also important to note that changes in GABAergic connectivity,[30–32] dentate gyrus inputs,[26] cellular geometry,[33] and alterations of intrinsic currents[29] can also modulate granule cell and network excitability. More importantly, physiological and anatomical observations can now be modeled in silico to test new hypotheses and guide future experimental work.

Most excitatory synapses with granule cell apical dendrites are axo-spinous, and that is also true for basal dendrites.[12] However, direct connections of excitatory synapses onto the dendritic shaft are up to four times more common on basal dendrites than on apical dendrites.[12] Similarly, in epileptic pilocarpine-treated rats, dendrites of hilar ectopic granule cells receive a disproportionately large fraction of mossy fiber synapses directly with the dendritic shaft.[19] It is unclear why these aberrant targets in the hilus are more likely to receive excitatory synapses on shafts versus spines, and the functional consequences of these excitatory shaft synapses remain to be elucidated.

The functional effects of basal dendrites are difficult to isolate and test because surgically resected tissue available for study from patients with temporal lobe epilepsy and animal models typically display mossy fiber sprouting into the molecular layer,[34,35] where apical dendrites are located. In these cases, aberrant recurrent excitation among granule cells could be attributable to sprouted mossy fibers that synapse with apical dendrites, basal dendrites, or both.[12,36] Control adult macaque monkeys, on the other hand, have few if any mossy fiber projections into the molecular layer, and approximately 10% of their granule cells have basal dendrites[5] (compared to control adult rodents, in which they rarely occur[9,37,38]). In hippocampal slices from macaque monkeys, mossy fibers were antidromically stimulated and synaptic responses recorded to compare recurrent excitation in granule cells with and without basal dendrites.[39] Excitatory postsynaptic currents were significantly more likely to be evoked and amplitudes were larger in granule cells with basal dendrites. However, since the stimulation paradigm might have activated other axons besides mossy fibers, the possibility of input from CA3 pyramidal cells or mossy cells to basal dendrites could not be excluded. Recordings and intracellular labeling of monosynaptically coupled pairs would be a more rigorous and direct test of the strength and efficacy of unitary synaptic events generated in basal dendrites. Even without those data, currently available functional evidence is consistent with anatomical results showing that mossy fibers synapse with basal dendrites—and thereby produce recurrent excitation among granule cells. These electrophysiological observations have also been modeled and predict virtually similar output—namely, enhanced excitation.[28]

Inhibitory Synapses

In addition to excitatory synapses, basal dendrites in epileptic pilocarpine-treated rats receive GABAergic input[12] (Fig. 36–2). The relative proportion of GABAergic versus glutamatergic synapses is different in apical versus basal dendrites. GABAergic synapses account for 20% and 28% of all synapses with granule cell apical dendrites in control and epileptic rats, respectively.[40] In contrast, GABAergic synapses

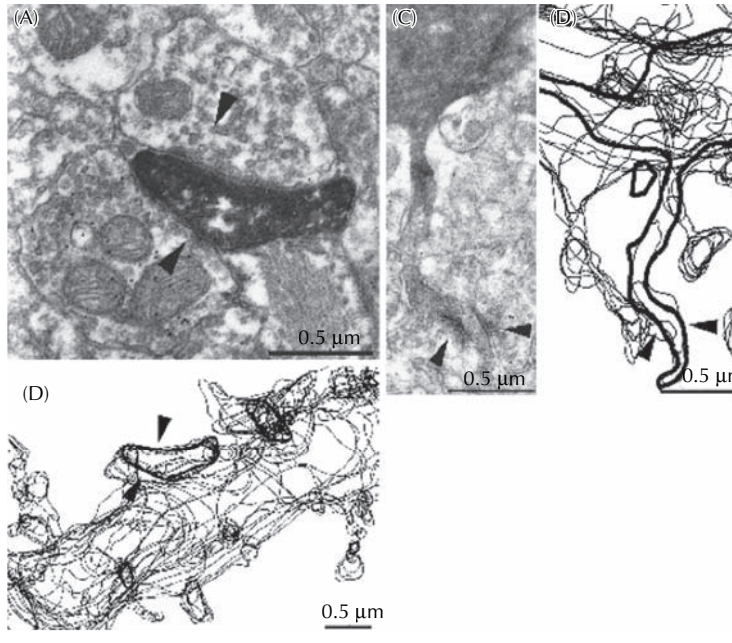

Figure 36–2. Gamma-aminobutyric acid-negative and GABA-positive axon terminals synapse (arrowheads) with the same spine of granule cell basal dendrites in epileptic rats. **A.** Electron micrograph of a spine of basal dendrite #1 labeled with an electron-dense reaction product. Gamma-aminobutyric acid immunoreactivity is indicated by small black particles, which are 10 nm diameter colloidal gold. **B.** Reconstructed segment of basal dendrite #1. The bold contours are of the basal dendrite profile shown in **A**. **C.** Electron micrographs of basal dendrite #2 labeled with an electron-dense reaction product, which is lighter in the spine head than in the shaft. Gamma-aminobutyric acid immunoreactivity is indicated by small black particles, which are 10 nm diameter colloidal gold. **D.** Reconstructed segment of a basal dendrite. The bold contours are of the basal dendrite profile shown in **C**. From ref. 12.

account for only 7% of all synapses with hilar basal dendrites[12] (Fig. 36–3). Most GABAergic synapses are onto the dendritic shafts of basal dendrites, but there is a relatively large proportion of such synapses with spines, similar to the situation on apical dendrites.[36,41] A variety of different types of GABAergic interneurons are found in the dentate gyrus, but specific sources of interneuron input to basal dendrites have not yet been identified. The presence of inhibitory synapses with granule cell basal dendrites in epileptic pilocarpine-treated rats has special significance. The presence of such synapses confirms the long suspected but only indirectly supported hypothesis that GABAergic synaptogenesis occurs in mature epileptic animals.[42] These observations contribute to recent accumulating evidence that in epileptic tissue, surviving interneurons in the dentate gyrus sprout axons and form new synapses with granule cells.[40,43] Although basal dendrites receive these GABAergic synapses, their numbers are relatively low compared to those of the sprouted excitatory synapses. The strength and efficacy of inhibitory input to basal dendrites have not yet been evaluated.

TIME COURSE FOR BASAL DENDRITE FORMATION FOLLOWING SEIZURE ACTIVITY

Dashtipour et al.[44] were the first to address the issue of the time course for the development of basal dendrites following seizures. Using retrograde labeling with biocytin injected into the CA3 region in hippocampal slice preparations, they observed labeled granule cells with basal dendrites 7 days after pilocarpine-induced status epilepticus in rats. At the earliest time point examined in that study (3 days postseizures), no basal dendrites were found. This study indicated that basal dendrites may form on dentate granule cells as early as 1 week following pilocarpine-induced seizures.[44] However, the method that was used in this study required that a granule cell axon be present in the CA3

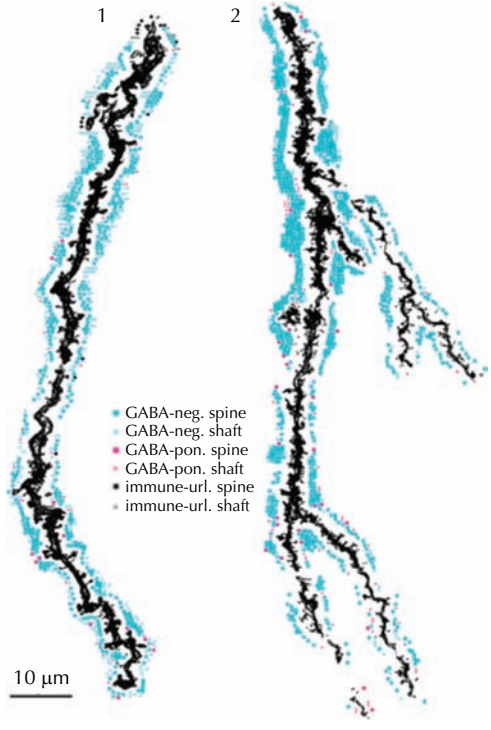

Figure 36–3. Summary of synapses with granule cell basal dendrites #1 and #2 from epileptic rats. Proximal is up, distal is down. Synapses are indicated by markers. Most synapses are with GABA-negative spines. "Immuno.-unk." synapses of basal dendrite #1 were in tissue sections that were used for the reconstruction but were not processed for immunocytochemistry. From ref.12.

region where biocytin was injected. Thus, this method would not label newborn granule cells at this time point (they require about 2 weeks to grow their axons into CA3).[45] Therefore, this study suggested that basal dendrites appeared relatively quickly after seizures (compared to mossy fiber sprouting), but it was not determined whether these dendrites originated from newborn granule cells.

DO BASAL DENDRITES ARISE FROM NEWLY GENERATED NEURONS?

Most granule cells with hilar basal dendrites are located at the hilar border of the granule cell layer,[9] and granule cell neurogenesis occurs in adults at this same location.[46–48] Therefore, it seemed logical to hypothesize that the newly generated granule cells developed hilar basal dendrites and the more mature granule cells at the molecular layer border did not display these basal dendrites. Testing the first part of this hypothesis required the use of a newborn cell marker that labels the cell body, axon, and dendrites. Doublecortin was selected for this purpose because it is a protein found in dentate newborn neurons for up to 3 weeks after their birth and is effective in labeling their growth cones, processes, and perikaryal cytoplasm.[49–52] However, one confounding problem with its use was that 31% to 55% of newborn doublecortin-labeled granule cells exhibit a basal dendrite.[51,52] Such doublecortin-labeled basal dendrites are transient.[37,53]

To determine whether basal dendrites arise from newborn neurons, doublecortin-labeled granule cells were examined 30 days after the induction of seizures. Light microscopic preparations showed that the basal dendrites from doublecortin-labeled granule cells of epileptic rats are significantly longer than those found in control rats.[54] It was also found that 20% of newborn neurons in the epileptic rat have a basal dendrite that enters the hilus at an angle greater than 30° from its cell body. In control rats, the dendrites that emanate from the basal portion of newborn neurons are typically at an angle less than 30° from their cell body and frequently do not travel in the hilus but instead curve back into the granule cell layer (i.e., form "recurrent" basal dendrites).[54] These data on doublecortin-labeled basal dendrites suggested that seizures induced morphological changes in the normally transient basal dendrite, and these changes (in length and in the angle of penetration into the hilus) may have significance for the persistence of these basal dendrites.

Another significant difference between epileptic and control animals was the presence of synapses on basal dendrites. Electron microscopy of doublecortin-labeled basal dendrites from epileptic rats showed that they had synapses.[15] This observation suggested that excitatory inputs were targeting the basal dendrites of immature granule cells as shown for mature granule cells (Fig. 36–4). In contrast, the doublecortin-labeled basal dendrites from newly generated granule cells from animals that are not epileptic lacked synapses on their basal dendrites.[15] These results were confirmed in a subsequent study in which the doublecortin-labeled basal dendrites from epileptic animals were examined on each of the first 5 days after seizures

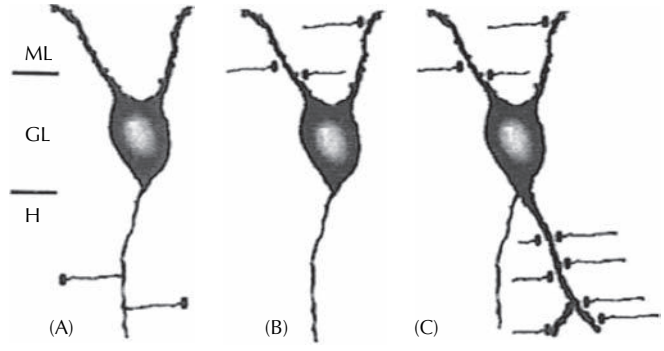

Figure 36–4. Schematic diagram of granule cells in the dentate gyrus of the hippocampal formation. **A.** A normal granule cell with its dendrites in the molecular layer (ML), cell body in the granule cell layer (GL), and axon terminals (filled circles) in the hilus (H). **B.** A granule cell from an epileptic rat showing mossy fiber sprouting (three axon terminals in the ML). Synapses made by these axon terminals provide the basis for robust excitatory responses following antidromic activation of granule cells and form a recurrent excitatory circuit. **C.** A granule cell with a hilar basal dendrite in the hilus that is postsynaptic to mossy fiber collaterals (seven axon terminals) and sprouted mossy fibers in the ML (three axon terminals). From ref. Shapiro and Ribak.[58]

were induced.[16] No synapses were found on doublecortin-labeled basal dendrites on the first 3 days following pilocarpine-induced seizures.[16] However, developing synapses were observed as early as 4 days after seizures on doublecortin-labeled basal dendrites.[16] Therefore, synapses are found on doublecortin-labeled hilar basal dendrites at both early (4–5 days after seizures) and later (30 days after seizures) time points. The fact that basal dendrites are found to have synapses after seizures (see the section "Synaptic Connections of Hilar Basal Dendrites") and have synapses on newly generated granule cell basal dendrites suggests that the early-formed synapses may persist, perhaps by the release of trophic factors at these synapses. In addition, we hypothesized that the synaptic targeting of basal dendrites of newly generated granule cells contributed to the persistence of hilar basal dendrites on neurons born after pilocarpine-induced seizures. Consistent with this hypothesis is the fact that no synapses are observed on doublecortin-labeled basal dendrites from control rats,[15] and such dendrites in nonepileptic animals are known to be transient structures.[37,52,55]

These data on the time course for the development of hilar basal dendrites indicate that basal dendrites may arise from seizure-induced de novo granule cells.[16] A recent study by Walter et al.[56] confirmed this observation and also showed that granule cells generated about a week prior to seizures may form hilar basal dendrites following seizure induction. It was suggested that those granule cells were still immature at the time seizures were induced.[56,57] Together, these studies indicate that newly generated granule cells are a major part of the population of granule cells that have hilar basal dendrites following seizures. However, it remains to be shown how the basal dendrites grow into the hilus following seizures. The following section addresses this topic.

WHAT GUIDES THE BASAL DENDRITES TO GROW INTO THE HILUS?

Shapiro and Ribak[58] initially hypothesized that hilar basal dendrites from newborn neurons in epileptic animals grow along an ectopic glial scaffold. This conjecture was based on the fact that the basal dendrites were found closely apposed to the horizontal processes of the radial glial-like cells that extend into the hilus[54] (Fig. 36–5). After seizures, these radial glial-like cells have a hypertrophied appearance indicative of an inflammatory response in this region.[58] In control rats, the radial glial-like cells rarely extend lengthy horizontal processes into the hilus. The same radial glial-like cells have vertical processes extending through the granule cell layer, and these vertical processes provide a scaffold for the apical dendrites of newborn neurons to grow through the granule cell layer.[59,60] Thus, under normal conditions, the apical dendrite of newborn neurons grows along the radial glial-like process through the

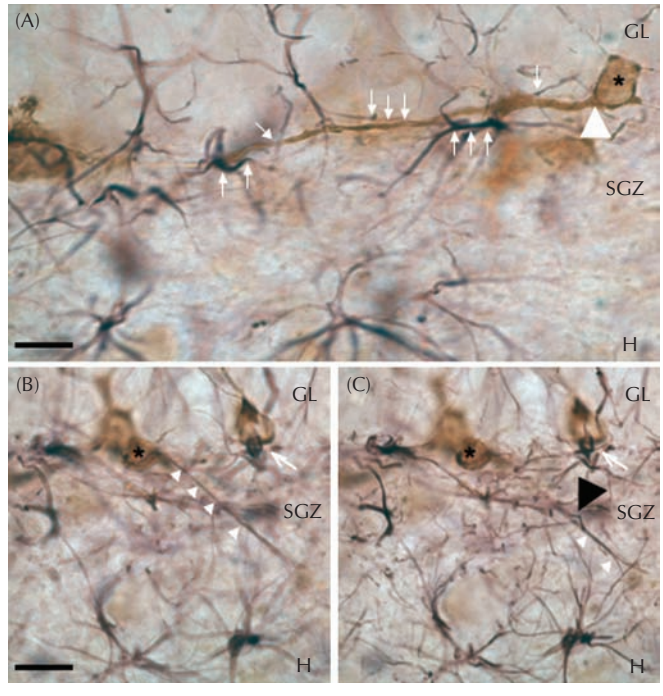

Figure 36–5. Light micrographs of doublecortin-labeled newborn neurons with basal dendrites adjacent to GFAP-labeled processes from the epileptic rats. **A.** A doublecortin-labeled newborn neuron (asterisk) at the border between the subgranular zone (SGZ) and the granule cell layer (GL). Note the basal dendrite (white arrowhead) emanating from the basal portion of the cell body and extending horizontally along the base of the GL. Two GFAP-positive astrocytes can be seen along the extent of the basal dendrite with their processes (white arrows) adjacent to the basal dendrite. **B,C.** Two different planes of focus of a doublecortin-labeled newborn neuron (asterisk) located in the SGZ. Note the basal dendrite (white arrowheads) emanating from the basal portion of this cell and extending into the hilus. This basal dendrite is adjacent to GFAP-labeled processes and can best be visualized in **C**, where a kink in the GFAP-labeled process (black arrowhead) reveals how close the dendrite is to the GFAP-labeled process. Scale bars: 8 μm. From ref. Shapiro et al.[54]

granule cell layer; following seizures, these radial glial-like cells appear hypertrophied and sprout a horizontal process that forms an ectopic glial scaffold to guide the aberrant growth of basal dendrites into the hilus. Therefore, the extension of both the radial glial-like cell's horizontal process and the granule cell's basal dendrite into the hilus can be thought of as a seizure-induced change.

More recently, additional support for this hypothesis was provided by Foresti et al.,[61] who showed that after seizures, the radial glial-like cells at the border between the granule cell layer and the subgranular zone express the chemokine receptor CCR2. Such expression is rarely seen in control animals.[61] Moreover, the horizontal processes from the radial glial-like cells that orient toward the hilus also show expression of CCR2.[61] Previous studies have shown that CCR2 expression on neuroblasts, together with that of its ligand CCL2, guides the migration of neuroblasts to sites of inflammation.[62–65] These data suggest that this chemokine/receptor complex is involved in the ectopic growth and migration of immature neurons, and thus may play a role in extension of newborn granule cell basal dendrites into the hilus.

DISCLOSURE STATEMENT

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REFERENCES

1. Cajal SR. *Histologie du Systeme Nerveux de l'Homme et des Vertebres*. Vol. 2. Paris: Maloine; 1911.
2. Dashtipour K, Yan XX, Dinh TT, Okazaki MM, Nadler JV, Ribak CE. Quantitative and morphological analysis of dentate granule cells with recurrent basal dendrites from normal and epileptic rats. *Hippocampus*. 2002;12:235–244.

3. Ribak CE, Tran PH, Spigelman I, Okazaki MM, Nadler JV. Status epilepticus-induced hilar basal dendrites on rodent granule cells contribute to recurrent excitatory circuitry. *J Comp Neurol*. 2000;428: 240–253.
4. Yan XX, Spigelman I, Tran PH, Ribak CE. Atypical features of rat dentate granule cells: recurrent basal dendrites and apical axons. *Anat Embryol*. 2001;203: 203–209.
5. Seress L, Mrzljak L. Basal dendrites of granule cells are normal features of the fetal and adult dentate gyrus of both monkey and human hippocampal formations. *Brain Res*. 1987;405:169–174.
6. Seress L, Frotscher M. Morphological variability is a characteristic feature of granule cells in the primate fascia dentata: a combined Golgi/electron microscope study. *J Comp Neurol*. 1990;293:253–267.
7. Franck JE, Pokorny J, Kunkel DD, Schwartzkroin PA. Physiologic and morphologic characteristics of granule cell circuitry in human epileptic hippocampus. *Epilepsia*. 1995;36:543–558.
8. von Campe G, Spencer DD, de Lanerolle NC. Morphology of dentate granule cells in the human epileptogenic hippocampus. *Hippocampus*. 1997;7: 472–488.
9. Spigelman I, Yan X-X, Obenaus A, Lee EYS, Wasterlain CG, Ribak CE. Dentate granule cells form novel basal dendrites in a rat model of temporal lobe epilepsy. *Neuroscience.* 1998;86:109–120.
10. Buckmaster PS, Dudek FE. In vivo intracellular analysis of granule cell axon reorganization in epileptic rats. *J Neurophsyiol*. 1999;81:712–721.
11. Pekcec A, Potschka H. Newborn neurons with hilar basal dendrites hallmark epileptogenic networks. *Neuroreport.* 2007;18:585–589.
12. Thind KK, Ribak CE, Buckmaster PS. Synaptic input to dentate granule cell basal dendrites in a rat model of temporal lobe epilepsy. *J Comp Neurol*. 2008;509:190–202.
13. Díaz-Cintra S, Xue B, Spigelman I, Van K, Wong AM, Obenaus A, Ribak CE. Dentate granule cells form hilar basal dendrites in a rat model of hypoxia-ischemia. *Brain Res.* 2009;1285:182–187.
14. Jessberger S, Zhao C, Toni N, Clemenson GD Jr, Li Y, Gage FH. Seizure-associated, aberrant neurogenesis in adult rats characterized with retrovirus-mediated cell labeling. *J Neurosci*. 2007;27:9400–9407.
15. Shapiro LA, Ribak CE. Newly born dentate granule neurons after pilocarpine-induced epilepsy have hilar basal dendrites with immature synapses. *Epilepsy Res*. 2006;69:53–66.
16. Shapiro LA, Figueroa-Aragon S, Ribak CE. Newly generated granule cells show rapid neuroplastic changes in the adult rat dentate gyrus during the first five days following pilocarpine-induced seizures. *Eur J Neurosci*. 2007;26:583–592.
17. Patel LS, Wenzel HJ, Schwartzkroin PA. Physiological and morphological characterization of dentate granule cells in the p35 knock-out mouse hippocampus: evidence for an epileptic circuit. *J Neurosci*. 2004;24: 9005–9014.
18. Dashtipour K, Tran PH, Okazaki MM, Nadler JV, Ribak CE. Ultrastructural features and synaptic connections of hilar ectopic granule cells in the rat dentate gyrus are different from those of granule cells in the granule cell layer. *Brain Res*. 2001;890:261–271.
19. Pierce JP, Melton J, Punsoni M, McCloskey DP, Scharfman HE. Mossy fibers are the primary source of afferent input to ectopic granule cells that are born after pilocarpine-induced seizures. *Exp Neurol*. 2005;196:316–331.
20. Buckmaster PS, Strowbridge BW, Schwartzkroin PA. A comparison of rat hippocampal mossy cells and CA3 pyramidal cells. *J Neurophysiol*. 1993;70:1281–1299.
21. Buckmaster PS, Wenzel HJ, Kunkel DD, Schwartzkroin PA. Axon arbors and synaptic connections of hippocampal mossy cells in the rat in vivo. *J Comp Neurol*. 1996;366:270–292.
22. Frotscher M, Seress L, Schwerdtfeger WK, Buhl E. The mossy cells of the fascia dentata: a comparative study of their fine structure and synaptic connections in rodents and primates. *J Comp Neurol*. 1991;312: 145–163.
23. Buckmaster PS, Amaral DG. Intracellular recording and labeling of mossy cells and proximal CA3 pyramidal cells in macaque monkeys. *J Comp Neurol*. 2001;430:264–281.
24. Li XG, Somogyi P, Ylinen A, Buzsáki G. The hippocampal CA3 network: an in vivo intracellular labeling study. *J Comp Neurol*. 1994;339:181–208.
25. Siddiqui AH, Joseph SA. CA3 axonal sprouting in kainate-induced chronic epilepsy. *Brain Res*. 2005;1066: 129–146.
26. Dimoka A, Courellis SH, Marmarelis VZ, Berger TW. Modeling the nonlinear dynamic interactions of afferent pathways in the dentate gyrus of the hippocampus. *Ann Biomed Eng*. 2008;36:852–864.
27. Dyhrfjeld-Johnsen J, Santhakumar V, Morgan RJ, Huerta R, Tsimring L, Soltesz I. Topological determinants of epileptogenesis in large-scale structural and functional models of the dentate gyrus derived from experimental data. *J Neurophysiol*. 2007;97: 1566–1587.
28. Morgan RJ, Soltesz I. Nonrandom connectivity of the epileptic dentate gyrus predicts a major role for neuronal hubs in seizures. *Proc Natl Acad Sci USA*. 2008;105:6179–6184.
29. Howard AL, Neu A, Morgan RJ, Echegoyen JC, Soltesz I. Opposing modifications in intrinsic currents and synaptic inputs in post-traumatic mossy cells: evidence for single-cell homeostasis in a hyperexcitable network. *J Neurophysiol*. 2007;97:2394–2409.
30. Szabadics J, Soltesz I. Functional specificity of mossy fiber innervation of GABAergic cells in the hippocampus. *J Neurosci*. 2009;29:4239–4251.
31. Naylor DE, Liu H, Wasterlain CG. Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci*. 2005;25:7724–7733.
32. Jedlicka P, Deller T, Schwarzacher SW. Computational modeling of GABA(A) receptor-mediated paired-pulse inhibition in the dentate gyrus. *J Comput Neurosci*. 2010;29(3):509–519.
33. Chauvet GA, Berger TW. Hierarchical model of the population dynamics of hippocampal dentate granule cells. *Hippocampus.* 2002;12:698–712.
34. Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol*. 1989;26:321–330.
35. Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF. Synaptic reorganization by mossy fibers in human epileptic fascia dentata. *Neuroscience.* 1991:42:351–363.

36. Buckmaster PS, Yamawaki R, Zhang GF. Axon arbors and synaptic connections of a vulnerable population of interneurons in the dentate gyrus in vivo. *J Comp Neurol.* 2002;445:360–373.
37. Seress L, Pokorny J. Structure of the granular layer of the rat dentate gyrus. A light microscopic and Golgi study. *J Anat*. 1981;133:181–195.
38. Kobayashi M, Buckmaster PS. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. *J Neurosci*. 2003;23:2440–2452.
39. Austin JE, Buckmaster PS. Recurrent excitation of granule cells with basal dendrites and low interneuron density and inhibitory postsynaptic current frequency in the dentate gyrus of macaque monkeys. *J Comp Neurol*. 2004;476:205–218.
40. Thind KK, Yamawaki R, Phanwar I, Zhang G, Wen X, Buckmaster PS. Initial loss but later excess of GABAergic synapses with dentate granule cells in a rat model of temporal lobe epilepsy. *J Comp Neurol*. 2010;518:647–667.
41. Fifkova E, Eason H, Schaner P. Inhibitory contacts on dendritic spines of the fascia dentata. *Brain Res.* 1992;577:331–336.
42. Davenport CJ, Brown WJ, Babb TL. Sprouting of GABAergic and mossy fiber axons in dentate gyrus following intrahippocampal kainate in the rat. *Exp. Neurol*. 1990;109:180–190.
43. Zhang W, Yamawaki R, Wen X, Uhl J, Diaz J, Prince DA, Buckmaster PS. Surviving hilar somatostatin interneurons enlarge, sprout axons, and form new synapses with granule cells in a mouse model of temporal lobe epilepsy. *J Neurosci*. 2009;29:14247–14256.
44. Dashtipour K, Wong AM, Obenaus A, Spigelman I, Ribak CE. Temporal profile of hilar basal dendrite formation on dentate granule cells after status epilepticus. *Epilepsy Res*. 2003;54:141–151.
45. Zhao C, Teng EM, Summers RG Jr, Ming GL, Gage FH. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci*. 2006;26:3–11.
46. Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol.* 1965;124:319–335.
47. Kempermann G, Kuhn HG, Gage FH. Experience-induced neurogenesis in the senescent dentate gyrus. *J Neurosci*. 1998;18:3206–3212.
48. Cameron HA, McKay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J Comp Neurol*. 2001;435:406–417.
49. Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet MC, Friocourt G, McDonnell N, Reiner O, Kahn A, McConnell SK, Berwald-Netter Y, Denoulet P, Chelly J. Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron*. 1999;23:247–256.
50. Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH. Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development.* 2003;130:391–399.
51. Rao MS, Shetty AK. Efficacy of doublecortin as a marker to analyze the absolute number and dendritic growth of newly generated neurons in the adult dentate gyrus. *Eur J Neurosci*. 2004;19:234–246.
52. Ribak CE, Korn MJ, Shan Z, Obenaus A. Dendritic growth cones and recurrent basal dendrites are typical features of newly generated dentate granule cells in the adult hippocampus. *Brain Res*. 2004;1000:195–199.
53. Jones SP, Rahimi O, O'Boyle MP, Diaz DL, Claiborne BJ. Maturation of granule cell dendrites after mossy fiber arrival in hippocampal field CA3. *Hippocampus*. 2003;13: 413–427.
54. Shapiro LA, Korn MJ, Ribak CE. Newly generated dentate granule cells from epileptic rats exhibit elongated hilar basal dendrites that align along GFAP-immunolabeled processes. *Neuroscience*. 2005;136: 823–831.
55. Seress L, Ribak CE. Postnatal development of the light and electron microscopic features of basket cells in the hippocampal dentate gyrus of the rat. *Anat Embryol*. 1990;181:547–565.
56. Walter C, Murphy BL, Pun RY, Spieles-Engemann AL, Danzer SC. Pilocarpine-induced seizures cause selective time-dependent changes to adult-generated hippocampal dentate granule cells. *J Neurosci.* 2007;27: 7541–7552.
57. Kron MM, Zhang H, Parent JM. The developmental stage of dentate granule cells dictates their contribution to seizure-induced plasticity. *J Neurosci*. 2010;30:2051–2059.
58. Shapiro LA, Ribak CE. Integration of newly born granule cells into adult brains: hypotheses based on normal and epileptic rodents. *Brain Res Rev*. 2005;48:43–56.
59. Shapiro LA, Korn MJ, Shan Z, Ribak CE. GFAP-expressing radial glia-like cell bodies are involved in a one-to-one relationship with doublecortin-immunolabeled newborn neurons in the adult dentate gyrus. *Brain Res.* 2005;1040:81–91.
60. Seki T, Namba T, Mochizuki H, Onodera M. Clustering, migration, and neurite formation of neural precursor cells in the adult rat hippocampus. *J Comp Neurol*. 2007;502:275–290.
61. Foresti ML, Arisi GM, Katki K, Montañez A, Sanchez RM, Shapiro LA. Chemokine CCL2 and its receptor CCR2 are increased in the hippocampus following pilocarpine-induced status epilepticus. *J Neuroinflammation*. 2009;6:40.
62. Ji JF, He BP, Dheen ST, Tay SS. Expression of chemokine receptors CXCR4, CCR2, CCR5 and CX3CR1 in neural progenitor cells isolated from the subventricular zone of the adult rat brain. *Neurosci Lett*. 2004;355:236–240.
63. Belmadani A, Tran PB, Ren D, Miller RJ. Chemokines regulate the migration of neural progenitors to sites of neuroinflammation. *J Neurosci*. 2006;26:3182–3191.
64. Tran PB, Banisadr G, Ren D, Chenn A, Miller RJ. Chemokine receptor expression by neural progenitor cells in neurogenic regions of mouse brain. *J Comp Neurol*. 2007;500:1007–1033.
65. Yan YP, Sailor KA, Lang BT, Park SW, Vemuganti R, Dempsey RJ. Monocyte chemoattractant protein-1 plays a critical role in neuroblast migration after focal cerebral ischemia. *J Cereb Blood Flow Metab*. 2007;27: 1213–1224.

Chapter 37

Perturbations of Dendritic Excitability in Epilepsy

Cha-Min Tang
Scott M. Thompson

The dendrite is where thousands of excitatory and inhibitory synaptic inputs are received by the neuron. But rather than just being a simple antenna, the dendrite is also the location where these inputs actively interact with intrinsic conductances. The interactions are complex and are still incompletely understood. The synaptic inputs are distributed in time and space, and each of the many intrinsic dendritic conductances is also distributed in its own unique spatial pattern. The interactions lead to signal transformations whose significance may be best appreciated in terms of elementary steps in signal processing and computation. Under pathological conditions, changes to these interactions may result in aberrant excitability and contribute to neurological disease.

Rather than compiling a list of dendritic conductances and their linkages with epilepsy, which is done in other chapters of this book, the purpose of this chapter is to integrate these results, with an emphasis on how perturbations of the elementary steps in dendritic integration affect the way neurons process their inputs and promote aberrant neuronal excitability.

A BRIEF HISTORY OF THE ACTIVE DENDRITE

Our view of the role of dendrites in epilepsy has evolved with increased understanding of the roles dendrites play in normal physiology.

For most of the previous century, the dendritic arbor was viewed as a structure that gathered and faithfully funneled inputs to the soma and axon hillock, where nonlinear processing—in the form of action potential initiation—was thought to reside. This view of the dendrite as a passive antenna does not suggest that the dendrite plays a pivotal role in neuronal hyperexcitability. The possibility that apical dendrites of pyramidal neurons are active was first demonstrated by Spencer and Kandel when they described events they called *fast prepotentials*.[1] Similar *dendritic spikes* were reported from neocortical neurons by Purpura.[2] The concept of the active dendrite was further advanced with the demonstration of calcium electrogenesis in dendrites by Llinas, Schwartzkroin, Wong, Prince, and Sakmann and their colleagues.[3–6] Magee and Johnston further extended the concept of the active dendrite by demonstrating high levels of expression of sodium channels throughout the apical trunk in hippocampal pyramidal neurons.[7] Stuart and Sakmann and others showed that action potentials can propagate bidirectionally in the dendritic arbor.[8] This bidirectional signaling provided the means for coincidence detection, which was postulated to serve an important role in triggering acute and long-term changes in excitability such as spike timing-dependent plasticity.[9] Several other members of Johnston's and Sakmann's groups also investigated the properties of calcium electrogenesis at the apical tuft region of layer 5 pyramidal neurons, showing that these calcium channels help amplify synaptic inputs from the more distal branches[10–12] and that backpropagating action potentials (bAPs) can trigger calcium spikes localized to the apical tuft.[13] Next, Schiller and colleagues demonstrated the ability of *N*-methyl-D-aspartate (NMDA) receptors to generate local spikes on tertiary basal dendrites of cortical pyramidal neurons.[14] The capacity of NMDA receptors for regenerative depolarization under appropriate conditions provided an additional mechanism of excitability that is unique to dendrites and is not observed in other excitable membranes such as axons and muscle. Thus, over a span of 20 to 30 years, the view of the dendrite as a passive antenna evolved to that of a highly excitable structure that rivaled the soma. This change in perspective also led to a greater interest in and understanding of the role of dendritic excitability in epilepsy.

ELECTRICAL COMPARTMENTALIZATION OF DENDRITES: THE INTERSECTION OF FORM AND FUNCTION

The organization of this chapter is based on the premise that dendrites serve signal processing and computational needs that are fundamental to the function of the nervous system. Because nontrivial computation generally requires some form of nonlinear operation, it is informative to examine the source of nonlinearity in dendritic integration. One obvious source of nonlinearity is active conductances. A less obvious but important determinant of nonlinearity is electrical compartmentalization.

Electrical compartmentalization is like the parentheses in mathematics. It separates the variables that are included in an operation from those that are excluded. The ability to segregate variables and "bind" them for nonlinear operations is as fundamental to orderly signal processing as it is in mathematics. At the level of the dendrite, binding of synaptic inputs can be implemented by electrical compartmentalization within different regions of the dendritic arbor and individual dendritic segments.[15–17] Electrical compartmentalization can be functionally defined as a condition in which local interactions within a confined space occur quasi-independently of the influence of the rest of the cell. At rest, all dendritic regions are close to isopotential. During activity, a region of the dendritic arbor (i.e., a *compartment*) may be at a membrane potential that is significantly different from the potential of other nearby dendrites and the cell body. For example, nearly synchronous activity in a set of synaptic inputs that arrive on a single dendritic segment can trigger a variety of active electrical responses, such as plateau potentials.[15] These responses functionally bind together the original inputs and are amplified and transmitted to the soma reliably. In contrast, inputs arriving on different dendritic branches, or arriving with insufficient temporal synchrony to allow such binding, fail to trigger active dendritic responses; consequently, they will not be amplified and will exert lesser influence on the output of the neuron.

Electrical compartmentalization is determined by three factors: the passive electronic properties of the dendritic arbor, the expression of active conductances on these structures,

and the temporospatial pattern of excitation. Factors favoring compartmentalization within the dendritic tree are high output impedance of the dendritic segment and high expression of regenerative voltage-dependent conductances. Electrical compartmentalization can be dynamic, and changes in the extent of ongoing synaptic activity (both excitatory and inhibitory) can change the impedance of the dendrite. Because the voltage-dependent blockade of NMDA receptors by Mg^{2+} can effectively add a nonlinear excitability to the dendrite, the tempospatial pattern of synaptic excitation within a dendritic domain is a strong determinant of the extent of the compartment.

Two functional dendritic compartments will be discussed in detail in the context of epileptogenesis. One is the compartment comprising the individual terminal dendritic branches, which receive >80% of synaptic inputs to pyramidal neurons.[18] The other is the region on the distal apical trunk, near the base of the apical dendritic tuft, which has a high propensity for generating calcium spikes.[6,19]

Experimental observation has shown that distal and proximal dendritic compartments express distinct intrinsic conductances. In fact, dendritic information processing can be modeled as a two-layer system of distal and apical domains, each with distinct functions that are connected to the soma.[20]

THE TERMINAL DENDRITE AS AN ELECTRICAL COMPARTMENT

The terminal dendrite of cortical and hippocampal pyramidal neurons has attributes that favor electrical compartmentalization. Contrary to frequent belief, the dendritic arbor of pyramidal neurons does not mimic the arbor of a typical tree. Whereas the diameter of branches of a tree gradually tapers with each division and becomes progressively shorter, the diameter of the terminal dendritic branch of pyramidal neurons is markedly thinner than its parent branch and the terminal branch is typically the longest segment of the dendritic arbor.[19] Interestingly, the diameter of the terminal dendrite is not tapered, in contrast to the apical trunk or the branches of a tree; it is <1 μm in diameter both where it joins the main apical trunk and at its distal tip 100 μm away. This geometry is well suited for electrical compartmentalization, because synaptic currents are gathered from a large surface area within a high-resistance anatomically defined region. Thus, even a relatively modest level of synaptic current flowing into the long terminal dendritic process produces significant depolarization over a considerable portion of its length. This depolarization can then secondarily trigger the activation of voltage-dependent sodium and calcium conductances and the unblocking of glutamate-bound—but Mg^{2+}-blocked—NMDA receptors.[14,15] The result is a regenerative spike in the thin terminal dendrite that appears as a slow plateau potential at the soma (Fig. 37–1A). Calcium imaging suggests that the spike originates from and is largely confined to the terminal dendritic compartment (Fig. 37–1B).

The regenerative depolarization of these thin distal processes is mediated by a combination of NMDA and voltage-gated channels. The relative contribution of NMDA and voltage-gated calcium conductances is hard to determine precisely. We will therefore use the term *plateau potential* to refer these regenerative all-or-none depolarizations. The impedance mismatch of the terminal dendrite where it joins the main apical trunk results in marked attenuation of the distal depolarization—which allows the terminal dendrite to function quasi-independently of the apical trunk.

Perturbations Affecting the Thin Distal Electrical Compartment

What factors limit the generation of dendritic plateau potentials? The answer includes both extrinsic and intrinsic conductances. Modeling of the excitability of the thin terminal dendrites shows that the level of GABAergic (GABA, gamma-aminobutyric acid) inhibition is a very potent modulator of plateau potential generation.[21] A local inhibitory current 20-fold lower in amplitude than the current underlying the plateau potential is sufficient to prevent its initiation. Further, the impact of a conductance on excitability of the dendrite will depend, in part, on the level of its expression relative to the input impedance/leak conductance of the electrical compartment. The steepness of the negative slope of the current–voltage activation relationship of the NMDA and

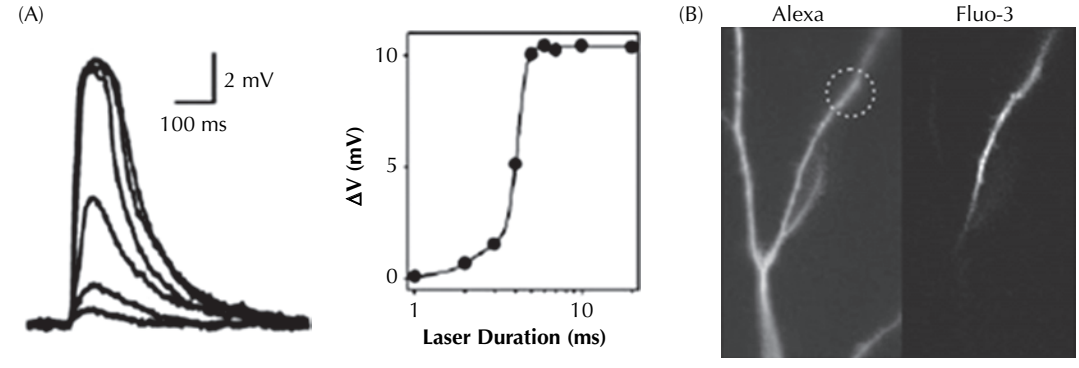

Figure 37–1. Compartmentalized and spiking property of terminal dendrites. **A.** Progressive increase in the strength of glutamate photolysis stimulus directed at a thin terminal dendrite leads to a nonlinear response. **B.** Calcium imaging with Fluo-3 during such a plateau potential reveals that the electrogenesis is largely confined to the terminal dendritic compartment (dotted circle).

voltage-dependent Ca^{2+} channels (VDCCs) that underlie plateau potential generation are also critical. One possible explanation for the extraordinary sensitivity of the plateau potential to inhibition is that the negative slope of the NMDA and VDCC current–voltage activation relationship is not steep compared to that of sodium channels at the axon initial segment. Intrinsic inhibitory voltage-dependent conductances may similarly exert powerful control over the excitability of the terminal dendrites. These conductances include I_A and I_h, which are activated at or near rest, and SK-type calcium-activated K^+ channels (I_{SK}), which are activated subsequent to depolarization. All three channel types are found in abundance in the thin distal dendrites. This distribution of channels creates a system that is normally well balanced but is vulnerable to overexcitation when inhibition is reduced. Thus, factors that attenuate I_A, I_h, and I_{SK} will lead to aberrant excitability of distal dendrites that subsequently feed into the burst-generating apical trunk compartment of the dendritic arbor.

Attenuation of I_A Lowers the Threshold for Regenerative Spiking

I_A is a rapidly inactivating voltage-gated K^+ conductance that is mediated by Kv4.2 potassium channels in pyramidal cell dendrites. Its expression on apical trunk dendrites has been directly measured by patch-clamp methods and has been found to increase with distance from the soma up to at least 350 μm.[22] On the thin terminal dendrite, however, the expression of I_A must be inferred by indirect means because it is not possible to record from these thin structures directly. Fluorescent monitoring of intracellular calcium levels in response to somatically evoked bAPs has provided a convenient indirect measure for the presence of I_A.[23] Under control conditions, the bAP-evoked calcium signal in thin oblique dendrites was similar to that observed in the apical trunk. After application of the I_A blocker, 4-aminopyridine (4AP), the calcium signal was markedly increased in the oblique dendrites to a much greater extent than in the trunk dendrite.[23] This observation confirms that I_A is expressed on the oblique dendrites. But it may lead to overestimation of the density of I_A in the oblique dendrite, because I_A can exert highly nonlinear control over the generation of NMDA and calcium channel-dependent spikes (Fig. 37–2). Because the threshold for NMDA spike generation is significantly lower in the oblique dendrites than in the main apical trunk due to cable properties, increased sensitivity to I_A as indirectly monitored by calcium influx does not necessarily mean that there is increased expression of I_A in the oblique dendrites. Regardless of the absolute density of I_A on the thin terminal dendrites, I_A exerts powerful control over regenerative excitation. Indeed, when NMDA receptor-mediated glutamate responses in thin oblique dendrites are examined in the presence of an alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate

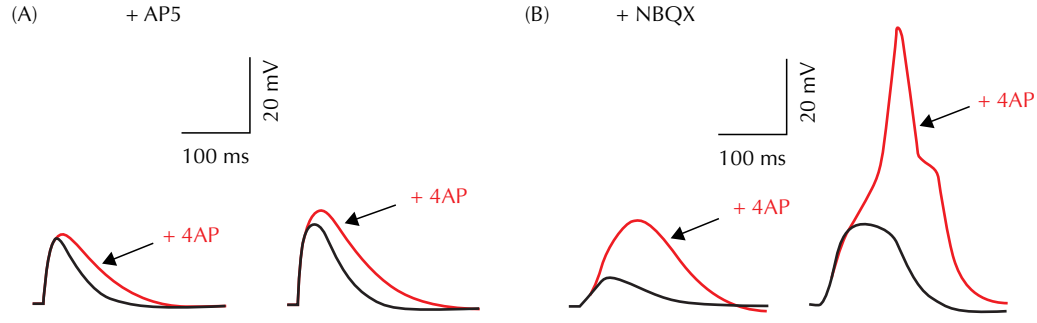

Figure 37–2. Disparate effect of I_A on regenerative and nonregenerative dendritic excitation. **A.** A hippocampal CA1 oblique dendrite in an acute hippocampal slice is stimulated by photolysis of caged glutamate at two intensities in the presence of an NMDA receptor antagonist (AP5, black). The stimulation is then repeated in the presence of 4AP (red). **B.** An oblique dendrite is similarly stimulated at two intensities in the presence of an AMPA receptor antagonist (NBQX) (black), and stimulation is then repeated in the presence of 4AP (red).

(AMPA) receptor antagonist (Fig. 37–2B), removing the inhibitory influence of I_A (by applying 4AP) has a much more dramatic effect on regenerative spike initiation than on AMPA-mediated depolarization (Fig. 37–2A).

I_A is a highly regulated conductance in dendrites. Its activity is altered by the recent history of activation, by the baseline membrane potential, and by the activity of a number of protein kinases.[24–26] I_A inactivates rapidly at voltages close to the resting membrane potential. High-frequency inputs will therefore inactivate I_A and release the regenerative capacity of the terminal dendrites from its inhibitory influence. High-frequency inputs are thus far more effective in triggering plateau potentials.[27] Tight regulation of I_A provides hints to its central role in controlling excitability within the dendritic arbor and the development of short- and long-term plasticity.[28] Most recent attention has focused on the role played by I_A in the apical trunk in regulating the extent to which somatic action potentials can backpropagate into the distal dendritic arbor. In addition to changes in bAP propagation, however, it is important to remember that changes in the properties of I_A can also promote epilepsy by altering the compartmentalization and regenerative activity of distal dendrites.

Factors that attenuate or downregulate the activity of I_A are well recognized as proconvulsants. Blockade of I_A with 4AP is a well-established in vitro model of epilepsy.[29] Local application of 4AP to the distal apical dendrite of CA1 pyramidal neurons causes the afterhyperpolarization to change to an afterdepolarization and the normal single evoked spike to change to bursts of spikes.[30] In the pilocarpine model of temporal lobe epilepsy (TLE), attenuation of I_A and increased excitability of pyramidal cell dendrites are believed to be secondary to extracellular-signal-regulated kinase (ERK)-dependent phosphorylation of the Kv4.2 channels.[31] In another study of the chronic phase of the Li-pilocarpine animal model, there is downregulation of Kv4.2 channel expression.[32] Surgical tissue obtained from patients with hippocampal sclerosis showed decreased immunoreactivity to Kv4.2 in the dendritic region of the CA1 and CA3 hippocampus[33] but increased immunoreactivity in the soma of the surviving neurons. Unfortunately, the limited resolution of light microscopic methods did not allow a determination of whether the decrease was localized to the apical trunk or the thin tertiary branches, or whether it was due to an overall loss of dendrites.

While these studies show a solid correlation between attenuated dendritic I_A and epilepsy, one is still faced with the classic dilemma of not knowing whether the change in I_A is the cause or the result of epilepsy. It remains important to determine whether the perturbation is localized to the apical trunk, or to the terminal dendrite, or occurs throughout the dendritic arbor. The action of I_A on the backpropagation of action potentials (APs) and the interaction between the soma and the apical tuft are well documented and will be discussed later.

Whether perturbation of I_A on the terminal dendrites contributes significantly to the epileptic conditions described above is not known. The observations shown in Fig. 37–2 provide one plausible pro-convulsant mechanism. I_A can regulate the threshold for regenerative NMDA and calcium spikes. This issue requires further investigation, for if hyperexcitability were to originate from the terminal dendrite, it would involve mechanisms that are distinct from those of APs propagating backward from the apical trunk and soma.

Downregulation of I_h Increases Input Impedance

I_h is a mixed sodium and potassium cation conductance activated by membrane hyperpolarization and gated by intracellular cyclic nucleotides. I_h is mediated by the hyperpolarization-activated cyclic nucleotide-gated (HCN) family of ion channels, which are highly expressed on distal dendrites of CA1 and cortical pyramidal neurons.[34,35] In conjunction with potassium channels, I_h controls the resting membrane potential especially for dendrites of pyramidal neurons farther away from the soma.[36] Activation of this conductance leads to membrane depolarization with a reversal potential near –30 mV. Because its activation would be expected to move the membrane potential toward –30 mV, it may be considered excitatory. But I_h also significantly reduces the input impedance of the distal dendrites, thereby decreasing the efficacy of excitatory synaptic inputs. In this respect, I_h may be considered a stabilizing or inhibitory conductance. These opposing actions on membrane excitability have led to conflicting opinions as to the role of I_h in epilepsy. Whether the net effect of I_h is increased or decreased excitability depends on a combination of factors, including the preexisting input impedance, resting membrane potential, membrane time constant, timing of synaptic inputs, neuronal subtype, and the age of the animal[37] (see Chapter 7 by Poolos in this edition). Like I_A, I_h may serve different roles in different locations of the neurons. Because of the high baseline input impedance of the distal dendritic compartment, the stabilizing action of increased shunting by I_h is likely to be more important than its depolarizing action. I_h-mediated decreases in input impedance attenuate the depolarization of glutamatergic inputs and speed the decay of excitatory synaptic events. The net result is reduced summation of excitatory postsynaptic potentials (EPSPs).[34,38]

A large body of evidence supports the idea that I_h is predominantly a stabilizing conductance with respect to epilepsy. In the kainate model of TLE, decreased I_h conductance is linked to the latent period of epileptogenesis.[39] In a hippocampal deafferentation model of epilepsy created by lesioning the entorhinal cortex, there is a decrease in the expression of HCN1 channels.[40] Also consistent with this notion is the observation that two anti-convulsants, lamotrigine and acetazolamide, both enhance I_h,[41,42] although questions remain about whether it is their action on I_h that provides them with their anti-convulsant actions.[43] The more general question of whether promoting I_h is pro- or anti-convulsant does not have a simple answer. Different seizure types respond differently to I_h modulation. In the rat febrile seizure model, I_h is enhanced and its blockade reduces hyperexcitability.[43] Interestingly, I_h has also been implicated in thalamically generated absence epilepsy. In a genetic model of absence epilepsy in rats, a rapid decline in the expression of HCN1 channels precedes the onset of seizures.[44] Similarly, enhancing I_h prevents experimentally induced thalamic hyperexcitability.[45]

Another possibility is that I_h serves as a shunt to maintain the membrane potential of distal dendrites at a steady value and decreases its time constant. This action may be beneficial by acting as a bias current to regulate the voltage-dependent block of NMDA receptors by Mg^{2+}. Because of the high affinity of NMDA receptors for glutamate, subthreshold synaptic excitation of terminal dendrites results in an appreciable fraction of NMDA receptors on that branch that are in the bound-but-blocked state. In this state, the NMDA receptor is not conducting current; however, it reverts to a conducting state if the membrane becomes sufficiently depolarized. I_h is ideally suited for such a depolarizing purpose. Because the bound-but-blocked state of the NMDA receptor contains information about the recent activity at that receptor (dating back hundreds of milliseconds), it may provide an energy-efficient, high-capacity mechanism for short-term memory.[46]

Downregulation of SK Channels Leads to Prolonged Spikes in Terminal Dendrites

I_A and I_h play a role in limiting dendritic depolarization and the initiation of regenerative dendritic events such as plateau potentials. SK-type Ca^{2+} activated K^+ channels, in contrast, are critical for termination of plateau potentials. Although single Ca^{2+}-activated K^+ channels have not been observed in recordings from the main apical trunk, there is evidence that Ca^{2+}-activated K^+ channels are expressed on oblique and thin terminal dendrites. SK-type channels have also been detected in dendritic spines, where they are activated by NMDA receptor-mediated Ca^{2+} influx.[47] Activation of voltage-dependent calcium channels, and relief of NMDA channel block by Mg^{2+} during the depolarization of the plateau potential, lead to large and relatively long-lasting elevations of the intracellular concentration of Ca^{2+} at the site of active initiation.[15] This elevated Ca^{2+} activates SK-type channels that are responsible for termination of the plateau potential.[27] Addition of the toxin apamin, a selective SK channel blocker, or chelation of intracellular calcium markedly increases the duration of the plateau potential without affecting its amplitude.

We have obtained evidence that alterations in SK channel function may contribute to epileptogenesis after traumatic brain injury. In many neurological conditions associated with abnormal excitability, such as posttraumatic epilepsy, neural damage leads to cellular degeneration and loss of nerve tracts. In addition, rapid acceleration/deceleration of the brain leads to axonal injury due to damaging shear forces. One consequence of these injuries is to produce chronic partial deafferentation of large populations of neurons. In a study of the effects of chronic deafferentation resulting from Schaffer collateral transections in hippocampal slice cultures, we observed increased excitability in area CA1 beginning with a delay of several days after transection. Comparable hyperexcitability is observed in the neocortex after deafferentation and axonal injury produced by cortical undercuts.[48] Hyperexcitability in denervated CA1 cells is accompanied by a marked prolongation of plateau potentials due to a functional downregulation of repolarizing SK channels.[49] The molecular mechanisms remain unclear, but decreases in mRNA levels or protein expression could not be detected, suggesting posttranslational regulation of channel conductance, trafficking, or Ca^{2+} sensitivity. Interestingly, SK channel enhancers such as 1-ethyl-2-benzimidazolinone (EBIO) have recently been shown to be effective against an in vitro model of epilepsy.[50]

Why does prolongation of plateau potentials lead to hyperexcitability? Excitatory postsynaptic potentials occurring in distal thin and oblique dendrites have a relatively low probability of triggering APs compared to main apical dendrites.[50] Plateau potentials in terminal apical dendrites or oblique dendrites elicit APs in <10% of trials in normally innervated CA1 cells. Similarly, strong activation of temporoammonic inputs to distal apical dendrites in stratum lacunosum/molecular is relatively ineffective in triggering somatic APs.[51] Both pharmacological prolongation of plateau potentials with SK channel blockers and deafferentation-induced prolongation of plateau potentials markedly facilitate AP initiation.[27] One week after deafferentation, >80% of terminal apical and oblique dendrites display APs as the result of plateau potential initiation. Although AP initiation can occur in apical trunk dendrites, the threshold for initiation is higher than at the soma and the axon hillock.[52] Facilitation of AP initiation by prolonged plateau potentials in chronically deafferented dendrites results from a lowering of the effective threshold for dendritic initiation of APs. In a population of neurons coupled by recurrent excitatory synapses, such as those in the hippocampus, such maladaptive plasticity will be amplified in a feedforward synergistic manner and promote hyperexcitability and epileptiform discharge.

THE APICAL TRUNK AS AN INDEPENDENT ELECTRICAL COMPARTMENT

From a morphological standpoint, the apical trunk, with its direct high-conductance connection to the soma, is an unlikely structure to behave as an independent electrical compartment. But dual electrode recordings have clearly shown that the distal apical trunk can generate electrical behavior that is independent of that of the soma.[53] In contrast to the

thin terminal dendritic compartment, which is created in large part by its passive cable properties, electrical compartmentalization of the apical tuft at the distal apical trunk is largely created by the distribution of active intrinsic conductances. The high expression of VDCCs near the apical tuft allows the regenerative calcium current produced there to overwhelm and escape the electrotonic control of the soma. The expression of VDCCs is lower in the region between the tuft and the soma in layer 5 pyramidal neurons.[6] This spatially restricted expression, combined with the tapering geometry of the apical trunk, normally limits the ability of calcium spikes generated at the apical tuft to propagate regeneratively to the soma. The distinct electrical excitability of the thin terminal dendrites and the apical tuft in response to focal photolysis is illustrated in Fig. 37–3.[15] These dendritic calcium spikes, even when confined to the apical tuft, are still able to drive action potential firing at the soma.

Wong and Stewart showed that depolarizing current injection into the apical trunk of guinea pig CA1 pyramidal neurons initiated burst firing, whereas current injection at the soma elicited single APs.[19] These observations show the electrical independence of the apical trunk and its special role in driving burst firing that are particularly powerful in recruiting downstream neurons within a neural network.

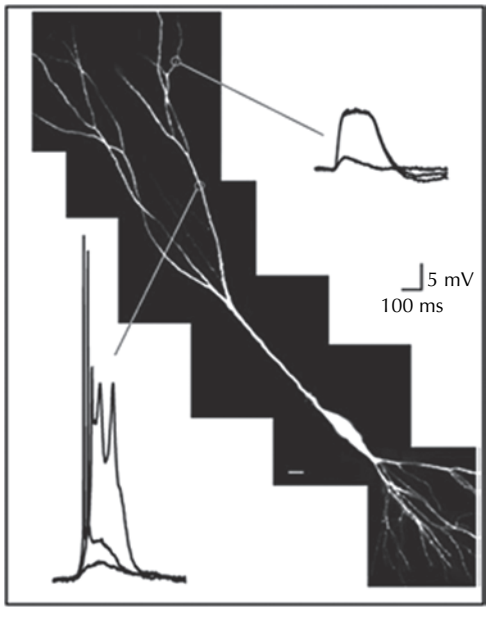

Figure 37–3. Distal and proximal dendritic compartments. Photolysis of caged glutamate directed at the terminal dendrite evokes a spatially restricted plateau potential. Photolysis directed at the main apical trunk near the base of the tuft evokes higher-amplitude depolarizations that are dominated by calcium spikes.

Perturbations of the Apical Trunk Compartment

With the demonstration of dendritic calcium spikes, considerable attention was given to the linkage between these calcium spikes and burst firing patterns that are closely associated with epileptiform discharges. In particular, Wong and Prince[54] noticed the parallels between the dendritic calcium spikes and the intrinsic burst discharges displayed by CA3 pyramidal cells in acutely prepared hippocampal brain slices, in which a burst of 2–10 high-frequency APs rides on a slow depolarizing envelope of about 20 mV in amplitude and about 100 ms in duration. This discharge bore considerable resemblance to the so-called paroxysmal depolarization shift, the discharge displayed by pyramidal cells during interictal electroencephalographic (EEG) activity in various models of epilepsy.[55] Performing the first direct recordings from dendrites, Wong and Prince[56] showed that burst discharges can be elicited from CA1 cell dendrites in response to direct depolarization, much like those described previously in the large dendrites of alligator Purkinje cells by Llinas and Nicholson.[3] Nevertheless, these dendritic bursts are not normally elicited by orthodromic synaptic stimuli. Wong and Prince showed that the short-latency hyperpolarization produced by the feedforward inhibitory postsynaptic potential (IPSP) prevents synaptically evoked bursting under normal conditions. Convulsants such as penicillin, bicuculline, or picrotoxin diminish this IPSP (because they are $GABA_A$ receptor antagonists) and thereby disinhibit this endogenous burst capacity of the apical dendrites.

Computer modeling studies suggested that intrinsically bursting neurons are pivotal in the generation of epileptiform activity.[57,58] In vitro studies of convulsant-induced discharge in the neocortex also showed that cells with intrinsic burst firing were critical for the initiation of epileptiform events.[59–61] In the pilocarpine model of TLE, 54% of CA1 pyramidal cells,

which normally fire in a regular mode, are persistently converted to a bursting mode after an episode of status epilepticus induced by the convulsant.[62] Evidence suggests that T-type calcium channels located in the apical dendrites are drivers of de novo burst firing in the pilocarpine model of hippocampal epilepsy. Burst firing in this model could be suppressed by focally applying the putative T-type calcium channel blocker Ni^{2+} to the apical dendrites but not to the soma. Severing the distal apical dendrites approximately150 μM from the pyramidal layer also abolished this activity.[63]

COUPLING BETWEEN DIFFERENT DENDRITIC COMPARTMENTS

Coupling between the soma, apical tuft, and terminal dendritic compartments is mediated by both passive and active mechanisms. Due to the asymmetric geometry of the dendritic arbor, passive cable theory predicts that the reliability of regenerative spikes propagation will be different, depending on the direction of propagation.[64] The smaller diameter of higher-order dendritic branches creates a significant impedance mismatch at the branch point. Signals leaving thin branches would experience a significant drop in impedance as they enter the larger branch. This mismatch would result in significant voltage attenuation, whereas little attenuation would be expected for signals traveling from the larger branch toward the thinner branch. Thus, under normal conditions, short-duration plateau potentials in the terminal dendrites are ineffective in generating somatic APs,[15] and sodium and calcium spikes generated at the apical tuft may not always propagate to the soma. In contrast to poor orthodromic propagation, cable theory predicts that back-propagation of APs from the soma toward the dendrites should be more reliable. Contrary to this prediction, however, direct patch-clamp recordings from the distal apical tufts of pyramidal neurons revealed that bAPs only partially propagate into the dendritic arbor. The bulk of the evidence suggests that two dendritic conductances, I_A and I_h, actively regulate the extent of bAP invasion of distal dendrites, thereby explaining the discrepancy between the prediction of cable theory and actual experimental observation. The fast kinetics of I_A and the Kv4.2 potassium channel are well suited to attenuate the fast kinetics of the bAP.[22] In contrast, the slower kinetics of I_h and the HCN family of channels have been shown to be important in the regulation of bursts of bAPs in layer 5 pyramidal neurons.[65] It is important to note, however, that this understanding has been challenged by a recent report in which voltage-sensitive dyes were used to monitor membrane depolarizations. With this technique, robust invasion of bAPs into the very tips of thin terminal dendrites was observed.[66]

What functional advantages might the tight regulation of AP backpropagation in the dendritic arbor serve? Two ideas have been proposed: control of synaptic plasticity and control of dendritic excitability. Precise coincident activation of a presynaptic glutamatergic synaptic input and the firing of an AP of the postsynaptic neuron underlie a form of synaptic plasticity called *spike timing-dependent plasticity* (STDP).[9,67] Robust AP backpropagation enables the somatic depolarization of the postsynaptic cell to reach the synaptic site. It has been suggested that regulation of the coupling between the soma and the apical tuft via the bAP is one means for regulating synaptic plasticity.[24–26] As discussed above, I_A limits synaptic plasticity by regulating AP backpropagation. Conversely, synaptic plasticity is accompanied by regulation of I_A. The Kv 4.2 channels underlying I_A are phosphorylated by a number of activity-dependent kinases, which shift their activation to more depolarized potentials and effectively decrease their activation.[24]

Robust AP backpropagation into the distal dendrite may also trigger calcium spikes in the apical tuft and plateau potentials in the thin terminal dendrites.[12,65] Bursts of four to five bAPs over a narrow frequency range (10–20 Hz) are particularly effective in eliciting large calcium spikes in the apical tufts of layer 5 pyramidal neurons.[12] These calcium spikes at the apical tuft can, in turn, lead to burst firing at the axon hillock.

Perturbations of Coupling Between the Soma and the Apical Tuft

As discussed earlier in this chapter, conditions associated with attenuated I_A or I_h are strongly linked to epilepsy. Studies of these conditions postulated that the key mechanism responsible

for the epilepsy was an increase in somato-dendritic coupling. In support of this hypothesis, dual recording from the soma and the apical tuft were used to measure the increased somato-dendritic coupling and the lowering of the frequency threshold for generating dendritic calcium spikes in the rat absence epilepsy model due to decreased HCN1 channels.[44] Similarly, in the pilocarpine model of TLE, with its decreased expression of Kv4.2 and increased phosphorylation of the Kv4.2 channels, evidence was obtained indicating that there is increased penetration of bAPs into the dendritic arbor.[31] Despite these findings, it is still not possible to conclude definitively that altered somato-dendritic coupling is the sole consequence of altered I_A and I_h. Loss of I_A and I_h also increases coupling between the terminal dendrite and apical tuft compartments, directly increases the intrinsic excitability of all dendritic compartments, and increases coupling from the apical tuft to the soma. To address these issues, it will be interesting to focally apply blockers of these two conductances to different regions of the dendritic arbor and/or examine dendritic excitability in response to orthodromic, physiological synaptic stimuli.

THE EPILEPTIC NEURON VERSUS THE EPILEPTIC NETWORK

While it is generally accepted that epilepsy exists in many forms and has many different pathophysiological mechanisms, opinions over the years have shifted between two extreme emphases: the epileptic neuron and the epileptic network. One would think that epilepsy associated with dendritic hyperexcitability would clearly place this mechanism in the epileptic neuron category. But this categorization can still be problematic because the changes in the expression of dendritic conductances could be a primary event—but also could be secondary to changes in dendritic input. In certain examples, such as in the rat absence epilepsy model due to decreased HCN1 expression, it is possible to determine which event comes first.[44] But in other cases, it is not clear whether the changes in I_A expression associated with epilepsy are the result of repeated seizures. In yet other cases, such as the downregulation of SK channels following deafferentation, the change in dendritic excitability is secondary to injury, but the dendrite is still the primary source of aberrant hyperexcitability, so aberrant excitability in this model would fall best in the epileptic neuron category. This issue is reminiscent of the controversy about the origin of the paroxysmal depolarization shift. One school of thought postulated that the paroxysmal depolarization shift is an abnormal intrinsic dendritic event,[56] whereas another postulated that it is secondary to a giant synaptic potential.[68] After 30 years, this issue, and many others relating to the role of dendrites in epilepsy, remain incompletely understood.

Controversies in epilepsy were an important early driving force in the study of dendrites. We now hope that recent rapid advances in techniques to study dendritic excitability will lead to a better understanding of many epilepsies.

DISCLOSURE STATEMENT

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REFERENCES

1. Spencer WA, Kandel ER. Electrophysiology of hippocampal neurons. IV. Fast prepotentials. *J Neurophysiol*. 1961;24:272–285.
2. Purpura D. Comparative physiology of dendrites. In: Quarton G, Melnechuk T, Schmitt F, eds. *The Neurosciences: A Study Program*. New York: Rockefeller University Press;1967:372–393.
3. Llinas R, Nicholson C. Electrophysiological properties of dendrites and somata in alligator Purkinje cells. *J Neurophysiol*. 1971;34:532–551.
4. Schwartzkroin PA, Slawsky M. Probable calcium spikes in hippocampal neurons. *Brain Res.* 1977;135:157–161.
5. Wong RK, Prince DA, Basbaum AI. Intradendritic recordings from hippocampal neurons. *Proc Natl Acad Sci USA*. 1979;76:986–990.
6. Schiller J, Schiller Y, Stuart G, Sakmann B. Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *J Physiol.* 1997;505:605–616.
7. Magee JC, Johnston D. Characterization of single voltage-gated Na^+ and Ca^{2+} channels in apical dendrites of rat CA1 pyramidal neurons. *J Physiol*. 1995;487:67–90.
8. Stuart GJ, Sakmann B. Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature*. 1994;367:69–72.
9. Abbott LF, Nelson SB. Synaptic plasticity: taming the beast. *Nat Neurosci Suppl*. 2000;3:1178–1183.

10. Magee JC, Avery RB, Christie BR, Johnston D. Dihydropyridine-sensitive, voltage-gated Ca^{2+} channels contribute to the resting intracellular Ca^{2+} concentration of hippocampal CA1 pyramidal neurons. *J Neurophysiol*. 1996;76:3460–3470.
11. Markram H, Sakmann B. Calcium transients in dendrites of neocortical neurons evoked by single subthreshold excitatory postsynaptic potentials via low-voltage-activated calcium channels. *Proc Natl Acad Sci USA*.1994;91:5207–5211.
12. Larkum ME, Kaiser KM, Sakmann B. Calcium electrogenesis in distal apical dendrites of layer 5 pyramidal cells at a critical frequency of backpropagating action potentials. *Proc Natl Acad Sci USA*. 1999;96:14600–14604.
13. Larkum ME, Zhu JJ, Sakmann B. A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature*. 1999;398:338–341.
14. Schiller J, Major G, Koester HJ, Schiller Y. NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature*. 2000;404:285–289.
15. Wei DS, Mei YA, Baga A, Kao JPY, Thompson SM, Tang C-M. Compartmentalized and binary behavior of terminal dendrites in hippocampal pyramidal neurons. *Science.* 2001;293:2272–2275.
16. Polsky A, Mel BW, Schiller J. Computational subunits in thin dendrites of pyramidal cells. *Nat Neurosci*. 2004;7:621–627.
17. Losonczy A, Magee JC. Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron*. 2006;50:291–307.
18. Bannister NJ, Larkman AU. Dendritic morphology of CA1 pyramidal neurons from the rat hippocampus. I. Branching patterns. *J Comp Neurol.* 1995;360: 150–160.
19. Wong RK, Stewart M. Differing firing patterns generated in dendrites and somata of CA1 pyramidal neurons in guinea-pig hippocampus. *J Physiol.* 1992;457:675–687.
20. Poirazi P, Brannon T, Mel BW. Pyramidal neurons as two-layer neural network. *Neuron*. 2003;37:989–999.
21. Rhodes P. The properties and implications of NMDA spikes in neocortical pyramidal cells. *J Neurosci*. 2006;26:6704–6715.
22. Hoffman DA, Magee JC, Colbert C, Johnston D. K^+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature.* 1997;387:869–875.
23. Frick A, Magee J, Koester HJ, Migliore M, Johnston D. Normalization of Ca^{2+} signals by small oblique dendrites of CA1 pyramidal neurons. *J Neurosci*. 2003;23: 3243–3250.
24. Hoffman DA, Johnston D. Downregulation of transient K^+ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. *J Neurosci*. 1998;18:3521–3528.
25. Frick A, Johnston D. Plasticity of dendritic excitability. *J Neurobiol*. 2005;64:100–115.
26. Frick A, Magee J, Johnston D. LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. *Nat Neurosci*. 2004;7:126–135.
27. Cai S, Liang CW, Muralidharan S, Kao JPY, Tang C-M, Thompson SM. Unique roles of SK and Kv4.2 potassium channels in dendritic integration. *Neuron*. 2004;44:351–364.
28. Losonczy A, Makara JK, Magee JC. Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature*. 2008;452:436–442.
29. Rutecki PA, Lebeda FJ, Johnston D. 4-Aminopyridine produces epileptiform activity in hippocampus and enhances synaptic excitation and inhibition. *J Neurophysiol*. 1987;57:1911–1924.
30. Magee JC, Curruth M. Dendritic voltage-gated ion channels regulate the action potential firing mode of hippocampal CA1 pyramidal neurons. *J Neurophysiol*. 1999;82:1895–1901.
31. Bernard C, Anderson A, Becker A, Poolos NP, Beck H, Johnston D. Acquired dendritic channelopathy in temporal lobe epilepsy. *Science*. 2004;305: 532–535.
32. Su T, Cong WD, Long YS, Luo AH, Sun WW, Deng WY, Liao WP. Altered expression of voltage-gated potassium channel 4.2 and voltage-gated potassium channel 4-interacting protein, and changes in intracellular calcium levels following lithium-pilocarpine-induced status epilepticus. *Neuroscience*. 2008;157: 566–576.
33. Aronica E, Boer K, Doorn KJ, Zurolo E, Spliet WGM, van Rijen PC, Baayen JC, Gorter JA, Jeromin A. Expression and localization of voltage dependent potassium channel Kv4.2 in epilepsy associated focal lesions. *Neurobiol Dis*. 2009;36:81–95.
34. Magee JC. Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *J Neurosci*. 1998;18: 7613–7624.
35. Williams SR, Stuart GJ. Site independence of EPSP time course is mediated by dendritic I(h) in neocortical pyramidal neurons. *J Neurophysiol*. 2000;83: 3177–3182.
36. Magee JC. Voltage-gated ion channels in dendrites. In: Stuart G, Spruston N, Hausser M., eds. *Dendrites.* New York: Oxford University Press; 1999:139–160.
37. Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I. Persistent modified h-channel after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat Med*. 2001;7:331–317.
38. Wang Z, Xu N-L, Wu C-P, Duan S, Poo M-M. Bidirectional changes in spatial dendritic integration accompanying long-term synaptic modification. *Neuron*. 2003;37:463–472.
39. Shah MM, Anderson AE, Leung V, Lin X, Johnston D. Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. *Neuron*. 2004;44: 495–508.
40. Brauer AU, Savaskan NE, Kole MHP, Plaschke M, Monteggia LM, Nestler EJ, Simburger E, Deisz RA, Ninnemann O, Nitsch R. Molecular and functional analysis of hyperpolarization-activated pacemaker channels in the hippocampus after entorhinal cortex lesion. *FASEB J*. 2001;15:2689–2701.
41. Poolos NP, Migliore M, Johnston D. Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. *Nat Neurosci*. 2002;5:767–774.
42. Munsch T, Pape HC. Modulation of the hyperpolarization-activated cation current of rat thalamic relay neurons by intracellular pH. *J Physiol*. 1999;519: 493–504.

43. Chen K, Aradi I, Santhakumar V, Soltesz I. H-channel in epilepsy: new targets for seizure control? *Trends Pharmacol Sci*. 2002;23:552–527.
44. Kole MHP, Brauer AU, Stuart GJ. Inherited cortical HCN1 channel loss amplifies dendritic calcium electrogenesis and burst firing in a rat absence epilepsy model. *J Physiol*. 2007;578:507–525.
45. Luthi A, McCormick DA. Modulation of a pacemaker current through Ca(2+)-induced stimulation of cAMP production. *Nat Neurosci*. 1999;2:634–641.
46. Santos M, Mohammadi M, Tang C-M. Dendritic hold and read: a gated short-term memory mechanism for temporal processing. SFN abstract 2009;
47. Giessel AJ, Sabatini BL. M1 muscarinic receptors boost synaptic potentials and calcium influx in dendritic spines by inhibiting postsynaptic SK channels. *Neuron*. 2010;68:936–947.
48. Prince DA, Tseng GF. Epileptogenesis in chronically injured cortex: in vitro studies. *J Neurophysiol*. 1993;69:1276–1291.
49. Cai X, Wei D-S, Bagal A, Mei Y-A, Kao JPY, Thompson SM, Tang C-M. Hyperexcitability of distal dendrites in hippocampal pyramidal cells following chronic partial deafferentation. *J Neurosci*. 2007;27:59–68.
50. Pan Y-Z, Karr L, Rutecki P. Ictal activity induced by group I metabotropic glutamate receptor activation and loss of afterhyperpolarizations. *Neuropharmacology*. 2010;59:86–92.
51. Jarsky T, Roxin A, Kath WL, Spruston N. Conditional dendritic spike propagation following distal synaptic activation of hippocampal CA1 pyramidal neurons. *Nat Neurosci*. 2005;8:1667–1676.
52. Golding NL, Spruston N. Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. *Neuron*. 1998;21:1189–1200.
53. Stuart G, Schiller J, Sakmann B. Action potential initiation in neocortical pyramidal neurons. *J Physiol*. 1997;505:671–732.
54. Wong RK, Prince DA. Participation of calcium spikes during intrinsic burst firing in hippocampal neurons. *Brain Res*. 1978;159:385–390.
55. Ayala GF, Dichter M, Gumnit RJ, Matsumoto H, Spencer WA. Genesis of epileptic interictal spikes. New knowledge of cortical feedback systems suggests a neurophysiological explanation of brief paroxysms. *Brain Res*. 1973;52:1–17.
56. Wong RK, Prince DA. Dendritic mechanisms underlying penicillin-induced epileptiform activity. *Science*. 1979;204:1228–1231.
57. Traub RD, Wong RK. Cellular mechanism of neuronal synchronization in epilepsy. *Science*. 1982;216:745–747.
58. Miles R, Wong RK. Synchronized afterdischarges in the hippocampus: contribution of local synaptic interactions. *Neuroscience*. 1984;12:1179–1189.
59. Chagnac-Amitai Y, Connors BW. Synchronized excitation and inhibition driven by intrinsically bursting neurons in neocortex. *J Neurophysiol*. 1989;62:1149–1162.
60. Jensen MS, Yaari Y. Role of intrinsic burst firing, potassium accumulation, and electrical coupling in the elevated potassium model of hippocampal epilepsy. *J Neurophysiol*. 1997;77:1224–1233.
61. Sanabria ER, Su H, Yaari Y. Initiation of network bursts by Ca^{2+}-dependent intrinsic bursting in the rat pilocarpine model of temporal lobe epilepsy. *J Physiol*. 2001;532:205–216.
62. Su H, Sochivko D, Becker A, Chen J, Jiang Y, Yaari Y, Beck H. Upregulation of a T-type Ca^{2+} channel causes a long-lasting modification of neuronal firing mode after status epilepticus. *J Neurosci*. 2002;22:3645–3655.
63. Yaari Y, Yue C, Su H. Recruitment of apical dendritic T-type Ca^{2+} channels by backpropagating spikes underlies de novo intrinsic bursting in hippocampal epileptogenesis. *J Physiol*. 2007;580:435–450.
64. Rall W, Rinzel J. Branch input resistance and steady attenuation for input to one branch of a dendritic neuron model. *Biophys J*. 1973;13:648–687.
65. Berger T, Senn W, Luscher H-R. Hyperpolarization-activated current I_h disconnects somatic and dendritic spike initiation zones in layer V pyramidal neurons. *J Neurophysiol*. 2003;90:2428–2437.
66. Holthoff K, Zecevic D, Konnerth A. Rapid time course of action potentials in spines and remote dendrites of mouse visual cortex neurons. *J Physiol*. 2010;588:1085–1096.
67. Debanne D, Gähwiler BH, Thompson SM. Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. *J Physiol*. 1998;507:237–247.
68. Johnston D, Brown TH. Giant synaptic potential hypothesis for epileptiform activity. *Science*. 1981;211:294–297.

Chapter 38

Neurogenesis and Epilepsy

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Michelle M. Kron

Medial temporal lobe epilepsy (mTLE) is a common and often intractable form of epilepsy. Approximately 50 million people suffer from different epilepsies worldwide,[1] 30%–40% of whom may continue to have poorly controlled seizures despite therapy.[2,3] Medial TLE is estimated to be the most common cause of intractable epilepsy in this population.[4] In addition to chronic seizures, the long-term morbidity of mTLE includes an increased incidence of depression[5,6] and problems with learning and memory[7,8] that may progress despite adequate seizure control.[9] Thus, progress in the study of mTLE is critical for developing better therapies to ease the large burden of this disorder.

Humans with mTLE often have an initial precipitating event, followed by a latent period and subsequent development of epilepsy later in life. This knowledge has led to the development of the most common animal models of mTLE. In these models, a prolonged seizure (termed *status epilepticus,* SE) is induced by either electrical stimulation or a chemoconvulsant, leading to injury as the initial precipitating event. After a seizure-free latent period, spontaneous seizures develop and persist for the lifetime of the animal.[10,11] The two most commonly used chemconvulsant-induced SE models of mTLE are the kainic acid and pilocarpine models.

ADULT NEUROGENESIS IN THE EPILEPTIC BRAIN

Neurogenesis persists throughout adulthood in mammals, specifically in the subgranular zone of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the forebrain lateral ventricles. Neural progenitor cells migrate from the dentate subgranular zone to the granule cell layer, and from the SVZ through the rostral migratory stream into the olfactory bulb. While the majority of cells are generated early in life, new dentate granule cells (DGCs) arise at a lower rate throughout adulthood and into senescence in both rats[12,13]

and humans.[14,15] Adult-born neurons make up about 6% of the granule cell layer in rats,[16] and these cells are thought to integrate into hippocampal circuitry and acquire characteristics of mature DGCs.[17,18] Additionally, bromodeoxyuridine (BrdU) labeling studies indicate that DGCs born during adulthood that become integrated into circuits will survive to maturity. These DGCs are very stable and may replace DGCs born during development.[19] Adult-born neurons are thought to play an important role in certain types of learning and memory or in regulating anxiety.[20–25]

In the rat pilocarpine model of mTLE, the rostral SVZ exhibits increased neurogenesis within weeks following prolonged seizure activity.[26] Neuroblasts generated in the SVZ migrate more rapidly to the olfactory bulb, and some exit the migratory stream prematurely.[26] As assessed by the expression of Ki-67, an endogenous cell proliferation marker, or short-pulse BrdU mitotic labeling, the dentate gyrus also responds to SE by increasing cell proliferation in the subgranular zone.[27,28] After either pilocarpine- or kainic acid-induced SE, dentate gyrus cell proliferation increases 5- to 10-fold after a latent period of several days and persists for several weeks.[27,29] In the dentate, this early proliferative response seems to be mediated by radial glial-like neural progenitor cells,[30] as well as through activation of transit-amplifying cells that are actively proliferating before SE.[31] Interestingly, even single seizure-like discharges modestly increase DGC neurogenesis.[32] Between 75% and 90% of cells newly generated after SE express mature DGC markers within 4 weeks,[27,28] and SE appears to accelerate the maturation and integration of adult-born DGCs.[33]

Approximately 3–4 weeks after an SE episode, proliferation rates return to baseline levels.[27] In fact, chronic mTLE is associated with a decrease in neurogenesis, as levels are substantially below baseline by 5 months after kainic acid-induced SE.[34] These findings may also be relevant to human epilepsy, as the dentate gyrus of children who have had frequent seizures shows decreased numbers of proliferating cells and immature neurons.[35] Potential reasons for this decrease include exhaustion of the progenitor pool, loss of needed growth/trophic factors, or altered cellular interactions (reviewed in ref. 36).

MORPHOLOGICAL ABNORMALITIES IN MTLE: ROLE OF NEUROGENESIS

The epileptic hippocampus in human mTLE is associated with numerous cellular abnormalities, including CA1 and CA3 pyramidal cell loss as well as hippocampal astrogliosis. Damage to the hilus of the dentate gyrus (DG), known as *endfolium sclerosis*, is the most commonly observed lesion in the brains of patients with mTLE.[37] Status epilepticus provoked by kainic acid or pilocarpine mimics this phenotype, destroying about half of the neurons in the dentate hilus.[38,39] The human DG also shows distinct morphological abnormalities, including mossy fiber sprouting, granule cell layer dispersion, ectopically located DGCs, and DGCs with very prominent hilar basal dendrites (HBDs).[40,41] In rodent models of SE such as the pilocarpine model, hippocampal pathways exhibit structural plasticity mirroring these changes.[11]

Mossy Fiber Sprouting

Mossy fibers are the axons of the hippocampal DGCs. The mossy fiber pathway normally projects to the pyramidal cells and interneurons of hippocampal area CA3 as well as to the dentate hilus. In the nonepileptic brain, this pathway is thought to make few, if any, recurrent synapses onto granule cells. A common feature of human mTLE,[40,41] however, and of animal models of temporal lobe epilepsy, is the development of numerous mossy fiber–granule cell synapses.[42,43]

In the epileptic human DG, Timm staining, dynorphin immunoreactivity, and biocytin cell fills reveal that mossy fibers sprout into the dentate inner molecular layer.[41,44,45] In the rat pilocarpine model of mTLE, Timm staining, used to visualize the zinc present in mossy fiber axons, also reveals significant amounts of mossy fiber sprouting in the dentate inner molecular layer.[11] Electron microscopy studies demonstrate that mossy fibers synapse onto neighboring DGCs, creating recurrent excitatory synapses.[42]

Over a dozen years ago, we first hypothesized that mossy fiber sprouting in experimental mTLE arises from adult-born rather

than preexisting DGCs.[27] When we used irradiation to kill adult-born cells in the setting of SE, however, we did not block inner molecular layer mossy fiber sprouting 4 weeks later, suggesting that DGCs generated after SE do not send axons aberrantly into the dentate inner molecular layer. To address this question more definitively, we recently used retroviral reporter labeling to birth date DGCs in combination with low-dose irradiation to transiently suppress DGC neurogenesis.[46] At 4 weeks after pilocarpine-induced SE in adult animals, we found that neither neonatally generated DGCs nor those born after SE contributed to sprouting; instead, only DGCs that were 2–4 weeks old at the time of SE showed aberrant axonal reorganization. This finding is consistent with retroviral reporter labeling studies from another group who observed that adult-generated DGCs born 4 weeks before kainic acid-induced SE contributed to inner molecular layer mossy fiber sprouting.[47] When we labeled adult-born DGCs 4 days after SE but allowed the animals to survive for 10 (instead of 4) weeks, in contrast, we found that the adult-born DGCs contributed robustly to mossy fiber sprouting[46] (Fig. 38–1).

The finding of mossy fiber remodeling only by developing or newborn, and not mature, DGCs has key mechanistic implications for understanding seizure-induced DGC plasticity. Rather than recapitulating development, mossy fiber sprouting after SE appears to involve an alteration of ongoing development. This sprouting is thought to be progressive in nature, beginning 2 weeks after SE and peaking at around 100 days post-SE.[11,48] The idea that successive generations of adult-born DGCs sprout aberrantly as they develop is consistent with this progression of mossy fiber reorganization for several months after SE.[11] In fact, the timing of transiently increased neurogenesis for 2–3 weeks after the initial seizures[27] followed by a potential suppression of neurogenesis chronically,[34] along with the delay in adult-born neurons manifesting aberrant axonal outgrowth, fits well with a model in which most or all of the newborn DGCs eventually sprout. Such a time course would lead to a peak in mossy fiber sprouting at about 2–3 months after SE.

Hilar Ectopic Granule Cells

The vast majority of neurons born in the subgranular zone during adult life migrate into the granule cell layer. After SE, many DGCs migrate instead into the dentate hilus or through the granular layer into the molecular layer.[27,34,49–51] These ectopic cells are found in rodent models of epilepsy[27,49,50] (Fig. 38–2), and similar ectopically located granule-like neurons appear in the epileptic human hippocampus.[51,52]

Hilar ectopic granule cells may result from abnormal migratory behavior of DGC

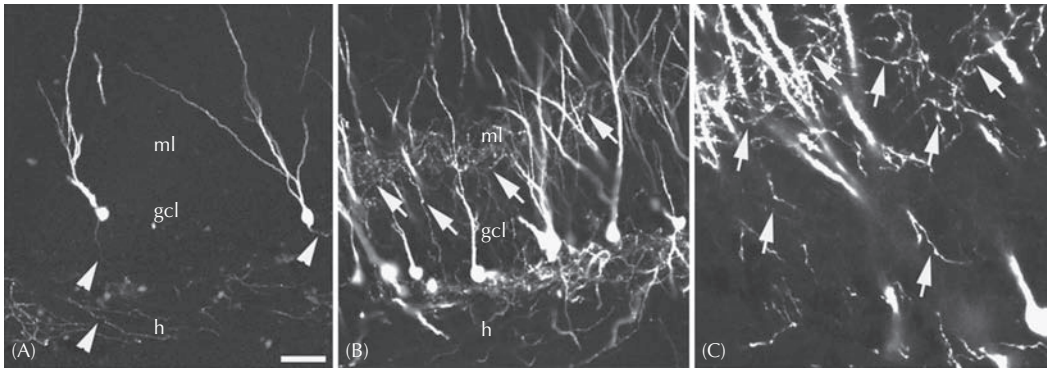

Figure 38–1. Mossy fiber sprouting by adult-born DGCs. **A–C.** Confocal images of coronal brain sections through the dentate gyrus of adult rats injected with retrovirus carrying a green fluorescent protein reporter (RV-GFP) and immunostained for GFP. A control received an injection of RV-GVP 4 days after saline treatment and survived for 10 weeks (**A**). Note the labeled DGC bodies in the granule cell layer (gcl), dendrites in the molecular layer (ml), and axons (arrowheads) in the hilus (h). An epileptic rat received a RV-GFP injection 4 days after pilocarpine treatment and survived for 10 weeks (**B**). Note the labeled mossy fibers (arrows) coursing through the inner ml orthogonal to the larger-caliber DGC dendrites. **C.** A higher-magnification view of the dentate gyrus in **B**. Scale bar (in **A**): 25 μm for **A**,**B**; 15 μm for **C**.

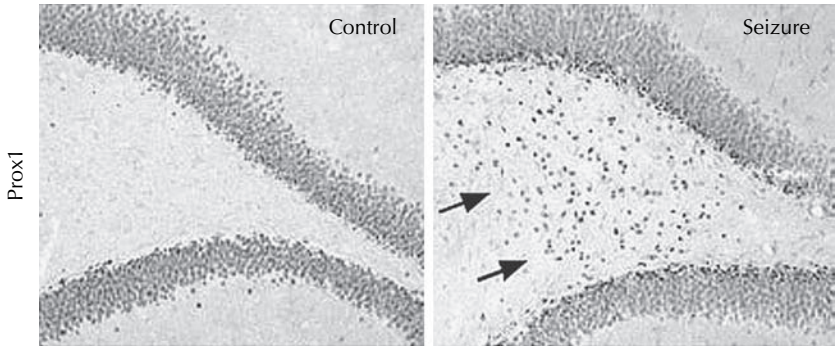

Figure 38–2. Hilar ectopic DGCs in experimental mTLE. Prox1 immunolabeling of DGCs from adult rats 35 days after saline (Control, left panel) or pilocarpine treatment (Seizure, right panel). Note the abundant Prox1-immunoreactive DGCs in the hilus of the epileptic rat (arrows).

progenitors after epileptogenic insults, as SE appears to cause aberrant chain migration of DGC progenitors to the hilus and the molecular layer.[51] Some propose a critical period after the birth of adult-generated neurons during which they are vulnerable to being recruited into epileptogenic neuronal circuits,[53] and indeed, we find that only DGCs generated after SE migrate ectopically.[46] One proposed cause of the aberrant migration is loss of the migration guidance cue Reelin, which is expressed in the adult rodent hippocampus.[54]

Hilar Basal Dendrites

The persistence of HBDs, normally a feature of only immature DGCs, may be a mechanism contributing to the hyperexcitability of adult-born DGCs in epilepsy. The percentage of granule cells with HBDs is probably substantially higher in persons with mTLE,[55] and this finding is recapitulated in several animal models in which prolonged seizures induce an increased percentage of granule cells with basal dendrites located at the hilar border and extending into the granule cell layer.[56,57] Many of these basal dendrites have numerous spines, suggesting that they are postsynaptic to axon terminals.[49,50,58] Using Thy1-GFP mice, Walter and colleagues[53] found that almost 50% of immature granule cells in mice exposed to pilocarpine-induced SE exhibited HBDs, and newborn cells were even more severely impacted than immature cells. In the rat pilocarpine model, about a third of adult-born DGCs that are 2 weeks old at the time of SE, or born 4 days after SE, develop HBDs.[46]

Electron microscopy demonstrates that HBDs form asymmetric synapses and are innervated by mossy fibers, potentially creating recurrent excitatory circuits.[59,60] Hilar basal dendrites can form on granule cells as early as 1 week following SE. Although the molecular mechanisms for the persistence of these HBDs are not well defined, they may involve changes in the glial scaffold.[61]

FUNCTIONAL IMPLICATIONS OF DGC ABNORMALITIES IN MTLE

A large body of information supports the hypothesis that cellular abnormalities such as mossy fiber sprouting, ectopic DGCs, and HBDs contribute to epileptogenesis in experimental and human mTLE. The functional implications of these abnormalities, however, and the contribution of adult-born DGCs to intact or epileptic hippocampal network function remain unclear. Here we describe the potential effects on hippocampal function of the different types of seizure-induced plasticity associated with aberrant neurogenesis.

Mossy Fiber Sprouting

Seizures in mTLE have been proposed to result from hyperexcitability due to aberrant excitatory recurrent axon collaterals between

granule cells.[62,63] Integration of light microscopic and electron microscopic data suggests that the majority of synapses formed by mossy fibers in the granule cell and molecular layers are with other granule cells, leading to recurrent excitation.[42,62,63] Additionally, evidence suggests that normal gamma-aminobutyric acid (GABA) inhibition is diminished by mossy fiber terminals, further contributing to hyperexcitability.[64] Recent data also suggest that interventions to block mossy fiber sprouting reduce the severity of seizures.[65]

However, further levels of complexity likely underlie epileptic pathogenesis. Although the aberrantly sprouted mossy fibers clearly form at least some recurrent excitatory synapses with other granule cells, some work suggests that DGC sprouting contributes to the synaptic drive onto inhibitory interneurons.[66] Anatomical analysis of epileptic rat hippocampi reveals that some aberrant granule cell axons densely innervate inhibitory neurons.[42,67] Furthermore, the density of mossy fiber sprouting may not be associated with the total number of lifetime seizures or the seizure frequency in experimental or human TLE.[68] These data suggest that other mechanisms in addition to mossy fiber sprouting might contribute to enhanced hippocampal excitability during epileptogenesis.

HBDs and Hilar Ectopic Granule Cells

Many studies have found that compared with controls, an increased number of granule cells in epileptic rats extended a basal dendrite into the hilus, providing a potential route for recurrent excitation.[59,60] Modeling studies suggest that even a relatively small percentage of DGCs, if hyperinnervated by excitatory input (so-called *hub* cells), could lead to spontaneous seizures.[69] Adult-born DGCs with long HBDs that receive excessive excitatory input may be just such cells.

Hilar ectopic granule cells themselves are also thought to be hyperexcitable. They have been shown to be postsynaptic to mossy fibers and have less inhibitory input on their somata and proximal dendrites than DGCs in the granule cell layer.[49,70] This finding is consistent with results showing that hilar ectopic granule cells are more excitable than granule cells in the granule cell layer, and they burst fire in synchrony with spontaneous, rhythmic bursts of area CA3 pyramidal cells that survive SE.[50] Consistent with these data is the finding that ablating neurogenesis after SE, the time when ectopic cells form,[46] attenuates subsequent epileptogenesis, with a reduction in the frequency and severity of spontaneous recurrent seizures.[71]

One study, however, suggests the opposite, namely, that adult-born neurons that integrate normally may compensate for hyperexcitability after an epileptogenic insult. Jakubs and colleagues[72] induced SE with electrical stimulation and then labeled adult-born neurons with retrovirus-expressing green fluorescent protein (GFP). They recorded from the GFP-labeled cells in acute hippocampal slices and found that they showed increased inhibitory drive and decreased excitatory input compared to GFP-labeled cells in controls and mature DGCs in epileptic rats. However, they only studied adult-born neurons that integrated normally. These findings suggest that increased DGC neurogenesis after SE leads to heterogeneous populations of adult-born DGCs, some of which integrate aberrantly and may become hyperexcitable, while others integrate normally and may restore inhibition (Fig. 38–3).

COMORBIDITIES ASSOCIATED WITH MTLE

The comorbidities associated with epilepsy include cognitive, behavioral, and emotional difficulties. Persons with epilepsy are at increased risk for depression, anxiety, sleep disturbances, and cognitive impairment (reviewed in ref. 73). Cognitive impairments include problems with memory, verbal fluency, and executive function.[7,74] Approximately 70% of patients with mTLE have problems with memory function, which represents the most common cognitive impairment in this group.

Neurons generated during epileptogenic insults may impact learning in several ways, including interference with normal network function caused by aberrantly integrated neurons (Fig. 38–3). This idea is supported by the finding that inhibition of seizure-induced neurogenesis with valproic acid (acting as a histone deacetylase inhibitor) protects epileptic animals from deficits in hippocampal-dependent object recognition.[75] Alternatively, suppression

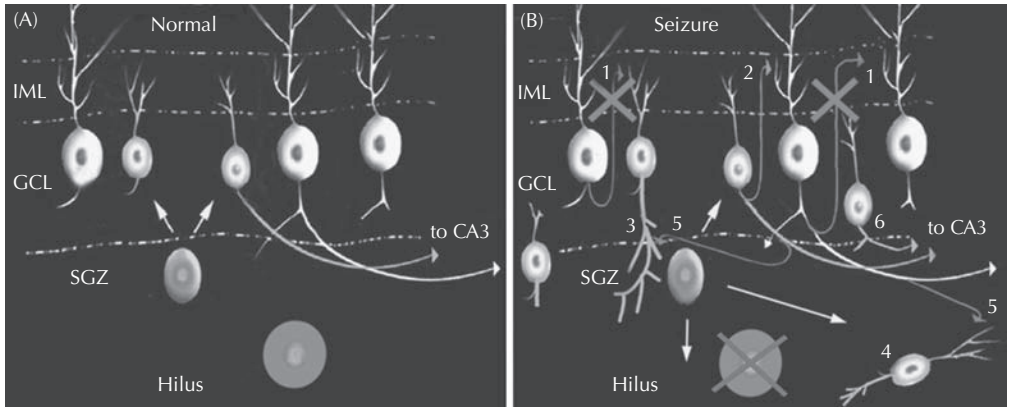

Figure 38–3. Aberrant neurogenesis in experimental mTLE. Schematic of the intact adult dentate gyrus (**A**) with a DGC progenitor in the subgranular zone (SGZ) giving rise to immature DGCs (arrows). In the epileptic dentate gyrus (**B**), mossy fiber sprouting does not come from mature DGCs (1), but instead arises from developing or newborn DGCs (2), which also display HBDs (3), ectopic migration (4), and possibly axonal sprouting onto DGCs with HBDs or ones that are ectopic (5). Some newborn DGCs appear to integrate normally and may restore inhibition (6). IML, inner molecular layer; GCL, granule cell layer.

of neurogenesis during chronic epilepsy could interfere with learning and memory.[34]

Depression is another important comorbidity in epilepsy (reviewed in ref. 76). Adult neurogenesis is unlikely to be related to the development of depression per se, but some studies suggest that the production of DGCs is necessary for an adequate response to antidepressants in rodent models of depression (reviewed in ref. 77). Even more compelling is recent work suggesting that loss of adult-born DGCs leads to an anxiety phenotype.[24] Thus, both cognitive and emotional behaviors have been linked to the production of adult-born DGCs. In summary, altered adult neurogenesis in the chronic epileptic state deserves further study to evaluate its potential role in the development of mTLE and its associated comorbidities.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Brodie MJ, Shorvon SD, Canger R, Halasz P, Johannessen S, Thompson P, et al. Commission on European Affairs: appropriate standards of epilepsy care across Europe. ILEA. *Epilepsia*. 1997;38(11): 1245–1250.
2. Engel J Jr, Williamson PD, Wieser HG. Mesial temporal lobe epilepsy. In: Engel J Jr, Pedley TA, eds. *Epilepsy: A Comprehensive Textbook*. Philadelphia: Lippincott-Raven; 1997:2417–2426.
3. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N Engl J Med*. 2000;342(5):314–319.
4. Engel J Jr. Etiology as a risk factor for medically refractory epilepsy: a case for early surgical intervention. *Neurology*. 1998;51(5):1243–1244.
5. Helmstaedter C, Sonntag-Dillender M, Hoppe C, Elger CE. Depressed mood and memory impairment in temporal lobe epilepsy as a function of focus lateralization and localization. *Epilepsy Behav*. 2004;5(5):696–701.
6. Mazza M, Orsucci F, De Risio S, Bria P, Mazza S. Epilepsy and depression: risk factors for suicide? *Clin Ter*. 2004;155(10):425–427.
7. Elger CE, Helmstaedter C, Kurthen M. Chronic epilepsy and cognition. *Lancet Neurol*. 2004;3(11): 663–672.
8. Helmstaedter C, Kurthen M, Lux S, Reuber M, Elger C. Chronic epilepsy and cognition: a longitudinal study in temporal lobe epilepsy. *Ann Neurol*. 2003;54(4):425–432.
9. Blume WT. The progression of epilepsy. Epilepsia. 2006;47 Suppl 1:71–78.
10. Arida RM, Scorza FA, Peres CA, Cavalheiro EA. The course of untreated seizures in the pilocarpine model of epilepsy. *Epilepsy Res*. 1999;34(2–3):99–107.
11. Mello LE, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, et al. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia*. 1993;34(6):985–995.
12. Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci*. 1996;16(6):2027–2033.
13. Schlessinger AR, Cowan WM, Gottlieb DI. An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. *J Comp Neurol*. 1975;159(2):149–175.

14. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, et al. Neurogenesis in the adult human hippocampus. *Nat Med*. 1998;4(11): 1313–1317.
15. Kempermann G, Jessberger S, Steiner B, Kronenberg G. Milestones of neuronal development in the adult hippocampus. *Trends Neurosci*. 2004;27(8):447–452.
16. Cameron HA, McKay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J Comp Neurol*. 2001;435(4):406–417.
17. Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H. GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature*. 2006;439(7076):589–593.
18. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. *Nature*. 2002;415(6875):1030–1034.
19. Dayer A, Ford A, Cleaver K, Yassaee M, Cameron H. Short-term and long-term survival of new neurons in the rat dentate gyrus. *J Comp Neurol*. 2003;460(4): 563–572.
20. Clelland CD, Choi M, Romberg C, Clemenson GD Jr, Fragniere A, Tyers P, et al. A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science*. 2009;325(5937):210–213.
21. Doetsch F, Hen R. Young and excitable: the function of new neurons in the adult mammalian brain. *Curr Opin Neurobiol*. 2005;15(1):121–128.
22. Garthe A, Behr J, Kempermann G. Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. *PLoS One*. 2009;4(5): e5464.
23. Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T, Yamaguchi M, et al. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci*. 2008;11(10):1153–1161.
24. Revest JM, Dupret D, Koehl M, Funk-Reiter C, Grosjean N, Piazza PV, et al. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. *Mol Psychiatry*. 2009;14(10):959–967.
25. Saxe MD, Battaglia F, Wang JW, Malleret G, David DJ, Monckton JE, et al. Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc Natl Acad Sci USA*. 2006;103(46):17501–17506.
26. Parent JM, Valentin VV, Lowenstein DH. Prolonged seizures increase proliferating neuroblasts in the adult rat subventricular zone–olfactory bulb pathway. *J Neurosci*. 2002;22(8):3174–3188.
27. Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J Neurosci*. 1997;17(10):3727–3738.
28. Jessberger S, Romer B, Babu H, Kempermann G. Seizures induce proliferation and dispersion of doublecortin-positive hippocampal progenitor cells. *Exp Neurol*. 2005;196(2):342–351.
29. Gray WP, Sundstrom LE. Kainic acid increases the proliferation of granule cell progenitors in the dentate gyrus of the adult rat. *Brain Res*. 1998;790(1–2):52–59.
30. Huttmann K, Sadgrove M, Wallraff A, Hinterkeuser S, Kirchhoff F, Steinhauser C, et al. Seizures preferentially stimulate proliferation of radial glia-like astrocytes in the adult dentate gyrus: functional and immunocytochemical analysis. *Eur J Neurosci*. 2003;18(10): 2769–2778.
31. Parent JM, Tada E, Fike JR, Lowenstein DH. Inhibition of dentate granule cell neurogenesis with brain irradiation does not prevent seizure-induced mossy fiber synaptic reorganization in the rat. *J Neurosci*. 1999;19(11):4508–4519.
32. Bengzon J, Kokaia Z, Elmer E, Nanobashvili A, Kokaia M, Lindvall O. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *Proc Natl Acad Sci USA*. 1997;94(19):10432–10437.
33. Overstreet-Wadiche LS, Bromberg DA, Bensen AL, Westbrook GL. Seizures accelerate functional integration of adult-generated granule cells. *J Neurosci*. 2006;26(15):4095–4103.
34. Hattiangady B, Rao MS, Shetty AK. Chronic temporal lobe epilepsy is associated with severely declined dentate neurogenesis in the adult hippocampus. *Neurobiol Dis*. 2004;17(3):473–490.
35. Mathern GW, Leiphart JL, De Vera A, Adelson PD, Seki T, Neder L, et al. Seizures decrease postnatal neurogenesis and granule cell development in the human fascia dentata. *Epilepsia*. 2002;43(suppl 5):68–73.
36. Hattiangady B, Shetty AK. Implications of decreased hippocampal neurogenesis in chronic temporal lobe epilepsy. *Epilepsia*. 2008;49(suppl 5):26–41.
37. Margerison JH, Corsellis JA. Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain*. 1966;89(3):499–530.
38. Buckmaster PS, Dudek FE. Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol*. 1997;385(3):385–404.
39. Okazaki MM, Molnar P, Nadler JV. Recurrent mossy fiber pathway in rat dentate gyrus: synaptic currents evoked in presence and absence of seizure-induced growth. *J Neurophysiol*. 1999;81(4):1645–1660.
40. Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol*. 1989;26(3): 321–330.
41. Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF. Synaptic reorganization by mossy fibers in human epileptic fascia dentata. *Neuroscience*. 1991;42(2):351–363.
42. Okazaki MM, Evenson DA, Nadler JV. Hippocampal mossy fiber sprouting and synapse formation after status epilepticus in rats: visualization after retrograde transport of biocytin. *J Comp Neurol*. 1995;352(4): 515–534.
43. Sutula T, He XX, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science*. 1988;239(4844):1147–1150.
44. de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res*. 1989;495(2): 387–395.
45. Houser CR, Miyashiro JE, Swartz BE, Walsh GO, Rich JR, Delgado-Escueta AV. Altered patterns of dynorphin immunoreactivity suggest mossy fiber reorganization in human hippocampal epilepsy. *J Neurosci*. 1990;10(1):267–282.
46. Kron MM, Zhang H, Parent JM. The developmental stage of dentate granule cells dictates their contribution to seizure-induced plasticity. *J Neurosci*. 2010;30(6):2051–2059.

47. Jessberger S, Zhao C, Toni N, Clemenson GD Jr, Li Y, Gage FH. Seizure-associated, aberrant neurogenesis in adult rats characterized with retrovirus-mediated cell labeling. *J Neurosci*. 2007;27(35):9400–9407.
48. Cavalheiro EA, Leite JP, Bortolotto ZA, Turski WA, Ikonomidou C, Turski L. Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures. *Epilepsia*. 1991;32(6):778–782.
49. Dashtipour K, Tran PH, Okazaki MM, Nadler JV, Ribak CE. Ultrastructural features and synaptic connections of hilar ectopic granule cells in the rat dentate gyrus are different from those of granule cells in the granule cell layer. *Brain Res*. 2001;890(2): 261–721.
50. Scharfman HE, Goodman JH, Sollas AL. Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. *J Neurosci*. 2000;20(16):6144–6158.
51. Parent JM, Elliott RC, Pleasure SJ, Barbaro NM, Lowenstein DH. Aberrant seizure-induced neurogenesis in experimental temporal lobe epilepsy. *Ann Neurol*. 2006;59(1):81–91.
52. Houser CR. Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. *Brain Res*. 1990;535(2):195–204.
53. Walter C, Murphy BL, Pun RYK, Spieles-Engemann AL, Danzer SC. Pilocarpine-induced seizures cause selective time-dependent changes to adult-generated hippocampal dentate granule cells. *J Neurosci*. 2007;27(28):7541–7552.
54. Gong C, Wang TW, Huang HS, Parent JM. Reelin regulates neuronal progenitor migration in intact and epileptic hippocampus. *J Neurosci*. 2007;27(8): 1803–1811.
55. von Campe G, Spencer DD, de Lanerolle NC. Morphology of dentate granule cells in the human epileptogenic hippocampus. *Hippocampus*. 1997;7(5): 472–488.
56. Dashtipour K, Yan X-X, Dinh TT, Okazaki MM, Nadler JV, Ribak CE. Quantitative and morphological analysis of dentate granule cells with recurrent basal dendrites from normal and epileptic rats. *Hippocampus*. 2002;12(2):235–244.
57. Ribak CE, Tran PH, Spigelman I, Okazaki MM, Nadler JV. Status epilepticus-induced hilar basal dendrites on rodent granule cells contribute to recurrent excitatory circuitry. *J Comp Neurol*. 2000;428(2): 240–253.
58. Shapiro LA, Ribak CE. Newly born dentate granule neurons after pilocarpine-induced epilepsy have hilar basal dendrites with immature synapses. *Epilepsy Res*. 2006;69(1):53–66.
59. Buckmaster PS, Dudek FE. In vivo intracellular analysis of granule cell axon reorganization in epileptic rats. *J Neurosci*. 1999;81(2):712–721.
60. Spigelman I, Yan XX, Obenaus A, Lee EY, Wasterlain CG, Ribak CE. Dentate granule cells form novel basal dendrites in a rat model of temporal lobe epilepsy. *Neuroscience*. 1998;86(1):109–120.
61. Shapiro LA, Ribak CE. Integration of newly born dentate granule cells into adult brains: hypotheses based on normal and epileptic rodents. *Brain Res Brain Res Rev*. 2005;48(1):43–56.
62. Tauck DL, Nadler JV. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. *J Neurosci.* 1985;5(4):1016–1022.
63. Buckmaster PS, Zhang GF, Yamawaki R. Axon Sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. *J Neurosci*. 2002;22(15):6650–6658.
64. Buhl EH, Otis TS, Mody I. Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. *Science*. 1996;271(5247):369–373.
65. Zeng LH, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J Neurosci*. 2009;29(21):6964–6972.
66. Sloviter RS, Zappone CA, Harvey BD, Frotscher M. Kainic acid-induced recurrent mossy fiber innervation of dentate gyrus inhibitory interneurons: possible anatomical substrate of granule cell hyperinhibition in chronically epileptic rats. *J Comp Neurol*. 2006;494(6):944–960.
67. Kotti T, Riekkinen PJ Sr, Miettinen R. Characterization of target cells for aberrant mossy fiber collaterals in the dentate gyrus of epileptic rat. *Exp Neurol*. 1997;146(2):323–330.
68. Pitkanen A, Nissinen J, Lukasiuk K, Jutila L, Paljarvi L, Salmenpera T, et al. Association between the density of mossy fiber sprouting and seizure frequency in experimental and human temporal lobe epilepsy. *Epilepsia*. 2000;41(suppl 6):S24–S29.
69. Morgan RJ, Soltesz I. Nonrandom connectivity of the epileptic dentate gyrus predicts a major role for neuronal hubs in seizures. *Proc Natl Acad Sci USA*. 2008;105(16):6179–6184.
70. Pierce JP, Melton J, Punsoni M, McCloskey DP, Scharfman HE. Mossy fibers are the primary source of afferent input to ectopic granule cells that are born after pilocarpine-induced seizures. *Exp Neurol*. 2005;196(2):316–331.
71. Jung KH, Chu K, Kim M, Jeong SW, Song YM, Lee ST, et al. Continuous cytosine-b-D-arabinofuranoside infusion reduces ectopic granule cells in adult rat hippocampus with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. *Eur J Neurosci*. 2004;19(12):3219–3226.
72. Jakubs K, Nanobashvili A, Bonde S, Ekdahl CT, Kokaia Z, Kokaia M, et al. Environment matters: synaptic properties of neurons born in the epileptic adult brain develop to reduce excitability. *Neuron*. 2006;52(6):1047–1059.
73. Jacobs MP, Leblanc GG, Brooks-Kayal A, Jensen FE, Lowenstein DH, Noebels JL, et al. Curing epilepsy: progress and future directions. *Epilepsy Behav.* 2009;14(3):438–445.
74. Helmstaedter C, Kockelmann E. Cognitive outcomes in patients with chronic temporal lobe epilepsy. *Epilepsia*. 2006;47(suppl 2):96–98.
75. Jessberger S, Nakashima K, Clemenson GD Jr, Mejia E, Mathews E, Ure K, et al. Epigenetic modulation of seizure-induced neurogenesis and cognitive decline. *J Neurosci.* 2007;27(22):5967–5975.
76. Kanner AM. Depression and epilepsy: a review of multiple facets of their close relation. *Neurol Clin*. 2009;27(4):865–880.
77. Sahay A, Hen R. Adult hippocampal neurogenesis in depression. *Nat Neurosci*. 2007;10(9):1110–1115.

Chapter 39

Temporal Lobe Epilepsy and the BDNF Receptor, TrkB

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INTRODUCTION

One of the most difficult but most important goals of epilepsy research today is to address the underlying mechanisms that contribute to seizures. Another important issue is understanding epileptogenesis—the transformation of the normal brain to a chronic epileptic state. If these issues can be effectively addressed, the resultant insights may lead to the development of better antiepileptic drugs (AEDs) to treat seizures and antiepileptogenic drugs that can prevent the disease. Current AEDs target many ion channels known to contribute to neuronal excitability. This broad "net" can help reduce seizures, but it also leads to side effects. Further, sometimes seizures cannot be controlled even with high doses of these drugs (pharmacoresistance). Mesial temporal lobe epilepsy (MTLE) is a good example of a condition with a complex set of causes and a subset of patients who are pharmacoresistant. Therefore, understanding the mechanisms of MTLE has been a subject of avid interest. Combining a clear clinical characterization of MTLE with insights from research using animal models,

as discussed below, has revealed a potential control point for new antiepileptic and antiepileptogenic therapy: the neurotrophic factor brain-derived neurotrophic factor (BDNF) and its receptor, TrkB.

MTLE: A PROGRESSIVE DISORDER IN HUMANS AND ANIMAL MODELS

Mesial temporal lobe epilepsy, the most common form of partial epilepsy, is a progressive disorder in a substantial number of affected individuals.[1,2] The progression is evident in part as persistence of disabling seizures despite anticonvulsant therapy, the refractoriness to therapy sometimes arising years after onset of the disorder.[3] Progression is also evident as destruction of hippocampus and parahippocampal gyrus and temporal lobes, as revealed in multiple magnetic resonance imaging (MRI) analyses conducted over time in patients with MTLE compared to normal controls.[4] Additional evidence of progressive atrophy of neocortical gray matter has been demonstrated by an independent group of investigators using MRI analyses in a cross-sectional study of MTLE patients compared to age-matched controls as well as a longitudinal study in which patients served as their own controls.[5]

What underlies the progression of MTLE in this subset of affected individuals? An astute clinician, Sir William Gowers, proposed that seizures themselves contributed to the progression of epilepsy.[6] Discovery of the kindling phenomenon, by Graham Goddard and colleagues,[7] almost a century later, validated Gowers' idea. In this model, repeated induction of brief focal seizures by chemical or electrical stimuli eventually results in longer and more severe focal and tonic-clonic seizures. Once established, this enhanced sensitivity to electrical stimulation persists for the life of the animal. While 15 or so stimulations (e.g., in the amygdala) are required to induce this lifelong enhanced sensitivity, additional stimulations (80+) lead to the emergence of spontaneous seizures[8,9] and destruction of cortical gray matter.[8] That is, periodic induction of isolated seizures (e.g., at daily intervals) in an animal model is sufficient to induce a progressive increase in the severity of evoked seizures, emergence of spontaneous seizures, and destruction of cortical gray matter.[10] Indeed, a progressive increase in seizure frequency is evident in a diversity of additional animal models, including models in which epilepsy is induced by stroke, or status epilepticus.[11–13] Whether seizures themselves cause the progression in these models is currently under investigation. That said, study of the kindling model clearly demonstrates that recurrent isolated seizures, not simply status epilepticus, are sufficient to produce spontaneous recurrent seizures and destruction of cortical gray matter.[7–10] Given the presence of recurrent seizures in a subset of humans with medically refractory MTLE and progressive increase in seizure frequency in animal models (e.g., status epilepticus and stroke), it seems plausible that recurrent seizures per se constitute one factor contributing to the progression of the epileptic state.

SEIZURES: A PATHOLOGICAL FORM OF NEURONAL ACTIVITY

Elucidating the molecular mechanisms by which recurrent seizures promote worsening of the epileptic condition, evident as spontaneous recurrent seizures and destruction of cortical gray matter, may provide novel targets for drugs aimed at limiting worsening of the condition in humans and also provide clues to the underlying cellular mechanisms. The recurrent isolated seizures that cause worsening of seizures and death of cortical neurons in the kindling model consist of an abnormal form of neuronal activity. Thus, the question arises as to how fleeting changes in neuronal activity (i.e., isolated recurrent seizures) produce lasting changes in brain structure and function (i.e., more severe seizures and destruction of neurons). Similar questions are being posed with respect to physiological forms of activity, the idea being that fleeting experiences are associated with fleeting patterns of neuronal activity. For example, how are fleeting visual experiences during development transformed into the lasting structural and functional modifications that underlie normal vision? How are brief experiences transformed into a memory that persists for a lifetime? Gene transcription is one molecular mechanism by which fleeting experiences can be encoded as persistent

changes in neuronal structure and function. This "answer," in turn, raises the question of which particular gene(s) might underlie the lasting modifications of neuronal structure and function that culminate in worsening of the epileptic condition in MTLE.

BDNF: AN ATTRACTIVE CANDIDATE GENE

If gene transcription provides a molecular mechanism underlying worsening of MTLE, what are the features of that particular gene (or genes), the transcription of which might lead to the lasting modifications of neuronal structure and function underlying worsening of MTLE? Important criteria for such a gene include the requirements that its expression is regulated by seizure activity, and that structural and functional consequences of its expression in model systems mimic features of an epileptic brain.

One gene meeting those criteria encodes BDNF. Yves Barde and colleagues[14] purified BDNF protein from pig brain extracts in their search for a factor that supported the survival and neurite outgrowth of embryonic chick sensory neurons. Subsequently, BDNF was found to activate a receptor tyrosine kinase named TrkB.[15] Packaged in dense core vesicles in axon terminals, BDNF is a 14 kDa secreted protein that binds to the ectodomain of TrkB, thereby inducing receptor dimerization, activation of receptor tyrosine kinase activity, and phosphorylation of select tyrosines in the cytoplasmic domain—which creates docking sites for adaptor proteins (shc) or enzymes (PLCγ1) that couple these receptors to intracellular signaling cascades. Relatives of BDNF include the other neurotrophins: nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). Likewise, relatives of TrkB include two other receptor tyrosine kinases, TrkA and TrkC; NGF serves as the ligand for TrkA, BDNF and NT-4 for TrkB, and NT-3 for TrkC.

Seizure-regulated expression of BDNF, together with its effects in simplified model systems, make the genes for BDNF and its receptor (TrkB) attractive candidates for promoting worsening of MTLE. Seizures induce dramatic increases in BDNF mRNA and protein expression in both animal models and humans with epilepsy.[16–21] Moreover, activation of TrkB by BDNF produces structural plasticities of the hippocampal dentate granule cells[22] similar to those identified in the epileptic brain.[23–27] In addition, BDNF promotes enhanced efficacy of excitatory synapses connecting principal neurons, a form of long-term potentiation[28–36] that is a type of plasticity identified at synapses of animal models of epilepsy.[37] Brain-derived neurotrophic factor-mediated activation of TrkB can also compromise gamma-aminobutyric acid (GABA)-mediated inhibition.[38]

EVIDENCE IMPLICATING BDNF AND TrkB IN MTLE

Animal Models In Vivo

Because blood-brain barrier-permeable drugs that selectively inhibit the activation of TrkB by BDNF have not been available, elucidating a causal role of BDNF and TrkB signaling in animal models of MTLE has required the development of genetically modified mice. Although mice lacking both *BDNF* alleles in the germline die in the neonatal period, elimination of just one *BDNF* allele results in a striking inhibition of development of kindling.[39] An alternative approach utilized *Trk receptor bodies*, recombinant proteins that bind and thereby scavenge neurotrophin ligands selectively for either TrkB or its relatives, TrkA and TrkC. Intraventricular infusion of TrkB receptor bodies markedly inhibits the development of kindling in adult rats, whereas infusion of TrkA or TrkC receptor bodies has no effect.[40] These findings demonstrate that scavenging BDNF de novo in the adult brain inhibits kindling development, suggesting that the consequences of absent BDNF during development are not sufficient to explain the impaired kindling found by Kokaia et al.[39] Moreover, these findings suggest that the neurotrophin receptor pivotal for seizure progression in this model is TrkB, not TrkA or TrkC. This suggestion was reinforced by the discovery that a conditional deletion of TrkB from subsets of central nervous system (CNS) neurons eliminated all behavioral evidence of seizure-induced progression of epilepsy in the kindling model[41] (Fig. 39–1). This conditional deletion of TrkB is the sole genetic or pharmacological perturbation known to eliminate all vestiges of behavioral evidence of kindling

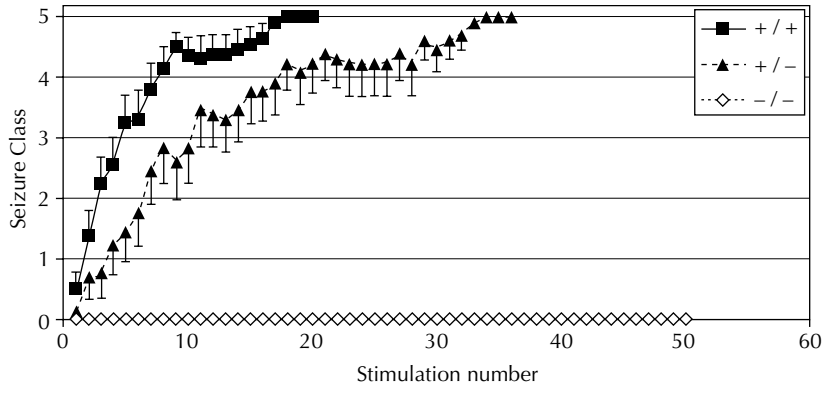

Figure 39–1. Inhibition of kindling by conditional deletion of TrkB. Kindling was compared in three groups of mice: $TrkB^{-/-}$, $TrkB^{-/+}$, and $TrkB^{+/+}$. The seizure score was determined in response to each kindling stimulation using the Racine scale (Stages 1–5). The seizure score increased progressively with kindling stimulation in $TrkB^{+/+}$ mice but was reduced in $TrkB^{-/+}$ mice, and no seizures were elicited in $TrkB^{-/-}$ mice. From ref. 41.

development. Taken together, these findings provide compelling evidence that TrkB is necessary for seizure-induced progression of epilepsy in the kindling model and suggest that BDNF contributes, at least in part, to activation of TrkB in this condition.

In additional studies, the issue of whether increasing expression of BDNF or TrkB is sufficient to induce hyperexcitability and limbic epilepsy has been addressed. Indeed, intrahippocampal infusion of BDNF, or transgenic overexpression of either BDNF or TrkB in transgenic mice, proved sufficient to increase seizure susceptibility or severity or induce seizures outright.[42–45] These findings support the conclusion that enhanced BDNF-mediated activation of TrkB is *sufficient* to induce hyperexcitability and limbic epilepsy. The evidence of both the sufficiency and necessity of BDNF and TrkB signaling in these animal models in vivo raises the question of whether this signaling pathway may be instructive for seizure-induced progression of MTLE. If so, then seizures themselves would be expected to produce enhanced activation of TrkB.

Limbic Seizures Enhance TrkB Activation

The occurrence of enhanced TrkB activation as a consequence of seizures would strengthen the likelihood that signaling activated by TrkB serves an instructive role in seizure-induced progression of MTLE. If seizures induce enhanced activation of TrkB, then elucidating the anatomical locale of this enhancement could pinpoint a specific population of neurons at which to investigate the cellular consequences of the enhanced activation of TrkB. And if seizures induce enhanced activation of TrkB, this would raise the question of the molecular mechanism by which seizures lead to enhanced activation of TrkB in vivo.

How does one measure activation of a receptor tyrosine kinase like TrkB? In contrast to ionotropic receptors for glutamate or GABA, the activation of which can be measured with electrophysiological techniques, a commonly used method for measuring activation of receptor tyrosine kinases utilizes antibodies that recognize phosphorylated tyrosines in the cytoplasmic domain of these proteins. Because activation of TrkB involves phosphorylation of specific tyrosine residues in its cytoplasmic domain,[46] the availability of antibodies that selectively recognize the phosphorylated form of Trk receptors (p-Trk) provides a surrogate measure of TrkB activation in Western blot studies of membrane fractions or in immunohistochemical studies. Immunohistochemical evidence of increased TrkB activation, evident as increased p-Trk immunoreactivity in the mossy fiber pathway of hippocampus, has been shown following induction of seizures by diverse patterns of electrical stimulation and diverse chemoconvulsants in rats and mice.[41,47–49] Interestingly, the biochemical and immunohistochemical evidence of TrkB

activation is evident beginning several hours after the occurrence of seizures and dissipates within 1 week.[47]

Potential Cellular Consequences of TrkB Activation Induced by Seizures

The demonstration that seizure-induced progression of epilepsy is inhibited in the TrkB and BDNF mutant mice, together with evidence of enhanced TrkB activation in diverse seizure models, suggests that the cellular consequences of enhanced activation of TrkB may promote progression of MTLE. The anatomical localization of the increased p-Trk immunoreactivity to the mossy fiber pathway directed our study of potential cellular consequences of TrkB activation to this locale. Both ex vivo and in vivo studies of animal models raise the possibility that long-term potentiation (LTP) of excitatory synapses between principal cells may contribute to limbic epileptogenesis[37]; that is, potentiation of these synapses may facilitate propagation of seizure activity through synaptically coupled neuronal populations throughout the limbic system and beyond. In support of this hypothesis, we showed that development of LTP of the synapses made by mossy fiber axons of the dentate granule cells with CA3 pyramidal cells requires TrkB kinase activity.[50] Further, study of hippocampal slices isolated from animals following induction of seizures in vivo (in the kainic acid model) revealed that the mossy fiber–CA3 synapse had undergone LTP.[51] The requirement for TrkB-dependent signaling for LTP of this synapse, together with immunohistochemical evidence of increased TrkB activation in the mossy fiber pathway in sections ex vivo from these models,[46,47] suggests that activation of TrkB in vivo may contribute to the LTP of this synapse induced by seizures.

The results of these in vitro and ex vivo electrophysiological studies in animal seizure models are consistent with those of earlier studies of hippocampal slices isolated from naive rats; those studies revealed that transient tissue exposure to recombinant BDNF led to potentiation of the mossy fiber–CA3 synapse.[52] Potentiation was long-lasting, continuing long after exposure to BDNF, as shown also for BDNF-induced potentiation in area CA1.[32] The effects of BDNF in area CA3 appeared to be specific to the mossy fiber input, because activation of the recurrent collaterals or input from fibers in the fimbria did not exhibit synaptic potentiation. Furthermore, the intrinsic properties of CA3 neurons did not appear to be affected by this treatment,[53] and fiber volleys were also unaffected by BDNF.[52] Potentiation was Trk-dependent, as shown by its blockade by the antagonist K252a.[52]

Notably, enhanced excitability in models of epilepsy is often accompanied (and likely caused) by both enhanced function of excitatory synapses and impaired function of inhibitory synapses. Might enhanced activation of TrkB also compromise inhibitory function and thereby contribute to the increased excitability seen in limbic epilepsy? One interesting possibility is that enhanced TrkB activation reduces expression of the K-Cl cotransporter, KCC2, resulting in accumulation of $[Cl^-]_i$ and a shift of E_{GABA} in a depolarizing direction.[54] Direct study of human epileptic tissue,[55,56] as well as extensive study of diverse in vivo and in vitro models,[54,57–61] suggests that reduced expression of KCC2 and resulting accumulation of $[Cl^-]_i$ is an important molecular and cellular mechanism contributing to limbic epilepsy. Further, in vitro studies demonstrate that TrkB-mediated activation can suppress KCC2 expression.[54,57] Whether TrkB-mediated activation is responsible for reductions of KCC2 expression described in the kindling and pilocarpine models[57,60] in vivo is unclear.

WHAT IS THE MOLECULAR MECHANISM MEDIATING TrkB ACTIVATION BY SEIZURES IN VIVO?

The localization of the increased p-Trk immunoreactivity to the mossy fiber pathway provided us with the opportunity to examine the molecular mechanism by which seizures induced the increased p-Trk immunoreactivity. The striking seizure-induced increases in BDNF immunoreactivity in the mossy fibers occurred with a time course similar to that of increased p-Trk immunoreactivity, thus supporting the idea that BDNF was responsible for the increase in p-Trk immunoreactivity. Unexpectedly, seizures induced increased p-Trk immunoreactivity in the mossy fiber

pathway in conditional BDNF null mutant mice similar to that seen in wild-type control mice.[41] What mediated the increased TrkB activation in the mossy fiber pathway following seizures in the *BDNF* $^{-/-}$ mice? The increased expression of NT-3 protein in the *BDNF* $^{-/-}$ mice is an interesting possibility, both because of NT-3's ability to activate TrkB[15,62,63] and because of the localization of NT-3 to the dentate granule cells,[64,65] whose mossy fiber axons coincide with the spatial distribution of the activated TrkB. Alternatively, transactivation of TrkB by zinc may contribute. *Transactivation* refers to the process whereby a given receptor and its downstream signaling are activated by a stimulus that does not interact directly with the receptor,[66] a mechanism distinct from activation of TrkB by neurotrophins. The seizure-induced activation of TrkB is localized to the mossy fiber pathway,[67–69] and the mossy fiber axons contain the highest concentration of zinc in mammalian forebrain.[70] Zinc is packaged in synaptic vesicles together with glutamate and is released with glutamate by physiological stimulation.[71] We demonstrated that zinc transactivates TrkB by a neurotrophin-independent and Src-dependent mechanism that is regulated by neuronal activity.[50] One functional consequence of zinc-mediated transactivation of TrkB is LTP of the mossy fiber–CA3 pyramid synapse. While zinc can transactivate TrkB in vitro, whether endogenous zinc contributes to TrkB activation in the hippocampal mossy fiber pathway following seizures in vivo awaits further study.

THE POTENTIAL ROLE OF BDNF IN CATAMENIAL EPILEPSY

The discussion above provides compelling arguments for a role of BDNF and TrkB in limbic epileptogenesis and MTLE. In addition, BDNF and TrkB signaling can potentially answer some questions that have puzzled scientists who study epilepsy, such as the reason a subset of patients appear to become progressively worse. For example, pharmacoresistance may develop, progressive structural deterioration may occur, as shown by neuroimaging, or cognitive side effects may become increasingly severe. Another common question—albeit one that seems very different—is the reason the reproductive endocrine system has such powerful effects on seizures. Below, we discuss this question as it relates to BDNF and suggest that although progression in MTLE and the effects of reproductive steroids on seizures seem very different, both may be explained, at least in part, by the effects of BDNF.

One of the reasons the relationship between reproductive hormones and seizures has remained unclear is that effects of estrogen, progesterone, and testosterone, the major reproductive steroid hormones, are usually inconsistent. The reasons for the lack of consistency are unclear and may be based in biological complexity, or there simply could be an appearance of inconsistency because of differences in experimental procedures across studies.[72]

The clinical observations that suggest a role of reproductive steroids in epilepsy are diverse. They include reports of changes in seizures at times of life when the reproductive status is altered, such as puberty, when epilepsy is often diagnosed for the first time.[73] In pregnancy or at menopause, other times of life when large fluctuations in levels of reproductive steroids occur, there also are changes in seizure severity in women with epilepsy,[74,75] although they are not universal. Other clinical data that support an influence of reproductive hormones on epilepsy are based on studies that have evaluated circulating levels of specific reproductive hormones, such as estrogen and progesterone, and have shown that when these levels fluctuate, seizures often fluctuate in severity.[72,76–79]

Here we address the effects of reproductive steroids on seizures by focusing on seizures during the menstrual cycle, a condition that is easier to study than puberty or menopause because it occurs at a relatively stable time of life (adulthood) and is relatively short in length (28 days). In addition, the menstrual cycle is repetitive, providing scientific advantages because measures can be repeated. However, studies of seizures during the menstrual cycle present some challenges. One problem is the ability to distinguish the effects of hormones like estrogen and progesterone from other changes that occur during the menstrual cycle, such as changes in luteinizing hormone or follicle stimulating hormone. Fluid retention, which often occurs during the perimenstrual period, can have effects on seizures that are independent of the effects of reproductive steroid levels.[80–82] In addition,

there are potential confounds of seizures in studies of the menstrual cycle in women with epilepsy, because there can be acute effects of a seizure on the endocrine system (even a single seizure), such as elevation of prolactin.[82] Effects of repeated seizures also have consequences for the endocrine system. Long-term, chronic seizures appear to have adverse effects on reproductive function, such as irregular or anovulatory cycles and polycystic ovarian syndrome (PCOS).[74,83] These adverse effects have been suggested to be due to AEDs such as valproic acid, but there is evidence that seizures also contribute to the disruption of the menstrual cycle and PCOS.[84,85] A strong argument for a role of chronic seizures in reproductive dysfunction (including irregular cycles and PCOS), instead of a role of AEDs, is that laboratory animals show signs of acyclicity and PCOS after repeated seizures—without ever being treated with AEDs.[85–89] Another challenge to research is that the endocrine systems of women and laboratory rodents are very different. The differences are exemplified by a comparison of the ovarian cycle of women and the laboratory rat. As shown in Fig. 39–2A, the rat cycle (estrous cycle) is only 4 days, in contrast to the 28-day menstrual cycle in women. In addition, there is primarily one surge in estrogen during the estrous cycle in rats that

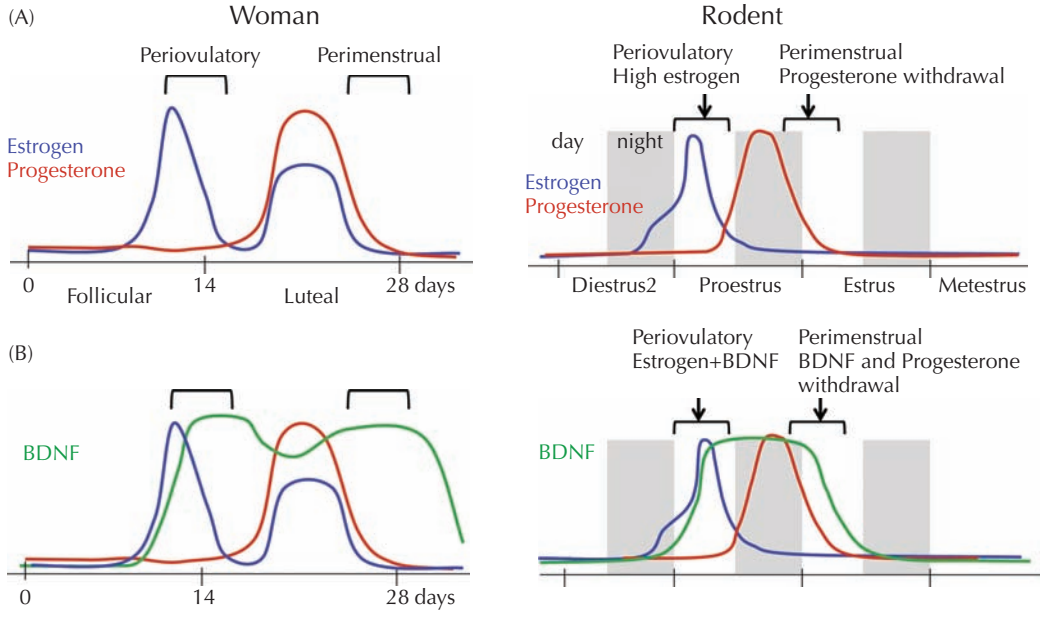

Figure 39–2. Explanations for catamenial epilepsy.

Top: The estrogen/progesterone hypothesis for catamenial epilepsy is shown schematically. The changes in serum estrogen and progesterone levels during the ovarian cycle are diagrammed for the rat (A) and the woman (B). In the rat, there is a 4-day cycle called the *estrous cycle*. In women, the 28-day cycle is divided into a follicular and a luteal phase. In catamenial epilepsy, the periovulatory and perimenstrual periods of the menstrual cycle are typically the times of the menstrual cycle when seizures worsen; the analogous times are marked for the rodent and human cycles.[72] One of the most common hypotheses to explain catamenial epilepsy is based on the idea that estrogen is pro-convulsant and progesterone has the opposite effect.[72,77] In addition, when progesterone levels fall dramatically at the end of the luteal phase, increased excitability has been demonstrated, and this contributes to perimenstrual seizure exacerbation.[72,76,77,78,145,146]

Bottom: An alternative hypothesis for catamenial epilepsy based on the role of BDNF in actions of estrogen and progesterone. A. The rise in hippocampal BDNF during the estrous cycle in the female rat is shown schematically, based on immunocytochemistry,[99] and illustrates a long-lasting elevation in BDNF after the preovulatory surge in estrogen. B. Based on the rodent data, a schematic is shown that illustrates the predicted rise in BDNF levels that would occur in women, that is, a long-lasting rise in BDNF following the preovulatory rise in serum estrogen levels. Because estrogen also rises during the luteal phase, BDNF could remain elevated during the entire luteal phase and the perimenstrual period. This prediction has support from measurements of serum BDNF in women, which is high during the luteal phase relative to the follicular phase.[112] Note that the times when BDNF is elevated and progesterone is low correspond well to the periovulatory and perimenstrual periods, in both rats and women, providing an alternative hypothesis for catamenial epilepsy.

occurs just before ovulation, but in women, there is a preovulatory surge as well as a secondary increase in estrogen between days 14 and 28, the luteal phase (Fig. 39–2).

CATAMENIAL EPILEPSY: DEFINITION AND POTENTIAL MECHANISMS

Catamenial epilepsy refers to seizures that increase in frequency or severity at specific stages of the menstrual cycle[90] and has been known for a long time. One of the first descriptions was published by Gowers in the same monograph that is discussed in the first section of this chapter.[6] In the last few decades, as the effects of hormones on excitability became better documented, there has been a more focused discussion about hormone-sensitive seizures. The ensuing clinical research studies that defined the incidence of catamenial epilepsy have not always been in agreement, with many investigators not completely convinced that catamenial epilepsy is a robust, reliable phenomenon[91] and others reporting, in contrast, a substantial prevalence— approximately one-third of women with epilepsy.[92]

Bäckstrom[93] was one of the first to document seizures in women that varied during the menstrual cycle. Both the seizures and the response to AEDs seemed to fluctuate, and did so mostly at two times of the menstrual cycle: (1) in midcycle, when ovulation occurs, and (2) at the end of the cycle, when menstruation begins. The times when seizures worsened did not coincide exactly with the time of ovulation or the onset of menses, leading to the terms *periovulatory* and *perimenstrual* to describe the two patterns.

Bäckstrom[93] suggested that the fluctuations in seizures were due to changes in serum levels of estrogen and progesterone, because of the large increase in circulating estrogen concentration that occurs just before ovulation, and a dramatic fall in progesterone levels at the end of the menstrual cycle (Fig. 39–2A). Bäckstrom suggested that the relative levels of estrogen and progesterone (the estrogen/progesterone ratio) helped explain the rise and fall in seizures: high ratios explaining seizure exacerbation and low ratios correlating with times when seizures were controlled by AEDs or were less severe/less frequent.

Herzog and colleagues[90] described three patterns of catamenial epilepsy: (1) seizures that worsen during the periovulatory period, (2) seizures that worsen during the perimenstrual period, and (3) seizures that worsen during anovulatory cycles when there is a failure of luteal rise in progesterone. The third seizure pattern helped to describe some women with epilepsy who developed irregular cycles or reproductive dysfunction in the course of epilepsy and experienced more severe or frequent seizures. Herzog and colleagues[90] echoed the suggestion of Bäckstrom that high estrogen levels were responsible for periovulatory seizures, and that the dramatic fall in progesterone late in the menstrual cycle caused perimenstrual seizure exacerbation. Furthermore, anovulatory cycles, associated with exacerbation of seizures, were attributed to low levels of progesterone.[90]

The basis for the idea that estrogen and progesterone levels explain seizure frequency was based on an emerging understanding of estrogen and progesterone actions in the brain at the time. To a large extent, experimental data suggested that estrogen was excitatory, and some investigators showed that it facilitated seizures or was pro-convulsant.[72] In contrast, progesterone appeared to be inhibitory, an effect mediated by its metabolite allopregnanolone (see below). As a result, progesterone or allopregnanolone were considered anti-convulsant. These ideas were attractive as a parsimonious way to explain clinical data and were consistent with neuroendocrinological findings outside of the field of epilepsy. However, the actions of estrogen and progesterone can also be potentially explained by their effects on BDNF.

ALTERNATIVE EXPLANATIONS FOR CATAMENIAL EPILEPSY BASED ON THE INTERPLAY BETWEEN ESTROGEN, PROGESTERONE, AND BDNF

The Role of BDNF in the Actions of Estrogen

It has been known for some time that estrogen targets growth factors, and in early life this interaction is thought to be critical for

appropriate neurodevelopment.[94] However, it was only recently suggested that estrogen may target growth factors in the brain, and specifically the neurotrophins.[94] In 1995, the link between estrogen and BDNF in the brain became better defined when Sohrabji and colleagues identified an estrogen response-like element on the gene for BDNF, and showed that ovariectomy of adult female rats reduced BDNF levels in the brain and estrogen treatment reversed the effect[95] (see also ref. 96). These studies raised the following question: do physiological fluctuations in estrogen also induce BDNF synthesis? In addition, where in the brain would estrogen-induced BDNF synthesis occur? One would think that it would occur wherever BDNF protein synthesis normally occurs, such as in the mossy fiber axons of dentate gyrus granule cells, one of the areas of the brain where BDNF protein expression is most robust.[16,97,98]

To answer these questions, intact female rats were compared at various stages of the estrous cycle. Expression of BDNF protein, evaluated with immunocytochemistry using hippocampal sections, increased in the mossy fiber pathway on proestrous morning, the time when serum estradiol peaks during the estrous cycle.[99] These data supported the hypothesis that periodic increases in circulating estradiol during the normal estrous cycle led to an increase in BDNF protein content in areas of the brain where it normally was synthesized.

Remarkably, the peak of BDNF levels in the mossy fiber pathway was actually not on proestrous morning, when serum concentrations of estradiol are highest, but the next day, long after serum levels of estradiol returned to baseline in the rat.[99] Therefore, BDNF levels were increased with a time course that outlasted the preovulatory surge in serum estradiol concentration. Furthermore, BDNF levels were highest at the time of the estrous cycle that is analogous to the perimenstrual period, when it is most common for seizures to worsen in women with epilepsy.[100]

These data suggested a potential explanation for catamenial seizure exacerbation: elevated BDNF levels increase seizure susceptibility. The locations where BDNF increased supported that idea, because BDNF protein was elevated in areas that are thought to be important in seizure generation, such as hippocampus. In addition, the amygdala showed an increase in BDNF protein by immunocytochemistry (Scharfman et al., unpublished). It is especially interesting that the areas where BDNF was increased contribute to temporal lobe epilepsy (TLE), because catamenial epilepsy appears to be most common in TLE.[100]

The increase in BDNF levels that occurred in the mossy fiber pathway of female rats provided an opportunity to test the hypothesis that increased levels of BDNF exert functional effects that are consistent with a pro-convulsant action.[16,97,98] Therefore, we made recordings in hippocampal slices and compared slices that were prepared from female rats during different estrous cycle stages. We found that the increase in mossy fiber BDNF was correlated with an increase in mossy fiber transmission. The nature of the increase in mossy fiber transmission was interesting because it suggested hyperexcitability. However, this type of hyperexcitability was not simulated by perfusion of slices with convulsants, such as $GABA_A$ receptor antagonists, so it seemed to be a novel form of hyperexcitability.[53] Most convulsants, such as the $GABA_A$ receptor antagonist bicuculline, induce area CA3 population discharges that occur spontaneously and rhythmically. In contrast, female rats with elevated mossy fiber BDNF protein did not demonstrate spontaneous burst discharges in area CA3 (or any other area of the slice). Instead, hyperexcitability was manifested in a different way. Stimulation of the mossy fibers at 1 Hz using pairs of stimuli (40 ms interstimulus interval at 50% maximum stimulus strength) produced multiple population spikes (after each stimulus), with only three to six pairs of stimuli required.[52,53,99] The population spikes that followed each stimulus occurred in trains with approximately 10–20 ms between each spike, and the entire train did not last more than 100 ms. Therefore, the hyperexcitability was different from the synchronized population bursts that occur in area CA3 after bicuculline exposure.[53] Spreading depression often followed the evoked trains of population spikes that were elicited by 1 Hz stimulation of the mossy fibers.[52,53,99] These data were interesting because both the pattern of population spike trains after stimulation and the spreading depression episodes were also observed after exposure of male rat slices to recombinant BDNF.[52] However, the female rat slices had no exposure to recombinant BDNF. All of the effects that were recorded in slices from

female rats at cycle stages with high mossy fiber BDNF protein expression were similar to the effects that had been previously described in slices from male rats exposed to recombinant BDNF.[52,99] Importantly, the effects that were evident in slices from female and male rats were blocked by K252a, a Trk receptor antagonist, consistent with the interpretation that BDNF was responsible.[99]

These data suggested that during every estrous cycle, the rise in serum levels of estradiol triggers BDNF synthesis in pathways like the mossy fibers, and the net effect is increased susceptibility to epileptiform activity. Therefore, it seemed plausible that actions of estrogen that increased seizure susceptibility in catamenial epilepsy are mediated by BDNF. Actions of estrogen could explain both periovulatory seizure exacerbation and the increase in seizures during the perimenstrual period because BDNF protein is elevated in the rat at the analogous times of the estrous cycle, that is, proestrous morning and estrous morning. Furthermore, Trk-dependent epileptiform activity was evoked by mossy fiber stimulation on both proestrous and estrous mornings. The experiments in female rats also suggested a potential reason why perimenstrual seizures might be more common in women than periovulatory seizures: BDNF levels were highest in the female rat at the time that is analogous to the perimenstrual period, estrous morning, and stimulus-evoked epileptiform activity in the female rat was most severe at that time.[99]

Many neuromodulators are altered on proestrous morning in the female rat, not only BDNF. For example, there are changes in norepinephrine and corticosterone levels.[101,102] Therefore, it was important to use another approach so that we could dissociate the effects of estrogen from the potential effects of norepinephrine and corticosterone. Therefore, ovariectomized rats were treated with a series of injections that simulates the rise in 17β-estradiol (estradiol) during the estrous cycle.[103] These experiments demonstrated that estradiol treatment per se could reproduce the effects observed in the intact rat, and supported the hypothesis that estradiol was responsible for the increase in mossy fiber BDNF and increased mossy fiber transmission in the intact rat. Together these experiments suggest new mechanisms that mediate the effects of estrogen in catamenial epilepsy. Estrogen may increase seizure susceptibility indirectly by inducing BDNF synthesis. In turn, BDNF exerts effects that facilitate seizures.

It is important to note that the effects of estrogen on BDNF may not be mediated by the estrogen-response element on the BDNF gene. It has been suggested that estrogen acts on GABAergic neurons to cause disinhibition.[104–107] Therefore, BDNF levels may increase because of activity-dependent expression.[107]

The reason BDNF levels remain high long after estrogen levels return to normal during the estrous cycle is unclear. One contributing factor to the long-lasting nature of increased BDNF expression may be effects of progesterone, which rises immediately after estrogen during the estrous cycle (Fig. 39–2A) and therefore is increased at a time that is well suited for a modulatory action. Although progesterone is commonly thought to counteract estrogen action, the actions of progesterone during the estrous cycle can facilitate estrogen action, at least with respect to reproductive behavior.[72,108] The experiments that have addressed the effects of progesterone on BDNF, however, have produced variable results. One study showed that BDNF was increased by progesterone acting at progesterone receptors.[109] Another study showed that ovariectomized animals treated with estrogen exhibited increased BDNF expression, and a subsequent injection of progesterone inhibited that effect.[110] Other studies have found little effect of progesterone on BDNF.[111]

Importantly, evaluation of BDNF levels in women suggests that cycle-dependent changes in serum BDNF in women are similar to changes in BDNF levels in hippocampus of female rats. Thus, analysis of serum BDNF in normal women showed that BDNF levels were high at ovulation and during the luteal phase relative to the follicular phase.[112] Although BDNF levels in brain were not studied, the data from serum samples are consistent with the idea that the ovulatory and luteal rise in estrogen in women is associated with an increase in BDNF levels in the brain.

In light of the data from the female rat, one explanation for catamenial epilepsy would be that estrogen and progesterone modulate BDNF synthesis, with estrogen and progesterone potentially increasing BDNF synthesis and allopregnanolone keeping any pro-convulsant

effects in check. This hypothesis would explain the three types of catamenial epilepsy suggested by Herzog and colleagues, as shown in Fig. 39–2B. During the periovulatory period, pro-convulsant effects of BDNF would emerge because of an increase in BDNF synthesis induced by the preovulatory surge in estrogen. During the luteal phase, high levels of allopregnanolone would be likely to keep the effects of elevated BDNF, that is, an increased predisposition for seizures, in check. However, during the perimenstrual period when progesterone levels fall, seizure exacerbation would be predicted because BDNF levels would still be high, but the effects of BDNF would no longer be counterbalanced by enhanced inhibition (Fig. 39–2). This view suggests that estrogen and progesterone are modulators of BDNF and do not cause the fluctuation in seizures during the menstrual cycle by direct effects.

A key component of this hypothesis is the argument that BDNF has effects that facilitate seizures and can explain many of the actions of estrogen. There is a great deal of data to support this idea. Effects of estrogen are mainly inferred from studies of the major bioactive form of estrogen, 17β-estradiol (estradiol), which is produced in the periphery (primarily in the ovaries) and also can be produced centrally from cholesterol metabolism. The effects of estradiol are extremely diverse both inside and outside the CNS, but they appear to be mediated entirely by three receptors: a membrane-bound receptor (mER) and two types of receptors that can migrate to the nucleus (nuclear receptors; ERα and ERβ) and act on target genes.

During the 1960s and 1970s, investigators showed that estradiol increases excitatory (glutamatergic) transmission, and some of the data suggested that the facilitation of glutamatergic transmission was strong enough to lead to seizures.[113,114] One of the first studies that supported this view used a bolus of estradiol applied directly to the cortex in anesthetized cats and found that the cortical electroencephalographic (EEG) activity increased, similar to a seizure.[113] Since that time, estradiol has been shown to produce many effects in the brain. Almost all of them are consistent with a net excitatory effect. For example, estradiol increases glutamatergic transmission,[115] facilitates LTP,[115,116] and induces dendritic spine growth.[117] The increase in glutamatergic transmission and LTP is potentially mediated by several mechanisms, including actions that are presynaptic as well as postsynaptic.[118,119]

Interestingly, many of the effects of estradiol can also be induced by BDNF.[108,120] For example, exposure of hippocampal slices to recombinant BDNF potentiates glutamatergic transmission,[32,52,53] and BDNF is required for LTP in hippocampus.[33,35,50,121] In addition, BDNF has potent effects on *N*-methyl-D-aspartate (NMDA) receptors and dendritic spine morphology, like those of estradiol, although the effects on spines may not be identical to those that have been reported for estradiol.[122] Estradiol also increases the rate of postnatal neurogenesis,[123] which is an effect of BDNF as well.[124] Estradiol decreases GABAergic transmission by decreasing levels of the synthetic enzyme for GABA, glutamic acid decarboxylase (GAD),[125] and via other mechanisms[104,126]; BDNF can also reduce GABAergic inhibition, although a specific action on GAD has not been identified.[38,127,128] Studies of BDNF in vivo in the context of seizures and epilepsy demonstrated that it is potentially pro-convulsant (as discussed above). Therefore, the actions of estrogen could be mediated, at least in part, by BDNF.

The time required for estradiol to exert its effects—relative to the time required for BDNF-dependent effects—is important to consider if one is to argue that BDNF contributes to the effects of estrogen during the menstrual cycle. Although the exact timing of estrogen and BDNF actions during the ovarian cycle are not clear in rodents or in women, from what is known, it is possible that BDNF mediates actions of estrogen. Thus, even the "rapid" effects of estrogen in rodents during the periovulatory period that are detected 12–18 h after the start of the preovulatory surge in estrogen can be explained by estrogen-induced BDNF synthesis because 12–18 h is long enough for induction of protein synthesis.

Importantly, the effects of estradiol do not seem to be universally "convulsant" (for review, see ref. 72). One explanation is that estrogen has a pro-convulsant effect because of its induction of BDNF synthesis, and estrogen also exerts an effect that is indirectly anti-convulsant because it leads to an increase in levels of neuropeptide Y (NPY), which has effects that are usually anti-convulsant.[129,130] The effect of estrogen on NPY levels could be mediated by BDNF, because

BDNF induces NPY synthesis following TrkB activation.[131–134] Therefore, some of the reasons estrogen is convulsant could be due to its effects on BDNF synthesis, and some of the reasons it is not convulsant could also be due to BDNF-mediated effects (see also ref. 135).

The actions of estrogen that are protective are also relevant to a discussion of epilepsy, particularly TLE, because limbic pathology (e.g., neuronal loss) is a characteristic of TLE that appears to be influenced by estradiol. However, effects of estradiol on neuronal damage after experimental seizures seem to vary from study to study.[72,108,120] Interestingly, the same is true for BDNF. Brain-derived neurotrophic factor can exacerbate toxicity to convulsants such as kainic acid in culture,[136] similar to estradiol, but it also appears to protect neurons against excitotoxicity in other experiments, which is also similar to the results of some studies on the effects of estradiol.[137] One explanation that has been used to explain the varied effects of estradiol is that high doses or chronic treatment can downregulate estrogen receptors (ERs)[138–140] (see also ref. 141). The same explanation has been used to explain the varied effects of BDNF: high doses decrease TrkB receptors at the plasma membrane.[45]

The Role of BDNF in the Actions of Progesterone

If the effects of estrogen on seizures can potentially be explained, at least in part, by BDNF, is the same true for progesterone? Many would think not, because the effects of progesterone on seizures that many investigators consider to be most robust are mediated by allopregnanolone, which binds directly to the $GABA_A$ receptor. However, the effects of BDNF may still play a role here, because BDNF can alter several aspects of GABAergic transmission.

Effects of progesterone are mediated by membrane and nuclear receptors analogous to estrogen. Whereas actions of estrogens can be mediated by different forms (estrone, estriol, estradiol), the effects of progesterone are due to progesterone itself, as well as its metabolites. The effects of progesterone at progesterone receptors (PRs) have effects that alter excitability,[142,143] but most is known about the metabolite allopregnanolone. Allopregnanolone is an allosteric modulator of the $GABA_A$ receptor; when allopregnanolone binds, the effects of GABA at $GABA_A$ receptors are potentiated.[144,145] Unlike effects of estradiol and BDNF, which seem to be very sensitive to experimental conditions, the anticonvulsant effects of allopregnanolone are very consistent from one experimental paradigm to the next.[76]

The available clinical data support the view that allopregnanolone controls seizures during the luteal phase of the menstrual cycle in women because allopregnanolone levels rise at that time. In addition, increased seizure severity during the periovulatory, perimenstrual, and anovulatory cycles can be explained by low levels of allopregnanolone. Therefore, progesterone, or other drugs that simulate neurosteroids acting at $GABA_A$ receptors, such as ganaloxone, have been suggested as AEDs.[146]

Although fluctuations in allopregnanolone levels can provide an explanation for the three types of catamenial epilepsy, there may be an influence of BDNF on the effects of allopregnanolone that is important to consider. For example, the $\alpha 2$, $\beta 2/3$, and $\gamma 2$ subunits of $GABA_A$ receptors are downregulated when neurons are exposed to BDNF in culture, which increases excitability.[147] However, cell surface expression of δ subunits increase in response to BDNF,[149] which should enhance the effects of allopregnanolone, not diminish them, because δ subunits typically facilitate for actions of allopregnanolone at $GABA_A$ receptors.[149] The effects of BDNF on $GABA_A$ receptors are complex because it appears that BDNF not only modulates receptor subunit expression, but also influences GABAergic transmission by increasing GABAergic synapse density[150] and modulating GABA release[151] and KCC2 expression.[152]

Perimenstrual seizure exacerbation has been explained not only by low levels of allopregnanolone, but also by the rapid reduction in progesterone levels at the end of the ovarian cycle.[146] This condition of *progesterone withdrawal* is associated with seizure susceptibility, which has been explained by an increase in $\alpha 4$ subunits of $GABA_A$ receptors.[153] Brain-derived neurotrophic factor has been shown to increase $\alpha 4$ expression by activating the transcription factor Egr3.[154] Therefore, the elevation of BDNF during the luteal phase of the menstrual cycle could lead to $\alpha 4$ upregulation by the end of the luteal phase, explaining the increase in

seizure susceptibility during the perimenstrual period. In addition, BDNF may contribute to progesterone "withdrawal" in other ways. For example, falling levels of allopregnanolone at the end of the menstrual cycle would be likely to cause disinhibition, which could lead to an increase in BDNF levels because BDNF synthesis is activity-dependent. An increase in BDNF protein would increase excitability by the facilitatory effects of BDNF on synaptic transmission.

Brain-derived neurotrophic factor may have other effects that increase excitability by actions on $GABA_A$ receptor subunits besides δ or α4 subunits. For example, it has been shown that upregulation of BDNF decreases α1 subunits.[155] This is important because of the evidence, in an animal model of epilepsy, that increasing α1 receptors reduces seizures.[156]

SUMMARY

Actions of estrogen and progesterone have been used to explain the changes in seizure frequency or severity in women with catamenial epilepsy. An alternative hypothesis is that BDNF is responsible, because estrogen causes an increase in BDNF expression that can have both acute effects on excitability and delayed, indirect effects by changing GABAergic transmission and $GABA_A$ receptors. Allopregnanolone may hold many of the excitatory effects in check during the luteal phase, but two phases of the cycle may be unprotected, the periovulatory and perimenstrual phases. This hypothesis helps explain why progesterone therapy may not always be efficacious in catamenial epilepsy and suggests that control of BDNF would be a logical complementary strategy.

CONCLUDING REMARKS

The evidence from diverse methods, and diverse animal models, strongly suggests that BDNF contributes to epilepsy, and does so in many ways. In acute seizures, BDNF is likely to play a role because of its rapid actions at many excitatory synapses and possibly at inhibitory synapses. In epileptogenesis, BDNF appears to be part of a cascade that initiates—and then perpetuates—the process of epileptogenesis. The activity dependence of BDNF and its specific expression in parts of the brain that regulate seizure susceptibility are two of many specific characteristics of BDNF and TrkB that are likely to make BDNF so influential in epileptogenesis. However, the role of BDNF is not only important in acute seizures and epileptogenesis, but is also likely to be important in chronic epilepsy, where factors that trigger seizures, such as fluctuations in gonadal hormones, may do so because, at least in part, they increase BDNF synthesis. In light of the multitude of pro-convulsant effects of BDNF, the molecular mechanisms that control BDNF synthesis and molecular mechanisms of TrkB signaling are attractive targets for anti-convulsant and anti-epileptogenic drug development.

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REFERENCES

1. Cascino GD. Temporal lobe epilepsy is a progressive neurologic disorder. *Neurology*. 2009;72:1718–1719.
2. Pitkänen A, Sutula TP. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal lobe epilepsy. *Lancet Neurol*. 2002;1: 173–181.
3. Berg AT, Vickrey BG, Testa FM, Levy SR, Shinnar S, DiMario F, Smith S. How long does it take for epilepsy to become intractable? A prospective investigation. *Ann Neurol.* 2006;60:73–79.
4. Coan AC, Appenzeller S, Bonilha L, Li LM, Cendes F. Seizure frequency and lateralization affect progression of atrophy in temporal lobe epilepsy. *Neurology.* 2009;73:834–842.
5. Bernhardt BC, Worsley KJ, Kim H, Evans AC, Betnasconi A, Bernasconi N. Longitudinal and cross-sectional analysis of atrophy in pharmacoresistant temporal lobe epilepsy. *Neurology.* 2009;72: 1747–1754.
6. Gowers WR. *Epilepsy and Other Chronic Convulsive Diseases*. London: J.A. Churchill; 1881.
7. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol.* 1969;25:295–330.
8. Pinel JP, Rovner LI. Experimental epileptogenesis: kindling-induced epilepsy in rats. *Exp Neurol.* 1978;58: 190–202.
9. Sayin U, Osting S, Hagen J, Rutecki P, Sutula T. Spontaneous seizures and loss of axo-axonic and

axo-somatic inhibition induced by repeated brief seizures in kindled rats. *J Neurosci*. 2003;23:2759–2768.
10. Kotloski R, Lynch M, Lauersdorf S, Sutula T. Repeated brief seizures induce progressive hippocampal neuron loss and memory deficits. *Prog Brain Res*. 2002;135: 95–110.
11. Williams PA, White AM, Clark S, Ferraro DJ, Swiercz W, Staley KJ, Dudek FE. Development of spontaneous recurrent seizures after kainate-induced status epilepticus. J Neurosci. 2009;29:2103–2112.
12. Noe F, Pool AH, Nissinen J, Gobbi M, Bland R, Rizzi M, Balducci C, Ferraguti F, Sperk G, During MJ, Pitkanen A, Vezzani A. Neuropeptide Y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. *Brain.* 2008;131: 1506–1515.
13. Kadam SD, White AM, Staley KJ, Dudek FE. Continuous electroencephalographic monitoring with radio-telemetry in a rat model of perinatal hypoxia-ischemia reveals progressive post-stroke epilepsy. *J Neurosci*. 2010;30:404–415.
14. Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. *EMBO J*. 1982;1:549–553.
15. Klein R, Nanduri V, Jing SA, Lamballe F, Tapley P, Bryant S, Cordon-Cardo C, Jones KR, Reichardt LF, Barbacid M. The TrkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell*. 1991;66:395–403.
16. Yan Q, Rosenfeld RD, Matheson CR, Hawkins N, Lopez OT, Bennett L, Welcher AA. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. *Neuroscience.* 1997;78: 431–448.
17. Wetmore C, Olson L, Bean AJ. Regulation of brain-derived neurotrophic factor (BDNF) expression and release from hippocampal neurons is mediated by non-NMDA type glutamate receptors. *J Neurosci*. 1994;14:1688–1700.
18. Gall CM, Isackson PJ. Limbic seizures increase neuronal production of messenger RNA for nerve growth factor. *Science.* 1989;245:758–761.
19. Mathern GW, Babb TL, Micevych PE, Blanco CE, Pretorius JK. Granule cell mRNA levels for BDNF, NGF, and NT-3 correlate with neuron losses or supragranular mossy fiber sprouting in the chronically damaged and epileptic human hippocampus. *Mol Chem Neuropathol*. 1997;30:53–76.
20. Murray KD, Isackson PJ, Eskin TA, King MA, Montesinos SP, Abraham LA, Roper SN. Altered mRNA expression for brain-derived neurotrophic factor and type II calcium/calmodulin-dependent protein kinase in the hippocampus of patients with intractable temporal lobe epilepsy. *J Comp Neurol*. 2000;418: 411–422.
21. Takahashi M, Hayashi S, Kakita A, Wakabayashi K, Fukuda M, Kameyama S, Tanaka R, Takahashi H, Nawa H. Patients with temporal lobe epilepsy show an increase in brain-derived neurotrophic factor protein and its correlation with neuropeptide Y. *Brain Res.* 1999;818:579–582.
22. Danzer SC, Crooks KR, Lo DC, McNamara JO. Increased expression of brain-derived neurotrophic factor induces formation of basal dendrites and axonal branching in dentate granule cells in hippocampal explant cultures. *J Neurosci*. 2002;22:9754–9763.
23. Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol*. 1989;26:321–330.
24. Represa A, Robain O, Tremblay E, Ben-Ari Y. Hippocampal plasticity in childhood epilepsy. *Neurosci Lett.* 1989;99:351–355.
25. Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF. Synaptic reorganization by mossy fibers in human epileptic fascia dentata. *Neuroscience.* 1991;42:351–363.
26. Houser CR. Morphological changes in the dentate gyrus in human temporal lobe epilepsy. *Epilepsy Res Suppl*. 1992;7:223–234.
27. Franck JE, Pokorny J, Kunkel DD, Schwartzkroin PA. Physiologic and morphologic characteristics of granule cell circuitry in human epileptic hippocampus. *Epilepsia.* 1995;36:543–558.
28. McAllister AK. Subplate neurons: a missing link among neurotrophins, activity, and ocular dominance plasticity? *Proc Natl Acad Sci USA*. 1999;96: 13600–13602.
29. Xu B, Gottschalk W, Chow A, Wilson RI, Schnell E, Zang K, Wang D, Nicoll RA, Lu B, Reichardt LF. The role of brain-derived neurotrophic factor receptors in the mature hippocampus: modulation of long-term potentiation through a presynaptic mechanism involving TrkB. *J Neurosci*. 2000;20:6888–6897.
30. Muller D, Djebbara-Hannas Z, Jourdain P, Vutskits L, Durbec P, Rougon G, Kiss JZ. Brain-derived neurotrophic factor restores long-term potentiation in polysialic acid–neural cell adhesion molecule-deficient hippocampus. *Proc Natl Acad Sci USA.* 2000;97: 4315–4320.
31. Minichiello L, Korte M, Wolfer D, Kuhn R, Unsicker K, Cestari V, Rossi-Arnaud C, Lipp HP, Bonhoeffer T, Klein R. Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron.* 1999;24: 401–414.
32. Kang H, Schuman EM. Long-lasting neurotrophin enhancement of synaptic transmission in the adult hippocampus. *Science*. 1995;267:1658–1662.
33. Korte M, Kang H, Bonhoeffer T, Schuman E. A role for BDNF in the late-phase of hippocampal long-term potentiation. *Neuropharmacology*. 1998;37:553–559.
34. Schinder AF, Poo M. The neurotrophin hypothesis for synaptic plasticity. *Trends Neurosci*. 2000;23:639–645.
35. Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron.* 1996;16:1137–1145.
36. Minichiello L, Calella AM, Medina DL, Bonhoeffer T, Klein R, Korte M. Mechanism of TrkB-mediated hippocampal long-term potentiation. *Neuron*. 2002;36: 121–137.
37. Sutula T, Steward O. Facilitation of kindling by prior induction of long-term potentiation in the perforant path. *Brain Res*. 1987;420:109–117.
38. Tanaka T, Saito H, Matsuki N. Inhibition of $GABA_A$ synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. *J Neurosci*. 1997; 2959–2966.
39. Kokaia M, Ernfors P, Kokaia Z, Elmer E, Jaenisch R, Lindvall O. Suppressed epileptogenesis in BDNF mutant mice. *Exp Neurol*. 1995;133:215–224.
40. Binder DK, Routbort MJ, Ryan TE, Yancopoulos GD, McNamara JO. Selective inhibition of kindling

development by intraventricular administration of TrkB receptor body. *J Neurosci.* 1999;19:1424–1436.
41. He XP, Kotloski R, Nef S, Luikart BW, Parada LF, McNamara JO. Conditional deletion of TrkB but not BDNF prevents epileptogenesis in the kindling model. *Neuron.* 2004;43:31–42.
42. Lahteinen S, Pitkanen A, Koponen E, Saarelainen T, Castren E. Exacerbated status epilepticus and acute cell loss, but no changes in epileptogenesis, in mice with increased brain-derived neurotrophic factor signaling. *Neuroscience*. 2003;122:1081–1092.
43. Croll SD, Suri C, Compton DL, Simmons MV, Yancopoulos GD, Lindsay RM, Wiegand SJ, Rudge JS, Scharfman HE. Brain-derived neurotrophic factor transgenic mice exhibit passive avoidance deficits, increased seizure severity and in vitro hyperexcitability in the hippocampus and entorhinal cortex. *Neuroscience*. 1999;93:1491–1506.
44. Scharfman HE, Goodman JH, Sollas AL, Croll SD. Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. *Exp Neurol*. 2002;174:201–214.
45. Xu B, Michalski B, Racine RJ, Fahnestock M. The effects of brain-derived neurotrophic factor (BDNF) administration on kindling induction, Trk expression and seizure-related morphological changes. *Neuroscience.* 2004;126:521–531.
46. Segal RA, Bhattacharyya A, Rua LA, Alberta JA, Stephens RM, Kaplan DR, Stiles CD. Differential utilization of Trk autophosphorylation sites. *J Biol Chem*. 1996;271:20175–20181.
47. Binder DK, Routbort MJ, McNamara JO. Immunohistochemical evidence of seizure-induced activation of Trk receptors in the mossy fiber pathway of adult rat hippocampus. *J Neurosci*. 1999;19: 4616–4626.
48. He XP, Minichiello L, Klein R, McNamara JO. Immunohistochemical evidence of seizure-induced activation of TrkB receptors in the mossy fiber pathway of adult mouse hippocampus. *J Neurosci*. 2002;22:7502–7508.
49. He XP, Pan E, Sciarretta C, Minichiello L, McNamara JO. Disruption of TrkB-mediated phospholipase Cg signaling inhibits limbic epileptogenesis. *J Neurosci.* 30:6188–6196.
50. Huang YZ, Pan E, Xiong ZQ, McNamara JO. Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. *Neuron*. 2008;57:546–548.
51. Goussakov IV, Fink K, Elger CE, Beck H. Metaplasticity of mossy fiber synaptic transmission involves altered release probability. *J Neurosci*. 2000;20:3434–3441.
52. Scharfman HE. Hyperexcitability in combined entorhinal/hippocampal slices of adult rat after exposure to brain-derived neurotrophic factor. *J Neurophysiol*. 1997;78:1082–1095.
53. Scharfman HE. BDNF and the dentate gyrus mossy fibers: implications for epilepsy. In: Stanton PK, Bramham CR, Scharfman HE, eds. *Synaptic Plasticity and Transsynaptic Signaling.* New York: Springer; 2005:201–220.
54. Rivera C, Voipio J, Thomas-Crusells J, Li H, Emri Z, Sipila S, Payne JA, Minichiello L, Saarma M, Kaila K. Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. *J Neurosci*. 2004;24:4683–4691.
55. Huberfeld G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, Rivera C. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. *J Neurosci.* 2007;27:9866–9873.
56. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science.* 2002;298:1418–1421.
57. Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, Kokaia Z, Airaksinen MS, Voipio J, Kaila K, Saarma M. BDNF-induced TrkB activation down-regulates the K^+-Cl^- cotransporter KCC2 and impairs neuronal Cl^- extrusion. *J Cell Biol*. 2002;159:747–752.
58. Blaesse P, Airaksinen MS, Rivera C, Kaila K. Cation-chloride cotransporters and neuronal function. *Neuron.* 2009;61:820–838.
59. Woo NS, Lu J, England R, McClellan R, Dufour S, Mount DB, Deutch AY, Lovinger DM, Delpire E. Hyperexcitability and epilepsy associated with disruption of the mouse neuronal-specific K-Cl cotransporter gene. *Hippocampus*. 2002;12:258–268.
60. Li X, Zhou J, Chen Z, Chen S, Zhu F, Zhou L. Long-term expressional changes of Na^+-K^+-Cl^- co-transporter 1 (NKCC1) and K^+-Cl^- co-transporter 2 (KCC2) in CA1 region of hippocampus following lithium-pilocarpine induced status epilepticus (PISE). *Brain Res*. 2008;1221:141–146.
61. Pathak HR, Weissinger F, Terunuma M, Carlson GC, Hsu FC, Moss SJ, Coulter DA. Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. *J Neurosci*. 2007;27:14012–14022.
62. Soppet D, Escandon E, Maragos J, Middlemas DS, Reid SW, Blair J, Burton LE, Stanton BR, Kaplan DR, Hunter T. The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the TrkB tyrosine kinase receptor. *Cell*. 1991;65: 895–903.
63. Farinas I, Wilkinson GA, Backus C, Reichardt LF, Patapoutian A. Characterization of neurotrophin and trk receptor functions in developing sensory ganglia: direct NT-3 activation of TrkB neurons in vivo. *Neuron*. 1998;21:325–334.
64. Ernfors P, Wetmore C, Olson L, Persson H. Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. *Neuron.* 1990;5:511–526.
65. Maisonpierre PC, Belluscio L, Squinto S, Ip NY, Furth ME, Lindsay RM, Yancopoulos GD. Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science*. 1990;247:1446–1451.
66. Carpenter G. Employment of the epidermal growth factor receptor in growth factor-independent signaling pathways. *J Cell Biol*. 1999;146:697–702.
67. Binder DK, Routbort MJ, McNamara JO. Immunohistochemical evidence of seizure-induced activation of trk receptors in the mossy fiber pathway of adult rat hippocampus. *J Neurosci.* 1999;19: 4616–4626.
68. He XP, Minichiello L, Klein R, McNamara JO. Immunohistochemical evidence of seizure-induced activation of TrkB receptors in the mossy fiber pathway of adult mouse hippocampus. *J Neurosci*. 2002;22: 7502–7508.
69. He XP, Kotloski R, Nef S, Luikart BW, Parada LF, McNamara JO. Conditional deletion of TrkB but not

BDNF prevents epileptogenesis in the kindling model. *Neuron*. 2004;43:31–42.
70. Frederickson CJ, Danscher G. Zinc-containing neurons in hippocampus and related CNS structures. *Prog Brain Res*. 1990;83:71–84.
71. Frederickson CJ, Koh JY, Bush AI. The neurobiology of zinc in health and disease. *Nat Rev Neurosci*. 2005;6:449–462.
72. Scharfman HE, MacLusky NJ. The influence of gonadal hormones on neuronal excitability, seizures and epilepsy in the female. *Epilepsia*. 2006;47:1423–1440.
73. Wheless JW, Kim HL. Adolescent seizures and epilepsy syndromes. *Epilepsia*. 2002;43(suppl 3):33–52.
74. Harden CL. Issues for mature women with epilepsy. *Int Rev Neurobiol*. 2008;83:385–395.
75. Harden CL, Pulver MC, Ravdin L, Jacobs AR. The effect of menopause and perimenopause on the course of epilepsy. *Epilepsia*. 1999;40:1402–1407.
76. Reddy DS. Role of neurosteroids in catamenial epilepsy. *Epilepsy Res*. 2004;62:99–118.
77. Rogawski MA. Progesterone, neurosteroids, and the hormonal basis of catamenial epilepsy. *Ann Neurol*. 2003;53:288–291.
78. Maguire J, Mody I. Steroid hormone fluctuations and $GABA_AR$ plasticity. *Psychoneuroendocrinology*. 2009;34(suppl 1):S84–S90.
79. Velisek L, Veliskova J. New avenue of research: antiepileptic drug and estradiol neuroprotection in epilepsy. *Recent Pat CNS Drug Discov*. 2008;3:128–137.
80. Somjen GG. Ion regulation in the brain: implications for pathophysiology. *Neuroscientist*. 2002;8:254–267.
81. Schwartzkroin PA, Baraban SC, Hochman DW. Osmolarity, ionic flux, and changes in brain excitability. *Epilepsy Res*. 1998;32:275–285.
82. Chen DK, So YT, Fisher RS. Use of serum prolactin in diagnosing epileptic seizures: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology*. 2005;65:668–675.
83. Herzog AG. Disorders of reproduction in patients with epilepsy: primary neurological mechanisms. *Seizure*. 2008;17:101–110.
84. TaubŁll E, Isojärvi JI, Harbo HF, Pakarinen AJ, Gjerstad L. Long-term valproate treatment induces changes in ovarian morphology and serum sex steroid hormone levels in female Wistar rats. *Seizure*. 1999;8:490–493.
85. Scharfman HE, Kim M, Hintz TM, MacLusky NJ. Seizures and reproductive function: insights from female rats with epilepsy. *Ann Neurol*. 2008;64:687–697.
86. Edwards HE, Burnham WM, Ng MM, Asa S, MacLusky NJ. Limbic seizures alter reproductive function in the female rat. *Epilepsia*. 1999;40: 1370–1377.
87. Amado D, Cavalheiro EA. Hormonal and gestational parameters in female rats submitted to the pilocarpine model of epilepsy. *Epilepsy Res*. 1998;32:266–274.
88. Amado D, Cavalheiro EA, Bentivoglio M. Epilepsy and hormonal regulation: the patterns of GnRH and galanin immunoreactivity in the hypothalamus of epileptic female rats. *Epilepsy Res*. 1993;14:149–159.
89. Amado D, Verreschi IT, Berzaghi MP, Cavalheiro EA. Effects of intrahippocampal injection of kainic acid on estrous cycle in rats. *Braz J Med Biol Res*. 1987;20: 829–832.
90. Herzog AG, Klein P, Ransil BJ. Three patterns of catamenial epilepsy. *Epilepsia*. 1997;38:1082–1088.
91. French JA. Catamenial epilepsy: the elusive condition. *Epilepsy Curr*. 2005;5:113–114.
92. Herzog AG. Catamenial epilepsy: definition, prevalence pathophysiology and treatment. *Seizure*. 2008;17:151–159.
93. Bäckstrom T. Epilepsy in women. Oestrogen and progesterone plasma levels. *Experientia*. 1976;32: 248–249.
94. Toran-Allerand CD, Singh M, Setalo G. Novel mechanisms of estrogen action in the brain: new players in an old story. *Front Neuroendocrinol*. 1999;20:97–121.
95. Sohrabji F, Miranda RCG, Toran-Allerand CD. Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor. *Proc Natl Acad Sci USA*. 1995;92:11110–11114.
96. Singh M, Meyer EM, Simpkins JW. The effect of ovariectomy and estradiol replacement on brain-derived neurotrophic factor messenger ribonucleic acid expression in cortical and hippocampal brain regions of female Sprague-Dawley rats. *Endocrinology*. 1995;136:2320–2324.
97. Danzer SC, McNamara JO. Localization of brain-derived neurotrophic factor to distinct terminals of mossy fiber axons implies regulation of both excitation and feedforward inhibition of CA3 pyramidal cells. *J Neurosci*. 2004;24:11346–11355.
98. Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S. Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *J Neurosci*. 1997;17:2295–2313.
99. Scharfman HE, Mercurio TC, Goodman JH, Wilson MA, MacLusky NJ. Hippocampal excitability increases during the estrous cycle in the rat: a potential role for brain-derived neurotrophic factor. *J Neurosci*. 2003;23:11641–11652.
100. Quigg M, Smithson SD, Fowler KM, Sursal T, Herzog AG. Laterality and location influence catamenial seizure expression in women with partial epilepsy. *Neurology*. 2009;73:223–227.
101. Luine VN. Serotonin, catecholamines and metabolites in discrete brain areas in relation to lordotic responding on proestrus. *Neuroendocrinology* . 1993;57:946–954.
102. Haim S, Shakhar G, Rossene E, Taylor AN, Ben-Eliyahu S. Serum levels of sex hormones and corticosterone throughout 4- and 5-day estrous cycles in Fischer 344 rats and their simulation in ovariectomized females. *J Endocrinol Invest*. 2003;26:1013–1022.
103. Scharfman HE, Hintz TM, Gomez J, Malthankar-Phatak GH, Stormes KA, Luine VN, MacLusky NJ. Changes in hippocampal function of ovariectomized rats in response to estradiol replacement that simulates the preovulatory estrogen surge. *Eur J Neurosci*. 2007;26:2595–2612.
104. Murphy DD, Cole NB, Greenberger V, Segal M. Estradiol increases dendritic spine density by reducing GABA neurotransmission in hippocampal neurons. *J Neurosci*. 1998;18:2550–2559.
105. Hart SA, Patton JD, Woolley CS. Quantitative analysis of ERα and GAD colocalization in the hippocampus of the adult female rat. *J Comp Neurol*. 2001;440: 144–155.
106. Rudick CN, Woolley CS. Estrogen regulates functional inhibition of hippocampal CA1 pyramidal cells in the adult female rat. *J Neurosci*. 2001;21:6532–6543.

107. Blurton-Jones M, Kuan PN, Tuszynski MH. Anatomical evidence for transsynaptic influences of estrogen on brain-derived neurotrophic factor expression. *J Comp Neurol.* 2004;468:347–360.
108. Scharfman HE, MacLusky NJ. Estrogen and BDNF: complexity of steroid hormone–growth factor interactions. *Frontiers Neuroendocrinol*. 2006;27: 415–435.
109. Jodhka PK, Underwood WA, Lydon JP, Singh M. The differences in neuroprotective efficacy of progesterone and medroxyprogesterone acetate correlate with their effects on brain-derived neurotrophic factor expression. *Endcrinology*. 2009;150:3162–3168.
110. Frye CA, Rhodes ME. Estrogen-priming can enhance progesterone's anti-seizure effects in part by increasing hippocampal levels of allopregnanolone. *Pharmacol Biochem Behav*. 2005;81:907–916.
111. Coughan T, Gibson C, Murphy S. Progesterone, BDNF and neuroprotection in the injured cns. *Int J Neurosci*. 2009;119:1718–1740.
112. Begliuomini S, Casarosa E, Pluchino N, Lenzi E, Centofanti M, Freschi L, Pieri M, Genazzani AD, Luisi S, Genazzani AR. Influence of endogenous and exogenous sex hormones on plasma brain-derived neurotrophic factor. *Hum Reprod*. 2007;22:995–1002.
113. Marcus EM, Watson CW, Goldman PL. Effects of steroids on cerebral electrical activity. Epileptogenic effects of conjugated estrogens and related compounds in the cat and rabbit. *Arch Neurol*. 1966;15:521–532.
114. Lange SC, Julien RM. Re-evaluation of estrogen-induced cortical and thalamic paroxysmal EEG activity in the cat. *Electroencephalogr Clin Neurophysiol*. 1978;44:94–103.
115. Foy MR, Xu J, Xie X, Brinton RD, Thompson RF, Berger TW. 17β-Estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. *J Neurophysiol*. 1999;81:925–929.
116. Smith CC, McMahon LL. Estrogen-induced increase in the magnitude of long-term potentiation occurs only when the ratio of NMDA transmission to AMPA transmission is increased. *J Neurosci*. 2005;25:7780–7791.
117. Woolley CS. Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus. *Horm Behav*. 1998;34:140–148.
118. Bi R, Broutman G, Foy MR, Thompson RF, Baudry M. The tyrosine kinase and mitogen-activated protein kinase pathways mediate multiple effects of estrogen in hippocampus. *Proc Natl Acad Sci USA*. 2000;97:3602–3607.
119. Bi R, Foy MR, Vouimba RM, Thompson RF, Baudry M. Cyclic changes in estradiol regulate synaptic plasticity through the MAP kinase pathway. *Proc Natl Acad Sci USA*. 2001;98:13391–13395.
120. Scharfman HE, Maclusky NJ. Similarities between actions of estrogen and BDNF in the hippocampus: coincidence or clue? *Trends Neurosci*. 2005;28: 79–85.
121. Patterson SL, Grover LM, Schwartzkroin PA, Bothwell M. Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. *Neuron*. 1992;9:1081–1088.
122. Chapleau CA, Larimore JL, Theibert A, Pozzo-Miller L. Modulation of dendritic spine development and plasticity by BDNF and vesicular trafficking: fundamental roles in neurodevelopmental disorders associated with mental retardation and autism. *J Neurodev Disord*. 2009;1:185–196.
123. Galea LA, Spritzer MD, Barker JM, Pawluski JL. Gonadal hormone modulation of hippocampal neurogenesis in the adult. *Hippocampus.* 2006;16: 225–232.
124. Li Y, Luikart BW, Birnbaum S, Chen J, Kwon CH, Kernie SG, Bassel-Duby R, Parada LF. TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. *Neuron*. 2008;59:399–412.
125. Weiland NG. Glutamic acid decarboxylase messenger ribonucleic acid is regulated by estradiol and progesterone in the hippocampus. *Endocrinology*. 1992;131:2697–2702.
126. Schultz KN, von Esenwein SA, Hu M, Bennett AL, Kennedy RT, Musatov S, Toran-Allerand CD, Kaplitt MG, Young LJ, Becker JB. Viral vector-mediated overexpression of estrogen receptor-α in striatum enhances the estradiol-induced motor activity in female rats and estradiol-modulated GABA release. *J Neurosci*. 2009;29:1897–1903.
127. Murphy DD, Cole NB, Segal M. Brain-derived neurotrophic factor mediates estradiol-induced dendritic spine formation in hippocampal neurons. *Proc Natl Acad Sci USA*. 1998;95:11412–11417.
128. Olofsdotter K, Lindvall O, Asztely F. Increased synaptic inhibition in dentate gyrus of mice with reduced levels of endogenous brain-derived neurotrophic factor. *Neuroscience*. 2000;101:531–539.
129. Veliskova J, Velisek L. β-Estradiol increases dentate gyrus inhibition in female rats via augmentation of hilar neuropeptide Y. *J Neurosci*. 2007;27:6054–6063.
130. Ledoux VA, Smejkalova T, May RM, Cooke BM, Woolley CS. Estradiol facilitates the release of neuropeptide Y to suppress hippocampus-dependent seizures. *J Neurosci*. 2009;29:1457–1468.
131. Barnea A, Roberts J, Croll SD. Continuous exposure to brain-derived neurotrophic factor is required for persistent activation of TrkB receptor, the ERK signaling pathway, and the induction of neuropeptide Y production in cortical cultures. *Brain Res.* 2004;1020:106–117.
132. Croll SD, Wiegand SJ, Anderson KD, Lindsay RM, Nawa H. Regulation of neuropeptides in adult rat forebrain by the neurotrophins BDNF and NGF. *Eur J Neurosci*. 1994;6:1343–1353.
133. Nawa H, Bessho Y, Carnahan J, Nakanishi S, Mizuno K. Regulation of neuropeptide expression in cultured cerebral cortical neurons by brain-derived neurotrophic factor. *J Neurochem.* 1993;60: 772–775.
134. Wirth MJ, Patz S, Wahle P. Transcellular induction of neuropeptide Y expression by NT-4 and BDNF. *Proc Natl Acad Sci USA*. 2005;102:3064–3069.
135. Reibel S, Larmet Y, Carnahan J, Marescaux C, Depaulis A. Endogenous control of hippocampal epileptogenesis: a molecular cascade involving brain-derived neurotrophic factor and neuropeptide Y. *Epilepsia*. 2000;41(S6):S127–S133.
136. Rudge JS, Mather PE, Pasnikowski EM, Cai N, Corcoran T, Acheson A, Anderson K, Lindsay RM, Wiegand SJ. Endogenous BDNF protein is increased in adult rat hippocampus after a kainic acid induced excitotoxic insult but exogenous BDNF is not neuroprotective. *Exp Neurol*. 1998;149:398–410.

137. Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, Wang L, Blesch A, Kim A, Conner JM, Rockenstein E, Chao MV, Koo EH, Geschwind D, Masliah E, Chiba AA, Tuszynski MH. Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nat Med*. 2009;15: 331–337.
138. Lauber AH, Mobbs CV, Muramatsu M, Pfaff DW. Estrogen receptor messenger RNA expression in rat hypothalamus as a function of genetic sex and estrogen dose. *Endocrinology*. 1991;129:3180–3186.
139. Lauber AH, Romano GJ, Mobbs CV, Pfaff DW. Estradiol regulation of estrogen receptor messenger ribonucleic acid in rat mediobasal hypothalamus: an in situ hybridization study. *J Neuroendocrinol*. 1990;2:605–611.
140. Cheng G, Li Y, Omoto Y, Wang Y, Berg T, Nord M, Vihko P, Warner M, Piao YS, Gustafsson JA. Differential regulation of estrogen receptor ERα and ERβ in primate mammary gland. *J Clin Endocrinol Metab*. 2005;90:435–444.
141. Adams MM, Fink SE, Shah RA, Janssen WB, Hayashi S, Milner TA, McEwen BS, Morrison JH. Estrogen and aging affect the subcellular distribution of estrogen receptor-α in the hippocampus of female rats. *J Neurosci*. 2002;22:3608–3614.
142. Lonsdale D, Burnham WM. The anticonvulsant effects of progesterone and 5α-dihydroprogesterone on amygdala-kindled seizures in rats. *Epilepsia*. 2003;44:1494–1499.
143. Edwards HE, Epps T, Carlen PL, MacLusky N. Progestin receptors mediate progesterone suppression of epileptiform activity in tetanized hippocampal slices in vitro. *Neuroscience.* 2000;101:895–906.
144. Kokate TG, Banks MK, Magee T, Yamaguchi S, Rogawski MA. Finasteride, a 5α-reductase inhibitor, blocks the anticonvulsant activity of progesterone in mice. *J Pharmacol Exp*. Ther 1999;288:679–684.
145. Majewska MD, Harrison N, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* 1986;232: 1004–1007.
146. Reddy DS, Rogawski MA. Neurosteroid replacement therapy for catamenial epilepsy. *Neurotherapeutics*. 2009;6:392–401.
147. Brunig I, Penschuck S, Berninger B, Benson J, Fritschy JM. BDNF reduces miniature inhibitory postsynaptic currents by rapid downregulation of $GABA_A$ receptor surface expression. *Eur J Neurosci*. 2001;13:1320–1328.
148. Joshi S, Kapur J. Slow intracellular accumulation of $GABA_A$ receptor δ subunit is modulated by brain-derived neurotrophic factor. *Neuroscience.* 2009;164:507–519.
149. Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by subunit–containing GABAA receptors. *Proc. Natl. Acad. Sci. USA* 2003;100:14439–14444.
150. Marty S, Wehrle R, Sotelo C. Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. *J Neurosci*. 2000;20: 8087–8095.
151. Ohba S, Ikeda T, Ikegaya Y, Nishiyama N, Matsuki N, Yamada MK. BDNF locally potentiates GABAergic presynaptic machineries: target-selective circuit inhibition. *Cereb Cortex.* 2005;15:291–298.
152. Rivera C, Voipio J, Kaila K. Two developmental switches in GABAergic signalling: the K^+/Cl^- cotransporter KCC2 and carbonic anhydrase CAVII. *J Physiol*. 2005;562:27–36.
153. Smith SS, Shen H, Gong QH, Zhou X. Neurosteroid regulation of $GABA_A$ receptors: focus on the α4 and δ subunits. *Pharmacol Ther*. 2007;116:58–76.
154. Roberts DS, Hu Y, Lund IV, Brooks-Kayal AR, Russek SJ. Brain-derived neurotrophic factor (BDNF)-induced synthesis of early growth response factor 3 (Egr3) controls the levels of type a GABA receptor α4 subunits in hippocampal neurons. *J Biol Chem*. 2006;281:29431–29435.
155. Lund IV, Hu Y, Raol YH, Benham RS, Faris R, Russek SJ, Brooks-Kayal AR. BDNF selectively regulates $GABA_A$ receptor transcription by activation of the JAK/STAT pathway. *Sci Signal.* 2008;1:ra9.
156. Raol YH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, Russek SJ, Brooks-Kayal AR. Enhancing $GABA_A$ receptor α1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. *J Neurosci.* 2006;26:11342–11346.

Chapter 40

Alterations in the Distribution of $GABA_A$ Receptors in Epilepsy

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Changes in gamma-aminobutyric acid A receptors ($GABA_AR$) in epilepsy are particularly complex and intriguing because of the multiple subunits that can be altered. The numerous $GABA_AR$ subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3) typically form heteropentameric receptors that are generally composed of two α, two β, and either one γ, δ, or other more minor subunit. The different subunit combinations create a diversity of receptor subtypes that differ in their function, pharmacology, and regional and cellular localization (see refs. 1 and 2 for reviews). Such diversity provides challenges for understanding the functional effects of $GABA_AR$ subunit alterations in epilepsy, but it may also offer unique possibilities for treatment of this disorder.

MULTIPLE CHANGES IN $GABA_AR$ SUBUNITS IN EPILEPSY

Previous descriptions and reviews of the localization of $GABA_AR$ subunits in human temporal lobe epilepsy[3,4] and related animal models of acquired epilepsy[4–9] have emphasized the multiple changes that can occur in $GABA_AR$

subunit labeling in seizure disorders. Within the same tissue or model, some subunits exhibit increases, whereas others show decreases in expression. There is as yet no clear, unifying view of these changes and their functional significance, but new ideas are developing as the findings are considered in the context of other findings in the field. This review will focus on several of the most consistently observed alterations and their relationship to the rapidly expanding knowledge of $GABA_A$Rs and their functional characteristics.

In studies of $GABA_A$R subunit distributions in epilepsy, an increased expression of several subunits has been a predominant finding. In surgical tissue from patients with temporal lobe epilepsy, increased labeling of α2, γ2, β2, and β3 in granule cells of the dentate gyrus has been particularly striking.[3,4] Likewise, in animal models, increased expression of α4, γ2, and β subunits has been observed frequently in the hippocampal formation.[5,7,10,11] Such findings have generally been interpreted as a compensatory response to increased excitability of the region. However, decreased labeling of some subunits, beyond that associated with cell loss, has also been observed. Most notably, decreased expression of the α5 and δ subunits has been found in the hippocampus and dentate gyrus, respectively, in several animal models.[5,7,10,12–14]

FRAMEWORK FOR VIEWING $GABA_A$R SUBUNIT CHANGES IN EPILEPSY

Initially, the multiple subunit changes appeared random and unrelated. However, current views of $GABA_A$R subunit partnerships and function have suggested some interesting patterns. First, distinctions between tonic and phasic inhibition have provided a new perspective from which to view the changes. While phasic or synaptic inhibition has been the focus of GABAergic inhibition for many years and is critical for rapid, precisely timed inhibitory activity, it has become clear that tonic inhibition, generated by low, maintained levels of GABA, makes substantial contributions to the overall GABAergic inhibition in the brain and may be particularly critical for controlling the excitability of neuronal networks.[15–18]

Distinctions between phasic and tonic inhibition can be related to different $GABA_A$Rs that have their own unique characteristics and localization. The $GABA_A$Rs that mediate phasic inhibition are located directly at the postsynaptic density where they can respond to synaptically released GABA, whereas $GABA_A$Rs involved in tonic inhibition are located outside the synapse, at either peri- or extrasynaptic locations, where they are well positioned to respond to ambient levels of GABA within the extracellular space or to spillover of GABA at the synapse.[19,20] Consistent with their locations, $GABA_A$Rs that mediate phasic inhibition generally have relatively low affinity for GABA and desensitize rapidly, whereas those responsible for tonic inhibition have high affinity for GABA and slower rates of desensitization.[21,22]

These characteristics of $GABA_A$Rs are conveyed by their particular subunit composition. In the hippocampal formation, receptors mediating phasic inhibition generally contain the γ2 subunit,[23,24] whereas the major $GABA_A$Rs responsible for tonic inhibition contain either the δ or α5 subunit, along with appropriate subunit partners.[25–28] Importantly, some receptors that mediate tonic inhibition, such as those that contain the δ subunit, are exquisitely sensitive to modulators, including neurosteroids and low levels of ethanol that can greatly enhance $GABA_A$R function and zinc that can reduce such function.

As studies of the effects of subunit variations in normal $GABA_A$Rs have progressed, it has become evident that the alterations in $GABA_A$R subunit expression that are found in epilepsy could have a powerful impact on the kinetics of these receptors, the modulators and pharmacological agents that affect the receptors, and the overall response of the receptors during periods of increased network activity.

DECREASED EXPRESSION OF $GABA_A$R SUBUNITS IN PRINCIPAL CELLS

The α5 and δ subunits show striking decreases in labeling during the chronic period in several models of temporal lobe epilepsy. Interestingly, both subunits are key components of $GABA_A$Rs that mediate tonic inhibition in the hippocampus and dentate gyrus.

Decreased α5 Subunit Expression

Strong decreases in α5 mRNA and protein have been found in several epilepsy models, including both the kainate and pilocarpine models in rats[5,6,10,12–14] (Fig. 40–1A,B) and the pilocarpine model in mice (Peng and Houser, unpublished data) (Fig. 40–1C,D). In normal animals, the α5 subunit is most prominent in the hippocampus, with the highest levels of expression in CA1 and CA2 in the rat (Fig. 40–1A) and CA3 in the mouse (Fig. 40–1C). The labeling is concentrated in the dendritic layers, strata radiatum, and oriens, where it appears to be confined to pyramidal cell dendrites. In pilocarpine-treated rats, α5 labeling was decreased throughout the dendritic layers of the hippocampus (Fig. 40–1B). The decreases were greatest in CA2, large to moderate in CA1, and slight in CA3 (Fig. 40–1B). In the pilocarpine-treated mouse, a moderate, more uniform decrease in labeling was found throughout CA1–CA3 (Fig. 40–1D). Although some cell loss can occur in CA1 and CA3 in these animal models, the decrease in subunit labeling cannot be explained solely by such loss since other subunits were strongly labeled in remaining pyramidal cells in the region.[12] In addition, remaining cell bodies exhibited decreased mRNA labeling that could be detected with in situ hybridization methods.[12]

Decreased δ Subunit Expression

Labeling of the δ subunit is also substantially decreased in the hippocampal formation in several epilepsy models.[7,10] The changes are most marked in the dentate gyrus, where the δ subunit is normally expressed on granule cell somata and dendrites in the molecular layer (Fig. 40–2A–D). Although relatively low levels of δ subunit labeling are normally found in CA1 and the adjacent subiculum and entorhinal cortex, these regions frequently exhibit a further decrease in δ subunit labeling in epileptic animals, suggesting that the changes in δ subunit expression extend throughout much of the limbic region. Consistent with these findings, a microarray analysis identified the δ subunit mRNA as one of a broad group of mRNAs that was significantly decreased at 2 weeks after status epilepticus in the rat pilocarpine model.[29] Interestingly, mutations of the δ subunit gene have also been found in humans with at least two forms of generalized epilepsy.[30]

Considering the complementary patterns of α5 and δ subunits, with a predominance of

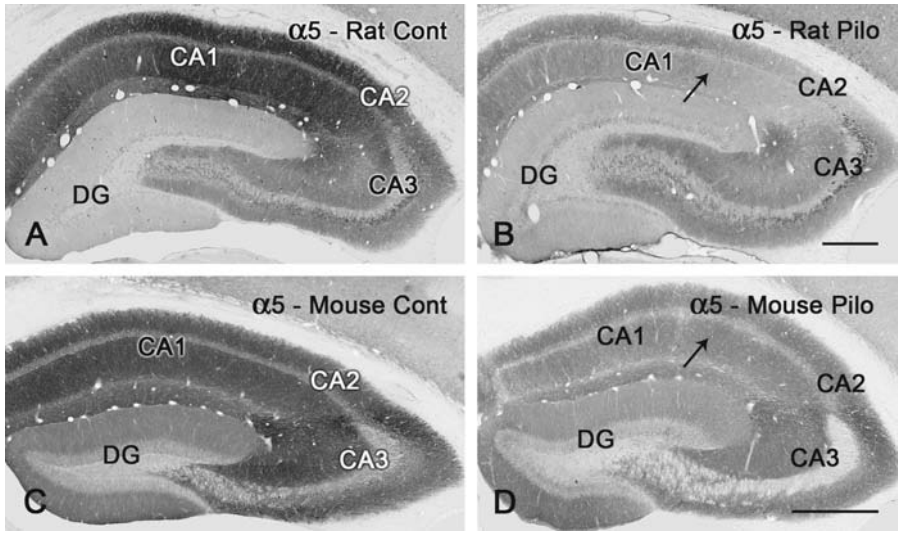

Figure 40–1. Alpha 5 subunit labeling is decreased in the hippocampus of pilocarpine-treated (Pilo) rats and mice compared to controls (Cont). **A,B.** In the rat, α5 subunit labeling is substantially decreased in dendritic layers of CA1 (arrow in **B**) and to an even greater extent in CA2. Less change is found in CA3 and the dentate gyrus (DG). **C,D.** In the mouse, a decrease in α5 subunit labeling is evident in dendritic regions (arrow in **D**) of the hippocampus (CA1–CA3). A slight decrease is also found in the dentate gyrus (DG). Scale bars: **A,B**, 500 μm; **C,D,** 500 μm. **A** and **B** adapted from ref. 12.

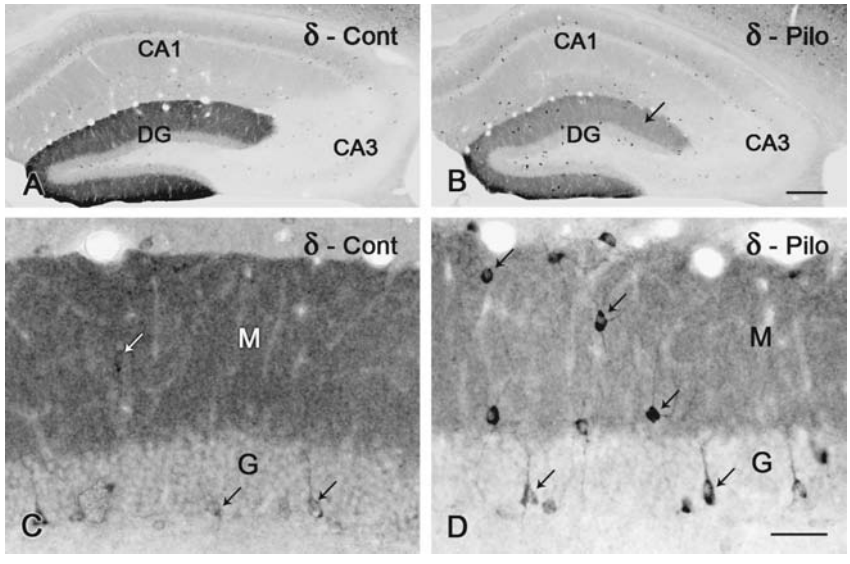

Figure 40–2. Delta subunit labeling is decreased in principal cells but increased in interneurons in pilocarpine-treated (Pilo) mice compared to controls (Cont). **A,B.** Diffuse labeling of the δ subunit is substantially decreased in the molecular layer (arrow in **B**) of the dentate gyrus (DG) in pilocarpine-treated mice. Less change is evident in CA1 and CA3, where δ subunit expression is normally low. **C,D.** In contrast, δ subunit labeling of interneurons (arrows) in the molecular layer (M) and along the base of the granule cell layer (G) is increased in pilocarpine-treated animals. Scale bars: **A,B**, 200 μm; **C,D**, 40 μm. Adapted from ref. 7.

α5 subunit labeling in the hippocampus and δ subunit labeling in the dentate gyrus, a marked decrease in subunits that mediate tonic inhibition occurs throughout the hippocampal formation.

Decreased α1 Subunit Expression

The α1 subunit is a third subunit that is decreased in some studies of epilepsy models. Utilizing single-cell mRNA amplification techniques, a significant decrease in α1 subunit mRNA has been identified in dissociated granule cells during the chronic period in the rat pilocarpine model.[11] A decrease in immunohistochemical labeling of the α1 subunit was also observed in the dentate gyrus in a group of drug-resistant epileptic rats following status epilepticus induced by electrical stimulation of the amygdala.[14] However, several other immunohistochemical and in situ hybridization studies of animal models have found either no significant change or an increase in α1 subunit expression in the dentate gyrus.[5,6,10,31] These discrepant findings could be related in part to the normal expression of the α1 subunit in both interneurons and principal cells.[32,33] In immunolabeled sections of the hippocampus, the α1 subunit is strongly expressed in interneurons, and the labeling of the interneurons and their processes could occlude labeling of principal cells, making changes in subunit expression in granule and pyramidal cells more difficult to detect. Likewise, immunoblot analyses of changes in the α1 subunit protein would not distinguish between cell type-specific changes. By contrast, in the biochemical studies of single cells,[11] the analysis was restricted to granule cells, thus perhaps making it easier to detect decreases in α1 mRNA in this class of neurons. However, in a study of human tissue, increased labeling of the α1 subunit was described on the cell bodies and proximal dendrites of dentate granule cells.[3] Thus, it remains difficult to reconcile the varying descriptions of α1 subunit changes in the epileptic hippocampus in different studies.

Functional Consequences of Decreased α5 and δ Subunit Expression in Principal Cells

The consistent decreases in α5 and δ subunit expression in principal cells in the hippocampus and dentate gyrus have led directly to the hypothesis that tonic inhibition could be

decreased in principal cells of the hippocampal formation. However, contrary to that prediction, a decrease in tonic inhibition has not been found. Indeed, tonic GABAergic inhibition was maintained or even greater in CA1 pyramidal cells of pilocarpine-treated rats than in control animals.[13] Likewise, in the rat model, tonic inhibition was maintained in granule cells within the granule cell layer and even increased in those aberrantly located in the hilus.[34] Tonic inhibition was thus maintained in dentate granule cells in both the mouse and rat pilocarpine models of recurrent seizures during the chronic period.[35,36]

The lack of noticeable changes in GABAergic tonic inhibition in the hippocampus and dentate gyrus following decreases in the α5 and δ subunits, respectively, is surprising and still unexplained. This could indicate that either relatively low levels of the subunits are required for maintaining tonic inhibition or compensation by other subunits has occurred. It also remains possible that tonic inhibition could be compromised in vivo even though such changes have not been detected with current in vitro analysis.

The $GABA_A$Rs that are mediating this retained tonic inhibition have not been determined with certainty. One possibility is that α5 and δ subunit-containing receptors substitute for each other. Thus, following a decrease in α5 subunit expression in the hippocampus, receptors containing the δ subunit might assume a greater role. Likewise, in the dentate gyrus, following the decrease in δ subunit expression, α5-containing receptors could become the primary mediator of tonic inhibition.[34] Such compensation has been described previously in mice that lack either the α5 or δ subunit.[28] Other $GABA_A$Rs that express only the α and β subunits also could be mediating tonic inhibition[37] in the seizure-prone animals.

However, in the mouse pilocarpine model, a change in the composition of $GABA_A$Rs that mediate tonic inhibition has also been suggested. In this epilepsy model, the γ2 subunit that is normally found in greatest abundance directly at synaptic contacts was found predominantly at perisynaptic sites in epileptic animals during the chronic period.[35] This change suggested that a receptor with an altered subunit composition, α4βγ2, could be mediating tonic inhibition in the dentate granule cells in the epileptic animals. This idea is supported by the finding of increased association of α4 and γ2 subunits in the dentate gyrus of epileptic animals.[36,38] Such a change in subunit composition could alter the kinetics and functional response of the receptor in critical ways.

Despite the apparent preservation of tonic inhibition in epileptic animals, functionally important alterations occur. In instances where the δ subunit is decreased, a marked decrease in modulation of the tonic current by neurosteroids is consistently found.[34–36] Thus, despite a maintained baseline level of tonic inhibition, the normal responsiveness of these receptors to physiological modulators is likely to be lost. Such alterations could be particularly important at times of stress or during hormonal fluctuations, precisely when an increase in tonic inhibition could be needed to control neuronal excitability.

The α5 subunit also contributes to $GABA_{A,slow}$ synaptic inhibition, and such inhibition has a strong influence on the ability of pyramidal cells to generate action potentials and thus influence network excitability.[39] It will be important to determine if this type of inhibition is decreased in animals with recurrent seizures, as it is in transgenic mice that are deficient in α5 subunit function.[40]

INCREASED EXPRESSION OF $GABA_A$R SUBUNITS IN PRINCIPAL CELLS

While several $GABA_A$R subunits exhibit increased expression in the hippocampus in epilepsy models and tissue from humans with temporal lobe epilepsy, increases in the α4 and γ2 subunits are among the most consistent.

The α4 subunit is a particularly plastic subunit, and increased α4 expression has been detected following rapid withdrawal from progesterone treatment,[41] following withdrawal from chronic intermittent ethanol treatment,[42,43] and in models of status epilepticus-induced epilepsy.[7,10,11,36] In the hippocampal formation, the α4 subunit is normally expressed at high levels in the molecular layer of the dentate gyrus, with lower levels of labeling in the hippocampus (Fig. 40–3A). Thus, throughout the hippocampal formation, the labeling pattern closely resembles that of the δ subunit. In epileptic animals, the labeling is

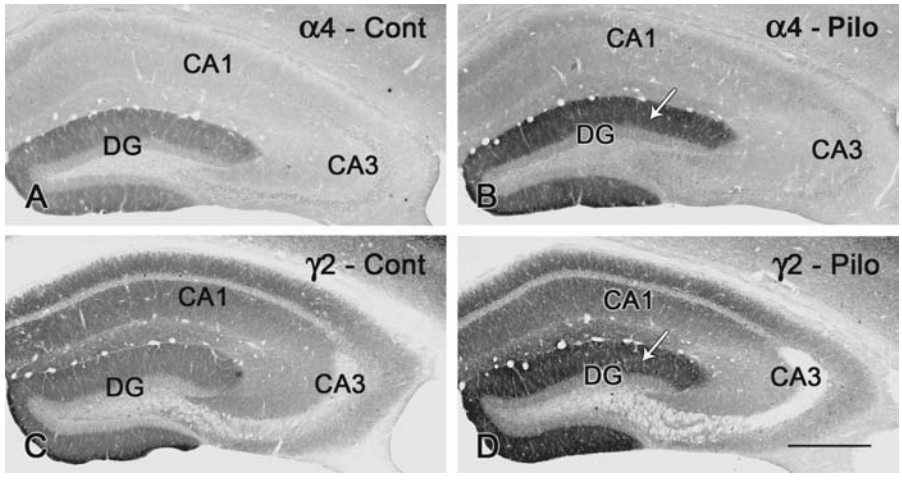

Figure 40–3. Alpha 4 and γ2 subunit labeling is increased in the hippocampal formation of pilocarpine-treated (Pilo) mice compared to controls (Cont). **A,B.** A substantial increase in α4 subunit labeling is present in the molecular layer (arrow in **B**) of the dentate gyrus (DG). A slight increase in α4 labeling is also evident in CA1. **C,D.** An increase in γ2 subunit labeling is most prominent in the dentate molecular layer (arrow in **D**) but is also evident in CA1. Scale bar: **A–D,** 500 μm.

increased substantially in the dentate molecular layer (Fig. 40–3B), in precisely the regions with decreased diffuse labeling of the δ subunit (Fig. 40–2B).

An increase in the γ2 subunit has also been observed in several epilepsy models,[7,10] and in most models, the increase has been particularly prominent in the molecular layer of the dentate gyrus (Fig. 40–3C,D).

Functional Consequences of Increased $GABA_A$R Subunit Expression in Principal Cells: Potential Alterations in Subunit Composition

The increased expression of these $GABA_A$R subunits has generally suggested a compensatory response of the receptors. However, in several instances, the partners of these subunits do not show parallel increases in expression. For example, the δ and α4 subunits are major partners in the forebrain, and yet the δ subunit is decreased while the α4 subunit is increased in the epilepsy models in which both subunits have been studied.[7,10] Likewise, the γ2 and α1 subunits are common partners, and yet the increase in γ2 labeling may be accompanied by a decrease in the α1 subunit in some cell types and models, and decreased association of α1 and γ2 has been reported in the dentate gyrus of epileptic rats.[38]

The concurrence of increases and decreases in different subunits within the same tissue suggests that the subunit composition of $GABA_A$Rs in these regions could be altered, with subunits that exhibit increased expression replacing those with decreased expression. Three $GABA_A$R subunits with unique interrelationships (α4, δ, γ2) are altered in several epilepsy models and could lead to $GABA_A$Rs with altered subunit composition. In the forebrain, the α4 and δ subunits have very similar anatomical distributions and are considered to be preferential partners.[44–46] However, α4 can also associate with the γ2 subunit. Thus, the δ and γ2 subunits may compete for partnership with the α4 subunit, and the presence of one of these subunits will exclude the other.[47–49] In δ subunit-deficient animals, expression of the γ2 subunit is increased, and its association with the remaining α4 subunit is likely to be increased.[46,49,50]

In pilocarpine-treated mice, while the δ subunit is decreased, both α4 and γ2 subunits are increased and could thus form a functional partnership. The time course of the changes in these three subunits supports this suggestion. An initial sharp decrease in α4 and γ2 subunits is followed a few days later by a gradual parallel increase in these two subunits, while the labeling intensity of the δ subunit remains decreased.[7]

However, electron microscopic studies of the α4 and γ2 subunits in normal mice have demonstrated different subcellular locations of these two subunits, with γ2 exhibiting strong synaptic localization (Fig. 40–4E) and α4 being preferentially located at nonsynaptic sites, including perisynaptic locations where the δ subunit is also localized[35] (Fig. 40–4A,C). Thus, if the partnership of the α4 and γ2 subunits were increased in the pilocarpine-treated mice, some change in their subcellular localization would be expected, with either the α4 subunit acquiring a synaptic localization or the γ2 subunit increasing at perisynaptic or extrasynaptic locations.

Ultrastructural studies suggest that a change in subunit localization indeed occurs.[35] First, a substantial decrease in δ subunit labeling in the pilocarpine-treated mice was observed, consistent with the light microscopic findings. A large

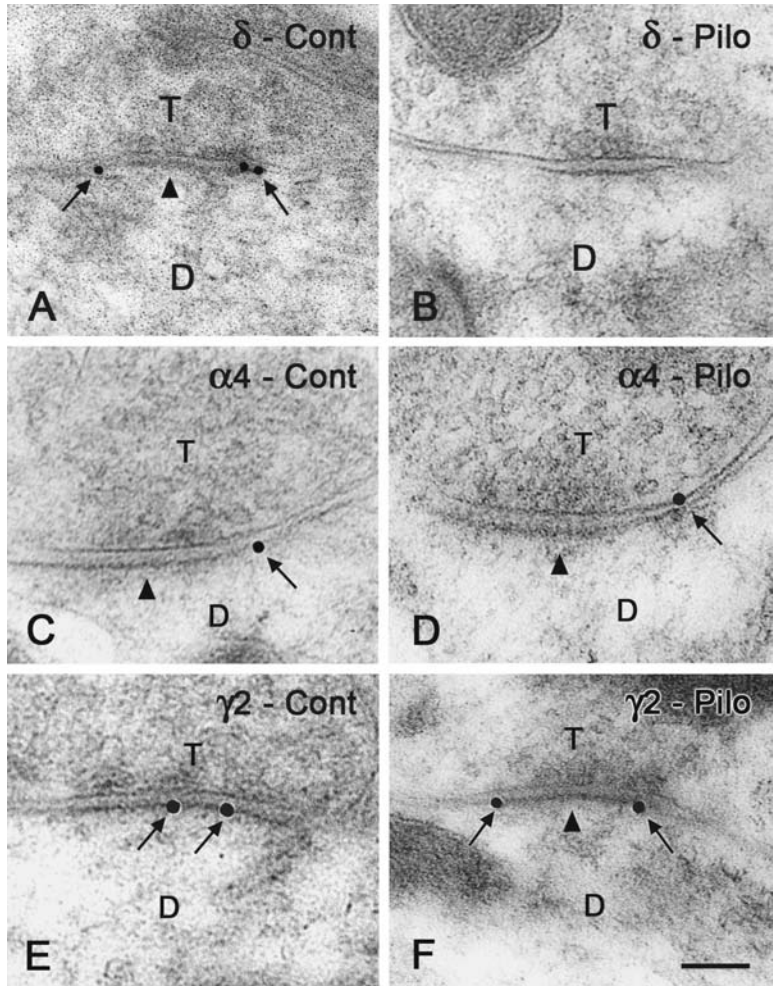

Figure 40–4. The subcellular localization of the δ, α4, and γ2 subunits in dendrites of granule cells suggests a preferential shift in the location of the γ2 subunit in pilocarpine-treated (Pilo) mice compared to controls (Cont). All electron micrographs illustrate synapses between a presynaptic axon terminal (T) and a postsynaptic dendrite (D). Arrowheads indicate the center of the synaptic contact, and arrows point to immunogold particles that label each subunit. **A,B.** The δ subunit is predominantly located at perisynaptic sites in control mice but is often absent at synapses in pilocarpine-treated mice. **C,D.** The α4 subunit is located primarily at the edge of synaptic contacts in control mice and remains at these locations in pilocarpine-treated mice. **E,F.** The γ2 subunit is located predominantly within or near the center of synaptic contacts in control mice but is frequently found at perisynaptic locations in pilocarpine-treated mice. Scale bar: 0.1 μm for all panels. Adapted from ref. 35.

(~62%) decrease in gold particles was found at perisynaptic locations, where the δ subunit is normally most abundant (Fig. 40–4A,B). In contrast, an increase in γ2 labeling was found at perisynaptic sites (Fig. 40–4F), where labeling of the α4 subunit was retained (Fig. 40–4D). Unexpectedly, while γ2 subunit labeling increased at perisynaptic locations, labeling directly at the synapse decreased (Fig. 40–4F). Such changes suggest that a subunit switch could have occurred, with the γ2 subunit forming a partnership with the α4 subunit in receptors at perisynaptic locations, where the δ subunit had previously been localized. Thus, the maintained tonic inhibition that has been found in dentate granule cells in these animals could be mediated in part by α4βγ2-containing perisynaptic receptors. While the similar subcellular localization of the α4 and γ2 subunits is not evidence of their presence in the same receptor, a similar location would be necessary and is consistent with an altered subunit partnership. In further support of a subunit switch, recent studies have demonstrated that a larger amount of the α4 subunit coimmunoprecipitated with the γ2 subunit in the hippocampus of epileptic animals compared to controls.[36,38]

Surprisingly, but in agreement with a decrease in γ2 labeling at the synapse, phasic inhibition at granule cell dendrites was decreased in the pilocarpine-treated mice.[35] In this instance, it appears that a shift in the γ2 subunit to perisynaptic locations, while possibly contributing to the preservation of tonic inhibition, may have compromised synaptic inhibition.

Analysis of similar subunit changes in other studies, using the rat pilocarpine model, has yielded somewhat different findings, although a change in receptor subunit composition is also suggested. In the rat model, ultrastructural studies demonstrated a shift of the α4 subunit from a perisynaptic to a synaptic location.[51] The reasons for the different findings in these studies remain unclear, but they could be related to species differences, the focus on dendritic synapses in the mouse and somatic synapses in the rat, or other technical issues. Consistent with possible species differences, a shift in α4 labeling from perisynaptic to synaptic locations was observed previously in a rat model of alcohol withdrawal syndrome in which a decrease in δ subunit expression also occurred in conjunction with an increase in the expression of α4 and γ2 subunits.[43] Despite some discrepancies in the epileptic animals, a marked decrease in modulation of inhibition by neurosteroids, due to the decrease in δ or the increase in α4 subunit expression, has been a consistent finding.[35,36,51]

Other studies have also suggested that the increase in α4 subunit expression could be associated with a change in subunit composition of $GABA_A$Rs, but they have emphasized the possible replacement of the α1 subunit by α4.[11,38,52] Comparison of the functional properties of $GABA_A$Rs in in vitro systems has revealed several interesting differences between receptors with the putative subunit compositions. In order to compare the kinetic properties of α4β3γ2 currents to those of α1β3γ2 currents, a rapid application of GABA was delivered to recombinant $GABA_A$Rs in HEK 293T cells.[53] A number of differences were identified, and two features were particularly striking. While both types of receptors were able to respond adequately to brief low-frequency stimulation, the α4β3γ2 receptors responded poorly to repetitive stimulation such as might occur during seizures. In addition, α4β3γ2 receptors were less effective than α1β3γ2 receptors after exposure to prolonged low levels of GABA, such as might be found in the extrasynaptic space. Thus, the investigators concluded that replacement of α1β3γ2 with α4β3γ2 receptors would be likely to impair inhibitory transmission.[53]

Such changes in $GABA_A$R responses would be consistent with the increased run-down of $GABA_A$ currents that has been observed in receptors from human temporal lobe epilepsy specimens and pilocarpine-treated rats following expression in oocytes. Similar use-dependent decreases (run-down) of $GABA_A$ currents were observed following repetitive activation of $GABA_A$Rs by application of GABA in CA1 pyramidal cells in pilocarpine-treated rats.[54] A decrease in the ratio of α1 to α4 subunits was also identified in the pilocarpine-treated animals compared to controls.[55] Thus, the receptors from the epileptic tissue, suggested to include α4βγ2 receptors, could function relatively normally under baseline conditions but failed to be effective when increased inhibitory responses were needed. While these electrophysiological changes were observed in phasic inhibition, responsiveness to spillover of GABA at perisynaptic sites could also be affected. Thus, it would be interesting to know if α4βγ2 receptors at perisynaptic locations might have

less than optimal function when compared to the original δ subunit-containing receptors.

These findings suggest that even an increase in abundance of specific subunits, such as the α4 subunit, could lead to compromised inhibition. Thus, increased expression of some $GABA_A$Rs may be less compensatory than was originally expected.

Other Proposed Effects of Altered $GABA_A$R Subunit Composition

Alterations in $GABA_A$R subunit composition could influence GABAergic function in additional ways. One particularly interesting possibility is that $GABA_A$R subunit changes may underlie the zinc-induced collapse of inhibition in kindled and pilocarpine-treated animals.[56,57] The hypothesis is that the reorganized mossy fibers in the inner molecular layer of the dentate gyrus may release zinc, along with glutamate, during repetitive firing at the time of seizure activity, and this could reduce $GABA_A$R activity at precisely the times when it is most needed to control excessive activity of dentate granule cells. Since the application of zinc did not affect $GABA_A$R responses in control animals, a change in $GABA_A$R subunit composition in the epileptic animal could be responsible for the altered inhibitory responses. The specific subunits that could underlie the altered zinc sensitivity in the epilepsy models have not been identified, although a shift from a predominance of α1- to α4-containing receptors has been suggested.[58]

While the emphasis in these studies has been on altered synaptic receptors, nonsynaptic receptors might be even more likely to be affected by the released zinc, as δ subunits are especially sensitive to the effects of zinc.[59,60] However, the reduced expression of the δ subunit in the dentate gyrus argues against a primary role for the δ subunit-containing receptors in the zinc-induced decrease in inhibition. Other possibilities for mediating the zinc effects include extrasynaptic αβ subunit-containing receptors that make modest contributions to tonic inhibition in normal animals and are quite sensitive to the influences of zinc.[37] Whether such receptors increase in the epilepsy models is unknown, but, as mentioned previously, such receptors remain a possible mediator of tonic inhibition in the epileptic animals.

ALTERED $GABA_A$R SUBUNIT EXPRESSION IN INTERNEURONS

While emphasis has been placed on altered $GABA_A$R subunits on principal cells, alterations have also been observed in remaining interneurons in the hippocampal formation. Strong labeling of the α1 subunit on persisting interneurons in surgical specimens from patients with temporal lobe epilepsy has indicated a preservation or possible increase in α1 subunit expression.[3] In the mouse pilocarpine model, an increase in δ subunit labeling occurs in many remaining interneurons in the hippocampus and dentate gyrus, and this contrasts with the substantial decrease in diffuse δ subunit labeling of granule cell dendrites in the dentate molecular layer[7] (Fig. 40–2D). The interneuron labeling increased progressively following the initial episode of status epilepticus and was maintained.

Consistent with an increase in δ subunit expression, initial findings suggest that some of the interneurons, including those in the molecular layer of the dentate gyrus, may exhibit increased tonic inhibition during the chronic period when the mice are experiencing recurrent seizures (W. Wei and I. Mody, unpublished findings). It is hypothesized that the enhanced tonic inhibition could lead to decreased excitability of the interneurons and thus less inhibitory control of their targets, such as in the dentate granule cells. These functional alterations could be particularly detrimental at the time of excessive stimulation of the granule cells when interneurons are normally recruited to control the increased input.

The differential changes in δ subunit expression in principal cells and interneurons in the epileptic animals are striking and raise many questions about the mechanisms underlying such cell-type specific changes. These changes also present challenges to pharmacological treatment since treatment that increases tonic inhibition in principal cells could also enhance tonic inhibition in interneurons, and the effects could counteract each other. However, the subunit composition of the δ subunit-containing receptors appears to differ in principal cells

and interneurons, with the δ subunit forming a strong partnership with the α4 subunit in granule cells but with the α1 subunit in interneurons.[61] Thus, pharmacological agents that would selectively target δ subunit-containing receptors in either principal cells or interneurons, and enhance or reduce their function, respectively, could be beneficial.

SUMMARY

Emerging Views and Questions

Several patterns are beginning to emerge from the numerous diverse changes that occur in $GABA_A$Rs in epilepsy. First, subunits that are primarily responsible for tonic inhibition show the most marked and consistent decreases in several models of acquired epilepsy, and appear more likely to be decreased than subunits of $GABA_A$Rs that mediate phasic inhibition. Yet, the preservation of GABA-mediated tonic current in principal cells of both the hippocampus and dentate gyrus, despite decreases in α5 and δ subunits, respectively, raises further questions. One suggestion has been that the tonic inhibition is maintained or enhanced in temporal lobe epilepsy as a homeostatic mechanism to counteract decreases in phasic inhibition.[18] In this view, an enhanced tonic current in principal cells, which was observed in response to increased GABA concentrations, could prevent or limit seizure activity and thus be compensatory.[13]

Alternatively, decreases in the α5 and δ subunits could represent critical changes that lead to the upregulation of other $GABA_A$R subunits and expression of altered or new nonsynaptic receptors with different functional properties. The kinetic properties of the receptors could be altered in ways that could reduce their effectiveness during increased stimulation. Likewise, even though capable of producing tonic inhibition, the replacement receptors could be unable to respond to specific modulators that normally influence the nonsynaptic receptors. Clearly, the decrease in δ subunit-containing receptors leads to reduced neurosteroid sensitivity that could make the receptor less sensitive to enhancement of tonic inhibition in response to stress and hormonal fluctuations.[18,62–64]

Such changes in subunit composition and the associated changes in functional properties of the receptors could alter their response to pharmacological agents, potentially reducing the response to some agents that are normally effective. However, once the composition and characteristics of the reorganized receptors are known, new pharmacological treatments could be designed.

Increases or decreases in $GABA_A$R subunit expression may not translate directly into either compensation or impairment, respectively, as the functional effects will depend on the resulting subunit composition in remaining receptors and the cell types in which the changes occur. Increased expression of the δ subunit in $GABA_A$Rs in interneurons could lead to suppressed activity in these neurons and their decreased inhibitory control of granule cells. However, the decrease of δ subunit in principal cells could lead to direct effects on these cells, including altered tonic inhibition and impaired modulation of such activity.

Several of the changes that are currently being identified in $GABA_A$Rs in epilepsy are precisely the types of alterations that might be anticipated in a disorder where network activity appears relatively normal for much of the time and then periodically shifts to excessive, synchronized activity, possibly due to the inability of $GABA_A$Rs to respond to increased demands.

Future Prospects and Challenges

Modulation of $GABA_A$R function continues to hold great promise for the treatment of epilepsy. As the mechanisms responsible for the changes in $GABA_A$R subunit expression are determined, ways of preventing these changes may be developed, with the hope of halting the epileptogenic process. In addition, as the subunit composition of $GABA_A$Rs in the epileptic regions is further elucidated, treatment approaches may be developed to target the reorganized receptors. Mechanisms responsible for dynamic changes in $GABA_A$R function are also being identified and could be particularly important for the development of novel treatment approaches. The high sensitivity of nonsynaptic receptors to specific modulators suggests that such agents could be developed to enhance $GABA_A$R function at times of stress or

excessive network activity.[65,66] Likewise, rapid changes in surface expression of $GABA_A$Rs could impair receptor function at the time of spontaneous seizures, as has been found during status epilepticus,[67,68] and new methods to limit internalization of receptor subunits could be therapeutic. Finally, phosphorylation of $GABA_A$Rs provides another avenue by which $GABA_A$R function can be rapidly altered.[69,70] All of these areas suggest new routes for $GABA_A$R-mediated treatment and, potentially, prevention of epilepsy.

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DISCLOSURE STATEMENT

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REFERENCES

1. Sieghart W, Sperk G. Subunit composition, distribution and function of $GABA_A$ receptor subtypes. *Curr Top Med Chem*. 2002;2:795–816.
2. Olsen RW, Sieghart W. $GABA_A$ receptors: subtypes provide diversity of function and pharmacology. *Neuropharmacology*. 2009;56:141–148.
3. Loup F, Wieser HG, Yonekawa Y, Aguzzi A, Fritschy J-M. Selective alterations in $GABA_A$ receptor subtypes in human temporal lobe epilepsy. *J Neurosci*. 2000;20:5401–5419.
4. Pirker S, Schwarzer C, Czech T, Baumgartner C, Pockberger H, Maier H, Hauer B, Sieghart W, Furtinger S, Sperk G. Increased expression of $GABA_A$ receptor β-subunits in the hippocampus of patients with temporal lobe epilepsy. *J Neuropathol Exp Neurol*. 2003;62:820–834.
5. Fritschy J-M, Kiener T, Bouilleret V, Loup F. GABAergic neurons and $GABA_A$-receptors in temporal lobe epilepsy. *Neurochem Int*. 1999;34:435–445.
6. Nishimura T, Schwarzer C, Gasser E, Kato N, Vezzani A, Sperk G. Altered expression of $GABA_A$ and $GABA_B$ receptor subunit mRNAs in the hippocampus after kindling and electrically induced status epilepticus. *Neuroscience*. 2005;134:691–704.
7. Peng Z, Huang CS, Stell BM, Mody I, Houser CR. Altered expression of the δ subunit of the $GABA_A$ receptor in a mouse model of temporal lobe epilepsy. *J Neurosci*. 2004;24:8629–8639.
8. Fritschy J-M. Epilepsy, E/I balance and $GABA_A$ receptor plasticity. *Front Mol Neurosci*. 2008;1:5.
9. Sperk G, Drexel M, Pirker S. Neuronal plasticity in animal models and the epileptic human hippocampus. *Epilepsia*. 2009;50(suppl 12):29–31.
10. Schwarzer C, Tsunashima K, Wanzenböck C, Fuchs K, Sieghart W, Sperk G. $GABA_A$ receptor subunits in the rat hippocampus II: altered distribution in kainic acid-induced temporal lobe epilepsy. *Neuroscience*. 1997;80:1001–1017.
11. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell $GABA_A$ receptor subunit expression and function in temporal lobe epilepsy. *Nat Med*. 1998;4:1166–1172.
12. Houser CR, Esclapez M. Downregulation of the α5 subunit of the $GABA_A$ receptor in the pilocarpine model of temporal lobe epilepsy. *Hippocampus*. 2003;13:633–645.
13. Scimemi A, Semyanov A, Sperk G, Kullmann DM, Walker MC. Multiple and plastic receptors mediate tonic $GABA_A$ receptor currents in the hippocampus. *J Neurosci*. 2005;25:10016–10024.
14. Bethmann K, Fritschy J-M, Brandt C, Loscher W. Antiepileptic drug resistant rats differ from drug responsive rats in $GABA_A$ receptor subunit expression in a model of temporal lobe epilepsy. *Neurobiol Dis*. 2008;31:169–187.
15. Mody I. Distinguishing between $GABA_A$ receptors responsible for tonic and phasic conductances. *Neurochem Res*. 2001;26:907–913.
16. Semyanov A, Walker MC, Kullmann DM, Silver RA. Tonically active $GABA_A$ receptors: modulating gain and maintaining the tone. *Trends Neurosci*. 2004;27:262–269.
17. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. *Nat Rev Neurosci*. 2005;6:215–229.
18. Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW. Extrasynaptic $GABA_A$ receptors: form, pharmacology, and function. *J Neurosci*. 2009;29:12757–12763.
19. Nusser Z, Sieghart W, Somogyi P. Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Comp Neurol*. 1998;18:1693–1703.
20. Wei W, Zhang N, Peng Z, Houser CR, Mody I. Perisynaptic localization of δ subunit-containing $GABA_A$ receptors and their activation by GABA spillover in the mouse dentate gyrus. *J Neurosci*. 2003;23:10650–10661.
21. Haas K, Macdonald RL. $GABA_A$ receptor subunit γ2 and δ subtypes confer unique kinetic properties on recombinant $GABA_A$ receptor currents in mouse fibroblasts. *J Physiol*. 1999;514:27–45.
22. Glykys J, Mody I. Activation of $GABA_A$ receptors: views from outside the synaptic cleft. *Neuron*. 2007;56:763–770.
23. Somogyi P, Fritschy J-M, Benke D, Roberts JDB, Sieghart W. The γ2 subunit of the $GABA_A$ receptor

is concentrated in synaptic junctions containing the α1 and β2/3 subunits in hippocampus, cerebellum and globus pallidus. *Neuropharmacology.* 1996;35: 1425–1444.
24. Sassoè-Pognetto M, Panzanelli P, Sieghart W, Fritschy J-M. Colocalization of multiple $GABA_A$ receptor subtypes with gephyrin at postsynaptic sites. *J Comp Neurol*. 2000;420:481–498.
25. Stell BM, Mody I. Receptors with different affinities mediate phasic and tonic $GABA_A$ conductances in hippocampal neurons. *J Neurosci.* 2002;22:1–5.
26. Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, Macdonald JF, Orser BA. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by α5 subunit-containing γ-aminobutyric acid type A receptors. *Proc Natl Acad Sci USA*. 2004;101:3662–3667.
27. Prenosil GA, Schneider Gasser EM, Rudolph U, Keist R, Fritschy J-M, Vogt KE. Specific subtypes of $GABA_A$ receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. *J Neurophysiol*. 2006;96:846–857.
28. Glykys J, Mann EO, Mody I. Which $GABA_A$ receptor subunits are necessary for tonic inhibition in the hippocampus? *J Neurosci*. 2008;28:1421–1426.
29. Elliott RC, Miles MF, Lowenstein DH. Overlapping microarray profiles of dentate gyrus gene expression during development- and epilepsy-associated neurogenesis and axon outgrowth. *J Neurosci*. 2003;23: 2218–2227.
30. Dibbens LM, Feng H-J, Richards MC, Harkin LA, Hodgson BL, Scott D, Jenkins M, Petrou S, Sutherland GR, Scheffer IE, Berkovic SF, Macdonald RL, Mulley JC. GABRD encoding a protein for extra- or peri-synaptic $GABA_A$ receptors is a susceptibility locus for generalized epilepsies. *Hum Mol Genet*. 2004;13:1315–1319.
31. Tsunashima K, Schwarzer C, Kirchmair E, Sieghart W, Sperk G. $GABA_A$ receptor subunits in the rat hippocampus III: altered messenger RNA expression in kainic acid-induced epilepsy. *Neuroscience*. 1997;80: 1019–1032.
32. Fritschy J-M, Mohler H. $GABA_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J Comp Neurol*. 1995;359:154–194.
33. Sperk G, Schwarzer C, Tsunashima K, Fuchs J, Sieghart W. $GABA_A$ receptor subunits in the rat hippocampus I: immunocytochemical distribution of 13 subunits. *Neuroscience*. 1997;80:987–1000.
34. Zhan RZ, Nadler JV. Enhanced tonic GABA current in normotopic and hilar ectopic dentate granule cells after pilocarpine-induced status epilepticus. *J Neurophysiol*. 2009;102:670–681.
35. Zhang N, Wei W, Mody I, Houser CR. Altered localization of $GABA_A$ receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *J Neurosci*. 2007;27:7520–7531.
36. Rajasekaran K, Joshi S, Sun C, Mtchedlishvilli Z, Kapur J. Receptors with low affinity for neurosteroids and GABA contribute to tonic inhibition of granule cells in epileptic animals. *Neurobiol Dis*. 2010;40:490–501.
37. Mortensen M, Smart TG. Extrasynaptic αβ subunit $GABA_A$ receptors on rat hippocampal pyramidal neurons. *J Physiol*. 2006;577:841–856.
38. Lund IV, Hu Y, Raol YH, Benham RS, Faris R, Russek SJ, Brooks-Kayal AR. BDNF selectively regulates $GABA_A$ receptor transcription by activation of the JAK/STAT pathway. *Sci Signal*. 2008;1:ra9.
39. Hentschke H, Benkwitz C, Banks MI, Perkins MG, Homanics GE, Pearce RA. Altered $GABA_{A,slow}$ inhibition and network oscillations in mice lacking the $GABA_A$ receptor β3 subunit. *J Neurophysiol*. 2009;102: 3643–3655.
40. Zarnowska ED, Keist R, Rudolph U, Pearce RA. $GABA_A$ receptor α5 subunits contribute to $GABA_{A,slow}$ synaptic inhibition in mouse hippocampus. *J Neurophysiol*. 2009;101:1179–1191.
41. Smith SS, Qi HG, Li X, Moran MH, Bitran D, Frye CA, Hsu FC. Withdrawal from 3α-OH-5α-pregnan-20-one using a pseudopregnancy model alters the kinetics of hippocampal $GABA_A$-gated current and increases the $GABA_A$ receptor α4 subunit in association with increased anxiety. *J Neurosci*. 1998;18:5275–5284.
42. Cagetti E, Liang J, Spigelman I, Olsen RW. Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of $GABA_A$ receptors. *Mol Pharmacol*. 2003;63:53–64.
43. Liang J, Zhang N, Cagetti E, Houser CR, Olsen RW, Spigelman I. Chronic intermittent ethanol-induced switch of ethanol actions from extrasynaptic to synaptic hippocampal $GABA_A$ receptors. *J Neurosci*. 2006;26:1749–1758.
44. Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR, McKernan RM. Preferential coassembly of α4 and δ subunits of the γ-aminobutyric $acid_A$ receptor in rat thalamus. *Mol Pharmacol*. 1999;56:110–115.
45. Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience.* 2000;101:815–850.
46. Peng Z, Hauer B, Mihalek RM, Homanics GE, Sieghart W, Olsen RW, Houser CR. $GABA_A$ receptor changes in δ subunit-deficient mice: altered expression of α4 and γ2 subunits in the forebrain. *J Comp Neurol*. 2002;446:179–197.
47. Quirk K, Whiting PJ, Ragan CI, McKernan RM. Characterisation of δ-subunit containing $GABA_A$ receptors from rat brain. *Eur J Pharmacol*. 1995;290: 175–181.
48. Araujo F, Ruano D, Vitorica J. Absence of association between δ and γ2 subunits in native $GABA_A$ receptors from rat brain. *Eur J Pharmacol*. 1998;347:347–353.
49. Tretter V, Hauer B, Nusser Z, Mihalek RM, Höger H, Homanics GE, Somogyi P, Sieghart W. Targeted disruption of the $GABA_A$ δ subunit gene leads to an up-regulation of γ2 subunit-containing receptors in cerebellar granule cells. *J Biol Chem*. 2001;276: 10532–10538.
50. Korpi ER, Mihalek RM, Sinkkonen ST, Hauer B, Hevers W, Homanics GE, Sieghart W, Luddens H. Altered receptor subtypes in the forebrain of $GABA_A$ receptor δ subunit-deficient mice: recruitment of γ2 subunits. *Neuroscience*. 2002;109:733–743.
51. Sun C, Mtchedlishvili Z, Erisir A, Kapur J. Diminished neurosteroid sensitivity of synaptic inhibition and altered location of the α4 subunit of $GABA_A$ receptors in an animal model of epilepsy. *J Neurosci*. 2007;27: 12641–12650.

52. Raol YH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, Russek SJ, Brooks-Kayal AR. Enhancing $GABA_A$ receptor α1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. *J Neurosci*. 2006;26:11342–11346.
53. Lagrange AH, Botzolakis EJ, Macdonald RL. Enhanced macroscopic desensitization shapes the response of α4 subtype-containing $GABA_A$ receptors to synaptic and extrasynaptic GABA. *J Physiol*. 2007;578:655–676.
54. Palma E, Roseti C, Maiolino F, Fucile S, Martinello K, Mazzuferi M, Aronica E, Manfredi M, Esposito V, Cantore G, Miledi R, Simonato M, Eusebi F. $GABA_A$-current rundown of temporal lobe epilepsy is associated with repetitive activation of $GABA_A$ "phasic" receptors. *Proc Natl Acad Sci USA*. 2007;104:20944–20948.
55. Mazzuferi M, Palma E, Martinello K, Maiolino F, Roseti C, Fucile S, Fabene PF, Schio F, Pellitteri M, Sperk G, Miledi R, Eusebi F, Simonato M. Enhancement of $GABA_A$-current run-down in the hippocampus occurs at the first spontaneous seizure in a model of temporal lobe epilepsy. *Proc Natl Acad Sci USA*. 2010;107:3180–3185.
56. Buhl EH, Otis TS, Mody I. Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. *Science*. 1996;271:369–373.
57. Cohen AS, Lin DD, Quirk GL, Coulter DA. Dentate granule cell $GABA_A$ receptors in epileptic hippocampus: enhanced synaptic efficacy and altered pharmacology. *Eur J Neurosci*. 2003;17:1607–1616.
58. Coulter DA. Mossy fiber zinc and temporal lobe epilepsy: pathological association with altered "epileptic" γ-aminobutyric acid A receptors in dentate granule cells. *Epilepsia*. 2000;41(suppl 6):S96–S99.
59. Saxena NC, Macdonald RL. Assembly of $GABA_A$ receptor subunits: role of the δ subunit. *J Neurosci*. 1994;14:7077–7086.
60. Smart TG, Hosie AM, Miller PS. Zn^{2+} ions: modulators of excitatory and inhibitory synaptic activity. *Neuroscientist*. 2004;10:432–442.
61. Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I. A new naturally occurring $GABA_A$ receptor subunit partnership with high sensitivity to ethanol. *Nat Neurosci*. 2007;10:40–48.
62. Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by δ subunit-containing $GABA_A$ receptors. *Proc Natl Acad Sci USA*. 2003;100:14439–14444.
63. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in $GABA_A$ receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci*. 2005;8:797–804.
64. Lawrence C, Martin BS, Sun C, Williamson J, Kapur J. Endogenous neurosteroid synthesis modulates seizure frequency. *Ann Neurol*. 2010;67:689–693.
65. Mody I. Extrasynaptic $GABA_A$ receptors in the crosshairs of hormones and ethanol. *Neurochem Int*. 2008;52:60–64.
66. Mortensen M, Ebert B, Wafford K, Smart TG. Distinct activities of GABA agonists at synaptic- and extrasynaptic-type $GABA_A$ receptors. *J Physiol*. 2010;588:1251–1268.
67. Naylor DE, Liu H, Wasterlain CG. Trafficking of $GABA_A$ receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci*. 2005;25:7724–7733.
68. Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J. Subunit-specific trafficking of $GABA_A$ receptors during status epilepticus. *J Neurosci*. 2008;28:2527–2538.
69. Terunuma M, Xu J, Vithlani M, Sieghart W, Kittler J, Pangalos M, Haydon PG, Coulter DA, Moss SJ. Deficits in phosphorylation of $GABA_A$ receptors by intimately associated protein kinase C activity underlie compromised synaptic inhibition during status epilepticus. *J Neurosci*. 2008;28:376–384.
70. Houston CM, He Q, Smart TG. CaMKII phosphorylation of the $GABA_A$ receptor: receptor subtype- and synapse-specific modulation. *J Physiol*. 2009;587:2115–2125.

Chapter 41

$GABA_A$ Receptor Plasticity during Status Epilepticus

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Status epilepticus (SE) is a prolonged, self-sustained seizure that can manifest as a prolonged convulsion, subtle facial or limb twitching, or simply altered mental status, always in association with a persistent electroencephalographic (EEG) seizure pattern. In clinical trials, convulsive seizures lasting longer than 5 min are often treated as SE,[1] whereas epidemiological studies have defined 30 min of continuous convulsive seizures or intermittent seizures without recovery of consciousness as SE.[2] There are 126,000 to 195,000 episodes of SE associated with 22,000 to 42,000 deaths each year in the United States when SE is defined as 30 min of seizures.[2] As many as 50% of patients older than 65 years of age who suffer from SE die within 30 days of the episode. Although mortality is far less common in younger patients, they are at risk for neurological morbidity, such as injury to the hippocampus[3] and neuropsychological dysfunction.[4] Prolonged seizures can also lead to significant systemic complications, including pulmonary congestion and edema, cardiac arrhythmias, hypotension, elevation of body temperature, hypoglycemia, acidosis, and rhabdomylosis. These systemic complications tend to worsen as more seizures occur.[5]

It has long been recognized that SE is a dynamic and rapidly evolving condition.[5,6] Ongoing seizures rapidly modify neuronal activity and synaptic function.[7] This rapid neuronal plasticity is manifest in changes in behavioral seizures, EEG patterns, sensitivity

Table 41–1 **Characteristics, Clinical Manifestations, and Treatment of SE**

	Early/Impending/ Convulsive	Refractory/Subtle	Late/Established
Clinical manifestation	Generalized convulsions	Subtle motor manifestations	Coma
EEG	Repeated seizures	Seizures merge	Periodic discharges and suppression mixed with seizures
Systemic effects	Hyperventilation, hypertension		Respiratory depression
Brain damage	No evidence	Starting	Increases with time
Treatment	Lorazepam/ diazepam	Benzodiazepines fail:? phenytoin/levetiracetam/ valproic acid	Anesthetics:? propofol, midazolam, barbiturates
Mortality (adults)	19.6%[8]	?	31.4%[8]
Animal models: behavior	Stage 1–2 seizures	10 min after first stage 5 seizure	60 min after first stage 5 seizure
Animal models: EEG	Discrete seizures	Continuous seizures	Seizures with suppression

Source: Modified from refs. 2 and 8.

to drugs, and evolution of neuronal injury and death. Although these changes are continuous, it is convenient to divide SE into stages for the purposes of investigation and treatment (Table 41–1).

ANIMAL MODELS OF SE

Animal models and in vitro cell culture models have proved very useful in understanding the cellular and molecular mechanisms underlying the seizures of SE (for review see ref. 9). In experimental animals, SE can be induced by either administration of chemo-convulsants or electrical stimulation of limbic structures. In addition, SE can be induced by activation of muscarinic receptors by the administration of the cholinergic agonist pilocarpine,[10] without or with the use of lithium (4 mEq/kg, 4–20 h prior to pilocarpine injection[11,12]), or by organophosphates, which inhibit the enzyme cholinesterase. Also, SE can be produced by stimulation of glutamatergic receptors by kainic acid. In addition, prolonged self-sustaining seizures of SE can be induced by electrical stimulation of limbic structures such as the hippocampus, entorhinal cortex, or amygdala.[13,14] The behavioral seizures and EEG in the animal models of SE evolve over time, with many similarities to the progression of human SE. Furthermore, the efficiency of benzodiazepines in controlling the seizures of SE diminishes with the duration of SE.[15–20]

Two in vitro models that mimic the repeated bursting activity of neurons characteristic of SE include the use of reduced/zero magnesium and high potassium concentrations in the media of hippocampal or cortical neuronal cultures. Reducing magnesium below physiological levels induces recurrent bursting in hippocampal slices and cultured neurons.[21–24] Elevating extracellular potassium results in prolonged depolarization and also induces spontaneous seizures.[25]

Metabolic mapping and EEG studies during SE demonstrate activation of the hippocampus, subiculum, entorhinal cortex, and parts of the thalamus and cortex.[26] The circuitry linking the entorhinal cortex, hippocampus, and subiculum forms a reentrant path facilitating recurrent seizures. The entorhinal cortex is connected by the perforant path to hippocampal dentate granule cells (DGCs), which project to CA3 pyramidal neurons via mossy fibers. CA3 pyramidal neurons, in turn, project to CA1 pyramidal neurons via Schaffer collaterals.[27] Axons of CA1 pyramidal neurons innervate the subiculum, which connects to layers IV and II of the

entorhinal cortex, thus forming a loop that can mediate self-sustaining seizures. Neurons in the CA1 region and entorhinal cortex are capable of generating recurrent bursting patterns characteristic of seizures.[28,29] Given the centrality of the hippocampus in this reentrant circuit, many studies aimed at understanding the cellular and molecular mechanisms underlying the seizures of SE have focused on the hippocampus.

REDUCED GABAERGIC NEUROTRANSMISSION DURING SE

Reduced inhibitory neurotransmission mediated by gamma-aminobutyric acid (GABA) type A receptors (GABARs) is proposed to be one of the underlying mechanisms of the self-sustaining and progressive nature of SE. Benzodiazepines, such as diazepam and lorazepam, are the preferred drugs to treat the seizures of SE[30]; they act on GABARs, which mediate the majority of inhibitory neurotransmission in the forebrain. In addition to these first-line drugs, phenobarbital, pentobarbital, midazolam, and propofol, used in intensive care units to treat refractory SE,[31] also modulate GABARs.

GABARS

These are pentameric ligand-gated anion channels. The subunits are derived from α, β, γ, δ, and ε gene families, some of which have multiple members, such as α 1–6, β1–3, and γ1–3. The majority of the receptors appear to be composed of 2α, 2β, and γ or δ subunits. The sensitivity of the receptor to many of the aforementioned modulators depends on its subunit composition.[32] Subunit composition-dependent properties of GABARs relevant to SE and epilepsy are their sensitivity to benzodiazepines such as diazepam, lorazepam, and midazolam, to the divalent cation Zn^{2+}, and to neurosteroids. Diazepam sensitivity requires the presence of a γ2 subunit, and the relative affinity of diazepam to the receptor also depends on the subtype of the α subunit.[33,34] In contrast, sensitivity to Zn^{2+} is reduced by the presence of a γ subunit, but relative sensitivity to Zn^{2+} is also regulated by the subtype of α subunit.[35–37]. The presence of a δ subunit confers higher GABA and neurosteroid affinity to the receptor.[38,39] In addition to pharmacological modulation by different drugs, subunit composition influences the kinetic properties of the receptors. For example, δ subunit-containing receptors desensitize slowly and more incompletely than γ2 subunit-containing receptors.

Subunit composition also determines receptor targeting. The presence of a γ2 subunit is necessary for synaptic localization of GABARs,[40,41] whereas GABARs composed of the δ subunit remain exclusively in the peri- and extrasynaptic membrane.[42,43] Synaptic GABARs mediate fast inhibition in response to the high concentration of GABA released in the synaptic cleft, whereas extrasynaptic receptors respond to GABA spilled over from synapses and contribute to the persistent background inhibition commonly referred to as *tonic inhibition*.[44–47]

Specific subunit assembly is present in various brain regions. For example, in hippocampal DGCs and cerebellar granule neurons, the δ subunit associates with α4 or α6 subunits, respectively,[48–50] whereas most of the γ2 subunit-containing receptors in the hippocampus and thalamus contain an α1 subunit.[51–53]

GABAR-MEDIATED INHIBITION IS DECREASED DURING SE

During SE, GABAR-mediated inhibition is reduced in the hippocampi of animals. The GABA-evoked GABAR whole cell currents recorded from DGCs and CA1 pyramidal neurons were smaller in animals 45 min after the onset of SE than those of naive animals.[17,54] Decreased synaptic and/or tonic inhibition mediated by GABARs could contribute to this reduction of whole cell currents. To determine whether synaptic inhibition mediated by GABARs was diminished during SE, miniature inhibitory postsynaptic currents (mIPSCs) were recorded from DGCs or CA1 pyramidal neurons 30–60 min after the onset of lithium-pilocarpine-induced SE.[55–57] These studies revealed that the amplitude of mIPSCs

recorded from DGCs and CA1 pyramidal neurons from animals in SE was smaller than that of controls. Furthermore, the amplitude reduction in mIPSCs in DGCs occurred as early as 30 min after the onset of forelimb clonus.[58] In addition to decreased mIPSC amplitude, mIPSC frequency was reduced.[55] The reduced amplitude and frequency of mIPSCs recorded from DGCs of animals in SE suggest presynaptic and postsynaptic changes. Reduced GABA release from presynaptic terminals may result in decreased amplitude and frequency, whereas diminution in the number of functional receptors at the postsynaptic membrane could cause decreased amplitude. Whether GABA release from presynaptic terminals is diminished in animals in SE is not known. However, biochemical studies have revealed a decrease in the number of functional receptors on the postsynaptic membrane in the hippocampi of animals in SE.[55,57] A biotinylation assay used to study the expression of GABAR subunits in surface membrane proteins in the hippocampi of animals in SE revealed decreased expression of γ2, β2/3, and α1 subunits, which form the majority of synaptic receptors, along with decreased expression of the α2 and α4 subunits[55,57] (Fig. 41–1). Reduced surface expression could be due to genomic effects such as decreased transcription or translation of GABAR subunits or altered receptor trafficking to the membrane. However, the time course of the changes in surface expression was very rapid, suggesting altered trafficking to be a likely underlying factor. Therefore, later studies addressed whether SE changed receptor trafficking, that is, insertion, which adds new receptors to the surface membrane, and internalization, which removes existing receptors.

INCREASED INTERNALIZATION OF SYNAPTIC GABARS DURING SE

Internalization of the γ2 and β2/3 subunits increased in in vitro models of SE.[24,55] Incubation of cultured hippocampal neurons in

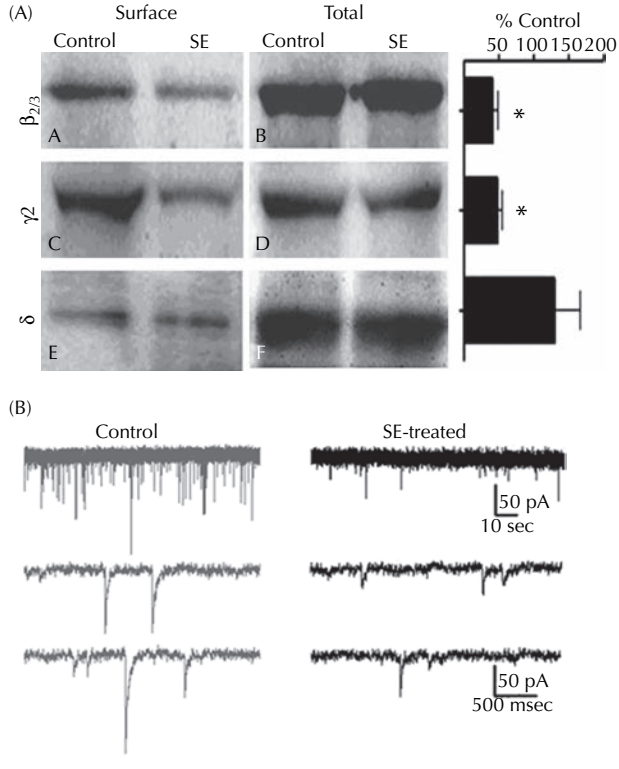

Figure 41–1. A. Decreased surface expression of the γ2 and β2/3 subunits in animals during SE. **B.** Reduced synaptic inhibition evident from decreased frequency and amplitude of mIPSCs in animals in SE.[55]

zero magnesium or high potassium-containing medium, which mimic the recurrent bursting characteristic of SE, resulted in increased internalization of the γ2 and β2/3 subunits.[24,55] Studies in animals in SE also suggested that increased internalization of GABARs likely reduced their surface expression. GABARs undergo clathrin-mediated internalization by their association with the AP2 complex of endocytotic machinery. In the hippocampi of animals in SE, an increased association between the β3 subunit of GABARs and the AP2 complex was observed,[57] which would facilitate internalization. Whether assembly and insertion of GABARs are also reduced during SE is not known.

Factors that may trigger increased internalization of GABARs during SE include neuronal firing, depolarization, *N*-methyl-D-aspartate (NMDA) receptor activation, and ligand binding. In studies of the mechanisms eliciting internalization of the γ2 subunit-containing receptors during bursting in cultured neurons, incubation with GABA did not accelerate receptor internalization.[55] Similarly, in organotypic hippocampal slice cultures, incubation with GABA in the presence of the uptake blocker NO711 did not decrease surface levels of the γ2 subunit, suggesting that ligand binding was unlikely to trigger increased internalization of GABARs during SE. In contrast, bursting-induced internalization of receptors was mimicked by NMDA receptor activation. In organotypic hippocampal slice cultures, incubation in a medium containing high extracellular potassium and NMDA decreased surface expression of the γ2 subunit. These studies suggested that activation of NMDA receptors, which occurs during SE,[16] contributes to accelerated internalization of the γ2 subunit.

SE-INDUCED DEPHOSPHORYLATION OF GABARS DECREASES THEIR CELL SURFACE STABILITY

A potential mechanism regulating the surface stability of GABARs is their phosphorylation and dephosphorylation.[59,60] Calcium influx through NMDA receptors activates second messengers such as protein kinase C (PKC) and calcineurin,[61,62] which can regulate phosphorylation of GABARs. In animals in SE, activity of PKCα, PKCβ, and PKCγ was decreased, and there was reduced phosphorylation of the β3 subunit. This was associated with increased interaction between the β3 subunit and the AP2 complex of clathrin endocytotic machinery.[57] Conversely, activation of PKC in hippocampal slices isolated from animals in SE reversed reduction of the β3 subunit surface expression. Thus, dephosphorylation of the β3 subunit facilitated its internalization during SE. However, it should be noted that PKC activity and its regulation of GABARs during SE is complex,[63] and in contrast to the report by Terunuma and colleagues,[57] other studies have shown an association between PKC activation and seizures.[64–66] The difference in findings may be due to multiple PKC isoforms.

In addition to regulation by PKC, GABAR surface stability is also determined by protein phosphatase II or calcineurin.[67] The activity of protein phosphatase II or calcineurin is increased during SE,[68,69] and treatment of hippocampal slices isolated from animals in SE with the calcineurin inhibitor FK506 prevented reduction in the phosphorylation of the β3 subunit. Whether it also reversed downregulation of surface expression of the β3 subunit is not known. However, progression of seizures was not attenuated in animals administered FK506 prior to the induction of SE. Therefore, in addition to phosphorylation-dependent mechanisms, other factors may also contribute to decreased GABAR function during SE. A reduction in the number of synaptic receptors observed in animal and cell culture models of SE may also explain reduced sensitivity to benzodiazepines (reviewed in ref. 70).

DECREASED BENZODIAZEPINE SENSITIVITY WITH SEIZURE PROGRESSION IN ANIMAL MODELS OF SE

Benzodiazepines are very effective in terminating early SE, but they become ineffective in controlling prolonged SE. In adult animals, diazepam was effective in controlling seizures when it was given 10 min after pilocarpine administration, but it failed after 45 min.[17] Correspondingly, diazepam enhancement of GABA-evoked currents was lower in DGCs

isolated 45 min after the onset of seizures; 1 μM diazepam enhanced GABA-evoked currents by 92 ± 6% in neurons from naive animals, whereas in neurons from SE animals, 3 μM diazepam enhanced GABAR currents by only 51 ± 8%.[17] Because benzodiazepines act on γ2 subunit-containing GABARs, reduced surface expression of these receptors, discussed above, could contribute to the loss of diazepam sensitivity associated with seizure progression.

TONIC INHIBITION MEDIATED BY GABARS DURING SE

As discussed above, the smaller amplitude of GABA-evoked whole cell currents recorded from DGCs and CA1 pyramidal neurons from animals 45 min after the onset of SE could also be due to reduced tonic inhibition. However, tonic currents recorded from DGCs of animals in SE remained unaltered or increased[55,56] (Fig. 41–2). In accordance, the number of surface-expressed δ subunit-containing receptors remained either unchanged or increased in the hippocampi of animals in SE.[55,57] The surface expression and internalization of δ subunit-containing GABARs also remained unaltered in cultured hippocampal neurons incubated in medium containing high extracellular potassium.[55] This could be due to slower constitutive internalization of the δ subunit[71] or signaling mechanisms that are distinct from those regulating the γ2 subunit-containing GABARs. Preserved surface expression of the δ subunit-containing receptors during SE is physiologically significant. GABARs containing a δ subunit have higher sensitivity to neurosteroids. In one study, neurosteroids were effective in treating pilocarpine- and kainite-induced SE.[72] In these animals, 5α3α or 5β3α isomers of tetrahydrodeoxycorticosterone (THDOC) and pregnanolone were potent in controlling SE when administered before pilocarpine. Furthermore, 5α3α pregnanolone was effective in aborting ongoing SE; in 50% of animals, 15 mg/kg 5α3α pregnanolone administered 15 min after the onset of seizures resulted in their termination.

GABAR FUNCTION IN CATAMENIAL EPILEPSY

In experimental animals, SE often results in the development of temporal lobe epilepsy.[73,74] A seizure-free period (latent period) spans the time between SE and the onset of a first spontaneous seizure,[75] and can range from months to years in human beings and from days to weeks in experimental animal models. GABAergic inhibition of hippocampal neurons undergoes a complex set of changes during this latent period in humans and in epileptic animals. These changes are discussed elsewhere in this book. We will focus on the neurosteroid modulation of GABARs, which is physiologically significant in the hormonal regulation of seizures in temporal lobe epilepsy.

In DGCs of epileptic animals, neurosteroid potentiation of whole cell GABAR currents, synaptic currents, and tonic currents is diminished.[76–79] Cyclic fluctuations in neurosteroid levels in women with epilepsy are often associated with periodic seizure exacerbation, often referred to as *catamenial epilepsy*.[80] In

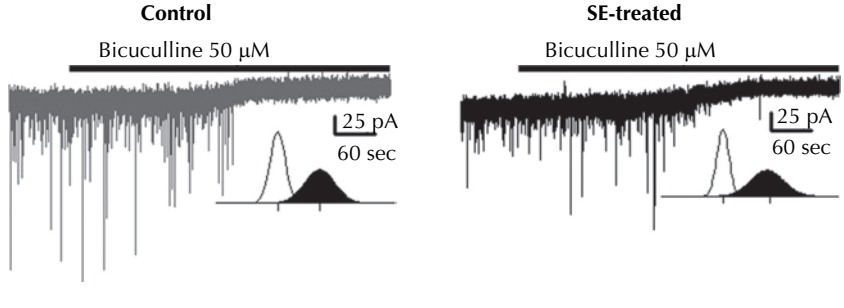

Figure 41–2. Tonic inhibition mediated by persistently open GABARs, measured in terms of the shift in the baseline holding current after application of bicuculline, was preserved in DGCs of animals in SE. Modified from ref. 55; synaptic currents have been removed to emphasize tonic inhibition.

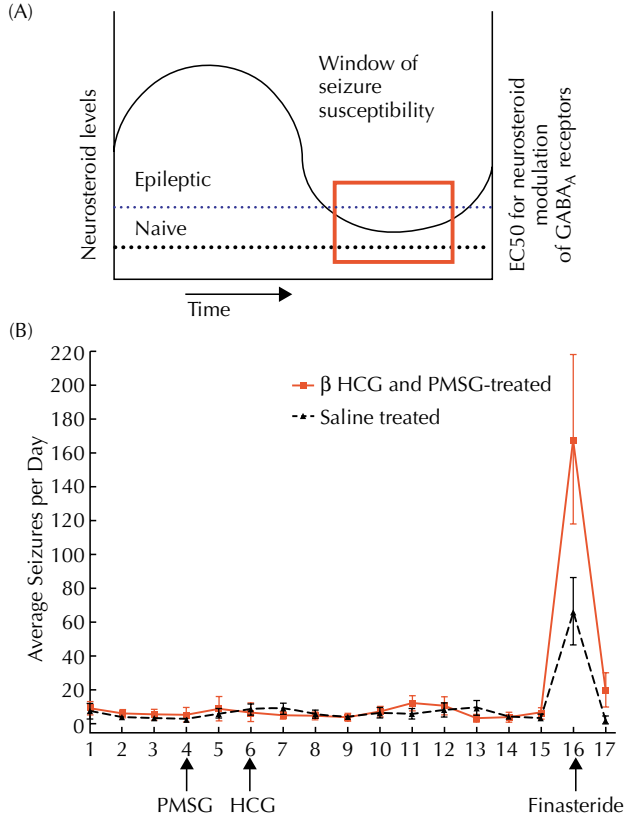

Figure 41–3. A. Schematic representation of neurosteroid levels and seizure susceptibility. **B.** Increased frequency of seizures following finasteride administration.[84] Animals were treated with pregnant mare serum gonadotropin (PMSG) and β-human chorionic gonadotropin (βHCG) to elevate their progesterone levels and then with finasteride to block neurosteroid synthesis. Seizure frequency increased following finasteride administration in animals with (βHCG- and PMSG-treated) or without increased progesterone levels (saline-treated).

a clinical study, exogenous administration of progesterone decreased seizure frequency in a woman with catamenial epilepsy.[81] However, administration of finasteride, a blocker of 5α-reductase, the enzyme catalyzing the rate-limiting step in the conversion of progesterone to allopregnanolone, abolished the anti-convulsant effects of progesterone, indicating that protective effects of progesterone were due to the synthesis of the neurosteroid allopregnanolone. In addition to anti-convulsant effects, allopregnanolone has action during the latent period[82]; inhibition of allopregnanolone synthesis accelerated development of spontaneous seizures following pilocarpine-induced SE.

Gonadal steroid hormones and neurosteroids regulate the expression of GABARs. Increased progesterone levels augment surface expression of δ subunit-containing receptors.[83] Progesterone levels are higher during the diestrus phase and decrease during the estrus phase, and levels of allopregnanolone fluctuate accordingly. A rapid decline in allopregnanolone levels during the estrus phase is thought to create a seizure susceptibility window (Fig. 41–3). However, a recent study in animals demonstrated that elevated progesterone levels are not necessary for seizures precipitation[84] (Fig. 41–3). Even at basal progesterone levels, inhibiting allopregnanolone synthesis greatly increased seizure frequency. Hence, endogenous neurosteroids are important regulators of seizure frequency.

CONCLUSIONS

Prolonged self-sustaining seizures, commonly referred to as *SE*, constitute a neurological

emergency because they can cause neuronal damage. Prompt treatment of these seizures with benzodiazepines can terminate SE. However, in many patients, benzodiazepines are not effective. Also, SE is a progressive condition in which refractoriness to benzodiazepines can develop over time. As seizures go on, there is reduced GABAergic inhibition of principal neurons in the hippocampus. This is due in part to reduced synaptic inhibition. Seizures accelerate the internalization of surface receptors in the synapses, perhaps via dephosphorylation. Interestingly, tonic inhibition and the number of diazepam-insensitive extrasynaptic receptors are preserved during SE. These insights into the mechanisms of SE suggest novel therapies targeting tonic inhibition. In addition, SE causes long-term changes in hippocampal GABARs; they become less sensitive to neurosteroids, which might contribute to seizure precipitation in epileptic animals.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Lowenstein DH. Status epilepticus: an overview of the clinical problem. *Epilepsia*. 1999;40(suppl 1):S3–S8.
2. DeLorenzo RJ, Hauser WA, Towne AR, Boggs JG, Pellock JM, Penberthy L, Garnett L, Fortner CA, Ko D. A prospective, population-based epidemiologic study of status epilepticus in Richmond, Virginia. *Neurology*. 1996;46:1029–1035.
3. VanLandingham KE, Heinz ER, Cavazos JE, Lewis DV. Magnetic resonance imaging evidence of hippocampal injury after prolonged focal febrile convulsions. *Ann Neurol*. 1998;43:413–426.
4. Dodrill CB, Wilensky AJ. Intellectual impairment as an outcome of status epilepticus. *Neurology*. 1990;40: 23–27.
5. Lothman E. The biochemical basis and pathophysiology of status epilepticus. *Neurology*. 1990;40:13–23.
6. Treiman DM. The role of benzodiazepines in the management of status epilepticus. *Neurology*. 1990;40(5 suppl 2):32–42.
7. Chen JW, Naylor DE, Wasterlain CG. Advances in the pathophysiology of status epilepticus. *Acta Neurol Scand*. 2007;115:7–15.
8. Lothman E. The biochemical basis and pathophysiology of status epilepticus. *Neurology*. 1990;40:13–23.
9. Kapur J. Pathophysiolgy of nonconvulsive status epilepticus. In: Kaplan PW, Drislane FW, eds. *Nonconvulsive Status Epilepticus*. New York, NY: Demos Medical Publishing; 2009:81–94.
10. Turski WA, Cavalheiro EA, Schwarz M, Czuczwar SJ, Kleinrok Z, Turski L. Limbic seizures produced by pilocarpine in rats: behavioural, electroencephalographic and neuropathological study. *Behav Brain Res*. 1983;9:315–335.
11. Jope RS, Morrisett RA, Snead OC. Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. *Exp Neurol*. 1986;91:471–480.
12. Honchar MP, Olney JW, Sherman WR. Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. *Science*. 1983;220:323–325.
13. Lothman EW, Bertram EH, Bekenstein JW, Perlin JB. Self-sustaining limbic status epilepticus induced by "continuous" hippocampal stimulation: electrographic and behavioral characteristics. *Epilepsy Res*. 1989;3: 107–119.
14. McIntyre DC, Nathanson D, Edson N. A new model of partial status epilepticus based on kindling. *Brain Res*. 1982;250:53–63.
15. Jones DM, Esmaeil N, Maren S, MacDonald RL. Characterization of pharmacoresistance to benzodiazepines in the rat Li-pilocarpine model of status epilepticus. Epilepsy Res. 2002;50:301–312.
16. Mazarati AM, Baldwin RA, Sankar R, Wasterlain CG. Time-dependent decrease in the effectiveness of antiepileptic drugs during the course of self-sustaining status epilepticus. *Brain Res*. 1998;814:179–185.
17. Kapur J, MacDonald RL. Rapid seizure-induced reduction of benzodiazepine and Zn^{2+} sensitivity of hippocampal dentate granule cell $GABA_A$ receptors. *J Neurosci*. 1997;17:7532–7540.
18. Goodkin HP, Liu X, Holmes GL. Diazepam terminates brief but not prolonged seizures in young, naive rats. *Epilepsia*. 2003;44:1109–1112.
19. Morrisett RA, Jope RS, Snead OC III. Effects of drugs on the initiation and maintenance of status epilepticus induced by administration of pilocarpine to lithium-pretreated rats. *Exp Neurol*. 1987;97:193–200.
20. Walton NY, Treiman DM. Response of status epilepticus induced by lithium and pilocarpine to treatment with diazepam. *Exp Neurol*. 1988;101:267–275.
21. Walther H, Lambert JDC, Jones RSG, Heinemann U, Hamon B. Epileptiform activity in combined slices of the hippocampus, subiculum and entorhinal cortex during perfusion with low magnesium medium. *Neurosci Lett*. 1986;69:156–161.
22. Sombati S, DeLorenzo RJ. Recurrent spontaneous seizure activity in hippocampal neuronal networks in culture. *J Neurophysiol*. 1995;73:1706–1711.
23. Mangan PS, Kapur J. Factors underlying bursting behavior in a network of cultured hippocampal neurons exposed to zero magnesium. *J Neurophysiol*. 2004;91:946–957.
24. Goodkin HP, Yeh JL, Kapur J. Status epilepticus increases the intracellular accumulation of $GABA_A$ receptors. *J Neurosci*. 2005;25:5511–5520.
25. Traynelis SF, Dingledine R. Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. *J Neurophysiol*. 1988;59:259–276.
26. VanLandingham KE, Lothman EW. Self-sustaining limbic status epilepticus. I. Acute and chronic cerebral metabolic studies: limbic hypermetabolism and neocortical hypometabolism. *Neurology*. 1991;41:1942–1949.
27. Amaral DG, Witter MP. The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience*. 1989;31:571–591.

28. Jones RS, Heinemann U. Synaptic and intrinsic responses of medical entorhinal cortical cells in normal and magnesium-free medium in vitro. *J Neurophysiol*. 1988;59:1476–1496.
29. Jones RS, Heinemann UF, Lambert JD. The entorhinal cortex and generation of seizure activity: studies of normal synaptic transmission and epileptogenesis in vitro. *Epilepsy Res Suppl*. 1992;8:173–180.
30. Lowenstein DH, Alldredge BK. Status epilepticus. *N Engl J Med*. 1998;338:970–976.
31. Bleck TP. Intensive care unit management of patients with status epilepticus. *Epilepsia*. 2007;48(suppl 8): 59–60.
32. Olsen RW, Sieghart W. $GABA_A$ receptors: subtypes provide diversity of function and pharmacology. *Neuropharmacology*. 2009;56:141–148.
33. Sieghart W, Sperk G. Subunit composition, distribution and function of $GABA_A$ receptor subtypes. *Curr Top Med Chem*. 2002;2:795–816.
34. Sigel E. Mapping of the benzodiazepine recognition site on $GABA_A$ receptors. *Curr Top Med Chem*. 2002;2:833–839.
35. Burgard EC, Tietz EI, Neelands TR, MacDonald RL. Properties of recombinant $GABA_A$ receptor isoforms containing the $\alpha5$ subunit subtype. *Mol Pharmacol*. 1996;50:119–127.
36. Knoflach F, Benke D, Wang Y, Scheurer L, Luddens H, Hamilton BJ, Carter DB, Mohler H, Benson JA. Pharmacological modulation of the diazepam-insensitive recombinant $GABA_A$ receptors $\alpha4\beta2\gamma2$ and $\alpha6\beta2\gamma2$. *Mol Pharmacol*. 1996;50:1253–1261.
37. Saxena NC, MacDonald RL. Properties of putative cerebellar $GABA_A$ receptor isoforms. *Mol Pharmacol*. 1996;49:567–579.
38. Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen RW, Homanics GE. Attenuated sensitivity to neuroactive steroids in $GABA_A$ receptor δ subunit knockout mice. *Proc Natl Acad Sci USA*. 1999;96:12905–12910.
39. Wohlfarth KM, Bianchi MT, Macdonald RL. Enhanced neurosteroid potentiation of ternary $GABA_A$ receptors containing the δ subunit. *J Neurosci*. 2002;22: 1541–1549.
40. Essrich C, Lorez M, Benson JA, Fritschy JM, Luscher B. Postsynaptic clustering of major $GABA_A$ receptor subtypes requires the $\gamma2$ subunit and gephyrin. *Nat Neurosci*. 1998;1:563–571.
41. Schweizer C, Balsiger S, Bluethmann H, Mansuy IM, Fritschy JM, Mohler H, Lnscher B. The $\gamma2$ subunit of $GABA_A$ receptors is required for maintenance of receptors at mature synapses. *Mol Cell Neurosci*. 2003;24:442–450.
42. Nusser Z, Sieghart W, Somogyi P. Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci*. 1998;18:1693–1703.
43. Wei W, Zhang N, Peng Z, Houser CR, Mody I. Perisynaptic localization of δ subunit-containing $GABA_A$ receptors and their activation by GABA spillover in the mouse dentate gyrus. *J Neurosci*. 2003;23: 10650–10661.
44. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. *Nat Rev Neurosci*. 2005;6:215–229.
45. Mtchedlishvili Z, Kapur J. High-affinity, slowly desensitizing $GABA_A$ receptors mediate tonic inhibition in hippocampal dentate granule cells. *Mol Pharmacol*. 2006;69:564–575.
46. Nusser Z, Mody I. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J Neurophysiol*. 2002;87:2624–2628.
47. Saxena NC, MacDonald RL. Assembly of $GABA_A$ receptor subunits: role of the δ subunit. *J Neurosci*. 1994;14:7077–7086.
48. Jones A, Korpi ER, McKernan RM, Pelz R, Nusser Z, Makela R, Mellor JR, Pollard S, Bahn S, Stephenson FA, Randall AD, Sieghart W, Somogyi P, Smith AJ, Wisden W. Ligand-gated ion channel subunit partnerships: $GABA_A$ receptor $\alpha6$ subunit gene inactivation inhibits δ subunit expression. *J Neurosci*. 1997;17: 1350–1362.
49. Peng Z, Hauer B, Mihalek RM, Homanics GE, Sieghart W, Olsen RW, Houser CR. $GABA_A$ receptor changes in δ subunit-deficient mice: altered expression of $\alpha4$ and $\gamma2$ subunits in the forebrain. *J Comp Neurol*. 2002;446:179–197.
50. Sun C, Sieghart W, Kapur J. Distribution of $\alpha1$, $\alpha4$, $\gamma2$, and δ subunits of $GABA_A$ receptors in hippocampal granule cells. *Brain Res*. 2004;1029:207–216.
51. Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR, McKernan RM. Preferential coassembly of $\alpha4$ and δ subunits of the $GABA_A$ receptor in rat thalamus. *Mol Pharmacol*. 1999;56:110–115.
52. Bencsits E, Ebert V, Tretter V, Sieghart W. A significant part of native $GABA_A$ receptors containing $\alpha4$ subunits do not contain γ or δ subunits. *J Biol Chem*. 1999;274:19613–19616.
53. Quirk K, Gillard NP, Ragan CI, Whiting PJ, McKernan RM. Model of subunit composition of $GABA_A$ receptor subtypes expressed in rat cerebellum with respect to their α and γ/δ subunits. *J Biol Chem*. 1994;269:16020–16028.
54. Kapur J, Coulter DA. Experimental status epilepticus alters $GABA_A$ receptor function in CA1 pyramidal neurons. *Ann Neurol*. 1995;38:893–900.
55. Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J. Subunit-specific trafficking of $GABA_A$ receptors during status epilepticus. *J Neurosci*. 2008;28:2527–2538.
56. Naylor DE, Liu H, Wasterlain CG. Trafficking of $GABA_A$ receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci*. 2005;25:7724–7733.
57. Terunuma M, Xu J, Vithlani M, Sieghart W, Kittler J, Pangalos M, Haydon PG, Coulter DA, Moss SJ. Deficits in phosphorylation of $GABA_A$ receptors by intimately associated protein kinase C activity underlie compromised synaptic inhibition during status epilepticus. *J Neurosci*. 2008;28:376–384.
58. Feng HJ, Mathews GC, Kao C, Macdonald RL. Alterations of $GABA_A$ receptor function and allosteric modulation during development of status epilepticus. *J Neurophysiol*. 2008;99:1285–1293.
59. Kittler JT, Moss SJ. Modulation of $GABA_A$ receptor activity by phosphorylation and receptor trafficking: implications for the efficacy of synaptic inhibition. *Curr Opin Neurobiol*. 2003;13:341–347.
60. Michels G, Moss SJ. $GABA_A$ receptors: properties and trafficking. *Crit Rev Biochem Mol Biol*. 2007;42:3–14.
61. Fukunaga K, Soderling TR, Miyamoto E. Activation of Ca^{2+}/calmodulin-dependent protein kinase II and

protein kinase C by glutamate in cultured rat hippocampal neurons. *J Biol Chem*. 1992;267:22527–22533.
62. Bannai H, Lévi S, Schweizer C, Inoue T, Launey T, Racine V, Sibarita JB, Mikoshiba K, Triller A. Activity-dependent tuning of inhibitory neurotransmission based on $GABA_A$ receptor diffusion dynamics. *Neuron*. 2009;62:670–682.
63. Merlin LR. Impact of protein kinase C activation on status epilepticus and epileptogenesis: oh, what a tangled web. *Epilepsy Curr*. 2008;8:101–103.
64. Daigen A, Akiyama K, Itoh T, Kohira I, Sora I, Morimoto K, Otsuki S. Long-lasting enhancement of the membrane-associated protein kinase C activity in the hippocampal kindled rat. *Jpn J Psychiatry Neurol*. 1991;45:297–301.
65. Guglielmetti F, Rattray M, Baldessari S, Butelli E, Samanin R, Bendotti C. Selective up-regulation of protein kinase C epsilon in granule cells after kainic acid-induced seizures in rat. *Brain Res Mol Brain Res*. 1997;49:188–196.
66. Fuortes MG, Faria LC, Merlin LR. Impact of protein kinase C activation on epileptiform activity in the hippocampal slice. *Epilepsy Res*. 2008;82:38–45.
67. Jovanovic JN, Thomas P, Kittler JT, Smart TG, Moss SJ. Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating $GABA_A$ receptor phosphorylation, activity, and cell-surface stability. *J Neurosci*. 2004;24:522–530.
68. Kurz JE, Sheets D, Parsons JT, Rana A, Delorenzo RJ, Churn SB. A significant increase in both basal and maximal calcineurin activity in the rat pilocarpine model of status epilepticus. *J Neurochem*. 2001;78:304–315.
69. Wang A, Chi Z, Wang S, Wang S, Sun Q. Calcineurin-mediated $GABA_A$ receptor dephosphorylation in rats after kainic acid-induced status epilepticus. *Seizure*. 2009;18:519–523.
70. Goodkin HP, Kapur J. The impact of diazepam's discovery on the treatment and understanding of status epilepticus. *Epilepsia*. 2009;50:2011–2018.
71. Joshi S, Kapur J. Slow intracellular accumulation of $GABA_A$ receptor δ subunit is modulated by brain-derived neurotrophic factor. *Neuroscience*. 2009;164:507–519.
72. Kokate TG, Cohen AL, Karp E, Rogawski MA. Neuroactive steroids protect against pilocarpine- and kainic acid-induced limbic seizures and status epilepticus in mice. *Neuropharmacology*. 1996;35:1049–1056.
73. Kapur J. Status epilepticus in epileptogenesis. *Curr Opin Neurol*. 1999;12:191–195.
74. Coulter DA. Chronic epileptogenic cellular alterations in the limbic system after status epilepticus. *Epilepsia.* 1999;40(suppl 1):S23–S33.
75. Dudek FE, Sutula TP. Epileptogenesis in the dentate gyrus: a critical perspective. In: Scharfman HE, ed. *Progress in Brain Research. The Dentate Gyrus: A Comprehensive Guide to Structure, Function, and Clinical Implications*. Amsterdam: Elsevier; 2007:755–773.
76. Mtchedlishvili Z, Bertram EH, Kapur J. Diminished allopregnanolone enhancement of $GABA_A$ receptor currents in a rat model of chronic temporal lobe epilepsy. *J Physiol*. 2001;537:453–465.
77. Sun C, Mtchedlishvili Z, Erisir A, Kapur J. Diminished neurosteroid sensitivity of synaptic inhibition and altered location of the α4 subunit of $GABA_A$ receptors in an animal model of epilepsy. *J Neurosci*. 2007;27:12641–12650.
78. Zhang N, Wei W, Mody I, Houser CR. Altered localization of $GABA_A$ receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *J Neurosci*. 2007;27:7520–7531.
79. Rajasekaran K, Joshi S, Sun C, Mtchedlishvilli Z, Kapur J. Receptors with low affinity for neurosteroids and GABA contribute to tonic inhibition of granule cells in epileptic animals. *Neurobiol Dis.* 2010;40(2):490–501.
80. Reddy DS, Rogawski MA. Neurosteroid replacement therapy for catamenial epilepsy. *Neurotherapeutics.* 2009;6:392–401.
81. Herzog AG, Frye CA. Seizure exacerbation associated with inhibition of progesterone metabolism. Ann Neurol. 2003;53:390–391.
82. Biagini G, Longo D, Baldelli E, Zoli M, Rogawski MA, Bertazzoni G, Avoli M. Neurosteroids and epileptogenesis in the pilocarpine model: evidence for a relationship between P450scc induction and length of the latent period. *Epilepsia*. 2009;50(suppl 1):53–58.
83. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in $GABA_A$ receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci.* 2005;8:797–804.
84. Lawrence C, Martin BS, Sun C, Williamson J, Kapur J. Endogenous neurosteroid synthesis modulates seizure frequency. *Ann Neurol*. 2010;67:689–693.

Chapter 42

Plasticity of $GABA_A$ Receptors Relevant to Neurosteroid Actions

Istvan Mody

It is now widely accepted that phasic (synaptic) and tonic (extrasynaptic) GABAergic influences on neuronal excitability are mediated by different receptors with different pharmacological profiles. This chapter will focus on the plasticity of the neurosteriod-sensitive δ subunit containing gamma-aminobutyric acid A ($GABA_A$) receptors (δ-$GABA_A$Rs) in animal models of temporal lobe epilepsies (TLE) and its possible consequences for modulating the excitability of the dentate gyrus.

$GABA_A$RS RESPONSIBLE FOR TONIC INHIBITION

The GABAergic system of the mammalian brain consists of neurons that release GABA and receptors that bind GABA. The GABA-releasing cells are extraordinarily diverse and highly specialized.[1] Some GABAergic cells control the activity in a local network (interneurons, INs), while others constitute the output of a well-defined structure (e.g., striatal medium spiny neurons, MSNs). The receptors for GABA are also diverse and specific.[2] $GABA_A$Rs are members of the Cys-loop receptor family and are present on virtually every neuron in the brain, performing different functions, depending on their synaptic or extrasynaptic localization.

In the mammalian central nervous system (CNS), GABA synapses were long known to generate fast, precisely timed activity in the form of inhibitory postsynaptic currents (IPSCs or phasic inhibition).[3] Over the past decade, diffusional inhibitory transmission, mediated by $GABA_A$Rs located outside the synapses and activated by the GABA levels present in the extracellular space or spilled over from other synapses, has triggered a great deal of interest.[4–9] This form of inhibition is generally referred to as *tonic inhibition*, while the conductance generated by the $GABA_A$Rs involved is known as *tonic conductance*.[10]

The five coassembled subunits of $GABA_A$Rs consist of at least three different proteins

selected from 19 subunits. These include α1–6, β1–4, γ1–3, δ, ε, θ, π, and ρ1–2.[11,12] The assembled subunits determine the specific anatomical localization of $GABA_A$Rs,[13] probably due to various anchoring and trafficking mechanisms specific to subcellular compartments or brain regions,[14] The receptors' developmental profiles, and their physiological and pharmacological properties, are also determined by their subunit composition[2,15] and constitute an area of great interest as highly specific drug targets in the CNS.[12] $GABA_A$Rs responsible for mediating a current that is "always on" should fulfill certain criteria. One of the first requirements would be to have a sufficiently high affinity for the agonist to be activated by the near-micromolar GABA concentrations present in the extracellular space,[16] This is in sharp contrast to the $GABA_A$Rs situated at synapses, which do not have to be of high affinity to react rapidly to fast rises in GABA to 1.5–3 mM that decay within a few hundred microseconds.[17] However, it is not trivial to establish the correct affinity of native $GABA_A$Rs. Another important factor in the tonic activation of $GABA_A$Rs is desensitization,[18] This common property of ligand-gated ion channels refers to their state characterized by long closed periods while the agonist is still bound. Simultaneous openings of a very large number of desensitizing receptors could easily sum to produce a tonic current, but receptors with little desensitization, devoid of such long nonconducting states, would certainly be better suited to mediate conductance in the continuous presence of a ligand.

The $GABA_A$Rs containing δ subunits in combination with either α4 or α6 and β2 or β3 subunits satisfy both the high-affinity and limited-desensitization criteria. Their half-maximal activation by GABA (EC_{50}) is in the tens of nanomolar range, well within the range of GABA found in the extracellular space,[19,20] The δ subunit-containing $GABA_A$Rs also have a low degree of desensitization.[21–23] In addition, these subunits have two other interesting properties that render them the prime mediators of tonic inhibition throughout the brain. The first is their extra- and perisynaptic ocalization. The δ subunit-containing $GABA_A$Rs are scattered over the surface of cerebellar granule cells[24] at locations far from the synapses (extrasynaptically). In the granule cells of the dentate gyrus, another area of the brain with high levels of δ subunits, the same receptors are localized somewhat closer to the outside edges of synapses (perisynaptically); this is an ideal location to sense GABA spilled over following vesicular release from nearby boutons or to be activated by the ambient levels of GABA present in the extracellular space.[25] The second property of the δ subunit-containing $GABA_A$Rs is the inefficiency of coupling GABA binding to channel gating; that is, GABA is a low-efficacy agonist at δ subunit-containing $GABA_A$Rs. It is not intuitively obvious why GABA should be a low-efficacy agonist at δ subunit-containing $GABA_A$Rs while their affinity for GABA is very high. But this interesting property means that the predominant mechanism for enhancing the function of these receptors may involve increasing the efficacy of GABA as an agonist instead of increasing their already exceptionally high affinity for GABA. This property may be critical in mediating the actions of the most potent positive endogenous modulators of $GABA_A$R function, that is, the actions of 3α-hydroxy ring A-reduced pregnane steroids (neurosteroids) that are brain-derived metabolites of ovarian steroids and corticosteroids with sedative-hypnotic, anti-convulsant, and anxiolytic effects.[26,27]

Neurosteroids act to enhance the efficacy of GABA at δ-$GABA_A$Rs.[22,23] The low efficacy of GABA at these receptors also means that there might be other compounds that are more efficacious than GABA and may thus have specific therapeutic application. The GABA agonist THIP (4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol, or Gaboxadol) is such a compound[28] and has been proposed for the treatment of insomnia[29] and premenstrual dysphoric disorder[30] through its actions at δ-$GABA_A$Rs. Physiological/pharmacological approaches and the use of null mutants have shown unequivocally that in several cell types, including the cerebellar granule cells,[31] dentate gyrus granule cells,[31] thalamic neurons,[32,33] layer 2/3 pyramidal cells,[34] interneurons of the dentate molecular layer,[35] and neurogliaform cells of the cerebral cortex,[36] these receptors underlie the generation of a tonic GABA-dependent conductance. In addition, $GABA_A$Rs containing α5 subunits have been shown to be critically involved in mediating tonic currents in CA1 and CA3 pyramidal cells (PCs)[37,38] and in cortical layer 5 PCs.[39] Receptors devoid of γ subunits, that is, containing only α and β subunits that are highly sensitive to Zn^{2+}, have also

been shown to contribute to the tonic current recorded in hippocampal neurons.[40] Moreover, ε subunit-containing $GABA_A$Rs may not even require GABA for ligand-independent openings that could underlie a tonic current.[41] Several types of tonically active receptors may be found on the same cell, but their activation may require different conditions.[10,42]

Of the many $GABA_A$R types capable of mediating a tonic conductance, only some may be active during a given condition. As conditions change around the neurons, for example through alterations of extracellular GABA levels,[42] the presence of modulators, changes in GABA uptake, changes in localization of the GABA source, or some other factors, different fractions of tonically active $GABA_A$Rs may contribute to the total measured tonic current.[10] It is very likely that in any given cell the openings of various types of $GABA_A$Rs sum up to generate a tonic conductance, albeit with different fractional contributions.

When compared to the charge carried by the phasically active (synaptic) channels, the charge carried by tonically active receptors is invariably greater by a margin of 3:1 to 5:1.[2,7] This is not to say that the phasic conductance does not have a role in controlling excitability, but its actions have to be considered in a highly timed fashion depending on the input received by the interneurons as well as by their targets. In contrast, the presence of uninterrupted GABA conductance will control the overall gain of the neuronal input-output.[5,7,43,44]

ALTERED TONICALLY ACTIVE $GABA_A$RS IN THE DENTATE GYRUS IN AN ANIMAL MODEL OF TLE

Numerous changes in $GABA_A$R subunits take place in humans with TLE and in animal models of the disease.[45–52] These changes are complex and include both increased and decreased expression of several $GABA_A$R subunits.[52] Functional consequences of these changes are difficult to predict based on the nature of the plasticity alone. The cell types and specific cellular domains (e.g., soma and dendrites) in which the alterations occur; the location of the subunits at synaptic, perisynaptic, or extrasynaptic sites; and the resulting subunit composition of the modified receptors will all influence the function of the $GABA_A$Rs altered during the process of TLE.

Certain patterns of $GABA_A$R subunit plasticity are present in several TLE models. A common finding is decreased expression of the δ subunit along with increased expression of γ2 and α4 subunits in the dentate gyrus[46,47,51,53] that could alter both tonic and phasic inhibition in the dentate gyrus. An alteration specific for tonic inhibition of the dentate gyrus granule cells in TLE is the shift of the tonically active $GABA_A$Rs from a predominantly δ-$GABA_A$R-mediated tonic conductance to that mediated by α5-$GABA_A$Rs.[53,54]

The finding that γ2 subunits have shifted to a perisynaptic location in pilocarpine-treated animals[53] may indicate that these subunits could also form partnerships with subunits other than α4. A considerable portion of the tonic inhibition in the normal dentate gyrus granule cells is mediated by $GABA_A$Rs composed of α5βγ2 subunits, as noted by single- and double-knockout animal studies.[55] In fact, the results of most knockout studies are consistent with a mutual replacement of δ-$GABA_A$R- and α5-$GABA_A$R-mediated tonic inhibition when one type of the receptor is downregulated or missing.[55] This is also the pattern in a rat TLE model, where there is clear upregulation of α5-$GABA_A$R-mediated tonic inhibition that is sensitive to the α5-$GABA_A$R-selective benzodiazepine receptor inverse agonist, L,655–70854. The switch from predominantly δ-$GABA_A$R-mediated to predominantly α5-$GABA_A$R-mediated tonic inhibition has several pharmacological implications. First, the GABA concentrations required for activation of the tonic conductance in dentate gyrus granule cells will most likely be different in control and TLE granule cells. Although because of associations with other neuron-specific proteins, and neuron-specific posttranslational modifications, the exact affinities for GABA of these two receptor types in situ may differ from those determined in expression systems, δ-$GABA_A$Rs are traditionally regarded as having a high affinity for GABA (EC_{50} <1 μM).[20,28,56] In contrast, α5-$GABA_A$Rs should respond only to about 10-fold higher GABA concentrations,[37,57] which leads some to postulate that these receptors contribute to tonic GABA currents only when extracellular GABA becomes relatively high. Unfortunately, there are no studies available on how these receptors are activated in vivo and

the corresponding ranges of GABA concentrations in the extracellular space. Experiments in brain slices may be biased by artificial changes to several cellular and molecular components that control the tonic GABA conductances. These considerations require further investigations, particularly since a blocker of one of the principal components in controlling extracellular GABA levels, the plasma membrane GABA transporter GAT-1,[58] is a clinically approved antiepileptic drug (AED).

The second main consequence of the switch from a δ-$GABA_A$R-mediated tonic inhibition to one mediated by α5-$GABA_A$Rs relates to a profound change in the pharmacological properties of tonic inhibition. $GABA_A$Rs containing δ subunits are considered to be insensitive to benzodiazepines, because the presence of γ2 subunits is required for benzodiazepine sensitivity[59] and the two subunits appear to be mutually exclusive.[60] However, one study has found cerebellar δ-$GABA_A$Rs to be sensitive to flunitrazepam at a very low (~200 nM) ambient GABA concentration that was already sufficient to activate tonic inhibition,[61] In contrast, α5-$GABA_A$Rs are sensitive to benzodiazepines, but not to the allosteric modulator zolpidem. It remains to be determined whether the function of the TLE dentate gate in vivo can be better enhanced by benzodiazepines than by zolpidem. The shift to an α5-$GABA_A$R-mediated tonic inhibition in the TLE dentate gyrus presents a possible problem for the use of α5-$GABA_A$R specific benzodiazepine-site inverse agonists, which are being developed by several pharmaceutical companies as potential cognitive enhancer drugs.[62] The use of such drugs in animal models of TLE may answer the question about the excessive involvement of α5-$GABA_A$Rs in tonic inhibition of epileptic granule cells. Our own findings[53] indicate that the contribution of α5-$GABA_A$Rs to tonic inhibition is not necessarily increased in pilocarpine-treated mice, since the diazepam potentiation of the tonic current did not increase in the epileptic animals, as would be expected from an upregulation of α5-$GABA_A$Rs following a loss of diazepam-insensitive (α4βδ) receptors. It is therefore possible that in dentate gyrus of epileptic animals, diazepam-insensitive receptors (α4βγ2)[63,64] mediate a portion of the tonic current originally mediated by δ-$GABA_A$Rs.[53]

The third important consequence is also pharmacological in nature, and it relates to the sensitivity of the tonic inhibition in TLE granule cells to neurosteroids. Neurosteroid enhancement of tonic inhibition was substantially reduced in epileptic animals,[53] most likely related to the loss of neurosteroid-sensitive δ subunits[31] and consistent with our earlier findings of decreased modulation of field potentials by tetrahydrodeoxycorticosterone (3α,21-dihydroxy-5α-pregnan-20-one; THDOC) in pilocarpine-treated mice.[51] Others have also found diminished sensitivity of $GABA_A$Rs to the neurosteroid allopregnanolone in acutely dissociated dentate granule cells from epileptic rats 4–6 weeks after status epilepticus.[65] Such deficits in the normal modulation of tonic inhibition in the dentate gyrus could limit the adaptive response of the GABA system to increased excitability, particularly at times of altered hormonal levels or increased stress.[30,66]

Finally, an alternative possibility may also be considered. Following loss of δ-$GABA_A$Rs[53] from the granule cells in TLE, α4 subunits may need to combine only with β subunits to form tonically active $GABA_A$Rs. Such αβ $GABA_A$Rs have been suggested to mediate tonic inhibition in the rat CA1 region, although they appear to account for only a small fraction of the tonic current.[40] A subpopulation of native α4-containing receptors in rat does not contain either δ or γ2,[67] and thus associations of α4 subunits with only β subunits or with other subunits cannot be ruled out, although in light of the findings in α5/δ double-knockout animals that have very little, if any, tonic current in dentate granule cells,[55] the contribution of αβ $GABA_A$Rs in these cells is unlikely.

POSSIBLE CONSEQUENCES OF ALTERED NEUROSTEROID PHARMACOLOGY IN THE DENTATE GYRUS AFTER TLE

In the previous section, the focus of neurosteroid-sensitive $GABA_A$R plasticity was on the principal cells of the dentate gyrus, the granule cells. However, molecular layer interneurons also show δ-$GABA_A$R-mediated tonic conductances, although the δ subunits are associated with α1 subunits in these neurons[35] rather than with α4 subunits, as in the granule cells. In contrast to the granule cells, the molecular layer interneurons do not lose the δ subunits. In fact,

there appears to be an upregulation in δ subunit staining in these cells in pilocarpine-treated mice.[51] We have recorded from these neurons after TLE induced by pilocarpine in mice and have established a significant increase in their tonic neurosteroid-sensitive GABA conductance (Wei and Mody, unpublished). Such an alteration may have significant consequences for the excitability of the dentate gate. In the chronic phase of TLE, the actions of neurosteroids and alcohol will no longer enhance the tonic GABAergic conductance of granule cells. In contrast, these compounds will potentiate an already larger than normal tonic GABA conductance in the feedforward interneurons, most likely resulting in disinhibition.

The effects of stress-related neurosteroids and ethanol on the function of the dentate gate in vivo remain to be determined. Nevertheless, we have carried out a set of simulations of dentate gyrus excitability (Santhakumar and Mody, unpublished) using a realistic neuron- and connectivity-based model of the dentate.[68] The model has shown that increasing a tonic GABA conductance alone in the molecular layer interneurons is not sufficient by itself to trigger repetitive activity in the dentate gyrus. However, enhancing this conductance further with the stress-related neurosteroid THDOC will result in the induction of repetitive discharges in the dentate gyrus, particularly when the altered tonically active $GABA_A$Rs of the granule cells are no longer neurosteroid sensitive, as occurs in TLE. This effect is intriguing, considering the considerable excitability-dampening effect of neurosteroids in the nonepileptic dentate gyrus where both interneuronal and granule cell tonic $GABA_A$ conductances are neurosteroid-sensitive. The diametrically opposite effects of THDOC before and after onset of TLE on perforant path-evoked field excitatory postsynaptic potentials (EPSPs) have previously been reported.[51]

An even more interesting scenario may emerge if it becomes evident that the molecular layer interneurons are in fact neurogliaform cells, which have recently been reported to have high expression of δ subunits and to be responsible for GABA volume transmission.[36] Lowering the excitability of these cells by neurosteroids in TLE may then lead to lowering of ambient GABA levels, which could affect the activation of the lower-affinity tonically active $GABA_A$Rs that have replaced the δ-$GABA_A$Rs of granule cells.

In summary, the complex changes in $GABA_A$Rs generating tonic and phasic inhibition in the dentate gyrus on principal cells and inhibitory interneurons alike will result in profound alterations in the excitability of this structure in association with TLE. In particular, the response of the dentate gate to endogenous (neurosteroids) and exogenous (e.g., alcohol, benzodiazepines) modulators will be affected, which could lead to distinct effects of these modulators in the epileptic brain that will not be encountered in controls. These findings and predictions may also be important to states comorbid with epilepsy, including stress, depression, and changes in the levels of ovarian hormones during the menstrual cycle, pregnancy, or menopause.

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REFERENCES

1. Freund TF, Buzsáki G. Interneurons of the hippocampus. *Hippocampus.* 1996;6:347–470.
2. Mody I, Pearce RA. Diversity of inhibitory neurotransmission through GABA(A) receptors. *Trends Neurosci.* 2004;27:569–575.
3. Mody I, De Koninck Y, Otis TS, Soltesz I. Bridging the cleft at GABA synapses in the brain. *Trends Neurosci.* 1994;17:517–525.
4. Mody I. Distinguishing between GABA(A) receptors responsible for tonic and phasic conductances. *Neurochem Res.* 2001;26:907–913.
5. Semyanov A, Walker MC, Kullmann DM, Silver RA. Tonically active GABA(A) receptors: modulating gain and maintaining the tone. *Trends Neurosci.* 2004;27:262–269.
6. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci.* 2005;6:215–229.

7. Cavelier P, Hamann M, Rossi D, Mobbs P, Attwell D. Tonic excitation and inhibition of neurons: ambient transmitter sources and computational consequences. *Prog Biophys Mol Biol.* 2005;87:3–16.
8. Semyanov AV. Diffusional extrasynaptic neurotransmission via glutamate and GABA. *Neurosci Behav Physiol.* 2005;35:253–266.
9. Vizi ES, Mike A. Nonsynaptic receptors for GABA and glutamate. *Curr Top Med Chem.* 2006;6:941–948.
10. Glykys J, Mody I. Activation of GABA(A) receptors: views from outside the synaptic cleft. *Neuron.* 2007;56: 763–770.
11. Sieghart W, Sperk G. Subunit composition, distribution and function of $GABA_A$ receptor subtypes. *Curr Top Med Chem.* 2002;2:795–816.
12. Whiting PJ. GABA-A receptor subtypes in the brain: a paradigm for CNS drug discovery? *Drug Discov Today.* 2003;8:445–450.
13. Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience.* 2000;101:815–850.
14. Moss SJ, Smart TG. Constructing inhibitory synapses. *Nat Rev Neurosci.* 2001;2:240–250.
15. Hevers W, Lüddens H. The diversity of GABA(A) receptors. Pharmacological and electrophysiological properties of $GABA_A$ channel subtypes. *Mol Neurobiol.* 1998;18:35–86.
16. Nyitrai G, Kekesi KA, Juhasz G. Extracellular level of GABA and Glu: in vivo microdialysis-HPLC measurements. *Curr Top Med Chem.* 2006;6:935–940.
17. Mozrzymas JW, Barberis A, Mercik K, Zarnowska ED. Binding sites, singly bound states, and conformation coupling shape GABA-evoked currents. *J Neurophysiol.* 2003;89:871–883.
18. Jones MV, Westbrook GL. The impact of receptor desensitization on fast synaptic transmission. *Trends Neurosci.* 1996;19:96–101.
19. Saxena NC, Macdonald RL. Assembly of GABA(A) receptor subunits: Role of the δ subunit. *J Neurosci.* 1994;14:7077–7086.
20. Wallner M, Hanchar HJ, Olsen RW. Ethanol enhances α4β3δ and α6β3δ GABA(A) receptors at low concentrations known to have effects in humans. *Proc Natl Acad Sci USA.* 2003;100:15218–15223.
21. Haas KF, Macdonald RL. GABA(A) receptor subunit gamma2 and delta subtypes confer unique kinetic properties on recombinant GABA(A) receptor currents in mouse fibroblasts. *J Physiol* (*Lond*). 1999;514(pt 1): 27–45.
22. Wohlfarth KM, Bianchi MT, Macdonald RL. Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit. *J Neurosci.* 2002;22: 1541–1549.
23. Bianchi MT, Macdonald RL. Neurosteroids shift partial agonist activation of GABA(A) receptor channels from low- to high-efficacy gating patterns. *J Neurosci.* 2003;23:10934–10943.
24. Nusser Z, Sieghart W, Somogyi P. Segregation of different GABA(A) receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci.* 1998;18:1693–1703.
25. Wei W, Zhang N, Peng Z, Houser CR, Mody I. Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. *J Neurosci.* 2003;23:10650–10661.
26. Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science.* 1986;232:1004–1007.
27. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci.* 2005;6:565–575.
28. Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA. Pharmacological characterization of a novel cell line expressing human α4β3δ GABA(A) receptors. *Br J Pharmacol.* 2002;136:965–974.
29. Wafford KA, Ebert B. Gaboxadol—a new awakening in sleep. *Curr Opin Pharmacol.* 2006;6:30–36.
30. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci.* 2005;8:797–804.
31. Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABA(A) receptors. *Proc Natl Acad Sci USA.* 2003;100:14439–14444.
32. Cope DW, Hughes SW, Crunelli V. GABA(A) receptor-mediated tonic inhibition in thalamic neurons. *J Neurosci.* 2005;25:11553–11563.
33. Bright DP, Aller MI, Brickley SG. Synaptic release generates a tonic GABA(A) receptor-mediated conductance that modulates burst precision in thalamic relay neurons. *J Neurosci.* 2007;27:2560–2569.
34. Drasbek KR, Jensen K. THIP, a hypnotic and antinociceptive drug, enhances an extrasynaptic GABA(A) receptor-mediated conductance in mouse neocortex. *Cereb Cortex.* 2006;16:1134–1141.
35. Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I. A new naturally occurring GABA(A) receptor subunit partnership with high sensitivity to ethanol. *Nat Neurosci.* 2007;10:40–48.
36. Olah S, Fule M, Komlosi G, et al. Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. *Nature.* 2009;461:1278–1281.
37. Caraiscos VB, Elliott EM, You T, et al. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by α5 subunit-containing γ-aminobutyric acid type A receptors. *Proc Natl Acad Sci USA.* 2004;101:3662–3667.
38. Glykys J, Mody I. Hippocampal network hyperactivity after selective reduction of tonic inhibition in GABA(A) receptor α5 subunit-deficient mice. *J Neurophysiol* 2006;95:2796–2807.
39. Yamada J, Furukawa T, Ueno S, Yamamoto S, Fukuda A. Molecular basis for the GABA(A) receptor-mediated tonic inhibition in rat somatosensory cortex. *Cereb Cortex.* 2007;17:653–660.
40. Mortensen M, Smart TG. Extrasynaptic α/β subunit GABA(A) receptors on rat hippocampal pyramidal neurons. *J Physiol.* 2006;577:841–856.
41. McCartney MR, Deeb TZ, Henderson TN, Hales TG. Tonically active GABA(A) receptors in hippocampal pyramidal neurons exhibit constitutive GABA-independent gating. *Mol Pharmacol.* 2007;71:539–548.
42. Scimemi A, Semyanov A, Sperk G, Kullmann DM, Walker MC. Multiple and plastic receptors mediate tonic GABA(A) receptor currents in the hippocampus. *J Neurosci.* 2005;25:10016–10024.
43. Mitchell SJ, Silver RA. Shunting inhibition modulates neuronal gain during synaptic excitation. *Neuron.* 2003;38:433–445.

44. Chadderton P, Margrie TW, Hausser M. Integration of quanta in cerebellar granule cells during sensory processing. *Nature.* 2004;428:856–860.
45. Rice A, Rafiq A, Shapiro SM, Jakoi ER, Coulter DA, DeLorenzo RJ. Long-lasting reduction of inhibitory function and gamma-aminobutyric acid type A receptor subunit mRNA expression in a model of temporal lobe epilepsy. *Proc Natl Acad Sci USA.* 1996;93: 9665–9669.
46. Schwarzer C, Tsunashima K, Wanzenböck C, Fuchs K, Sieghart W, Sperk G. GABA(A) receptor subunits in the rat hippocampus.2. Altered distribution in kainic acid-induced temporal lobe epilepsy. *Neuroscience.* 1997;80:1001–1017.
47. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat Med.* 1998;4:1166–1172.
48. Fritschy JM, Kiener T, Bouilleret V, Loup F. GABAergic neurons and GABA(A)-receptors in temporal lobe epilepsy. *Neurochem Int.* 1999;34:435–445.
49. Loup F, Wieser HG, Yonekawa Y, Aguzzi A, Fritschy JM. Selective alterations in GABA(A) receptor subtypes in human temporal lobe epilepsy. *J Neurosci.* 2000;20:5401–5419.
50. Houser CR, Esclapez M. Downregulation of the alpha5 subunit of the GABA(A) receptor in the pilocarpine model of temporal lobe epilepsy. *Hippocampus.* 2003;13:633–645.
51. Peng Z, Huang CS, Stell BM, Mody I, Houser CR. Altered expression of the delta subunit of the GABA(A) receptor in a mouse model of temporal lobe epilepsy. *J Neurosci.* 2004;24:8629–8639.
52. Coulter DA, Carlson GC. Functional regulation of the dentate gyrus by GABA-mediated inhibition. *Prog Brain Res.* 2007;163:235–243.
53. Zhang N, Wei W, Mody I, Houser CR. Altered localization of GABA(A) receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *J Neurosci.* 2007;27:7520–7531.
54. Zhan RZ, Nadler JV. Enhanced tonic GABA current in normotopic and hilar ectopic dentate granule cells after pilocarpine-induced status epilepticus. *J Neurophysiol.* 2009;102:670–681.
55. Glykys J, Mann EO, Mody I. Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? *J Neurosci.* 2008;28:1421–1426.
56. Storustovu SI, Ebert B. Pharmacological characterization of agonists at delta-containing GABA(A) receptors: functional selectivity for extrasynaptic receptors is dependent on the absence of gamma2. *J Pharmacol Exp Ther.* 2006;316:1351–1359.
57. Burgard EC, Tietz EI, Neelands TR, Macdonald RL. Properties of recombinant gamma-aminobutyric acid(A) receptor isoforms containing the α5 subunit subtype. *Mol Pharmacol.* 1996;50:119–127.
58. Chiu CS, Brickley S, Jensen K, et al. GABA transporter deficiency causes tremor, ataxia, nervousness, and increased GABA-induced tonic conductance in cerebellum. *J Neurosci.* 2005;25:3234–3245.
59. Pritchett DB, Sontheimer H, Shivers BD, et al. Importance of a novel GABA(A) receptor subunit for benzodiazepine pharmacology. *Nature.* 1989;338: 582–585.
60. Shivers BD, Killisch I, Sprengel R, et al. Two novel GABA(A) receptor subunits exist in distinct neuronal subpopulations. *Neuron.* 1989;3:327–337.
61. Santhakumar V, Hanchar HJ, Wallner M, Olsen RW, Otis TS. Contributions of the GABA(A) receptor alpha6 subunit to phasic and tonic inhibition revealed by a naturally occurring polymorphism in the alpha6 gene. *J Neurosci.* 2006;26:3357–3364.
62. Atack JR, Bayley PJ, Seabrook GR, Wafford KA, McKernan RM, Dawson GR. L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for α5-containing GABA(A) receptors. *Neuropharmacology.* 2006;51:1023–1029.
63. Wisden W, Herb A, Wieland H, Keinanen K, Lüddens H, Seeburg PH. Cloning, pharmacological characteristics and expression pattern of the rat GABA(A) receptor α4 subunit. *FEBS Lett.* 1991;289: 227–230.
64. Benke D, Michel C, Möhler H. GABA(A) receptors containing the α4-subunit. Prevalence, distribution, pharmacology, and subunit architecture in situ. *J Neurochem.* 1997;69:806–814.
65. Mtchedlishvili Z, Bertram EH, Kapur J. Diminished allopregnanolone enhancement of GABA(A) receptor currents in a rat model of chronic temporal lobe epilepsy. *J Physiol.* 2001;537:453–465.
66. Maguire J, Mody I. GABA(A)R plasticity during pregnancy: relevance to postpartum depression. *Neuron.* 2008;59:207–213.
67. Bencsits E, Ebert V, Tretter V, Sieghart W. A significant part of native gamma-aminobutyric acid(A) receptors containing alpha4 subunits do not contain gamma or delta subunits. *J Biol Chem.* 1999;274:19613–19616.
68. Dyhrfjeld-Johnsen J, Santhakumar V, Morgan RJ, Huerta R, Tsimring L, Soltesz I. Topological determinants of epileptogenesis in large-scale structural and functional models of the dentate gyrus derived from experimental data. *J Neurophysiol.* 2007;97: 1566–1587.

Chapter 43

$GABA_A$ Receptor Plasticity in Alcohol Withdrawal

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Igor Spigelman

INTRODUCTION

Alcohol, the fruit of the vine and the braumeister's ware, has been one of the most popular drugs in the world throughout history and one of the most abused. The development of dependence after chronic use of ethanol (EtOH) depends on two parallel effects of the drug on the brain each time it is used: stimulation of the reward pathway and subsequent triggering of a small but significant withdrawal. There is rebound hyperexcitability following the initial action of EtOH as a central nervous system (CNS) depressant and triggering of some adaptive process, that is, molecular changes associated with tolerance.[1,2] Each of these "mini-withdrawals" reflects transient plasticity in the brain affecting the balance of excitation and inhibition. The simplest description of the changes could be, for example, the ratio of glutamate and gamma-aminobutyric acid (GABA) neurotransmitter activities.

Whatever the mechanism, a fairly well-supported theory of how repeated use of EtOH leads to a dependent condition is that the chronic repetition of the mini-withdrawals leads to a persistent state of withdrawal (alcohol withdrawal syndrome, AWS) in which the withdrawals become more severe and long-lasting, eventually becoming permanent. In other words, repetition turns a relatively normal brain activity involving plasticity into a pathological condition of uncontrolled hyperactivity. This is reminiscent of the kindling phenomenon in epilepsy research, in which seizures can be triggered by subconvulsant stimuli after they have been repeated over and over[3,4]; eventually, seizures can become spontaneous, and once they do, they can occur for the rest of the person's life. One facet of human alcohol dependence is increased seizure susceptibility, and delirium tremens and frank seizures are triggered by withdrawal from EtOH in very heavy abusers.[5] Greater susceptibility and/or severity of seizures is produced by greater periods of EtOH abuse and by previous withdrawals and/or withdrawal seizures. When the number of previous exposures and

withdrawal episodes reaches a certain threshold, the severe withdrawal (AWS) becomes persistent, possibly permanent. This led to the conclusion of a kindling-like phenomenon in human EtOH dependence.[6–9] However, a significant reduction in seizure threshold can be measured during the mini-withdrawals experienced in rats after EtOH administration.[10,11] This suggests that seizure susceptibility is, first, an integral component of withdrawal. Second, the increased severity and persistence of seizure susceptibility are signs of and critical ingredients of alcohol dependence. Numerous animal models employ this kindling-like regimen of intermittent episodes of EtOH intoxication and withdrawal, termed *chronic intermittent ethanol* (CIE),[12–16] and we have been studying the CIE rat model for 20 years.[11] Indeed, there are many similarities between the development of drug dependence, especially alcoholism, and the kindling model of epilepsy, as well as with other models and theories of epileptogenesis.

Our research has established that an important aspect of alcohol's acute action on the brain is enhancement of inhibition mediated by GABA and especially $GABA_A$ receptors (GABAR), and that withdrawal includes as a critical component a reduction in GABAR-mediated inhibition. The behavioral changes of AWS can be explained by persistently reduced GABAR-mediated inhibition due to EtOH-induced plasticity of GABAR. When this becomes persistent due to the CIE treatment, the condition can be termed *aberrant plasticity*. We mentioned that the receptors for the very important rapid neurotransmitters glutamate, and especially GABA, are liable to aberrant plasticity and are in a position to do the most harm. In the case of CIE, the treated individual has all the signs of AWS, which is an extreme hyperexcitable condition. Increased seizure susceptibility would seem to be a critical aspect of epileptogenesis, but we do not know what additional factors, if any, are required to generate actual spontaneous seizures (epilepsy). The CIE rats (or mice) have not been observed to exhibit spontaneous seizures, but this has not been studied carefully enough to conclude that there are none.

The ratio of excitation to inhibition is so important that a new concept called *scaling* has gained prominence; in scaling, compensatory changes in either excitation or inhibition accompany any perturbation of the other process.[17] We provide examples in which the deciding factor for aberrant plasticity is reduced GABAergic inhibitory function, which seems particularly susceptible to derangement. These examples cover several chronic drug models as well as epilepsy (see the "Discussion" section below). It is known that application of GABAergic drugs, or even GABA itself, to the mammalian cerebral cortex produces withdrawal signs upon removal[18]; that even an hour's exposure can produce long-lasting focal seizures upon termination, the *GABA withdrawal syndrome*[18–20]; and that modified GABAR are found in many types of human and experimental epilepsy[21,22] (cf. Chapter 57, this volume). Our hypothesis concerning alcohol withdrawal is that the extrasynaptic GABAR subtypes containing the δ subunit that are most sensitive to low doses of EtOH[23–26] are the first targets of acute EtOH action and the first to show plasticity in the face of chronic EtOH stimulation. Alcohol is accepted to have a GABA-mimetic effect. However, some important effects of EtOH on GABA-mediated inhibition may be presynaptic.[27] Another important concept is that plasticity most often involves changes in protein trafficking[28,29] rather than gene expression, especially for the early events. This does not necessarily apply to the events leading to persistent alterations in drug dependence or epileptogenesis.

RESULTS

The CIE Model and Its Relationship to Human Alcoholism

Twenty years ago, Kokka and Olsen set out to establish a rat model of the kindling hypothesis of alcohol dependence in humans[6] and to investigate the possible role of $GABA_A$ receptors.[11] The CIE regimen, with 5–6 g/kg EtOH administered to rats by gavage per day for 60 days, was found to reduce the seizure threshold to the GABAergic convulsant drug pentylenetetrazol (PTZ), administered by slow tail vein injection, and this change lasted at least 40 days after EtOH was stopped; importantly, the persistence of the changes (*kindling*) was dependent on the intermittent drug administration regimen, since continuous administration of EtOH for several days without withdrawal led

to one big withdrawal, possibly including seizures, upon cessation but no detectable change at 2 days or any time thereafter.[11] In other words, the animals rapidly forgot about the EtOH exposure but not the multiple withdrawals. Other workers have demonstrated that the intermittent administration of EtOH, including periods of deprivation, can increase voluntary consumption (e.g., refs. 14 and 30).

We[31] showed that in CIE, GABAR binding was not much affected throughout the brain but that GABAR function, assessed with a neurochemical assay of GABA-stimulated[36] Cl^- flux in brain slices, was impaired specifically in hippocampal formation, but not in inferior colliculus, several lobes of cortex, thalamus, striatum, or cerebellum. Using extracellular electrode recording in hippocampal slices in collaboration with Dr. Igor Spigelman, we demonstrated a parallel reduction in paired-pulse inhibition[31] that was consistent with the increase in behavioral seizure susceptibility. Veatch and Gonzalez[32] presented similar evidence that intermittent EtOH with multiple withdrawals led to elevated excitability specifically in hippocampus, as detected by electroencephalography (EEG). We have further shown small changes in benzodiazepine (BZ) modulation of GABAR radioligand binding accompanied by a significant elevation in the GABAR α4 subunit mRNA assessed by in situ hybridization histochemistry; the increase was relatively larger in hippocampus than in thalamus, despite higher levels of the subunit in thalamus.[33] This is consistent with elevated BZ-insensitive GABAR and behavioral and cellular tolerance to BZ. Indeed, with intracellular sharp electrode recordings in hippocampal slices, we showed a reduction in allosteric modulation of GABAR-mediated postsynaptic potentials by BZ and steroids but not by EtOH. Ethanol enhancement of evoked synaptic potentials was, if anything, increased.[34] In situ hybridization and reverse transcriptase-polymerase chain reaction (RT-PCR) revealed several changes in GABAR subunits in CIE rat brain, including elevated γ2S in hippocampus and increased binding of the imidazobenzodiazepine radioligand [^{3}H]Ro15–4513 to diazepam-insensitive sites in cerebellum and forebrain, considered to involve the α6 and α4 subunits, respectively.[35] Similar increases in GABAR α4 subunit and smaller changes in some other subunits were observed by others in rodents treated with chronic EtOH (e.g., refs. 36 and 37). Measurements by most groups did not include significant withdrawal periods.

Using subunit-specific antibodies, we measured GABAR subunits by Western blotting in CIE rat hippocampus and demonstrated significant, persistent elevation in the α4 and γ2 subunits with a decrease in α1 and δ—in other words, a net "subunit switch" of α1 to α4 and δ to γ2. The same subunit changes have been reported for several animal models of temporal lobe epilepsy, as reported in Chapters 40, 41, and 44 of this volume, as we noted in the previous edition of *Jasper's Basic Mechanisms of the Epilepsies*.[21] Animals treated with CIE were shown to exhibit increased anxiety in the elevated plus maze assay, and behavioral tolerance to the sedative action of EtOH, BZ, and neurosteroids.[38] Steroids and BZ showed reduced enhancement of GABAR synaptic and tonic inhibitory currents in hippocampal neurons recorded by patch-clamp electrodes in CIE rats; sensitivity to the α4-selective agonists bretazenil and Ro15–4513 was not decreased but possibly increased; the GABA analogue THIP (tetrahydroisoxazolo-pyridin-3-ol) showed reduced activation of tonic currents but increased modulation of miniature inhibitory postsynaptic currents (mIPSCs).[39] These changes in cellular pharmacology are consistent with the net switch of GABAR subunits from α1 to α4. The mIPSCs showed less inhibitory charge transfer due to the contribution of GABAR α4 subunits to the synaptic current measured in CIE rats, as well as reduced tonic inhibitory currents, consistent with the hyperexcitable state.[38,39] Rats treated with CIE also exhibited decreased levels of endogenous neurosteroids in the hippocampus and impaired spatial learning.[40]

Importantly, changes in EtOH pharmacology were also found. We observed significant enhancement of GABAR-mediated tonic inhibitory currents in CA1 neurons and dentate gyrus granule cells by 50 mM EtOH in untreated rats, with a threshold effect at 10 mM.[41] This enhancement of GABAR-mediated tonic inhibitory currents by millimolar concentrations of EtOH was observed at the same time by Wei et al.[42] in dentate gyrus granule cells and by Hanchar et al.[24] in cerebellar granule cells. Rats treated with CIE showed a decreased amplitude of tonic inhibitory current and a correspondingly decreased enhancement

by low concentrations of EtOH. On the other hand, mIPSCs showed little effect of EtOH up to 100 mM in vehicle-treated control rats, but they were significantly enhanced by 10–30 mM EtOH in CIE rat hippocampus. We confirmed that in the absence of acute EtOH, reduced charge transfer of both synaptic and extrasynaptic GABAR currents was found in CIE rat hippocampal neurons compared to control neurons, consistent with the AWS state, and its associated increased anxiety and disturbed sleep. Further, immunostaining and electron microscopy showed that the α4 subunit, normally localized to the perisynaptic membrane and not to the synaptic membrane, was observed primarily in the synaptic region. The δ subunit is typically not found in the synaptic membrane but is instead localized perisynaptically in controls and did not change in CIE rats.[41] It seems that much of the altered physiology and pharmacology of hippocampal neurons in CIE can be explained by the changes in GABAR subunit composition observed, and this might also explain much of the behavioral phenotype and contribute to EtOH dependence.

The changes found after CIE treatment did not appear to involve any gross pathology in either brain or liver.[31] Microscopic examination of tissue sections revealed no evident changes in the morphology, number, and location of GABA-synthesizing neurons in hippocampus, thalamus, or neocortex.[33] Neuronal cell counts (by the UCLA School of Medicine pathology lab) were normal in the hippocampus and in several other regions of CIE rats; no increase in damaged or dying cells or inflammation markers was observed. Unbiased stereological cell counts in the nucleus accumbens of NeuN-stained sections showed no differences between CIE, single-dose EtOH, and vehicle-treated animals (Spigelman, Ahmad, and Olsen, unpublished). This is despite evidence that exposure to a single very high dose of EtOH with blood levels of over 300 mg/dL, as experienced in human binge drinking, or to a very high level of cumulative alcohol exposure, as in human chronic alcohol abuse, produced significant neuronal cell death.[43,44] We found no evidence for a significant increase in newborn neurons or for stem cell death in dentate gyrus of CIE rats versus normal controls (Spigelman, Olsen, and Crews, unpublished). Thus, in our hands, high blood levels of EtOH administered by gavage, exceeding 250 mg/dL for several hours but not exceeding 275 mg/dL,[45] were insufficient or too brief to produce the damage reported by other extreme exposures to alcohol. Nevertheless, CIE treatment is definitely a severe, abnormal stress to the brain.

In order to learn more about the mechanism of GABAR plasticity induced by CIE, we attempted to determine the minimum dose, duration, and frequency of EtOH administration required to produce the changes. We found that a single intoxicating dose of EtOH administered by gavage was able to induce many of the same changes in behavior, GABAR subunit composition, and hippocampal neuron pharmacology seen in CIE, but the changes were transient.[45] Thus, we showed that within 1 h the α4 and δ subunits, but not the α1 or γ2 subunits, were reduced at the cell surface, accompanied by loss of EtOH enhancement of tonic inhibitory currents but no change in synaptic pharmacology. Thus, the first target of EtOH action, the extrasynaptic δ subunit-containing GABAR,[25] are the first to respond with plastic changes. After 24 h but not at 1 h, one could detect increased cell surface and increased total levels of γ2 and α4 subunits, decreased levels of α1 subunit, and a tolerance to BZ enhancement of both extrasynaptic and synaptic currents. It appears that these changes are the result, at least in part, of altered gene expression. It is not known if these changes are triggered by the reduced tonic inhibition or even the reduced synaptic inhibition seen at several hours post-EtOH, or if altered protein synthesis may also be initiated by the EtOH exposure but requires a longer time to be measurable. Also, at 12–24 h, the animals exhibited tolerance to BZ-induced loss of righting reflex (LORR), and the synaptic currents became more sensitive to EtOH (as in CIE), but they returned to normal within a few days. The δ subunit remained low for 1–2 days and then returned to normal.[45] All the changes require repetition of the CIE regimen to become more persistent. These EtOH-induced plastic changes in GABAR are summarized in Fig. 43–1. Current studies are examining the precise time course of changes in GABAR subunits and function, demonstrating subunit partnerships using co-immunoprecipitation, and examining association of GABAR with other proteins such as trafficking chaperones and clustering factors, as well as kinases and phosphatases, looking for some causal relationships.

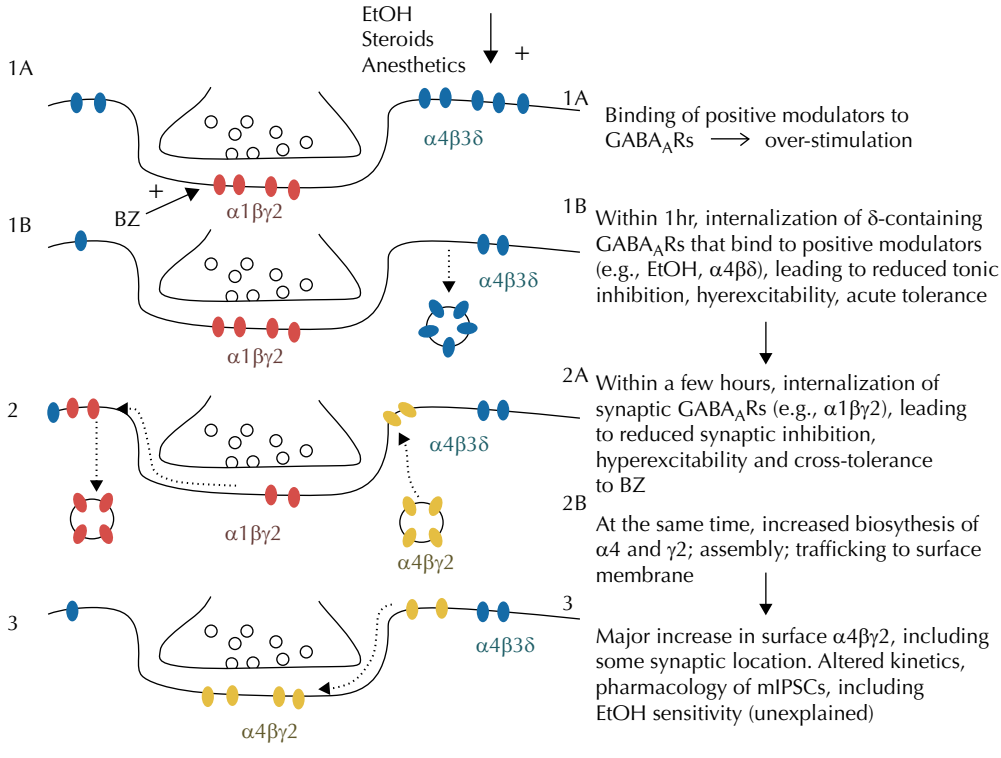

Figure 43–1. GABAR plasticity hypothesis.

One additional observation made about GABAR plasticity induced by CIE[46] demonstrated a correlation between the degree of tolerance induced for a series of GABAergic sedative-hypnotic drugs to produce LORR and the degree of tolerance induced for the same drugs to enhance GABAR-mediated tonic inhibitory currents in hippocampal neurons. Since the hippocampal neurons do not mediate LORR, or at least not all of the response, we suggest that EtOH-induced changes in extrasynaptic GABAR in other brain regions may be very relevant to the soporific action of these agents. Likewise, since one of the prevalent and problematic signs of alcoholism is insomnia, and resistance to commercial sleep aids, we suggest that those hypnotics that do not show complete tolerance to LORR and modulation of extrasynaptic GABAR in the CIE rat model might retain some efficacy to assuage the insomnia problems of human alcohol abusers.

In summary, the CIE model and our combination of behavioral, biochemical, and electrophysiological measurements have demonstrated the critical role of GABAR in many of the signs and symptoms of AWS and thus have implicated GABAR in EtOH dependence.

Remarkable plasticity of GABAR is induced by acute EtOH, and the biochemical mechanisms should be decipherable. The same changes are produced by CIE, but notably they become persistent, lasting up to 120 days post-EtOH.[45] Mechanisms for the change to persistence are under study. Approaches include extension of the CIE model to the mouse to allow use of genetically engineered animals, such as GABAR subunit knockouts and knock-ins. Knockdown of GABAR subunits in critical brain regions has been demonstrated in rats to reduce alcohol consumption.[47]

DISCUSSION

The CIE Model of Alcoholism and GABAR

Two conclusions have been presented about the rat CIE model of alcoholism and the

mechanistic role of GABAR. First, even a single rather large dose of EtOH can trigger within minutes to hours plastic changes in GABAR subunit composition and function, with behavioral correlates, lasting many hours to days, but transient, not persistent.[45] The model has recently been extended to the mouse and to primary cultured rat hippocampal neurons (Olsen and Spigelman, unpublished). Second, chronic administration of moderately high doses of EtOH involving intermittent episodes of intoxication and withdrawal leads to those same plastic changes in GABAR becoming persistent, reminiscent of the kindling of epileptic seizures.[41]

One might ask if the CIE model more closely follows the regimen of the human alcohol abuser who is continually imbibing or that of a binge-type drinker. Although our model requires blood alcohol levels sufficient to produce the mini-withdrawal (~3 h at ~50 mM), the CIE regimen is not exactly like binging since we administer alcohol every day, and we do not reach the blood levels shown in binge studies to be necessary for neuronal toxicity,[44] so we regard the model as more closely approximating the regimen of the alcohol abuser with sporadic use patterns, which could be fairly frequent, including almost every night but not drinking all day, every day.

We recently established the minimum dose, frequency, and duration of the regimen needed to produce long-lasting (>40 days after the last EtOH dose), if not permanent, changes in GABAR function and behavior. In the rat this amounts to about 2.5–3.0 g/kg per day, once every 2 days, for at least 15 doses (Spigelman and Olsen, unpublished). The major question under study, and of most relevance to the topic of this chapter, is how does the plasticity produced become permanent? This question appears to be highly analogous to the same question raised in epileptogenesis, and only with more understanding of the mechanisms of the change to persistent plasticity can and will cures to the process be developed. We have been examining the period of early plastic changes and later-period plasticity induced by CIE in hopes of determining, firstly, what is changed; secondly, what sorts of additional changes occur; and thirdly, how the plasticity becomes aberrant and persistent.

The CIE rats exhibit hyperexcitability in locomotion, rearing, and exploratory behavior. They have a quantitative reduction in seizure threshold to PTZ, increased anxiety, impaired hippocampal spatial memory, perturbed sleep patterns, and tolerance to the soporific actions of EtOH, BZs, neurosteroids, and several general anesthetics, including most commercial sleep aids. They are not altered in sensorimotor performance. Rodent studies using regimens analogous to CIE lead to increased voluntary consumption and presumably craving.[14,30] Using patch-clamp recording in hippocampal slices from CIE rats, we showed that neurons in CIE rat brain have reduced inhibitory synaptic and extrasynaptic (tonic) currents. They have reduced EtOH enhancement of GABAR-mediated tonic inhibitory currents, but no tolerance for EtOH enhancement of GABAR-mediated inhibitory synaptic currents and, ndeed, increased sensitivity. Cross-tolerance to BZ and steroids is seen in both synaptic and tonic GABAR currents, and a change in GABAR channel kinetics and pharmacology was observed that was consistent with the subunit switch in GABAR observed (replacement of normal subunit subtypes with the $\alpha4\beta\gamma2$ subtype). Synaptic inhibition is reduced in total charge transfer due to the more rapid decay of $\alpha4$- versus $\alpha1$-containing GABAR. The BZ-sensitive $\alpha1$ subunit and the EtOH-sensitive δ subunit are persistently downregulated.[41,43] In rats treated acutely with EtOH, these GABAR properties are similarly altered in some other regions, including basolateral amygdala (Spigelman, Liang, and Olsen, unpublished), and probably in ventral tegmentum.[48] In the nucleus accumbens, CIE-induced changes are persistent and pharmacologically similar to those in the hippocampus. However, single dose EtOH-induced alterations exhibit differences not observed in other brain regions (Spigelman, Liang, and Olsen, unpublished observations).

Animals treated with CIE show tolerance to the sedative-hypnotic action of GABAergic drugs, although to varying degrees, apparently related to the fraction of action of each drug on extrasynaptic tonic inhibition.[46] Thus, the symptoms, primarily alcoholic insomnia, can still be treated with GABAergic drugs that do not show much tolerance for action on the GABAR-mediated tonic inhibitory currents and in LORR behavior (e.g., propofol, gaboxadol, and barbiturates). Interestingly, the anti-convulsant actions of EtOH, and especially of neurosteroids, and most of the other GABAergic drugs, do not show much tolerance, and the same

can be said for the anxiolytic actions.[38,40] The modulation of GABAR-mediated synaptic currents by EtOH does not exhibit tolerance, and in fact, the synaptic currents in hippocampal neurons actually become more sensitive to EtOH.[34,41,45] This increased modulation of mIPSCs by EtOH is also seen in the GABAR α4 subunit knockout mouse and might account for the lack of reduction in many EtOH behaviors in this mouse.[49,50]

Therefore, we asked: what might be the subunit composition of GABARs accounting for this increased EtOH sensitivity of synaptic currents? In CIE we observed an increase in α4βγ2 GABAR, including movement of the α4 into the postsynaptic membrane. The δ subunit was not elevated and did not accumulate in the synaptic membrane, and the increased EtOH modulation of mIPSCs was also observed in the GABAR δ subunit knockout mouse.[41] The increased sensitivity to EtOH in mIPSCs in the α4 subunit knockout mouse rules out the α4βδ and α4βγ2 for the EtOH-sensitive GABAR pentamer, so we are examining other possibilities. One cannot help suggesting that some unknown factor(s) other than subunit composition alone might affect EtOH sensitivity. Perhaps this is related to subcellular location, and/or associated proteins, and/or some protein phosphorylation event(s).

Other Studies on Chronic EtOH and Other Drug-Induced GABAR Plasticity

GABAR subunit changes have been reported by others in chronic EtOH-treated animals or cells.[37] Mhatre et al.[51] demonstrated that chronic EtOH treatment in rats produced a hypersensitivity to the behavioral effects of the BZ alcohol antagonist Ro15–4513, followed by biochemical evidence[36] for upregulation of [^{3}H] Ro15–4513 binding to diazepam-insensitive (DZ-IS) sites (shown elsewhere to reflect α4- and α6-containing GABAR). We showed that CIE treatment led to elevated levels of α6 mRNA and protein in cerebellum as well as elevated γ2S mRNA in forebrain[35] and elevated α4 mRNA in hippocampus but not thalamus or cortex.[33] Western blots revealed elevated α4 and γ2 protein as well as reduced α1 and δ polypeptides in CIE hippocampus.[38] In retrospect, the elevated Ro15–4513 binding polypeptide reported in forebrain following chronic EtOH administration by Mhatre and Ticku[36] was undoubtedly the α4 subunit. Meanwhile, the lab of Morrow et al. showed that chronic EtOH administration led to elevated levels of α4 and reduction of α1 involving phosphorylation-regulated trafficking in some forebrain regions.[52,53] A few other subunits were altered, including especially elevated γ1. Similar subunit changes were observed in the lab of Biggio and colleagues for rats and neurons, with an emphasis on the requirement of several hours of withdrawal for EtOH-induced plastic changes in GABAR subunits and behaviors.[54] What falls out of the summarized literature is that chronic EtOH leads to upregulation of the GABAR α4 subunit, which appears to be a very "plastic" subunit, subject to changes in levels under many conditions of stress or overactivity.[21] The α4 subunit is available to replace other α subunits, and this leads to tolerance to sedative drugs given chronically but also to an abnormally excitable state. It suggests the possibility that in the epilepsy models there is overactivity of GABA-mediated inhibition leading to a similar increase in α4 that has some benefit but also produces hyperexcitability. Recent work describes how the GABAR α4 subunit is indeed regulated at the promoter level for gene expression by immediate early genes including heat shock proteins[55,56] and early growth response factors (Egr3),[57,58] which are elevated under various stressful and overstimulation conditions including alcohol exposure[56] and prolonged seizures.[57,58] The α4 subunit polypeptide can be partnered with γ or δ subunits, leading to different pharmacological and functional properties; thus, which of these isoforms is involved is likely very important. We have shown that in fact the α4βδ GABAR are downregulated and the α4βγ2 GABAR are upregulated,[38,41] and play an important role in AWS and EtOH dependence and provide a profound example of aberrant plasticity in the CIE model.

Interestingly, Hu and Ticku[59] were able to reproduce many of the effects of CIE on rats in vivo on cortical cultured neurons in vitro, including hypersensitivity to blockade of GABAR function by PTZ. Primary cultured neurons may therefore provide a good model of alcohol dependence allowing more defined studies of mechanism including GABAR

plasticity. It is critical to establish that primary cultured neurons can mimic as much as possible of the phenotype of the mature brain neurons and the critical plastic changes induced by EtOH. We have now demonstrated in primary cultured hippocampal neurons that one brief exposure to EtOH produces many changes in GABAR[60] that we found in vivo in rats.[45]

GABAR changes have been seen in animals or cells treated with chronic BZ.[61–66] In all of these examples, one can conclude that overstimulation of the receptor by any positive modulator or agonist leads to downregulation of the GABAR subtypes, and only those, that are sensitive to that ligand. In some cases, the function (chloride current) is monitored, whereas in other cases, BZ modulation of GABA site ligand binding is reduced (uncoupling). All of these examples are likely due to removal of the activated GABAR protein from the cell surface, which, depending on the time of the study, can result in reversible return to normal or, at longer times, in degradation of the receptor protein. The BZ effects are limited to action on the subtypes of GABAR that respond to BZ, namely, the γ2-containing isoforms containing α1, α2, α3, or α5.[62] The result is not just a loss of BZ modulation of GABAR function but also a silencing of the GABAR function at that site for a period of time ranging from hours to days (e.g., ref. 63). The loss of cell surface GABAR by endocytosis can be demonstrated. However, basal binding of BZ ligands may persist because the protein in intracellular pools can bind ligand but it is not subject to modulation by GABA in the test tube (uncoupled) because of the low pH of the endosome environment.[62,64,66] GABAR plasticity is also triggered by chronic[67,68] or acute administration of neurosteroids.[69,70] Chronic administration of steroid as a drug produces CNS depression with measurable withdrawal[68] that is accompanied by a tolerance of electrophysiologically recorded GABAR currents to steroids but more dramatically to BZ, accompanied by a switch in subunit composition from traditional BZ-sensitive synaptic GABAR (α1, α2, α3, α5) to the BZ-insensitive α4 subunit. There does not appear to be downregulation of the highly steroid-sensitive α4βδ GABAR subtypes. The in vitro and in vivo pharmacological changes as well as the withdrawal behavior are all reduced by preventing the increase in α4 subunit with anti-sense mRNA.[63]

The plasticity appears to be a variant on the usual mechanism of use-dependent downregulation. More importantly, the neurosteroids are endogenous ligands with in vivo mechanisms of homeostasis, including linkage to the endocrine system.[67,68,71,72] It might not be healthy if steroid-sensitive GABAR were downregulated every time they were modulated for more than a few seconds by endogenous modulatory neurosteroids. And what sort of severe overstimulation by GABA itself is required before downregulation is triggered?

Indeed, the function of GABAR is tightly coupled to the state of neurosteroid activity in the CNS. It appears that high levels of stimulation of the highly sensitive α4βδ type GABAR by neurosteroids leads to plasticity, probably involving mechanisms similar to those mentioned above. GABAR changes are observed in pregnancy and parturition,[72,73] at puberty,[74] and during the estrus cycle,[75] possibly related to premenstrual syndrome. Neurosteroids have even been postulated to mediate at least some of the effects of EtOH occurring minutes to hours after administration.[76] It is therefore likely that neurosteroids play a role in EtOH-induced plasticity of GABAR observed in AWS and dependence. These are early days in this field, but the implications for epileptogenesis are obvious (cf. Chapter 42, this volume).

GABAR Plasticity in Epilepsy

GABAR changes similar to those described in CIE have been reported after seizures, after status epilepticus, and in other epilepsy models.[21,22,77] Nusser et al.[78] demonstrated that experimental epilepsy leads to an increased number of synaptic GABAR measured by electron microscopy in rat hippocampus. Brooks-Kayal and Russek (Chapter 44, this volume)[79] showed that epileptic animals exhibit a switch in GABAR from α1 to α4 and that development of epileptogenesis could be inhibited by preventing the GABAR subunit switch.[80] Banerjee et al.[81] observed a decrease in allosteric modulation by neurosteroids of [^{35}S]TBPS binding to GABAR during absence seizures in rats only in the affected brain cells in thalamus. We interpreted this to reflect a switch in GABAR subunit composition involving both trafficking and gene expression. This conclusion was further supported by Banerjee et al.,[82]

who demonstrated rapid (within 1–2 h) changes in GABAR α1 and α4 subunit gene expression in thalamic neurons during absence seizures. Naylor et al.[83] demonstrated that status epilepticus produced downregulation in the γ2-containing synaptic GABAR, interpreted as overstimulation by massive GABA synaptic release; they also postulated a protection of extrasynaptic GABAR-mediating inhibitory tonic currents. Subunit-selective regulation of synaptic GABAR trafficking and localization has been demonstrated by Kapur and colleagues[84–86] (Chapter 41, this volume). The mechanism of status epilepticus-induced internalization of synaptic GABAR has been shown to involve increased dephosphorylation-regulated binding of GABAR to clathrin for endocytotic removal from the cell surface.[87] Status epilepticus-induced spontaneous seizures were shown to be accompanied by alterations in the synaptic versus perisynaptic localization of α4βγ2– and α4βδ-type GABAR[88] (Houser et al., this volume).

In conclusion, we suggest that the plasticity observed in alcohol withdrawal involving GABAR and especially extrasynaptic α4βδ-type GABAR is indicative of the common use of GABAR (and GLUR) plasticity in normal brain functions including learning and memory. Further, the mechanisms responsible for GABAR (and GLUR) plasticity have the propensity for aberrant plasticity leading to seizure susceptibility: a model of epileptogenesis? We continue to study this hypothesis. Current studies involve CIE treatment in the mouse and examination of genetically engineered animals (e.g., refs. 89 and 90) and a cultured rat hippocampal neuron model.[60] Finally, recent studies increasingly implicate changes in GABAR-mediated tonic inhibition in epilepsy physiology (see Chapters 40, 41, 42, and 44 in this volume).

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REFERENCES

1. Goldstein DB. Physical dependence on ethanol: its relation to tolerance. *Drug Alcohol Depend*. 1979;4: 33–39.
2. Chandler LJ, Harris RA, Crews FT. Ethanol tolerance and synaptic plasticity. *Trends Pharmacol Sci*. 1998;9:491–495.
3. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol*. 1969;25:295–308.
4. McNamara JO. Kindling: an anijmal model of complex partial epilepsy. *Ann Neurol*. 1984;16: 572–579.
5. Porter RJ, Mattson RH, Cramer JA, Diamond I, eds. *Alcohol and Seizures: Basic Mechanisms and Clinical Concepts*. Philadelphia: FA Davis; 1990.
6. Brown ME, Anton RF, Malcolm R, Ballenger JC. Alcohol detoxification and withdrawal seizures: clinical support for a kindling hypothesis. *Biol Psychiatry*. 1988;23:507–514.
7. Booth BM, Blow FC. The kindling hypothesis: further evidence from a U.S. national study of alcoholic men. *Alcohol.* 1993;28:593–598.
8. Becker HC. Kindling in alcohol withdrawal. *Alcohol Res Health*. 1998;22:25–33.
9. Breese GR, Overstreet DH, Knapp DJ. Conceptual framework for the etiology of alcoholism: a "kindling"/ stress hypothesis. *Psychopharmacology* (*Berl*). 2005;178:367–380.
10. McQuarrie DG, Fingl E. Effects of single doses and chronic administration of ethanol on experimental seizures in mice. *J Pharmacol Exp Ther.* 1958;124:264–273.
11. Kokka N, Sapp DW, Taylor AM, Olsen RW. The kindling model of alcohol dependence: similar persistent reduction in seizure threshold to pentylenetetrazol in animals receiving chronic ethanol or chronic pentylenetetrazol. *Alcohol Clin Exp Res.* 1993;17:525–531.
12. McCown TJ, Breese GR. Multiple withdrawals from chronic ethanol "kindles" inferior colliculus seizure activity: evidence for kindling of seizures associated with alcoholism. *Alcohol Clin Exp Res*. 1990;14: 394–399.
13. Ehlers CL, Slawecki CJ. Effects of chronic ethanol exposure on sleep in rats. *Alcohol.* 2000; 20:173–179.
14. Rimondini R, Arlinde C, Sommer W, Heilig M. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *FASEB J.* 2002;16:27–35.
15. Veatch LM, Becker HC. Electrographic and behavioral indices of ethanol withdrawal sensitization. *Brain Res*. 2002;946:272–282.
16. O'Dell LE, Roberts AJ, Smith RT, Koob GF. Enhanced alcohol self-administration after intermittent versus continuous alcohol vapor exposure. *Alcohol Clin Exp Res.* 2004;28:1676–1682.

17. Kilman V, van Rossum MCW, Turrigiano GG. Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic $GABA_A$ receptors clustered at neocortical synapses. *J Neurosci*. 2002;22:1328–1337.
18. Frey GD. γ-Aminobutyric acid changes in alcohol withdrawal. In: Porter RJ, Mattson RH, Cramer JA, Diamond I, eds. *Alcohol and Seizures: Basic Mechanisms and Clinical Concepts.* Philadelphia: FA Davis; 1990:87–101.
19. Brailowsky S, Kunimoto M, Menini C, Silva-Barrat, S, Riche D, Naquet TR. The GABA- withdrawal syndrome: a new model of focal epileptogenesis. *Brain Res*. 1988;442:175–179.
20. Silva-Barrat C, Champagnat J, Brailowsky S, Menini C, Naquet R. Relationship between tolerance to $GABA_A$ agonist and bursting properties in neocortical neurons during GABA-withdrawal syndrome. *Brain Res*. 1989;498:289–298.
21. Olsen RW, DeLorey TM, Gordey M, Kang MH. GABA receptor function and epilepsy. In: Delgado-Escueta AV, Wilson W, Olsen RW, Porter RJ, eds. *Jasper's Basic Mechanisms of the Epilepsies*. Vol. III. Philadelphia: Lippincott-Williams & Wilkins; 1999:499–510.
22. Coulter DA. Epilepsy-associated plasticity in GABA receptor expression, function, and inhibitory synaptic properties. *Int Rev Neurobiol*. 2002;45:237–252.
23. Sundstrom-Poromaa I, Smith AD, Gong QH, Sabado TN, Li X, Light A, Wiedmann M, Williams K, Smith SS. Hormonally regulated α4β2δ $GABA_A$ receptors are a target for alcohol. *Nat Neurosci*. 2002;5:721–722.
24. Hanchar HJ, Dodson PD, Olsen RW, Otis TS, Wallner M. Alcohol induced motor impairment caused by increased extrasynaptic $GABA_A$ receptor activity. *Nat Neurosci*. 2005;8:339–345.
25. Wallner M, Hanchar HJ, Olsen RW. Low dose alcohol actions on α4β3δ $GABA_A$ receptors are reversed by the behavioral alcohol antagonist Ro15–4513. *Proc Natl Acad Sci USA.* 2006;103:8540–8545.
26. Olsen RW, Hanchar HJ, Meera P, Wallner M. $GABA_A$ receptor subtypes: the "one glass of wine" receptors. *Alcohol*. 2007;41:201–209.
27. Siggins GR, Roberto M, Nie Z. The tipsy terminal: presynaptic effects of ethanol. *Pharmacol Ther*. 2005;107:80–98.
28. Fritschy JM, Brunig I. Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. *Pharmacol Ther*. 2003;98:299–323.
29. Kittler JT, Moss SJ. Modulation of $GABA_A$ receptor activity by phosphorylation and receptor trafficking: implications for the efficacy of synaptic inhibition. *Curr Opin Neurobiol*. 2003;13:341–347.
30. Simms JA, Steensland P, Medina B, Abernathy KE, Chandler LJ, Wise R, Bartlett SE. Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. *Alcohol Clin Exp Res*. 2008;32:1816–1823.
31. Kang MH, Spigelman I, Sapp DW, Olsen RW. Persistent reduction of $GABA_A$ receptor- mediated inhibition in rat hippocampus after chronic intermittent ethanol treatment. *Brain Res*. 1996;709:221–228.
32. Veatch LM, Gonzalez LP. Repeated ethanol withdrawal produces site-dependent increases in EEG spiking. *Alcohol Clin Exp Res*. 1996;20:262–267.
33. Mahmoudi M, Kang MH, Tillakaratne N, Tobin AJ, Olsen RW. Chronic intermittent ethanol treatment in rats increases $GABA_A$ receptor α4-subunit expression: possible relevance to alcohol dependence. *J Neurochem*. 1997;68:2485–2492.
34. Kang MH, Spigelman I, Olsen RW. Alterations in the sensitivity of $GABA_A$ receptors to allosteric modulatory drugs in rat hippocampus following chronic intermittent ethanol treatment. *Alcohol Clin Exp Res*. 1998;22:2165–2173.
35. Petrie J, Sapp DW, Tyndale RF, Park MK, Fanselow M, Olsen RW. Altered $GABA_A$ receptor subunit and splice variant expression in rats treated with chronic intermittent ethanol. *Alcohol: Clin Exp Res*. 2001;25: 819–828.
36. Mhatre MC, Ticku MK. Chronic ethanol administration alters $GABA_A$ receptor gene expression. *Mol Pharmacol*. 1992;42:415–422.
37. Kumar S, Fleming RL, Morrow AL. Ethanol regulation of γ-aminobutyric $acid_A$ receptors: genomic and nongenomic mechanisms. *Pharmacol Ther*. 2004;101: 211–226.
38. Cagetti E, Liang J, Spigelman I**,** Olsen RW. Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of $GABA_A$ receptors. *Mol Pharmacol.* 2003;63:53–64.
39. Liang J, Cagetti E, Olsen RW, Spigelman I. Altered pharmacology of synaptic and extrasynaptic $GABA_A$ receptors on hippocampal CA1 pyramidal neurons is consistent with subunit changes in a model of alcohol withdrawal and dependence. *J Pharmacol Exp Ther.* 2004;310:1234–1245.
40. Cagetti E, Pinna G, Guidotti A, Baicy K, Olsen RW. Chronic intermittent ethanol (CIE) administration in rats decreases levels of neurosteroids in hippocampus, accompanied by altered behavioral responses to neurosteroids and memory function. *Neuropharmacology.* 2004;46:570–579.
41. Liang J, Zhang N, Cagetti E, Houser CR, Olsen RW, Spigelman I. Chronic intermittent ethanol-induced switch of ethanol actions from extrasynaptic to synaptic hippocampal $GABA_A$ receptors. *J Neurosci.* 2006;26:1749–1758.
42. Wei W, Faria LC, Mody I. Low ethanol concentrations selectively augment the tonic inhibition mediated by δ subunit-containing $GABA_A$ receptors in hippocampal neurons. *J Neurosci.* 2004;24:8379–8382.
43. Collins MA, Zou JY, Neafsey EJ. Brain damage due to episodic alcohol exposure in vivo and in vitro: furosemide neuroprotection implicates edema-based mechanism. *FASEB J.* 1998;12:221–230.
44. Nixon K, Crews FT. Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. *J Neurochem*. 2002;83:1087–1093.
45. Liang J, Suryanarayanan A, Abriam A, Snyder B, Olsen RW, Spigelman I. Mechanisms of reversible $GABA_A$ receptor plasticity after ethanol intoxication. *J Neurosci*. 2007;27:12367–12377.
46. Liang J, Spigelman I, Olsen RW. Tolerance to sedative/hypnotic actions of GABAergic drugs correlates with tolerance to potentiation of extrasynaptic tonic currents in hippocampus of alcohol-dependent rats. *J Neurophysiol*. 2009;102:224–233.

47. Rewal M, Jurd R, Gill TM, He DY, Ron D, Janak PH. Alpha4-containing $GABA_A$ receptors in the nucleus accumbens mediate moderate intake of alcohol. *J Neurosci*. 2009;29:543–549.
48. Melis M, Camarini R, Ungless MA, Bonci A. Long-lasting potentiation of GABAergic synapses in dopamine neurons after a single in vivo ethanol exposure. *J Neurosci*. 2002;22:2074–2082.
49. Chandra D, Werner DF, Liang J, Suryanarayanan A, Spigelman I, Olsen RW, Harrison NL, Homanics GE. Normal acute behavioral responses to ethanol in $GABA_A$ receptor α4 subunit knockout mice. *Alcohol: Clin Exp Res*. 2008;32:10–18.
50. Liang J, Suryanarayanan A, Chandra D, Homanics GE, Olsen RW, Spigelman I. Functional consequences of $GABA_A$ receptor α4 subunit deletion on synaptic and extrasynaptic currents in mouse dentate granule cells. *Alcohol: Clin Exp Res*. 2008;32:19–26.
51. Mhatre M, Mehta AK, Ticku MK. Chronic ethanol administration increases the binding of the benzodiazepine inverse agonist and alcohol antagonist [^{3}H] Ro15–4513 in rat brain. *Eur J Pharmacol*. 1988;153: 141–145.
52. Kumar S, Kralic JE, O'Buckley TK, Grobin AC, Morrow AL. Chronic ethanol consumption enhances internalization of α1 subunit-containing $GABA_A$ receptors in cerebral cortex. *J Neurochem.* 2003;86: 700–708.
53. Kumar S, Suryanarayanan A, Boyd K, Commerford C, Lai M, Ren Q, Morrow AL. Ethanol reduces $GABA_A$ [alpha]1 subunit receptor surface expression by a PKC[gamma]- dependent mechanism in cultured cerebral cortical neurons. *Mol Pharmacol*. 2010;77: 793–803.
54. Sanna E, Mostallino MC, Busonero F, Talani G, Tranquilli S, Mameli M, Spiga S, Follesa P, Biggio G. Changes in GABA(A) receptor gene expression associated with selective alterations in receptor function and pharmacology after ethanol withdrawal. *J Neurosci*. 2003;23:11711–11724.
55. Ma L, Song L, Radoi GE, Harrison NL. Transcriptional regulation of the mouse gene encoding the alpha-4 subunit of the $GABA_A$ receptor. *J Biol Chem.* 2004;279: 40451–40461.
56. Pignataro L, Miller AN, Ma L, Midha S, Protiva P, Herrera DG, Harrison NL. Alcohol regulates gene expression in neurons via activation of heat shock factor 1. *J Neurosci*. 2007;27:12957–12966.
57. Roberts DS, Raol YH, Bandyopadhyay S, Lund IV, Budreck EC, Passini MA, Wolfe JH, Brooks-Kayal AR, Russek SJ. Egr3 stimulation of $GABR_A4$ promoter activity as a mechanism for seizure-induced up-regulation of GABA(A) receptor alpha4 subunit expression. *Proc Natl Acad Sci USA*. 2005;102: 11894–11899.
58. Roberts DS, Hu Y, Lund IV, Brooks-Kayal AR, Russek SJ. Brain-derived neurotrophic factor (BDNF)-induced synthesis of early growth response factor 3 (Egr3) controls the levels of type A GABA receptor alpha4 subunits in hippocampal neurons. *J Biol Chem*. 2006;281:29431–29435.
59. Hu XJ, Ticku MK. Functional characterization of a kindling-like model of ethanol withdrawal in cortical cultured neurons after chronic intermittent ethanol exposure. *Brain Res.* 1997;767:228–234.
60. Shen Y, Lindemeyer AK, Spigelman I, Sieghart W, Olsen RW, Liang J. Plasticity of $GABA_A$ receptors following ethanol pre-exposure in cultured hippocampal neurons. *Mol Pharmacol.* 2011;79:432–442.
61. Hu X, Ticku MK. Chronic benzodiazepine agonist treatment produces functional uncoupling of the GABA–benzodiazepine receptor ionophore complex in cortical neurons. *Mol Pharmacol*. 1994;45: 618–625.
62. Primus RJ, Yu J, Xu J, Hartnett C, Meyyappan M, Kostas C, Ramabhadran TV, Gallager DW. Allosteric uncoupling after chronic benzodiazepine exposure of recombinant $GABA_A$ receptors expressed in Sf9 cells: ligand efficacy and subtype selectivity. *J Pharmacol Exp Ther*. 1996;276:882–890.
63. Poisbeau P, Williams SR, Mody I. Silent $GABA_A$ synapses during flurazepam withdrawal are region-specific in the hippocampal formation. *J Neurosci*. 1997;17:3467–3475.
64. Ali N, Olsen RW. Chronic benzodiazepine treatment of cells expressing recombinant $GABA_A$ receptors uncouples allosteric binding: studies on possible mechanisms. *J Neurochem.* 2001;79:1100–1108.
65. Barnes EM. Assembly and intracellular trafficking of $GABA_A$ receptors. *Int Rev Neurobiol*. 2001;48:1–29.
66. Gravielle MC, Faris R, Russek SJ, Farb DH. GABA induces activity dependent delayed-onset uncoupling of GABA/benzodiazepine site interactions in neocortical neurons. *J Biol Chem.* 2005;280:20954–20960.
67. Smith SS, Gong QH, Hsu FC, Markowitz RS, ffrench-Mullen JM, Li X. $GABA_A$ receptor α4 subunit suppression prevents withdrawal properties of an endogenous steroid. *Nature.* 1998;392:926–930.
68. Smith SS, Gong QH, Li X, Moran MH, Bitran D, Frye CA, Hsu FC. Withdrawal from 3α-OH-5α-pregnan-20-one using a pseudopregnancy model alters the kinetics of hippocampal $GABA_A$-gated current and increases the $GABA_A$ receptor α4 subunit in association with increased anxiety. *J Neurosci.* 1998;18:5275–5284.
69. Gulinello M, Gong QH, Li X, Smith SS. Short-term exposure to a neuroactive steroid increases α4 $GABA_A$ receptor subunit levels in association with increased anxiety in the female rat. *Brain Res*. 2001; 910:55–66.
70. Shen H, Gong QH, Yuan M, Smith SS. Short-term steroid treatment increases delta $GABA_A$ receptor subunit expression in rat CA1 hippocampus: pharmacological and behavioral effects. *Neuropharmacology*. 2005;49:573–586.
71. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the $GABA_A$ receptor. *Nat Rev Neurosci*. 2005;6:565–575.
72. Biggio G, Purdy RH, eds. Neurosteroids and brain function. *Int Rev Neurobiol*. 2001;46:207–272.
73. Maguire J, Mody I. Neurosteroid synthesis-mediated regulation of $GABA_A$ receptors: relevance to the ovarian cycle and stress. *J Neurosci*. 2007;27:2155–2162.
74. Shen H, Gong QH, Aoki C, Yuan M, Ruderman Y, Dattilo M, Williams K, Smith SS. Reversal of neurosteroid effects at α4β2δ $GABA_A$ receptors triggers anxiety at puberty. *Nat Neurosci*. 2007;10:469–477.
75. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in $GABA_A$ receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci*. 2005;8:797–804.

76. Morrow AL, Khisti R, Tokunaga S, McDaniel JR, Matthews DB. GABAergic neurosteroids modulate selective ethanol actions: mechanisms and significance. In: Smith SS, ed. *Neurosteroid Effects in the Central Nervous System: The Role of the $GABA_A$ Receptor*. Boca Raton, FL: CRC Press; 2004:219–245.
77. Olsen RW, Avoli M. GABA and epileptogenesis. *Epilepsia*. 1997;38:399–407.
78. Nusser Z, Hajos N, Somogyi P, Mody I. Increased number of synaptic $GABA_A$ receptors underlies potentiation at hippocampal inhibitory synapses. *Nature*. 1998;395:172–177.
79. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell $GABA_A$ receptor subunit expression and function in temporal lobe epilepsy. *Nat Med*. 1998;4:1166–1172.
80. Raol YH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, Russek SJ, Brooks-Kayal AR. Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. *J Neurosci.* 2006;26:11342–11346.
81. Banerjee PK, Olsen RW, Tillakaratne NJK, Brailowsky S, Tobin AJ, Snead OC. Absence seizures decrease steroid modulation of *t*-[^{35}S]butyl bicylcophosphorothionate (TBPS) binding in thalamic relay neurons. *J Pharmacol Exp Ther.* 1998;287:766–772.
82. Banerjee PK, Tillakaratne NJK, Brailowsky S, Olsen RW, Tobin AJ, Snead OC. Alterations in $GABA_A$ receptor α1 and α4 subunit mRNA levels in thalamic relay nuclei following absence-like seizures in rats. *Exp Neurol*. 1998;154:213–223.
83. Naylor DE, Liu H, Wasterlain CG. Trafficking of $GABA_A$ receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci*. 2005;25:7724–7733.
84. Goodkin HP, Yeh JL, Kapur J. Status epilepticus increases the intracellular accumulation of GABA-A receptors. *J Neurosci*. 2005;25:5511–5520.
85. Sun C, Mtchedlishvili Z, Erisir A, Kapur J. Diminished neurosteroid sensitivity of synaptic inhibition and altered location of the alpha4 subunit of $GABA_A$ receptors in an animal model of epilepsy. *J Neurosci*. 2007;27:12641–12650.
86. Goodkin H, Joshi S, Mtchedlishvili Z, Brar J, Kapur J. Subunit-specific trafficking of GABA-A receptors during status epilepticus. *J Neurosci*. 2008;28:2527–2538.
87. Terunuma M, Xu H, Vithlani M, Sieghart W, Kittler J, Pangalos M, Haydon PG, Coulter DA, Moss SJ. Deficits in phosphorylation of GABA-A receptors by intimately associated protein kinase C activity underlie compromised synaptic inhibition during status epilepticus. *J Neurosci.* 2008;28:376–384.
88. Zhang N, Wei W, Mody I, Houser CR. Altered localization of $GABA_A$ receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *J Neurosci*. 2007:27:7520–7531.
89. Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen RW, Homanics GE. Attenuated sensitivity to neuroactive steroids in $GABA_A$ receptor δ subunit knockout mice. *Proc Natl Acad Sci USA*. 1999;96:12905–12910.
90. Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, Spigelman I, Houser CR, Olsen RW, Harrison NL, Homanics GE. $GABA_A$ receptor α4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and are required for the action of gaboxadol. *Proc Natl Acad Sci USA*. 2006;103:15230–15235.

Chapter 44

Regulation of $GABA_A$ Receptor Gene Expression and Epilepsy

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Results of research in animal models as well as from human retrospective studies suggest that an initial precipitating event such as status epilepticus (SE), stroke, or traumatic brain injury can increase the risk of later development of the recurrent spontaneous seizures that define epilepsy. The process by which a normal brain transforms into one capable of producing recurrent spontaneous seizures, known as *epileptogenesis*, is likely to be complex and multifactorial. Among the many changes that occur during epileptogenesis are alterations in expression of a wide variety of genes. Determining what molecular pathways regulate these changes in gene expression, and which of them are consequential or causative of disease, are two of the major challenges of research in this area, and are critical to effectively utilizing this information to develop new therapies for the prevention and treatment of epilepsy.

ROLE OF $GABA_A$ RECEPTORS IN EPILEPSY

Many laboratories, including our own, have focused on the role of gene regulation in determining changes in gamma-aminobutyric acid (GABA) receptor plasticity that begin during the latent period after brain insult and persist after the development of the epileptic state. GABA is the major inhibitory neurotransmitter in the mature brain, and several drugs that enhance GABAergic inhibition are commonly used as antiepileptic medications. Conversely, drugs that block GABAergic inhibition can induce seizures in animals, further supporting the potential importance of alterations in GABAergic transmission in the etiology of epilepsy.

Three types of GABA receptors, $GABA_A$, $GABA_B$, and $GABA_C$, are found in the mature central nervous system. $GABA_A$ and $GABA_C$ are ionotropic receptors, whereas $GABA_B$ is a metabotropic receptor. Most fast synaptic inhibition in the mature brain is mediated by $GABA_A$ receptors (GABARs), whereas slow inhibition is mediated by $GABA_B$ receptors. GABARs are composed of multiple subunits from a variety of subtypes (α1–6, β1–3, γ1–3, δ, ε, π, θ, and ρ1–3) that form a pentameric anion-selective channel.[1] GABAR subunit composition determines the intrinsic properties of each channel, including GABA affinity, kinetics, conductance, allosteric modulation,

probability of channel opening, interaction with modulatory proteins, and subcellular distribution.[2] The typical in vivo subunit composition is two α, two β, and one γ or δ subunit. Synaptic GABARs in cortex and hippocampus most commonly contain a γ subunit in combination with an α1 or α2 and β_x- subunit, whereas those located at perisynaptic and extrasynaptic sites contain predominantly a δ subunit in combination with an α4 and a β_x subunit or a γ subunit in combination with an α5 and a β_x subunit.[3] There is remarkable receptor heterogeneity, with subtype combinations varying in different brain regions, cell types, and membrane locations and during different times in ontogeny.[2,4–6]

Prolonged seizures (SE) result in alterations in the expression and membrane localization of several GABAR subunits (α1, α4, γ2, δ) in hippocampal dentate granule neurons.[7–9] These alterations, which are associated with changes in phasic and tonic GABAR-mediated inhibition, decreased GABAR modulation by benzodiazepines and neurosteroids, and increased inhibition by zinc, begin soon after SE and continue after animals become epileptic.[7–13] In the pilocarpine model of SE in adult rats, GABAR α1 subunit mRNA expression decreases and α4 subunit mRNA expression increases in dentate granule cells of the hippocampus, and animals uniformly go on to develop epilepsy. These changes in subunit mRNA expression correlate with a decreased presence of α1γ2-containing receptors[14] and an increased presence of α4γ2-containing receptors,[11,14] as well as an increase in perisynaptic localization of γ2 subunits, likely partnering with α4.[9] GABAR functional and subunit expression changes have also been observed in neurons from surgically resected hippocampus from patients with intractable temporal lobe epilepsy (TLE).[15–17] The changes in GABAR subunit expression and function in dentate granule cells of epileptic animals precede the development of epilepsy, suggesting that these changes contribute to the epileptogenic process. In contrast, neonatal SE (postnatal day 10) in rats results in increased GABAR α1 subunit expression and does not lead to the subsequent development of epilepsy.[18]

These studies suggest that GABAR subunit alterations may be critical contributors to epileptogenesis. To determine this more directly, we utilized gene transfer to mitigate GABAR subunit changes and examined the effect on epilepsy development. Specifically, we tested the hypothesis that the expression of higher α1 subunit levels would inhibit development of epilepsy after SE by using an adeno-associated virus (AAV) gene transfer vector (AAV2)-serotype 5 designed to express a bicistronic RNA that codes for both the GABAR α1 subunit and the reporter, enhanced yellow fluorescent protein (eYFP).[19] Expression of this RNA was placed under control of the GABAR α4 subunit gene (*GABRA4*) core promoter region, because it had been previously shown to be markedly activated in dentate gyrus following SE.[20] Thus, following SE, activity of the α4 promoter was upregulated, resulting in enhanced α1 transgene expression. Adeno-associated virus vectors containing either the α1/eYFP fused cDNA (AAV-α1) or the eYFP-reporter only (AAV-eYFP) were injected into dentate gyrus of adult rats, and SE was induced 2 weeks later by intraperitoneal injection of pilocarpine (385 mg/kg).[19] Rats injected with AAV-α1 showed threefold higher levels of α1 subunits in dentate gyrus by 2 weeks after SE compared to the control groups. Rats were continuously video-electroencephalogram (EEG) monitored to determine the latency for the development of spontaneous seizures. Injection of AAV-α1 resulted in a three-fold increase in the mean time to the first spontaneous seizure following SE, and only 39% of AAV-α1-injected rats were observed to develop spontaneous seizures in the first 4 weeks after SE compared to 100% of rats receiving sham injections. Because all groups of rats experienced similar SE after pilocarpine injection, these findings provide the first direct evidence that increasing the levels of a single GABAR subunit in dentate gyrus can inhibit the development of spontaneous seizures after SE. Together, these data support a role for GABAR α-subunit changes in the process of epileptogenesis.

MECHANISMS REGULATING $GABA_A$ RECEPTOR SUBUNIT EXPRESSION

α1 Subunit Regulation

Although viral gene transfer is a promising therapeutic avenue to modify the aberrant

gene expression associated with epileptogenesis, producing the optimal level of expression over a prolonged period can be challenging. Another potential approach is to modify the mechanisms regulating gene expression. Recent work in our laboratories has established cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) and inducible cAMP early repressor[14,21] as transcriptional regulators responsible for decreased GABAR α1 subunit mRNA and protein levels occurring after SE in the dentate gyrus. CREB is a stimulus-induced transcription factor that is activated by phosphorylation at its Ser 133 site. Phosphorylated CREB (pCREB) dimerizes, or forms heterodimers with CREB family members, and binds to cAMP response element (CRE) motifs on promoters that contain the consensus sequence TGACGTCA.[22] Along with the chromatin modifier, CREB binding protein (CBP), pCREB serves as an activator to increase transcription of its target genes. Transcriptional regulation through CREB has been implicated in mechanisms of cell survival, plasticity, and learning and memory.[22] Target genes of pCREB include CREB family members, which consist of cAMP response element modulator (CREM) and activating transcription factor. These paralogs and homologs of CREB also bind CRE elements to modulate the transcription of particular genes, often as heterodimers with CREB. The CREM gene produces many gene products, including truncated forms that are missing the activation domain and, hence, function as transcriptional repressors. A collection of such repressors, produced by the alternative use of an internal promoter region in a downstream intron of CREM, is inducible cAMP early repressor, a group of 4 proteins (α, β, γ, and δ).[23,24] The γ form is most prevalent and acts as a homodimer at the CRE site or heterodimerizes with CREB to directly block CREB-induced transcription.

The human α1 promoter *(GABRA1-p)* contains a functional CRE site,[25] and several studies using adult animal models of epilepsy suggest that seizures increase levels of the activated form of CREB (pCREB) and CREM/inducible cAMP early repressor (ICER) activity.[26,27] Our laboratories have found sustained increases in the levels of both pCREB and ICER in the dentate gyrus of the hippocampus 1–48 h after pilocarpine-induced SE.[14] Using chromatin immunoprecipitation (ChIP) and DNA pulldown studies, it was determined that there was also increased binding of pCREB and ICER to the endogenous *GABRA1-p* in dentate gyrus after SE.[14] Further, results of *GABRA1-p*/luciferase reporter assays in transfected primary hippocampal neurons show that overexpression of CREB and ICER produces robust decreases in *GABRA1-p* activity, and overexpression of ICER alone produces a marked decrease in the levels of endogenous α1 subunits at the cell surface.[21] These findings suggest that CREB and ICER are important regulators of seizure-induced changes in α1 subunit expression.

The excessive neuronal activity associated with SE stimulates many different signaling pathways that could lead to enhanced phosphorylation of CREB and expression of ICER.[28] We focused our studies on brain-derived neurotrophic factor (BDNF) as a potential regulator of ICER because BDNF expression increases markedly after SE[29–33] and because BDNF differentially regulates the abundance of both α1 and α4 subunits in cultured neurons.[32] Our results demonstrated that BDNF treatment of primary hippocampal neurons in culture produces changes in α subunit levels similar to those observed after SE. Twenty-four hours after BDNF treatment, α1 levels decrease 42% and α4 levels increase 120%.[32] We further found that BDNF regulates α1 subunit levels via activation of the Janus kinase (JAK)/signal transducer and activators of transcription (STAT) pathway, which, in turn, controls the synthesis of ICER, which then represses *GABRA1* transcription.

The JAK-STAT pathway can be activated by a variety of methods, including cytokines binding to their specific receptors, resulting in transphosphorylation of JAKs, which then lead to phosphorylation of STAT proteins.[34–38] Phosphorylation of STATs on tyrosine residues leads to STAT homo- or heterodimerization, translocation from the cytoplasm to the nucleus, and binding to specific DNA elements (STAT-recognition sites) to regulate target gene expression.[37,38] There is a STAT-recognition element in the ICER promoter, and using ChIP, we have shown that pSTAT3 association with this site is enhanced after SE in dentate gyrus.[14] Furthermore, siRNA knockdown of STAT3 inhibits BDNF-induced ICER increases, as does blockade of the JAK/STAT signaling pathway with pyridone 6 (P6) in

primary hippocampal cultures.[14] Most importantly, P6 administration in vivo into rat dentate gyrus prior to SE blocks both ICER induction and decreased transcription of *GABRA1*.[14] These findings suggest that the interplay of the CREB, JAK/STAT, and BDNF signaling pathways is critical for the decrease in α1 subunit levels that occurs in response to SE and that these pathways may provide novel therapeutic targets for epilepsy. In fact, several drugs that specifically inhibit the activity of JAK2 or block downstream STAT activation[39–42] have already been identified as potential agents in cancer chemotherapy and are in clinical trials. We are currently testing these agents to determine whether they may also provide alternative therapy for the future prevention or treatment of epilepsy.

α4 Subunit Regulation

GABARs that contain α4 subunits have unique pharmacological properties, such as insensitivity to benzodiazepines and increased sensitivity to zinc blockade.[43] Receptors containing α4 subunits are most often found with the δ rather than the γ subunit in combination with αβ. These α4βδ GABARs are localized to extrasynaptic sites and contribute to tonic inhibition. A minor population of α4βγ2 GABARs are found within dentate gyrus synapses, where they are proposed to affect both the rise time and decay of synaptic currents.[44] In addition to the decrease in α1 subunit expression, there is a marked increase in α4 subunit expression in dentate granule cells during epileptogenesis in TLE models[7–9] that results in an increase in the abundance of α4γ2-containing receptors[9,11,14] and a reduction in α1γ2-containing receptors.[14] The change in receptor subtype from α1βγ2 to α4βγ2 may contribute to epileptogenesis, as α4-containing GABARs have been shown to desensitize rapidly, especially when assembled with β3 subunits.[45] In addition, GABARs containing the α4 subunit are very sensitive to zinc blockade,[43] as are GABARs on dentate granule cells in the epileptic brain.[7]

Our studies have shown that the alteration in α4 levels after pilocarpine-induced SE is transcriptionally mediated via an increase in the expression of the transcription factor early growth response factor 3 (Egr3).[20] Egrs are a family of four proteins (Egr1, 2, 3, and 4) that share nearly identical zinc finger DNA binding domains and bind to a common Egr response element consensus sequence (ERE), GCG T/GGG GCG.[46] Our laboratories have shown induction of Egr family transcription factors after SE, with increases in protein levels of Egr3 and enhanced binding of Egr3 to the promoter of the endogenous α4 subunit gene (*GABRA4*) in the dentate gyrus of the hippocampus 24 h after pilocarpine-induced SE.[20] α_4 Subunit upregulation has also been demonstrated following withdrawal of progesterone-derived neurosteroids, such as allopregnanolone and pregnanolone,[47,48] resulting in enhanced neuronal excitability, seizure susceptibility, and benzodiazepine resistance. This increase in α4 subunit expression is thought to be a potential molecular basis of catamenial epilepsy, a neuroendocrine condition that occurs around the perimenstrual period and is characterized by neurosteroid withdrawal-linked seizure exacerbations in women with epilepsy. Recently, neurosteroid withdrawal-induced α4-subunit upregulation was found to be mediated by Egr3 inhibition, similar to the role played by this transcription factor in upregulating α4 after SE.[49]

Similar to its critical role in decreased expression of α1-containing GABARs, BDNF again is the endogenous signal that induces Egr3 synthesis and overexpression of α4 subunits—now, however, through different signaling pathways, protein kinase C (PKC) and mitogen activated protein kinase (MAPK).[32] In addition to its role in regulation of α1 and α4 subunit gene transcription after SE (for a summary, see Fig. 44–1), BDNF, acting via activation of TrkB receptors, has been shown to play an important role in determining the surface expression of the α2, β2,3 γ2, and δ subunits.[50–52] In combination, these findings establish BDNF's role as a multifunctional regulator of altered inhibition during epileptogenesis.

In addition to changes in α1 and α4 expression, an increase in γ2-subunit and a decrease in δ-subunit surface expression in dentate granule cells, with associated diminished neurosteroid sensitivity of tonic currents, has also been demonstrated in rodent models of TLE.[8,9,53,54] As for the α4 subunit, studies support an important role for the neurosteriods in the regulation of γ- and δ- subunit expression during the ovarian cycle and in pregnancy,[47,48,55,56] although the specific molecular mechanisms regulating

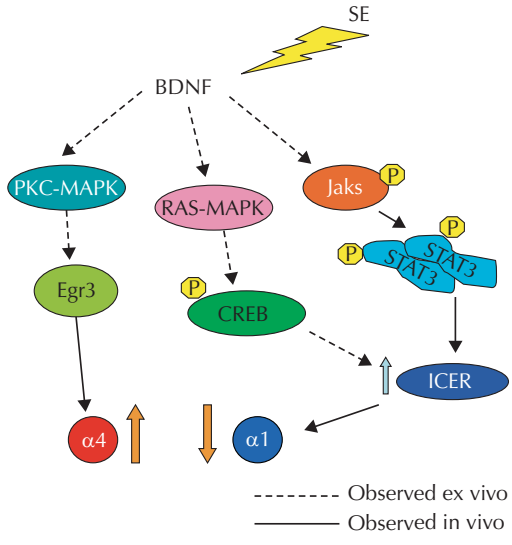

Figure 44–1. Differential expression of $GABA_A$ receptor α_4-subnits nits via BDNF-stimulated signal transduction pathways. Whether a $GABA_A$ receptor has an α1 or α4 subunit in its complex may have dramatic effects on brain inhibition. The results of our research show that BDNF may be responsible for flipping the switch in α subunit expression, with decreased α1 and increased α4, all in response to the activities of one signaling molecule, BDNF. Dramatic increases in the levels of BDNF associated with SE may drive distinct changes in gene expression through activation of at least three pathways: PKC/MAPK, Egr3, and JAK/STAT. Evidence to support this model comes from in vitro, ex vivo, and in vivo studies. From Brooks-Kayal AR, Raol YH, Russek SJ. Alteration of epileptogenesis genes, *Neurotherapeutics.* 2009;6(2):312–318.

changes in expression of these subunits during epileptogenesis remain to be determined.

CONCLUSION

The full range of gene expression changes that are involved in epileptogenesis and the molecular mechanisms that underlie them are just beginning to be characterized. Studies using animal models suggest that modulation in the transcription of a number of these genes via viral-mediated delivery of DNA binding proteins or RNA silencers, or the manipulation of their upstream regulation via selective inhibition of signal transduction pathways, may be useful therapeutic tools for the future treatment of epilepsy.

Recent work characterizing the functions of the BDNF, JAK/STAT, CREB/ICER, and Egr3 signaling pathways in GABAR α1 and α4 subunit changes in the dentate gyrus after SE provides an important lead for the future development of molecular therapies aimed at restoring the balance of excitation and inhibition in the nervous system. However, the upstream components of these pathways, and the exact means through which they confer vulnerability to epilepsy, must be further elucidated. Further, as these pathways regulate myriad genes with diverse functions, modulation of any of these pathways may have a multitude of downstream effects, many of which may involve cell- and region-specific responses throughout the brain. Therefore, the final impact of pathway blockade on epileptogenesis may be difficult to predict. For example, although the enhanced GABAR α1-subunit expression in dentate gyrus that results from JAK/STAT pathway blockade and subsequent ICER inhibition would be expected to have an antiepileptic effect, mutant mice lacking ICER have accelerated kindling[57] and develop more severe epilepsy following pilocarpine-induced SE.[58] Consistent with this finding, ICER-overexpressing mice show retardation of kindling development.[57] Whether the effects of acute and transient blockade of ICER upregulation at the time of SE specifically in the hippocampal formation will have an effect on epileptogenesis similar to that of constitutive under- or overexpression of ICER globally in the brain remains to be determined. Finally, as several of these signaling pathways have been implicated in the regulation of learning, memory, and cell survival, the effects of modulation of these pathways on these critical parameters will need to be closely monitored.

DISCLOSURE STATEMENT

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REFERENCES

1. Mehta A, Ticku M. An update on GABA(A) receptors. *Brain Res, Brain Res Rev.* 1999;29:196–217.

2. Vicini S. Pharmacologic significance of the structural heterogenetity of the $GABA_A$ receptor-chloride ion channel complex. *Neuropsychopharmacology.* 1991;4:9–15.
3. Glykys J, Mody I. The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. *J Physiol.* 2007;582:1163–1178.
4. Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen $GABA_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J Neurosci*. 1992;12:4151–4172.
5. Macdonald RL, Olsen RW. GABA(A) receptor channels. *Annu Rev Neurosci*. 1994;17:569–602.
6. Wisden W, Laurie D, Monyer M, Seeburg P. The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencehalon, diencephalon, mesencephalon. *J Neurosci*. 1992;12:1040–1062.
7. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat Med*. 1998;4:1166–1172.
8. Peng Z, Huang CS, Stell BM, Mody I, Houser CR. Altered expression of the delta subunit of the GABA(A) receptor in a mouse model of temporal lobe epilepsy. *J Neurosci.* 2004;24:8629–8639.
9. Zhang N, Wei W, Mody I, Houser CR. Altered localization of GABA(A) receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *J Neurosci*. 2007;27:7520–7531.
10. Cohen AS, Lin DD, Quirk GL, Coulter DA. Dentate granule cell GABA(A) receptors in epileptic hippocampus: enhanced synaptic efficacy and altered pharmacology. *Eur J Neurosci*. 2003;17:1607–1616.
11. Rajasekeran K, Joshi S, Sun C, Mtchedlishvilli A, Kapur J. Receptors with low affinity for neurosteroids and GABA contribute to toxic inhibition of granule cells in epileptic animals. *Neurobiol Dis.* 2010;40:490–501.
12. Mtchedlishviliz Z, Bertram EH, Kapur J. Diminished allopregnanolone enhancement of GABA(A) receptor currents in a rat model of chronic temporal lobe epilepsy. *J Physiol*. 2001;537:453–465.
13. Sun C, Mtchedlishvili Z, Erisir A, Kapur J. Diminished neurosteroid sensitivity of synaptic inhibition and altered location of the alpha4 subunit of GABA(A) receptors in an animal model of epilepsy. *J Neurosci*. 2007;27:12641–12650.
14. Lund IV, Hu Y, Raol YH, et al. BDNF selectively regulates GABA(A) receptor transcription by activation of the JAK/STAT pathway. *Sci Signal*. 2008;1:ra9.
15. Brooks-Kayal AR, Shumate MD, Jin H, et al. Human neuronal γ-aminobutyric $acid_A$ receptors: coordinated subunit mRNA expression and functional correlates in individual dentate granule cells. *J Neurosci*. 1999;19: 8312–8318.
16. Loup F, Wieser H, Yonekawa Y, Aguzzi A, Fritschy J. Selective alterations in GABA(A) receptor subtypes in human temporal lobe epilepsy. *J Neurosci*. 2000;20:5401–5419.
17. Shumate M, Lin D, Gibbs JW III, Holloway K, Coulter D. GABA(A) receptor function in epileptic human dentate granule cells: comparison to epileptic and control rat. *Epilepsy Res.* 1998;32:114–128.
18. Zhang G, Raol YS, Hsu FC, Brooks-Kayal AR. Long-term alterations in glutamate receptor and transporter expression following early-life seizures are associated with increased seizure susceptibility. *J Neurochem*. 2004;88:91–101.
19. Raol YH, Lund IV, Bandyopadhyay S, et al. Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. *J Neurosci.* 2006;26:11342–11346.
20. Roberts DS, Raol YH, Bandyopadhyay S, et al. Egr3 stimulation of GABRA4 promoter activity as a mechanism for seizure-induced up-regulation of GABA(A) receptor alpha4 subunit expression. *Proc Natl Acad Sci USA*. 2005;102:11894–11899.
21. Hu Y, Lund IV, Gravielle MC, Farb DH, Brooks-Kayal AR, Russek SJ. Surface expression of GABA (A) receptors is transcriptionally controlled by the interplay of CREB and its binding partner ICER. *J Biol Chem.* 2008;283(14):9328–9340. Epub 2008 Jan 7.
22. Lonze BE, Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. *Neuron.* 2002;35:605–623.
23. Jaworski J, Mioduszewska B, Sanchez-Capelo A, et al. Inducible cAMP early repressor, an endogenous antagonist of cAMP responsive element-binding protein, evokes neuronal apoptosis in vitro. *J Neurosci*. 2003;23:4519–4526.
24. Molina CA, Foulkes NS, Lalli E, Sassone-Corsi P. Inducibility and negative autoregulation of CREM: an alternative promoter directs the expression of ICER, an early response repressor. *Cell.* 1993;75:875–886.
25. Steiger JL, Russek SJ. GABA(A) receptors: building the bridge between subunit mRNAs, their promoters, and cognate transcription factors. *Pharmacol Ther*. 2004;101:259–281.
26. Fitzgerald LR, Vaidya VA, Terwilliger RZ, Duman RS. Electroconvulsive seizure increases the expression of CREM (cyclic AMP response element modulator) and ICER (inducible cyclic AMP early repressor) in rat brain. *J Neurochem*. 1996;66:429–432.
27. Lee B, Dziema H, Lee KH, Choi YS, Obrietan K. CRE-mediated transcription and COX-2 expression in the pilocarpine model of status epilepticus. *Neurobiol Dis*. 2007;25:80–91.
28. McNamara JO, Huang YZ, Leonard AS. Molecular signaling mechanisms underlying epileptogenesis. *Sci STKE*. 2006;2006:re12.
29. Altar CA, Laeng P, Jurata LW, et al. Electroconvulsive seizures regulate gene expression of distinct neurotrophic signaling pathways. *J Neurosci*. 2004;24: 2667–2677.
30. Binder DK, Croll SD, Gall CM, Scharfman HE. BDNF and epilepsy: too much of a good thing? *Trends Neurosci*. 2001;24:47–53.
31. Mudo G, Jiang XH, Timmusk T, Bindoni M, Belluardo N. Change in neurotrophins and their receptor mRNAs in the rat forebrain after status epilepticus induced by pilocarpine. *Epilepsia*. 1996;37: 198–207.
32. Roberts DS, Hu Y, Lund IV, Brooks-Kayal AR, Russek SJ. Brain-derived neurotrophic factor (BDNF)-induced synthesis of early growth response factor 3 (Egr3) controls the levels of type A GABA receptor alpha 4 subunits in hippocampal neurons. *J Biol Chem.* 2006;281:29431–29435.
33. Rudge JS, Mather PE, Pasnikowski EM, et al. Endogenous BDNF protein is increased in adult rat hippocampus after a kainic acid induced excitotoxic

insult but exogenous BDNF is not neuroprotective. *Exp Neurol*. 1998;149:398–410.
34. Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*. 1994;264:1415–1421.
35. Ihle JN. STATs: signal transducers and activators of transcription. *Cell*. 1996;84:331–334.
36. Schindler C, Darnell JE Jr. Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annu Rev Biochem*. 1995;64:621–651.
37. Zhong Z, Wen Z, Darnell J. STAT3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science*. 1994;264:95–98.
38. Zhong Z, Wen Z, Darnell J. STAT3 and STAT4: members of the family of signal transducers and activators of transcription. *Proc Natl Acad Sci USA*. 1994;91:4806–4810.
39. Amit-Vazina M, Shishodia S, Harris D, et al. Atiprimod blocks STAT3 phosphorylation and induces apoptosis in multiple myeloma cells. *Br J Cancer*. 2005;93:70–80.
40. Faderl S, Ferrajoli A, Harris D, et al. Atiprimod blocks STAT3 phosphorylation of JAK-STAT and inhibits proliferation of acute myeloid leukemia (AML) cells. *Leuk Res*. 2007;31:91–95.
41. Hussain S, Kong L, Jordan J, et al. A novel small molecule inhibitor of signal transducers and activators of transcription 3 reverses immume tolerance in malignant glioma patients. *Cancer Res*. 2007;67:9630–9636.
42. Dowlati A, Nethery D, Kern J. Combined inhibition of epidermal growth factor receptor and JAK/STAT pathways results in greater growth inhibition in vitro than single agent technology. *Mol Cancer Ther*. 2004;3:459–463.
43. White G, Gurley D. α Subunits influence Zn block of γ2 containing $GABA_A$ receptor currents. *Neuroreport*. 1995;6:461–464.
44. Jones-Davis D, Macdonald R. GABA(A) receptor function and pharmacology in epilepsy and status epilepticus. *Curr Opin Pharmacol*. 2003;2:12–18.
45. Lagrange AH, Botzolakis EJ, Macdonald RL. Enhanced macroscopic desensitization shapes the response of alpha4 subtype-containing $GABA_A$ receptors to synaptic and extrasynaptic GABA. *J Physiol*. 2007;578:655–676.
46. O'Donovan K, Tourtellotte W, Millbrandt J, Baraban J. The EGR family of transcription-regulatory factors: progress at the interface of molecular and systems neuroscience. *Trends Neurosci*. 1999;22:167–173.
47. Smith SS, Shen H, Gong QH, Zhou X. Neurosteroid regulation of GABA(A) receptors: focus on the alpha4 and delta subunits. *Pharmacol Ther*. 2007;116:58–76.
48. Reddy D. The role of neurosteroids in the pathophysiology and treatment of catamenial epilepsy. *Epilepsy Res*. 2009;85:1–30.
49. Gangisetty O, Reddy D. Neurosteroid withdrawal regulates GABA-A receptor α4-subunit expression and seizure susceptibility by activation of progesterone receptor-independent early growth response factor-3 pathway. *Neuroscience*. 2010;170:865–880.
50. Brunig I, Penschuck S, Berninger B, et al. BDNF reduces miniature inhibitory postsynaptic currents by rapid downregulation of GABA(A) receptor surface expression. *Eur J Neurosci*. 2001;13:1320–1328.
51. Kanematsu T, Yasunaga A, Mizoguchi Y, et al. Modulation of GABA(A) receptor phosphorylation and membrane trafficking by phospholipase C-related inactive protein/protein phosphatase 1 and 2A signaling complex underlying brain-derived neurotrophic factor-dependent regulation of GABAergic inhibition. *J Biol Chem*. 2006;281:22180–22189.
52. Joshi S, Kapur J. Slow intracellular accumulation of GABA(A) receptor delta subunit is modulated by brain-derived neurotrophic factor. *Neuroscience*. 2009;164:507–519.
53. Schwarzer C, Tsunashima K, Wanzenbock C, Fuchs K, Sieghart W, Sperk G. GABA(A) receptor subunits in the rat hippocampus II: altered distribution in kainic acid-induced temporal lobe epilepsy. *Neuroscience*. 1997;80:1001–1017.
54. Nishimura T, Schwarzer C, Gasser E, et al. Altered expression of GABA(A) and GABA(B) receptor subunit mRNAs in the hippocampus after kindling and electrically induced status epilepticus. *Neuroscience*. 2005;134:691–704.
55. Maguire J, Stell B, Rafizadeh M, Mody I. Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci*. 2005;8:797–804.
56. Maguire J, Mody I. GABA(A)R plasticity during pregnancy: relevance to postpartum depression. *Neuron*. 2008;59:207–213.
57. Kojima N, Borlikova G, Sakamoto T, et al. Inducible camp early repressor acts as a negative regulator for kindling epileptogenesis and long-term fear memory. *J Neurosci*. 2008;28:6459–6472.
58. Porter BE, Lund IV, Varodayan FP, Wallace RW, Blendy JA. The role of transcription factors cyclic-AMP responsive element modulator (CREM) and inducible cyclic-AMP early repressor (ICER) in epileptogenesis. *Neuroscience*. 2008;152(3):829–836.

Chapter 45

Chloride Homeostasis and GABA Signaling in Temporal Lobe Epilepsy

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Defects in GABAergic signaling have often been linked to the epilepsies. Suppressing fast inhibition mediated by gamma-aminobutyric acid A ($GABA_A$) receptors initiates interictal-like activities in healthy brain tissue,[1,2] and specific subgroups of interneurons seem to be especially sensitive to the neuronal death associated with temporal lobe epileptic syndromes.[3–5] However, defects in the neuronal homeostasis of chloride have only recently been linked to epileptiform activities. Intraneuronal levels of chloride control GABAergic signaling postsynaptically.[6] So, changes in chloride homeostasis can affect the strength and even the sign of GABAergic signals. We will describe work on tissue from patients with pharmacoresistant epilepsies of the temporal lobe that provided the first insight that chloride homeostasis might be altered in the epilepsies.[7,8] We will examine molecules that control chloride homeostasis, evidence that they are modulated by pathological stressors including denervation, anoxia and the sclerotic cell death associated with some focal epilepsies. We ask whether changes in chloride homeostasis contribute to ictal events, arguing that potassium efflux mediated by K-Cl cotransporters may contribute to prolonged ictal excitation. Finally, we examine how differences in chloride regulation may contribute to neonatal epilepsies and ask whether molecules targeting chloride homeostasis might be effective anti-epileptic drugs.

HUMAN INTERICTAL ACTIVITY AND CL^- HOMEOSTASIS

The first hint of a link between defects in Cl^- homeostasis and temporal lobe epilepsy (TLE)

emerged in work on slices of tissue from adult patients.[7] The subiculum, downstream from the sclerotic CA1 region, generated spontaneous interictal-like activity (Fig. 45–1). This population synchrony was suppressed by either GABAergic or glutamatergic antagonists, suggesting that both transmitter systems were involved in its expression. Depolarized reversal potentials for isolated GABA-mediated synaptic events in some subicular pyramidal cells suggested that Cl^- homeostasis was altered.

More specific evidence of changes in Cl^- homeostasis in brain tissue from patients with TLE, from in situ hybridization and immunostaining, suggests expression of two cotransporter molecules, NKCC1 and KCC2, may be altered. Expression of the Na-K-2Cl cotransporter, NKCC1,[9] which usually functions to import Cl^-, appears to be increased in epileptic tissue, while expression of the Cl^--extruding K-Cl cotransporter, KCC2,[10] seems to be reduced.[8,11,12] NKCC1 appears to be functional in tissue from adult TLE patients and contributes to the genesis of interictal activity.[8] Earlier work on human epileptic tissue showed some evidence suggestive of changes in Cl^- homeostasis.[13,14] Later results, from slice and animal models of focal epilepsies, confirmed that changes in Cl^- homeostasis can contribute to epileptiform activities by reducing the strength of hyperpolarizing GABAergic signaling, sometimes resulting in depolarizing responses.[15–17]

However, cellular studies in human tissue reveal a situation more complex than a uniform downregulation of KCC2 and upregulation of NKCC1. Firstly, GABA reversal potential (E_{GABA}) differs, suggesting that basal Cl^- homeostasis is not affected similarly in all neurons. Instead, there is a wide variation in driving force for GABAergic inhibition: in most principal cells it remains hyperpolarizing, while GABAergic events depolarize only a minority (~20%) of subicular pyramidal cells (Fig. 45–1). The proportion of cells depolarized during interictal events was similar to that of cells where E_{GABA} was depolarized with respect to resting potential.[8] Secondly, immunostaining reveals no KCC2 signal (Fig. 45-1A) in only a proportion of this minority of cells.[8] Perhaps low levels of KCC2 in some neurons of this interictal network cannot effectively assure hyperpolarizing responses to GABA, possibly KCC2 is expressed but inactivated by posttranscriptional mechanisms, or perhaps other Cl^--regulating molecules are involved.

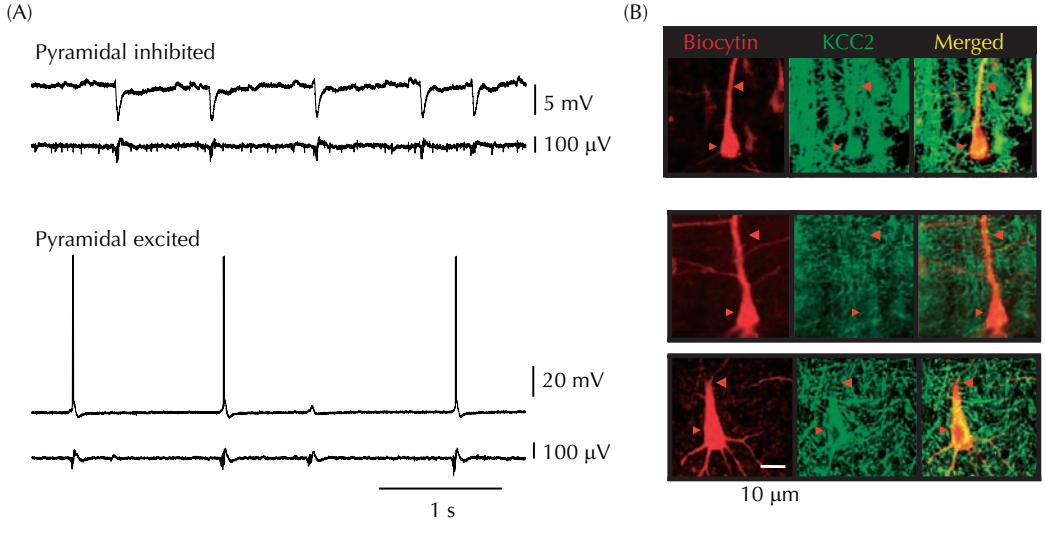

Figure 45–1. Correlation of pyramidal cell behavior during interical discharges with KCC2 expression in the human postoperative subiculum. **A.** Combined intracellular (top trace) and extracellular recordings (bottom trace) of a pyramidal cell inhibited by GABA (≈80%, upper recording) and a pyramidal cell depolarized and excited by GABA (≈20%, lower recording) during interictal events. **B.** Immunostaining for KCC2 (green) in cells identified by biocytin filling (red). All cells hyperpolarized during epileptiform events expressed KCC2 (yellow on merging, top cell). Most cells depolarized during interictal discharges did not express KCC2 (middle cell), but some of them have a clear staining for KCC2 (bottom cell). Modified from refs. 7 and 8.

Thirdly, while changes in Cl^- homeostasis seem to account for the generation of interictal-like activity in the subiculum, distinct mechanisms, possibly involving rearrangements in excitatory synaptic connectivity, may be responsible for a distinct interictal-like activity generated in the CA2 region.[18] Finally mechanisms of the interictal population synchrony remain to be explored. A population activity dependent on both GABAergic and glutamatergic signaling seems at first similar to the giant depolarizing potentials (GDPs) of immature hippocampus,[19] where interneurons may play a permissive role in rhythmogenesis.[20,21] However, interictal events of human epileptic tissue seem to be initiated by inhibitory cell firing,[22] suggesting that some interneurons should induce principal cell firing in the human epileptic subiculum.

GABA AND Cl^- REGULATION SYSTEMS

These data point to a defect in GABAergic signaling due to altered Cl^- homeostasis in some epilepsies. Neuronal Cl^- homeostasis depends in part on cation chloride cotransporters (CCCs). They are glycoproteins with 12 membrane-spanning segments and two cytosolic termini.[23,24] Adult pyramidal neurons are known to express the Na-K-2Cl cotransporter (NKCC1) and the K-Cl cotransporter isoforms, KCC2 and KCC3.[25] The KCC2 isoform is expressed only in central neurons. Alternatively spliced variants may support distinct regulatory mechanisms, via phosphorylation for instance, but their physiological role is unclear. The available evidence suggests that CCCs exist as homodimers in vivo and that dimerization probably plays a role in the regulation of their function.[26–28]

Neuronal CCCs are secondary transporters in that they do not consume adenosine triphosphate (ATP) but rather derive energy for ion transport from gradients established by the sodium-potassium adenosine triphosphatase (Na-K ATPase). Thus, Cl^- extrusion via KCCs is driven by the K^+ gradient, while NKCC1-mediated Cl^- uptake depends on the Na^+ gradient.[29–31] Since KCC2 operates close to its thermodynamic equilibrium, even a small increase in extracellular K^+ will reverse transport, from Cl^- efflux to influx. Even so, activity-dependent increases in internal Cl^- shift the equilibrium so that KCC2 may induce large transient increases in external K^+. Cation chloride cotransporters are electroneutral, with a stoichiometry of 1:1; K:Cl for KCCs and of 1:1:2; Na:K:Cl for NKCC1. Thus, electrophysiological methods cannot directly measure CCC transport. Most work has relied instead on Cl^--permeable channels, such as $GABA_A$ and glycine receptors, to estimate intracellular Cl^-, which has also been measured with specific optical probes.[32–34]

Synaptic events mediated by GABA or glycine have often been used to assess cotransporter function, but two reservations should be noted. First, while the basal inhibitory postsynaptic potential (IPSP) reversal potential is related to cotransporter action, function is better measured by imposing a defined Cl^- load on a neuron and measuring the consequent shift of E_{GABA}.[6,35,36] A second distinct point is that the direction, hyperpolarizing or depolarizing, of postsynaptic potential change provoked by a GABA- or glycine-mediated synaptic event does not completely describe its effects on postsynaptic excitability. The conductance increase due to receptor activation reduces local excitability at the synaptic site whether the membrane is depolarized or hyperpolarized.[37,38]

A differentially, selective pharmacology would facilitate work on the function of these cotransporters. The loop diuretic furosemide blocks both NKCC1 and KCCs with similar potency at millimolar (mM) concentrations, but also affects *N*-methyl-D-aspartate (NMDA) and $GABA_A$ receptors.[39] The diuretic bumetanide has a much higher affinity for NKCC1 than for KCC2, and 1–10 uM provides a selective inhibition.[24] Intracellular Cs^+, sometimes used in pipette solutions to enhance space clamp, is an antagonist of KCC2.[24,40]

NKCC1 and KCC2 seem to be expressed at distinct subcellular neuronal sites. Immunohistochemistry shows significant expression of KCC2 on somatic and dendritic membranes, including spines, but not at axonal sites.[41,42] This localization agrees with point measurements of E_{GABA}.[43–45] KCC2 expression by dendritic spines may contribute to morphogenic functions. KCC2 interacts with the cytoskeleton and may be involved in neuronal maturation[46] and specifically in spine formation.[47] Defining patterns of neuronal NKCC1 expression is difficult

due to the questionable specificity of available antibodies.[25]

Heterogeneous membrane expression of KCC2 and NKCC1, should impose gradients on subcellular Cl^- and so generate differences in basal E_{GABA} at different neuronal sites. Indeed, physiological data suggest that NKCC1-mediated Cl^- import may occur at the axon initial segment of mature neurons. Depolarized reversal potentials have been measured for GABAergic synaptic events induced by axo-axonic cells[43,45] and responses to GABA[44,48] at the axon initial segment. However, E_{GABA} is typically measured from somatic responses to the activation of GABAergic synapses, and axo-axonic inputs may have relatively small influence on this value. Other transporters, including the Cl^-/HCO_3^- exchanger, AE3, may also contribute to control of somatic levels of Cl^-.[44]

The $GABA_A$ receptor is permeable to HCO_3^- as well as Cl^-.[49,50] HCO_3^- carries significant current, which may exceed Cl^- currents in neurons with especially hyperpolarized resting potentials in vitro.[51] The resting membrane potential (V_m) is more positive in hippocampal neurons in vitro, so their E_{GABA} values are less strongly influenced by the HCO_3^- current.[6] Slice preparation may affect internal Cl^- values,[52] and of course, values of E_{GABA} determined for neurons in slices do not provide accurate data on E_{GABA} values in the intact animal, where Cl^- loads may be much higher.

Changes in Cl^--Regulating Systems in Pathological States

Neuronal Cl^- regulation is affected in multiple pathophysiological conditions.[53,54] KCC2 expression is downregulated, leading to a decreased efficacy of inhibition or even to excitatory actions of GABA, in response to kindling,[55] in models of concussion,[56] and by ischemia,[57–59] after axotomy,[60,61] after mechanical isolation of the neocortex,[16] and in nerve section models of chronic spinal pain.[62–64] Such trauma-induced downregulation of KCC2 is often accompanied by upregulation of NKCC1.[8,65]

Thus, the acquired epilepsies[17] may be a particular example of a more general response to brain trauma. Possibly, changes in KCC2 and NKCC1 expression and function participate in epileptogenesis; alternatively, they may be protective or adaptive mechanisms triggered by the trauma. Thus, the downregulation of KCC2 could usefully decrease energy expenditure in pathological states associated with an energy deficit.[25] In a similar way, Na-K ATPase is downregulated by neuronal damage.[66,67] Alternatively, changes in Cl^- homeostasis could contribute to more general processes of neuronal dedifferentiation induced by trauma. They may, for instance, tend to promote rewiring of damaged circuitry for recovery.[7,24]

In these diverse traumatic situations, KCC2 downregulation may be related to activation of the TrkB receptor by brain-derived neurotrophic factor (BDNF).[62] Exogenously applied BDNF was first shown to downregulate KCC2 via TrkB receptors in culture.[55] Work with animals expressing specific point mutations of the TrkB receptor has shown that both the Shc/FRS-2 (src homology 2 domain containing transforming protein/FGF receptor substrate 2) and PLCϒ–CREB (phospholipase Cγ-cyclic adenosine monophosphate response element binding) pathways must be activated to reduce KCC2 transcription. In contrast, the activation of Shc/FRS-2 alone via the TrkB receptor enhances KCC2 synthesis.[36] This observation points to divergent actions of BDNF on neuronal Cl^- regulation. It could explain how BDNF exerts opposing actions on KCC2 synthesis in mature and immature or in intact and damaged neurons.[65] The source of the BDNF involved in the different forms of trauma is not always clear. It is secreted by various types of neurons.[68] More likely however, may be that the BDNF involved in responses to deafferentation is liberated by activated microglia. In a nerve section model of spinal neuropathic pain, microglia migrate to sites of damage and liberate BDNF, thus altering Cl^- homeostasis via TrkB receptors.[62,63]

In these studies, traumatic stimuli reduce KCC2 transcription and thus the total cellular pool of the transporter, typically measured by immunoblots of the protein. However, KCC2 function depends on the fraction of cellular protein present in the cell membrane rather than on the total protein pool. Thus, as for other transporters, changes in membrane trafficking contribute crucially to KCC2 function.[69,70] Cotransporter function might also be modulated by changes in the intrinsic ion

transport rate, but details of whether and how this parameter is modulated are not yet clear.

NKCC1 and KCC2 function is also regulated by phosphorylation. For instance, the kinases WNK (with no lysine (K) kinase) and SPAK (Ste20p-related proline alanine-rich kinase)/OSR1 (oxidative-stress-responsive kinase 1) both activate NKCC1 and inhibit KCC2.[71–73] The phosphorylation state of KCC2 is changed by trauma, oxidative stress, or epilepsy.[69,74] It affects trafficking, including degradation,[69,70,74] and may alter the rate of cotransport by KCC2.[74] Early work stressed a reciprocal regulation in which phosphorylation activates NKCC1 and inhibits KCCs, while dephosphorylation inhibits NKCC1 and activates KCCs.[75] However, more recent work has shown that phosphorylation at different sites of the KCC2 molecule may exert opposing functional effects.[69,70]

Both short- and relatively long-term changes in transporter function can be explained by changes in membrane expression or cotransport rate due to phosphorylation state or by changes in expression due to altered transcription. But transporter function may be persistently altered over months and years after traumatic injuries and in the epilepsies.[7,8] One possible explanation is that of a maintained stimulus due perhaps to chronic inflammation. Maintained neuropathic pain is associated with the persistent release of pro-inflammatory cytokines and chemokines from glial cells.[76] Pro-inflammatory molecules are also involved in the pathogenesis of epilepsy and are present in the chronically epileptic brain.[77] Cells of the blood-brain barrier, whose permeability increases after a seizure, are targets for cytokine signaling.[78] Thus, inflammatory mechanisms may contribute to the evolution of chronic epilepsy.[79] Possibly pro-inflammatory molecules control, directly or indirectly, neuronal cotransporter function.

Cl^- HOMEOSTASIS AND ICTAL ACTIVITIES

Mechanisms of initiation of ictal events in focal epilepsies are not well understood. The human condition is quite well modeled by chronic animal models such as pilocarpine or kainate treatment.[80] They exhibit a similar pattern of sclerotic hippocampal cell death and show a delay between the initial convulsion and the emergence of recurring seizures. However, chronic epilepsy models have so far provided few insights into mechanisms of ictogenesis. Instead, most concepts derive from work on slices from healthy animals exposed to convulsants.[81–83]

Recent work on the genesis of epileptiform activities has emphasized a glial contribution,[84–86] and glial control of external levels of both potassium and glutamate may be compromised in an epileptic brain.[78,87] However, synaptic mechanisms involving both glutamatergic and GABAergic signaling certainly contribute to ictal discharges. Indeed, convulsants activate interneurons particularly strongly,[88,89] and ictal events are suppressed by agents, such as opiate receptor agonists, that selectively reduce interneuron activity.[81]

The chloride flux due to high-frequency activation of inhibitory synapses engages Cl^- homeostatic mechanisms. The cotransporters KCC2 and NKCC1 may then contribute to, and even favor, seizures. If Cl^- extrusion mechanisms cannot maintain low levels of intracellular chloride,[48,90,91] synaptic signals mediated by inhibitory cell firing may change from hyperpolarizing to depolarizing. Such a dynamic switch should enhance and prolong an ictal event. Furthermore, even if the polarity of GABAergic events is reversed, the KCC2 transporter continues to export not only Cl^- but also K^+ ions.[92] The strong activation of $GABA_A$ receptors during an ictal event leads to a large electrogenic uptake of Cl driven by the depolarizing HCO_3^- current (Fig. 45–2). The resulting surge in external K^+[93] adds to that due to massive neuronal firing. It increases neuronal excitability at both somatodendritic and axonal sites, with a consequent increase in antidromic firing.[94,95] The water influx into cells tends to reduce extracellular volume, enhances ephaptic neuronal interactions, and increases local concentrations of glutamate and K^+.[96]

A seizure-promoting action of KCC2 due to an increase in external K^+ is also consistent with the anti-convulsant actions of carbonic anhydrase (CA) inhibitors. Intracellular CA activity is needed to replenish the HCO_3^- and drive further Cl^- uptake.[50,93] A KCC2-mediated extracellular K^+ transient may also partly explain the anti-convulsant actions of furosemide.[97,98] However, elevated extracellular K^+[75] reverses

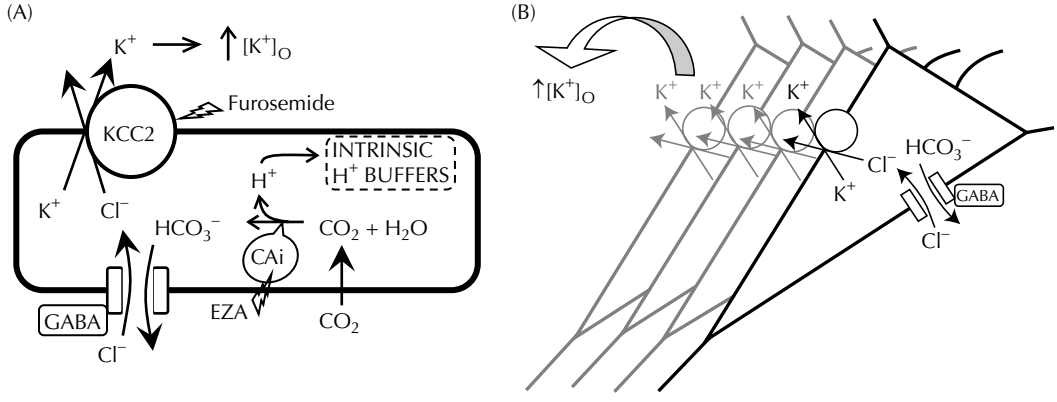

Figure 45–2. KCC2 in the generation of seizure-promoting $[K^+]_o$ transients. **A.** HCO_3^- efflux via a $GABA_A$ receptor channel causes depolarization of the membrane potential that drives a conductive uptake of Cl^-, and net hydration of CO_2 catalyzed by cytosolic carbonic anhydrase (CA) replenishes HCO_3^- during its efflux.[50] H^+ ions are produced at the same rate as HCO_3^- ions and bound by intrinsic cytosolic buffers, which is essential in the maintenance of the HCO_3^- electrochemical gradient during prolonged $GABA_A$ receptor activation. The HCO_3^--dependent intraneuronal accumulation of Cl^- drives K-Cl cotransport by KCC2, thereby giving rise to a net efflux of K^+. **B.** During intense activation of $GABA_A$ receptors in a population of neurons, the KCC2-mediated net efflux of K^+ can be large enough to lead to a large increase $[K^+]_o$, which is a characteristic feature of seizure activity. Note that the chain of events depicted in **A** can be blocked by furosemide or by membrane-permeable inhibitors of intracellular CA (CAi), such as ethoxyzolamide, both of which are known to exert anti-convulsant actions. Modified from ref. 93.

KCC2 cotransport of K^+ and Cl^-[99], so the surge in external K^+ should be self-limiting.

Cl^- REGULATION AND EPILEPTIFORM ACTIVITIES IN THE YOUNG

In contrast to the adult brain, seizure activity in neonatal rat hippocampus upregulates KCC2 activity via activation of TrkB receptors.[100] Interestingly, TrkB may also trigger events that enhance KCC2 expression in the normal neonate, initiating the hyperpolarizing shift of E_{GABA} during development.[101] In the neonate, BDNF-TrkB signaling induces an increase in membrane $GABA_A$ receptors, but it initiates a decrease in more mature neurons.[102] TrkB activation then synergistically enhances both the voltage and conductance effects of GABAergic inhibition in immature neurons but has opposite effects in the mature brain, possibly due to the activation of different signaling pathways.

In the adult, activity-dependent acidosis may be a key factor in seizure termination.[103] In contrast, neonatal seizures may be terminated in part by a seizure-induced increase in the efficacy of GABAergic inhibition.[100] We note that CA is not expressed by neonatal pyramidal neurons.[104] In its absence, transport mediated by KCC2 after strong GABAergic activity during a seizure should not produce a pro-convulsant increase in extracellular K^+. NKCC1 may play a key role in loading neonatal pyramidal cells with Cl^-, since the antagonist bumetanide seems to suppress neonatal seizures[105] and also reduces the resistance to pro-GABAergic drugs that occurs due to Cl accumulation during recurring ictal-like events in slices.[52]

MOLECULES REGULATING Cl^- HOMEOSTASIS AS TARGETS FOR ANTI-EPILEPTIC DRUGS

There is a strong need for new drug targets in TLE[106,107] Might pathways controlling Cl^-homeostasis be a useful target?

A compromised control of intracellular Cl may contribute to interictal rhythmogenesis. However, as we have discussed, residual cotransporter activity should tend to elevate extracellular K^+ in response to repetitive activation of inhibitory synapses and so contribute to the prolonged depolarization underlying an ictal event. Anti-epileptic drugs need to counter ictal rather than interictal events. Nevertheless, there has been interest in the diuretic molecule

bumetanide,[108] which can be used to block the Cl-importing cotransporter, NKCC1, without affecting the exporting transporter, KCC2.

Bumetanide should tend to shift the driving force for GABAergic actions in a hyperpolarizing direction. This action suffices to suppress interictal-like activity in slices of adult human epileptic tissue.[8] Similar results have been reported in different models of neonatal epilepsies.[52,109,110] However, bumetanide is reported not to have anti-ictal effects in chronically epileptic animals[111] and in some neonatal slice models.[111,112]

It has been suggested that compounds that selectively enhance KCC2 actions should increase the efficacy of postsynaptic inhibition and thereby act as anticonvulsant drugs.[108] Paradoxically, however, the role of KCC2 in promoting ictal discharges (Fig. 45–2) suggests that the opposite may be true. Indeed, furosemide, which inhibits both KCC2 and NKCC1, has anti-epileptic actions in focal cortical epilepsies,[97,98] although the high doses needed to block KCC2[93] probably preclude the use of this molecule as an anti-convulsant.

Proteins that regulate the expression, trafficking, and activity of the CCCs may offer alternative targets for anti-convulsant drugs. In practice, however, the importance of cotransporter function in regulating electrolyte balance and cell volume throughout the body implies that some means of targeting such molecules to neurons, or perhaps subsets of neurons, will also be needed

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Schwartzkroin PA, Prince DA. Penicillin-induced epileptiform activity in the hippocampal in vitro preparation. *Ann Neurol*. 1977;1:463–469.
2. Wong RK, Traub RD, Miles R. Cellular basis of neuronal synchrony in epilepsy. *Adv Neurol*. 1986;44:583–592.
3. Magloczky Z, Freund TF. Impaired and repaired inhibitory circuits in the epileptic human hippocampus. *Trends Neurosci*. 2005;28:334–340.
4. Cossart R, Bernard C, Ben-Ari Y. Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signaling in epilepsies. *Trends Neurosci*. 2005;28:108–115.
5. Knopp A, Frahm C, Fidzinski P, Witte OW, Behr J. Loss of GABAergic neurons in the subiculum and its functional implications in temporal lobe epilepsy. *Brain*. 2008;131:1516–1527.
6. Farrant M, Kaila K. The cellular, molecular and ionic basis of gaba(a) receptor signaling. *Prog Brain Res*. 2007;160:59–87.
7. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science*. 2002;298:1418–1421.
8. Huberfeld G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, Rivera C. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. *J Neurosci*. 2007;27:9866–9873.
9. Delpire E, Rauchman MI, Beier DR, Hebert SC, Gullans SR. Molecular cloning and chromosome localization of a putative basolateral Na(+)-K(+)-2Cl$^-$ cotransporter from mouse inner medullary collecting duct (mimcd-3) cells. *J Biol Chem*. 1994;269:25677–25683.
10. Payne JA, Stevenson TJ, Donaldson LF. Molecular characterization of a putative K-Cl cotransporter in rat brain. A neuronal-specific isoform. *J Biol Chem*. 1996;271:16245–16252.
11. Munoz A, Mendez P, DeFelipe J, Alvarez-Leefmans FJ. Cation-chloride cotransporters and GABA-ergic innervation in the human epileptic hippocampus. *Epilepsia*. 2007;48:663–673.
12. Palma E, Amici M, Sobrero F, Spinelli G, Di Angelantonio S, Ragozzino D, Mascia A, Scoppetta C, Esposito V, Miledi R, Eusebi F. Anomalous levels of Cl$^-$ transporters in the hippocampal subiculum from temporal lobe epilepsy patients make GABA excitatory. *Proc Natl Acad Sci USA*. 2006;103:8465–8468.
13. Kohling R, Lucke A, Straub H, Speckmann EJ, Tuxhorn I, Wolf P, Pannek H, Oppel F. Spontaneous sharp waves in human neocortical slices excised from epileptic patients. *Brain*. 1998;121(pt 6):1073–1087.
14. Schwartzkroin PA, Knowles WD. Intracellular study of human epileptic cortex: in vitro maintenance of epileptiform activity? *Science*. 1984;223:709–712.
15. Khalilov I, Holmes GL, Ben-Ari Y. In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. *Nat Neurosci*. 2003;6:1079–1085.
16. Jin X, Huguenard JR, Prince DA. Impaired Cl$^-$ extrusion in layer V pyramidal neurons of chronically injured epileptogenic neocortex. *J Neurophysiol*. 2005;93:2117–2126.
17. Pathak HR, Weissinger F, Terunuma M, Carlson GC, Hsu FC, Moss SJ, Coulter DA. Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. *J Neurosci*. 2007;27:14012–14022.
18. Wittner L, Huberfeld G, Clemenceau S, Eross L, Dezamis E, Entz L, Ulbert I, Baulac M, Freund TF, Magloczky Z, Miles R. The epileptic human hippocampal Cornu Ammonis 2 region generates spontaneous interictal-like activity in vitro. *Brain*. 2009;132:3032–3046.
19. Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL. Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiol*. 1989;416:303–325.
20. Marchionni I, Omrani A, Cherubini E. In the developing rat hippocampus a tonic $GABA_A$-mediated conductance selectively enhances the glutamatergic drive of principal cells. *J Physiol*. 2007;581:515–528.

21. Sipila ST, Huttu K, Soltesz I, Voipio J, Kaila K. Depolarizing GABA acts on intrinsically bursting pyramidal neurons to drive giant depolarizing potentials in the immature hippocampus. *J Neurosci*. 2005;25: 5280–5289.
22. Huberfeld G, Menendez de la Prida L, Pallud J, Cohen I, Le Van Quyen M, Adam C, Clemenceau S, Baulac M, Miles R. Glutamatergic pre-ictal discharges emerge at the transition to seizure in human epilepsy. *Nat Neurosci*. 2011;4:627–634.
23. Mercado A, Mount DB, Gamba G. Electroneutral cation-chloride cotransporters in the central nervous system. *Neurochem Res*. 2004;29:17–25.
24. Payne JA, Rivera C, Voipio J, Kaila K. Cation-chloride cotransporters in neuronal communication, development and trauma. *Trends Neurosci*. 2003;26:199–206.
25. Blaesse P, Airaksinen MS, Rivera C, Kaila K. Cation-chloride cotransporters and neuronal function. *Neuron.* 2009;61:820–838.
26. Blaesse P, Guillemin I, Schindler J, Schweizer M, Delpire E, Khiroug L, Friauf E, Nothwang HG. Oligomerization of KCC2 correlates with development of inhibitory neurotransmission. *J Neurosci*. 2006;26:10407–10419.
27. Casula S, Shmukler BE, Wilhelm S, Stuart-Tilley AK, Su W, Chernova MN, Brugnara C, Alper SL. A dominant negative mutant of the kcc1 K-Cl cotransporter: Both N- and C-terminal cytoplasmic domains are required for K-Cl cotransport activity. *J Biol Chem*. 2001;276:41870–41878.
28. Parvin MN, Gerelsaikhan T, Turner RJ. Regions in the cytosolic C-terminus of the secretory Na(+)-K(+)-2Cl(−) cotransporter NKCC1 are required for its homodimerization. *Biochemistry.* 2007;46:9630–9637.
29. Achilles K, Okabe A, Ikeda M, Shimizu-Okabe C, Yamada J, Fukuda A, Luhmann HJ, Kilb W. Kinetic properties of Cl uptake mediated by Na^+-dependent K^+-2Cl cotransport in immature rat neocortical neurons. *J Neurosci*. 2007;27:8616–8627.
30. Brumback AC, Staley KJ. Thermodynamic regulation of NKCC1-mediated Cl^- cotransport underlies plasticity of GABA(a) signaling in neonatal neurons. *J Neurosci*. 2008;28:1301–1312.
31. Russell JM. Sodium-potassium-chloride cotransport. *Physiol Rev*. 2000;80:211–276.
32. Arosio D, Ricci F, Marchetti L, Gualdani R, Albertazzi L, Beltram F. Simultaneous intracellular chloride and pH measurements using a GFP-based sensor. *Nat Methods*. 2010;7:516–518.
33. Kuner T, Augustine GJ. A genetically encoded ratiometric indicator for chloride: capturing chloride transients in cultured hippocampal neurons. *Neuron*. 2000;27:447–459.
34. Waseem T, Mukhtarov M, Buldakova S, Medina I, Bregestovski P. Genetically encoded Cl^- sensor as a tool for monitoring of Cl-dependent processes in small neuronal compartments. *J Neurosci Methods*. 2010;193:14–23.
35. Khirug S, Huttu K, Ludwig A, Smirnov S, Voipio J, Rivera C, Kaila K, Khiroug L. Distinct properties of functional KCC2 expression in immature mouse hippocampal neurons in culture and in acute slices. Eur J Neurosci. 2005;21:899–904.
36. Rivera C, Voipio J, Thomas-Crusells J, Li H, Emri Z, Sipila S, Payne JA, Minichiello L, Saarma M, Kaila K. Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. *J Neurosci*. 2004;24:4683–4691.
37. Gulledge AT, Stuart GJ. Excitatory actions of GABA in the cortex. *Neuron*. 2003;37:299–309.
38. Vida I, Bartos M, Jonas P. Shunting inhibition improves robustness of gamma oscillations in hippocampal interneuron networks by homogenizing firing rates. *Neuron*. 2006;49:107–117.
39. Staley KJ. Diuretics as antiepileptic drugs: should we go with the flow? *Epilepsy Curr/Am Epilepsy Soc*. 2002;2:35–38.
40. Williams JR, Payne JA. Cation transport by the neuronal K(+)-Cl(−) cotransporter KCC2: thermodynamics and kinetics of alternate transport modes. *Am J Physiol*. 2004;287:C919–931.
41. Baldi R, Varga C, Tamas G. Differential distribution of KCC2 along the axo-somato-dendritic axis of hippocampal principal cells. *Eur J Neurosci*. 2010;32: 1319–1325.
42. Gulyas AI, Sik A, Payne JA, Kaila K, Freund TF. The KCl cotransporter, KCC2, is highly expressed in the vicinity of excitatory synapses in the rat hippocampus. *Eur J Neurosci.* 2001;13:2205–2217.
43. Szabadics J, Varga C, Molnar G, Olah S, Barzo P, Tamas G. Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. *Science*. 2006;311: 233–235.
44. Khirug S, Yamada J, Afzalov R, Voipio J, Khiroug L, Kaila K. GABAergic depolarization of the axon initial segment in cortical principal neurons is caused by the Na-K-2Cl cotransporter NKCC1. *J Neurosci.* 2008;28:4635–4639.
45. Woodruff A, Xu Q, Anderson SA, Yuste R. Depolarizing effect of neocortical chandelier neurons. *Front Neural Circuits*. 2009;3:15.
46. Horn Z, Ringstedt T, Blaesse P, Kaila K, Herlenius E. Premature expression of KCC2 in embryonic mice perturbs neural development by an ion transport-independent mechanism. *Eur J Neurosci*. 2010;31:2142–2155.
47. Li H, Khirug S, Cai C, Ludwig A, Blaesse P, Kolikova J, Afzalov R, Coleman SK, Lauri S, Airaksinen MS, Keinanen K, Khiroug L, Saarma M, Kaila K, Rivera C. KCC2 interacts with the dendritic cytoskeleton to promote spine development. *Neuron*. 2007;56: 1019–1033.
48. Alger BE, Nicoll RA. GABA-mediated biphasic inhibitory responses in hippocampus. *Nature*. 1979;281: 315–317.
49. Kaila K. Ionic basis of $GABA_A$ receptor channel function in the nervous system. *Prog Neurobiol*. 1994;42: 489–537.
50. Kaila K, Voipio J. Postsynaptic fall in intracellular pH induced by GABA-activated bicarbonate conductance. *Nature*. 1987;330:163–165.
51. Kaila K, Voipio J, Paalasmaa P, Pasternack M, Deisz RA. The role of bicarbonate in $GABA_A$ receptor-mediated IPSPs of rat neocortical neurones. *J Physiol*. 1993;464:273–289.
52. Dzhala VI, Kuchibhotla KV, Glykys JC, Kahle KT, Swiercz WB, Feng G, Kuner T, Augustine GJ, Bacskai BJ, Staley KJ. Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. *J Neurosci.* 2010;30:11745–11761.
53. Delpire E, Mount DB. Human and murine phenotypes associated with defects in cation-chloride cotransport. *Annu Rev Physiol*. 2002;64:803–843.

54. Kahle KT, Staley KJ, Nahed BV, Gamba G, Hebert SC, Lifton RP, Mount DB. Roles of the cation-chloride cotransporters in neurological disease. *Nat Clin Pract Neurol*. 2008;4:490–503.
55. Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, Kokaia Z, Airaksinen MS, Voipio J, Kaila K, Saarma M. BDNF-induced Trkb activation down-regulates the K^+-Cl^- cotransporter KCC2 and impairs neuronal Cl^- extrusion. *J Cell Biol*. 2002;159:747–752.
56. Bonislawski DP, Schwarzbach EP, Cohen AS. Brain injury impairs dentate gyrus inhibitory efficacy. *Neurobiol Dis*. 2007;25:163–169.
57. Galeffi F, Sah R, Pond BB, George A, Schwartz-Bloom RD. Changes in intracellular chloride after oxygen-glucose deprivation of the adult hippocampal slice: effect of diazepam. *J Neurosci*. 2004;24:4478–4488.
58. Papp E, Rivera C, Kaila K, Freund TF. Relationship between neuronal vulnerability and potassium-chloride cotransporter 2 immunoreactivity in hippocampus following transient forebrain ischemia. *Neuroscience*. 2008;154:677–689.
59. Jaenisch N, Witte OW, Frahm C. Downregulation of potassium chloride cotransporter KCC2 after transient focal cerebral ischemia. *Stroke*. 2010;41:e151–159.
60. Nabekura J, Ueno T, Okabe A, Furuta A, Iwaki T, Shimizu-Okabe C, Fukuda A, Akaike N. Reduction of KCC2 expression and $GABA_A$ receptor-mediated excitation after in vivo axonal injury. *J Neurosci*. 2002;22:4412–4417.
61. Toyoda H, Ohno K, Yamada J, Ikeda M, Okabe A, Sato K, Hashimoto K, Fukuda A. Induction of NMDA and $GABA_A$ receptor-mediated Ca^{2+} oscillations with KCC2 mRNA downregulation in injured facial motoneurons. *J Neurophysiol*. 2003;89:1353–1362.
62. Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature*. 2005;438:1017–1021.
63. De Koninck Y. Altered chloride homeostasis in neurological disorders: a new target. *Curr Opin Pharmacol*. 2007;7:93–99.
64. Price TJ, Cervero F, Gold MS, Hammond DL, Prescott SA. Chloride regulation in the pain pathway. *Brain Res Rev*. 2009;60:149–170.
65. Shulga A, Thomas-Crusells J, Sigl T, Blaesse A, Mestres P, Meyer M, Yan Q, Kaila K, Saarma M, Rivera C, Giehl KM. Posttraumatic GABA(A)-mediated $[Ca^{2+}]$ increase is essential for the induction of brain-derived neurotrophic factor-dependent survival of mature central neurons. *J Neurosci*. 2008;28:6996–7005.
66. Pylova SI, Majkowska J, Hilgier W, Kapuscinski A, Albrecht J. Rapid decrease of high affinity ouabain binding sites in hippocampal CA1 region following short-term global cerebral ischemia in rat. *Brain Res*. 1989;490:170–173.
67. Ross ST, Soltesz I. Selective depolarization of interneurons in the early posttraumatic dentate gyrus: involvement of the Na(+)/K(+)-ATPase. *J Neurophysiol*. 2000;83:2916–2930.
68. Lessmann V, Brigadski T. Mechanisms, locations, and kinetics of synaptic BDNF secretion: an update. *Neurosci Res*. 2009;65:11–22.
69. Lee HH, Jurd R, Moss SJ. Tyrosine phosphorylation regulates the membrane trafficking of the potassium chloride cotransporter KCC2. *Mol Cell Neurosci*. 2010;45:173–179.
70. Lee HH, Walker JA, Williams JR, Goodier RJ, Payne JA, Moss SJ. Direct protein kinase C-dependent phosphorylation regulates the cell surface stability and activity of the potassium chloride cotransporter KCC2. *J Biol Chem*. 2007;282:29777–29784.
71. Gagnon KB, England R, Delpire E. Volume sensitivity of cation-Cl-cotransporters is modulated by the interaction of two kinases: Ste20-related proline-alanine-rich kinase and WNK4. *Am J Physiol*. 2006;290:C134–C142.
72. Kahle KT, Rinehart J, de Los Heros P, Louvi A, Meade P, Vazquez N, Hebert SC, Gamba G, Gimenez I, Lifton RP. WNK3 modulates transport of Cl^- in and out of cells: implications for control of cell volume and neuronal excitability. *Proc Natl Acad Sci USA*. 2005;102:16783–16788.
73. Rinehart J, Maksimova YD, Tanis JE, Stone KL, Hodson CA, Zhang J, Risinger M, Pan W, Wu D, Colangelo CM, Forbush B, Joiner CH, Gulcicek EE, Gallagher PG, Lifton RP. Sites of regulated phosphorylation that control K-Cl cotransporter activity. *Cell*. 2009;138:525–536.
74. Wake H, Watanabe M, Moorhouse AJ, Kanematsu T, Horibe S, Matsukawa N, Asai K, Ojika K, Hirata M, Nabekura J. Early changes in KCC2 phosphorylation in response to neuronal stress result in functional downregulation. *J Neurosci*. 2007;27:1642–1650.
75. Payne JA. Functional characterization of the neuronal-specific K-Cl cotransporter: implications for $[K^+]o$ regulation. *Am J Physiol*. 1997;273:C1516–C1525.
76. Abbadie C, Bhangoo S, De Koninck Y, Malcangio M, Melik-Parsadaniantz S, White FA. Chemokines and pain mechanisms. *Brain Res Rev*. 2009;60:125–134.
77. Fabene PF, Bramanti P, Constantin G. The emerging role for chemokines in epilepsy. *J Neuroimmunol*. 2010;224:22–27.
78. David Y, Cacheaux LP, Ivens S, Lapilover E, Heinemann U, Kaufer D, Friedman A. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *J Neurosci*. 2009;29:10588–10599.
79. Fabene PF, Navarro Mora G, Martinello M, Rossi B, Merigo F, Ottoboni L, Bach S, Angiari S, Benati D, Chakir A, Zanetti L, Schio F, Osculati A, Marzola P, Nicolato E, Homeister JW, Xia L, Lowe JB, McEver RP, Osculati F, Sbarbati A, Butcher EC, Constantin G. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. *Nat Med*. 2008;14:1377–1383.
80. Pitkanen A, Lukasiuk K. Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. *Epilepsy Behav*. 2009;14(suppl 1):16–25.
81. Avoli M, Louvel J, Kurcewicz I, Pumain R, Barbarosie M. Extracellular free potassium and calcium during synchronous activity induced by 4-aminopyridine in the juvenile rat hippocampus. *J Physiol*. 1996;493(pt 3):707–717.
82. Avoli M, Louvel J, Pumain R, Kohling R. Cellular and molecular mechanisms of epilepsy in the human brain. *Prog Neurobiol*. 2005;77:166–200.
83. Traynelis SF, Dingledine R. Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. *J Neurophysiol*. 1988;59:259–276.
84. Gomez-Gonzalo M, Losi G, Chiavegato A, Zonta M, Cammarota M, Brondi M, Vetri F, Uva L, Pozzan T,

de Curtis M, Ratto GM, Carmignoto G. An excitatory loop with astrocytes contributes to drive neurons to seizure threshold. *PLoS Biol*. 2010;8:e1000352.
85. Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M. An astrocytic basis of epilepsy. *Nat Med*. 2005;11:973–981.
86. Wetherington J, Serrano G, Dingledine R. Astrocytes in the epileptic brain. *Neuron.* 2008;58:168–178.
87. Oliet SH, Piet R, Poulain DA. Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. *Science.* 2001;292:923–926.
88. Fujiwara-Tsukamoto Y, Isomura Y, Kaneda K, Takada M. Synaptic interactions between pyramidal cells and interneurone subtypes during seizure-like activity in the rat hippocampus. *J Physiol*. 2004;557:961–979.
89. Ziburkus J, Cressman JR, Barreto E, Schiff SJ. Interneuron and pyramidal cell interplay during in vitro seizure-like events. *J Neurophysiol*. 2006;95: 3948–3954.
90. Staley KJ, Soldo BL, Proctor WR. Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors. *Science*. 1995;269:977–981.
91. Thompson SM, Gahwiler BH. Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus in vitro. *J Neurophysiol*. 1989;61:501–511.
92. Kaila K, Lamsa K, Smirnov S, Taira T, Voipio J. Long-lasting GABA-mediated depolarization evoked by high-frequency stimulation in pyramidal neurons of rat hippocampal slice is attributable to a network-driven, bicarbonate-dependent K^+ transient. *J Neurosci*. 1997; 17:7662–7672.
93. Viitanen T, Ruusuvuori E, Kaila K, Voipio J. The K^+-Cl cotransporter KCC2 promotes GABAergic excitation in the mature rat hippocampus. *J Physiol*. 2010;588: 1527–1540.
94. Pinault D, Pumain R. Ectopic action potential generation: its occurrence in a chronic epileptogenic focus. *Exp Brain Res*. 1985;60:599–602.
95. Stasheff SF, Hines M, Wilson WA. Axon terminal hyperexcitability associated with epileptogenesis in vitro. I. Origin of ectopic spikes. *J Neurophysiol*. 1993; 70:961–975.
96. Jefferys JG. Nonsynaptic modulation of neuronal activity in the brain: electric currents and extracellular ions. *Physiol Rev*. 1995;75:689–723.
97. Haglund MM, Hochman DW. Furosemide and mannitol suppression of epileptic activity in the human brain. *J Neurophysiol*. 2005;94:907–918.
98. Hochman DW, Baraban SC, Owens JW, Schwartzkroin PA. Dissociation of synchronization and excitability in furosemide blockade of epileptiform activity. *Science.* 1995;270:99–102.
99. Dietzel I, Heinemann U, Hofmeier G, Lux HD. Stimulus-induced changes in extracellular Na^+ and Cl^- concentration in relation to changes in the size of the extracellular space. *Exp Brain Res*. 1982;46:73–84.
100. Khirug S, Ahmad F, Puskarjov M, Afzalov R, Kaila K, Blaesse P. A single seizure episode leads to rapid functional activation of KCC2 in the neonatal rat hippocampus. *J Neurosci*. 2010;30:12028–12035.
101. Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. The K^+/Cl^- cotransporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*. 1999;397: 251–255.
102. Mizoguchi Y, Ishibashi H, Nabekura J. The action of BDNF on GABA(A) currents changes from potentiating to suppressing during maturation of rat hippocampal CA1 pyramidal neurons. *J Physiol*. 2003;548:703–709.
103. de Curtis M, Manfridi A, Biella G. Activity-dependent pH shifts and periodic recurrence of spontaneous interictal spikes in a model of focal epileptogenesis. *J Neurosci*. 1998;18:7543–7551.
104. Ruusuvuori E, Li H, Huttu K, Palva JM, Smirnov S, Rivera C, Kaila K, Voipio J. Carbonic anhydrase isoform VII acts as a molecular switch in the development of synchronous gamma-frequency firing of hippocampal CA1 pyramidal cells. *J Neurosci*. 2004;24:2699–2707.
105. Dzhala VI, Brumback AC, Staley KJ. Bumetanide enhances phenobarbital efficacy in a neonatal seizure model. *Ann Neurol*. 2008;63:222–235.
106. Baulac M, Pitkanen A. Research priorities in epilepsy for the next decade—a representative view of the European scientific community. *Epilepsia*. 2008;50:571–578.
107. Schuele SU, Luders HO. Intractable epilepsy: Management and therapeutic alternatives. *Lancet Neurol*. 2008;7:514–524.
108. Kahle KT, Staley KJ. The bumetanide-sensitive Na-K-2Cl cotransporter NKCC1 as a potential target of a novel mechanism-based treatment strategy for neonatal seizures. *Neurosurg Focus*. 2008; 25:E22.
109. Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, Delpire E, Jensen FE, Staley KJ. NKCC1 transporter facilitates seizures in the developing brain. *Nat Med.* 2005;11:1205–1213.
110. Nardou R, Ben-Ari Y, Khalilov I. Bumetanide, an NKCC1 antagonist, does not prevent formation of epileptogenic focus but blocks epileptic focus seizures in immature rat hippocampus. *J Neurophysiol*. 2009;101:2878–2888.
111. Brandt C, Nozadze M, Heuchert N, Rattka M, Loscher W. Disease-modifying effects of phenobarbital and the NKCC1 inhibitor bumetanide in the pilocarpine model of temporal lobe epilepsy. *J Neurosci*. 2010;30:8602–8612.
112. Kilb W, Sinning A, Luhmann HJ. Model-specific effects of bumetanide on epileptiform activity in the in-vitro intact hippocampus of the newborn mouse. *Neuropharmacology*. 2007;53:524–533.

Chapter 46

Astrocytes and Epilepsy

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Glia, Greek for "glue," was discovered by Rudolph Virchow, a German anatomist, in the mid-nineteenth century. The name reflects the original view that glia played merely a structural or metabolic support role for neurons. Glial cells, especially astrocytes, are much more than glue or merely quiescent and display their own set of activities. Studies over the last 20 years show that astrocytes perform a series of complex functions that go well beyond the uptake and recycling of neurotransmitters and the buffering of extracellular potassium.[1,2]

Morphologically, astrocytes are characterized by a highly ramified structure of thin processes with which they contact neurons, blood vessels, and other astrocytes. Astrocyte-astrocyte contacts mediate gap-junction coupling between adjacent glial cells to form a cellular network called *astrocytic syncytium*.[3] Although astrocytes form a highly interconnected network, they are structurally organized in nonoverlapping spatial domains with limited interdigitation of processes between adjacent cells.[4,5] Within its own domain of occupancy, each astrocyte can contact tens of thousands of synapses and hundreds of dendrites[4,5] (Fig. 46–1A). The contact between the astrocyte and the neuron is a highly dynamic structure,[6,7] and the extent of astrocytic coverage of the neuronal terminals is activity-dependent.[8]

While astrocytes have been considered nonexcitable cells, because unlike neurons they do not fire action potentials,[9] they display a form of excitability that is based on variations of the intracellular Ca^{2+} concentration.[10] Astrocytes express a plethora of receptors for

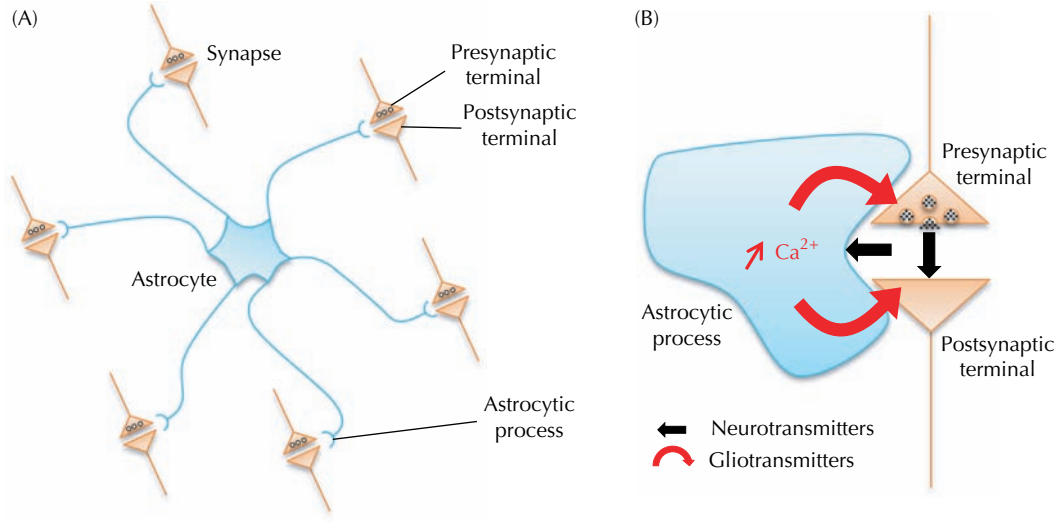

Figure 46–1. The tripartite synapse. **A.** A single astrocyte sends out processes that enwrap a number of synapses. One synapse results from the association between the pre- and postsynaptic terminals. **B.** At the synapse, neurotransmitters released from the presynaptic terminal activate an astrocyte, which responds with Ca^{2+} elevations. In turn, Ca^{2+} elevations lead to the release of neuroactive substances called *gliotransmitters.* which act to the synapse to regulate the presynaptic function and modulate the postsynaptic response.

neurotransmitters whose activation leads to increases in intracellular Ca^{2+} concentration that can propagate to neighboring astrocytes as an intercellular Ca^{2+} wave.[10–12] These Ca^{2+} increases promote the release of neuroactive substances, the so-called *gliotransmitters* (Fig. 46–1B). These gliotransmitters can control diverse brain processes, such as vasculature tone[13] and neuronal activity,[1,2] and can also modulate mammalian behavior such as sleep.[1,2]

In keeping with the currently accepted concept that astrocytes form an integral and active part of excitatory and inhibitory synaptic transmission and communicate back to synapses,[14] emerging evidence has suggested a critical role for these glial cells in the pathogenesis of neurological disorders such as epilepsy.[15–17] Astrocytes become reactive in the epileptic brain and show changes in the expression of metabolic enzymes such as glutamine synthetase and adenosine kinase leading to modification of neuronal excitability. Astrocytes also release glutamate through a Ca^{2+}-dependent mechanism that can synchronize neuronal firing and modulate neuronal excitability and synaptic transmission. In this chapter, we will focus on current lines of evidence suggesting the involvement of reactive astrocytes and gliotransmission in experimental studies of epilepsy, and possible underlying mechanisms.

CA^{2+} SIGNALS IN ASTROCYTES

In Vitro and In Situ Studies

The development of video imaging techniques and fluorescent indicators of Ca^{2+} has allowed us to observe dynamic spatiotemporal changes in Ca^{2+} concentration[18] in neurons and glial cells simultaneously. Unlike neurons, astrocytes do not produce action potentials, and thus they were thought to be quiescent. However, in the early 1990s, initial cell culture studies reported that the excitatory neurotransmitter glutamate elicited Ca^{2+} elevations in individual astrocytes that can propagate to neighboring astrocytes as an intercellular Ca^{2+} wave involving dozen of cells, suggesting long-distance communication between these cells.[10–12] Further evidence for neuronal activity-dependent Ca^{2+} elevations in astrocytes comes from studies showing that the stimulation of Schaffer collateral in hippocampal slices preparation also increases the intracellular Ca^{2+} concentration in these glials cells.[19]

The synaptic control of astrocytic Ca^{2+} signals is due to the fact that astrocytes express a wide range of functional receptors for different neurotransmitters.[20] Many of these receptors are of the metabotropic type, such as metabotropic glutamate receptor 5 (mGluR5) and metabotropic receptor activated by purines such as P_2Y_1. These receptors are associated with G proteins that, upon activation, stimulate phospholipase C and formation of diacylglycerol and inositol (1,4,5)-triphosphate (IP_3). In turn, IP_3 increases the intracellular concentration of Ca^{2+} through the release of Ca^{2+} from intracellular IP_3-sensitive Ca^{2+} stores.[21] Subsequently, several lines of evidence coming from brain slice preparations demonstrated that excitatory neuronal activity can trigger Ca^{2+} elevations in astrocytes.[21]

In Vivo Studies

Recent studies using two-photon microscopy[22,23] and specific fluorescent dyes that selectively label astrocytes[24] convincingly demonstrate that neuronal activity can trigger Ca^{2+} signals in astrocytes in vivo. Using these two revolutionary techniques, Hirase et al.[25] were the first to analyze changes in Ca^{2+} signals in cortical astrocytes from living anesthetized rat. More than 60% of the imaged astrocytes showed a complex pattern of changes in intracellular Ca^{2+} concentration. However, these changes occurred with relatively low frequency under basal conditions and showed a limited degree of correlation with nearby astrocytes.

The first evidence that astrocytes respond to neuronal activity by increasing their intracellular Ca^{2+} concentration in vivo was the observation that the application of gamma-aminobutyric acid A ($GABA_A$) receptor antagonists such as bicuculline[25] or picrotoxin,[26] which increase neuronal activity by triggering epileptic-like discharges, resulted in an increase in Ca^{2+} signaling in cortical astrocytes. Additionally, these Ca^{2+} signals were correlated between pairs of nearby astrocytes,[25] suggesting that in vivo neuronal activity leads to synchronous Ca^{2+} signals in multiple astrocytes. Subsequently, sensory stimulation was shown to increase Ca^{2+} signals in astrocytes in anesthetized animals. In the mouse, whisker,[27] limb,[28] and odor stimulation[29] causes Ca^{2+} elevations in astrocytes in the whisker barrel, the primary somatosensory cortex, and the olfactory bulb, respectively. Generally, these Ca^{2+} increases in astrocytes were delayed by a few seconds compared with the neuronal responses[27,30] and were significantly correlated with the strength of the sensory stimulation.[27] Moreover, a study in ferrets[30] demonstrates that visual stimulation can also induce Ca^{2+} signals in astrocytes in the visual cortex. Interestingly, this study found that astrocytes were even more sharply tuned for stimulus orientation and frequency than neurons.

More recently, neuronal activity in awake, behaving mice was shown to correlate with Ca^{2+} elevations in astrocytes. Two-photon microscopy through a cranial window of an awake, head-restrained mouse allowed to run on a Styrofoam ball was used to visualize Ca^{2+} signals in cortical neurons and astrocytes.[31] Repetitive Ca^{2+} signals were associated with the running behavior and were temporally correlated in multiple astrocytes over a distance of almost 100 μm.[31] Similar studies were performed in the cerebellum, where radial Bergmann glia signals were found to correlate with locomotor behavior and were sensitive to blockade of neuronal activity.[32] Interestingly, in these studies, Nimmerjahn et al.[32] described three different forms of Ca^{2+} signals; one of them was initiated during locomotor behavior and correlated with changes in blood perfusion, suggesting that these glia networks modulate macroscopic changes in brain dynamics and blood flow.[32]

In vivo pharmacological studies have been performed to elucidate the mechanisms underlying activity-mediated Ca^{2+} signals in astrocytes. In vivo application of the mGluR1 or mGluR5 antagonists LY367385 or 6-methyl-2-(phenylethynyl)-pyridine (MPEP), respectively, reduced the whisker activity-induced Ca^{2+} elevations in astrocytes, while the application of the ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) had no effect on the astrocytic Ca^{2+} response,[27] indicating a role for both mGluR1and mGluR5 but not for ionotropic receptors in astrocyte activation. Additionally, another in vivo study has shown that application of the astrocytic glutamate transporter inhibitor DL-threo-β-benzyloxyaspartate (TBOA) reduced Ca^{2+} elevations in astrocytes induced by visual stimulation,[30] suggesting a role for

glutamate transporters in the modulation of Ca^{2+} signals in astrocytes.

Together, these in vivo results demonstrate that, in response to sensory stimulations, astrocytes exhibit complex and extremely finely tuned intracellular Ca^{2+} signals generated by the synaptic release of glutamate that activates mGluRs and glutamate transporters at the astrocytic surface. More importantly, recent studies suggest that these astrocytic Ca^{2+} signals are important for the regulation of the arteriole diameter,[33–40] the modulation of the hemodynamic response that generates the intrinsic optical signal,[30] and the control of blood flow associated with motor behavior.[32] While astrocytic Ca^{2+} signals appear to play important physiological roles, as evidenced by the aforementioned studies, they also are activated by pathological neuronal activities, such as during epileptiform discharges,[25,26] and might participate in seizure generation and maintenance, as described later in this chapter.

ASTROCYTES RELEASE CHEMICAL TRANSMITTERS TO MODULATE NEURONAL AND SYNAPTIC FUNCTIONS

The functional consequence of the astrocytic Ca^{2+} signals is the release of gliotransmitters that have been shown to modulate neuronal and synaptic functions[1,2,41] (Fig. 46–1B). Several of these gliotransmitters, such as glutamate, adenosine triphosphate (ATP), adenosine, and D-serine, are released in a Ca^{2+}-dependent manner[21,42] through vesicle[43–46] and lysosome exocytosis.[47–49] Astrocytes in culture express the soluble *N*-ethylmaleimide-sensitive fusion protein receptor (SNARE) complex,[43,50–52] which is colocalized with small vesicles positive for vesicular glutamate transporters,[43,50–52] ATP-storing vesicles,[49,53] and D-serine-containing vesicles.[54] Furthermore, ultrastructural studies in situ have shown that astrocytic processes contain small synaptic-like vesicles with a diameter of 30 nm, which are located in close proximity to synapses.[43,44] While Ca^{2+}-dependent exocytosis represents the better-characterized pathways of astrocytic release of glutamate, ATP, adenosine, and D-serine, alternative release mechanisms have also been proposed. These mechanisms include reversal of glutamate transporters, connexin/pannexin hemichannels, pore-forming P_2X_7 receptors, and swelling-induced activation of volume-regulated anion channels.[55]

In accordance with the concept of the *tripartite synapse* (Fig. 46–1A,B), in which astrocytes are considered to be functionally associated with the pre- and postsynaptic nerve terminals as a third signaling element at the synapse,[56] it is now well accepted that astrocytes not only respond to neuronal activity but also regulate neuronal excitability, synaptic transmission, and behavior by releasing gliotransmitters.[1,2,14] The mechanisms and consequences of this bidirectional communication between neurons and astrocytes are grouped in a process called *gliotransmission*.[2] The better-characterized gliotransmitters involved in gliotransmission are discussed below.

Release of Glutamate

Glutamate, one of the first gliotransmitters released from astrocytes to be identified, has been reported to exert many effects on synaptic transmission and neuronal excitability. Several studies have shown that glutamate release from astrocytes through a Ca^{2+}-dependent mechanism activated receptors located at the presynaptic terminals. Through activation of mGluR1[57,58] or *N*-methyl-D-aspartate (NMDA) receptors,[44] astrocytes enhance the frequency of spontaneous and evoked excitatory synaptic currents. Additionally, astrocytic glutamate induces the depression or potentiation[59] of inhibitory synaptic transmission by activation of presynaptic mGluR2/3[60] or kainate receptors,[61] respectively. Moreover, it has been shown that Ca^{2+}-dependent glutamate release from astrocytes at single hippocampal synapses participated in the generation of long-term potentiation (LTP) through the activation of the presynaptic mGluR1.[58] Thus, all of these studies indicate that Ca^{2+}-dependent release of glutamate from astrocytes modulates synaptic strength and plasticity.

Furthermore, various studies have shown that glutamate release from astrocytes activates postsynaptic NMDA receptors to generate slow inward currents (SICs).[62] The observation that spontaneous Ca^{2+} spikes in astrocytes correlates in thalamic neurons with the detection of SICs suggests that these spontaneous

Ca^{2+} signals mediate the release of glutamate from astrocytes to modulate neuronal excitability. Following this observation, multiple experiments performed to determine the nature of these SICs have shown that different protocols used to induce Ca^{2+} signals in astrocytes also increased the occurrence of the SICs.[2,41] For example, ligands that induce astrocytic Ca^{2+} signals,[63–68] photolysis of caged Ca^{2+} that has been selectively loaded into astrocytes,[64,69] single-astrocyte depolarization,[70] and electrical stimulation of excitatory presynaptic terminals[64,69] all resulted in Ca^{2+} elevations in astrocytes and occurrence of SICs in nearby neurons. Additionally, SICs have been described in different brain regions[71] such as the thalamus, the cortex, the hippocampus, the nucleus accumbens, the olfactory bulb, and, more recently, the brainstem.[72] Typically, SICs are mediated by the activation of a specific group of extrasynaptic NMDA receptors containing the NR2B subunit since they are inhibited by ifenprodil, a selective antagonist of NR2B-NMDA receptors.[64] Furthermore, SICs are insensitive to tetrodotoxin (TTX) and tetanus neurotoxin (TeNT), two substances that block the generation of action potential and the synaptic release of neurotransmitters, respectively,[64] confirming their nonneuronal origin. A recent study also demonstrated that the generation of SICs depends on the duration and kinetics of the Ca^{2+} signals in astrocytes,[68] although Fiacco et al.[73] suggested an alternative interpretation. More importantly, when SICs reach sufficient amplitude, they can trigger burst of action potentials that occur with a high degree of synchronicity in hippocampal pyramidal neurons over short distances of 100 μm.[63,64] Thus, glutamatergic gliotransmission increases neuronal excitability and operates as a nonsynaptic mechanism for neuronal synchronization. This form of communication between neurons and astrocytes could represent a significant source of excitation during epileptic discharges, as discussed later in this chapter.

Release of D-Serine

The presence of serine racemase, an enzyme required for the conversion of L- to D-serine, in astrocytes has led to the idea that the amino acid might be a significant player in the regulation of the NMDA receptors by acting as the natural substrate for glycine binding sites on the receptor.[74,75] D-Serine-containing vesicles are released from astrocytes in a Ca^{2+}-dependent manner and act as a coagonist of the NMDA receptor.[54] In the supraoptic nucleus of the hypothalamus, the dynamic astrocytic coverage of synapses dependent on physiological signals influences extracellular levels of astrocytic D-serine and consequently leads to a certain form of metaplasticity.[76] For example, the high degree of synaptic coverage by astrocytes seen in virgin rodents induces LTP, whereas the reduced astrocytic coverage of synapses that occurs during lactation induces long-term depression.[76] More recent work has addressed the role of D-serine release in NMDA receptor-dependent LTP in the Schaffer collateral-CA1 synapses.[77] In this work, the selective inhibition of serine racemase in one astrocyte suppressed local LTP induction, demonstrating that one astrocyte is the direct source of D-serine in the hippocampus and can modulate synaptic input plasticity on nearby neurons.

Release of ATP

The release of ATP from astrocytes has been known for a long time and was initially proposed as a mechanism for the propagation of intercellular Ca^{2+} waves through the astrocytic syncytium.[3,11,78] First, ATP is released from astrocytes during Ca^{2+} wave propagation.[11,79] Second, the propagation can be abolished by antagonists of purinergic P_2Y receptors[11,79–81] or the ATP-degrading enzyme apyrase.[11,81] Third, visualization of the release of ATP demonstrates that its velocity correlates with that of the Ca^{2+} wave in astrocytes.[81] These results suggest that ATP could be an extracellular messenger and a primary signal for the Ca^{2+} wave propagation. While mechanisms of glutamate and D-serine release from astrocytes are experimentally well described, the mechanisms underlying the release of ATP from astrocytes are less well understood. The release of ATP is reduced by inhibitors of several anion channels,[82,83] gap junctions, hemichannels,[78,84,85] and pore-forming P_2X_7 receptors,[86] suggesting the involvement of different pathways in the release. Additionally, ATP release from astrocytes is partly Ca^{2+}- and SNARE proteins-dependent.[84,87] Further, astrocytes also display vesicles that contain

ATP, and vesicular adenosine triphosphatase (ATPase) inhibitors block the release of ATP.[53,87] More importantly, Pascual et al.[88] generated inducible transgenic mice that express a dominant-negative SNARE (dnSNARE) domain of vesicle-associated membrane protein 2 (VAMP2) selectively in astrocytes to suppress the exocytotic release of chemical transmitters from astrocytes. Interestingly, using these transgenic astrocyte-specific dnSNARE mice, it was shown that astrocytes regulate the activation of neuronal A1 receptors that are responsible for presynaptic inhibition of excitatory synaptic transmission in the hippocampus and cortex.[88–90] Bioluminescence imaging demonstrated that this molecular genetic manipulation led to reduced extracellular ATP, and pharmacological evidence was consistent with the notion that astrocyte-derived extracellular ATP is hydrolyzed to adenosine to cause a tonic suppression of synaptic transmission.[88] The mechanism of release of these purines has not been investigated further. The recent discovery of Sawada et al.[91] demonstrated that a novel member of an anion transporter family functioning as a vesicular nucleotide transporter was highly expressed in astrocytes. Together these findings raise the possibility that astrocytes release ATP by exocytosis. However, before such a conclusion is drawn, considerable additional studies will be required.

Once ATP is released from astrocytes, it can exert physiological effects modulating neuronal excitability. For example, in hypothalamic slices, astrocytes express α-1 adrenergic receptors, and in response to adrenergic input they release ATP, which acts on P_2X_7 receptors localized on nearby magnocellular neurosecretory cells (MNCs). As a result, there is an enhancement of AMPA receptor surface expression and an increase in the amplitude of miniature excitatory postsynaptic current in these cells.[92] More recent studies have confirmed postsynaptic effects of ATP on MNCs. Using combined two-photon Ca^{2+} imaging, photolysis of caged compounds, and electrophysiology, it has been shown that there is a mGluR1-dependent Ca^{2+} increase in astrocytes along with ATP release resulting in an increase in the amplitude of miniature excitatory postsynaptic currents of MNCs through the activation of postsynaptic P_2X_7 receptors.[93] Additionally, another study by Zhang et al.[94] in hippocampal cultures has shown that the release of ATP from stimulated astrocytes was able to depress glutamatergic neuronal transmission through the direct activation of P_2Y receptors. Interestingly, using hippocampal slices, Zhang et al.[94] also stated that the glutamatergic synaptic depression was due to the ATP metabolite adenosine, which acted on the A1 adenosine receptors. Altogether, these results suggest that ATP release from astrocytes can modulate neuronal excitability and synaptic transmission through direct and indirect actions. The indirect action of astrocytic ATP requires its conversion to adenosine, a metabolite known to have multiple effects on neuronal and synaptic functions, as described below.

Adenosine Derived from Astrocyte-Released ATP

Once ATP is released into the extracellular space, a variety of ectonucleotidases hydrolyze ATP to AMP and then a 5′-nucleotidase converts AMP to adenosine,[95] which is known to be a powerful modulator of synaptic activity via its actions on the G-protein-coupled adenosine receptor subtypes (A1, A2, and A3).[96] In the retina, adenosine derived from astrocyte-released ATP can activate A1 receptors coupled to K^+ channels, which hyperpolarize neurons and decrease their excitability.[97] In the hippocampus, the adenosine thus produced results in presynaptic inhibition of excitatory synaptic transmission mediated by the activation of A1 receptors.[88,94,98] Moreover, chelating Ca^{2+} in astrocytic syncytium and the use of glia-specific toxins interfere with the A1-dependent synaptic depression, confirming the glial origin of this process.[98] In addition to the mechanisms described above, astrocytes can directly release adenosine, especially in response to hypoxic stimulation,[99,100] even though release of adenosine is more typical of neurons.[101] In that case, the release of adenosine from astrocytes depends on export of adenosine through the equilibrative nucleoside transporters.

Recent work using dnSNARE animals has shown that astrocytic adenosine is important for sleep homeostasis by participating in the accumulation of sleep pressure and contributing to cognitive deficits associated with sleep loss.[90] Moreover, another study has shown that the dnSNARE animals displayed a 50%

reduction in surface expression of NMDA receptors, resulting in a decrease in cortical slow oscillations.[89] Thus, these studies identify astrocytes as a major regulator of the activation of neuronal A1 receptors, and thus presumably as a source of adenosine in the brain, and suggest that purinergic gliotransmission plays an important role in synaptic transmission, plasticity, and behavior.

REACTIVE ASTROCYTOSIS AND EPILEPSY

Reactive changes in astrocytes are frequently encountered in the hippocampus in association with temporal lobe epilepsy (TLE) in humans[102] and with animal models of epilepsy.[103,104] These reactive changes, termed *reactive astrocytosis*, generally involve increases in astrocyte size and number[103,104] and often occur together with neuronal loss and synaptic rearrangements.[103,105] Reactive astrocytes exhibit increased expression of glial cytoskeletal proteins, glial fibrillary acidic protein (GFAP), and vimentin, which are therefore used to assess the development of reactive astrocytosis.[106,107] Interestingly, recent studies have shown that reactive astrocytosis was also accompanied by a loss of astrocytic domain organization[108] and the generation of new astrocytes from stem cells.[109] More importantly, in addition to morphological changes, many proteins are up- or downregulated in reactive astrocytes, leading to changes in cellular functions.[17] Whether these functional changes modify seizure susceptibility is an intriguing notion receiving increased attention. In the following sections, we discuss recent evidence suggesting that changes in expression of two astrocyte-specific enzymes, glutamine synthetase (GS) and adenosine kinase (ADK), could promote seizures in the epileptic brain.

Reactive Astrocytosis and GS Downregulation in Epilepsy

In the brain, GS is expressed almost exclusively by astrocytes[110] and is responsible for the conversion of synaptically released glutamate into glutamine after the neurotransmitter is taken up by transporters into the synaptically associated glia[111] (Fig. 46–2). This glutamate-glutamine cycle normally serves as a major mechanism for ammonia detoxification in the brain and also as a buffered reservoir of a precursor (glutamine) for glutamate and GABA synthesis[112] (Fig. 46–2).

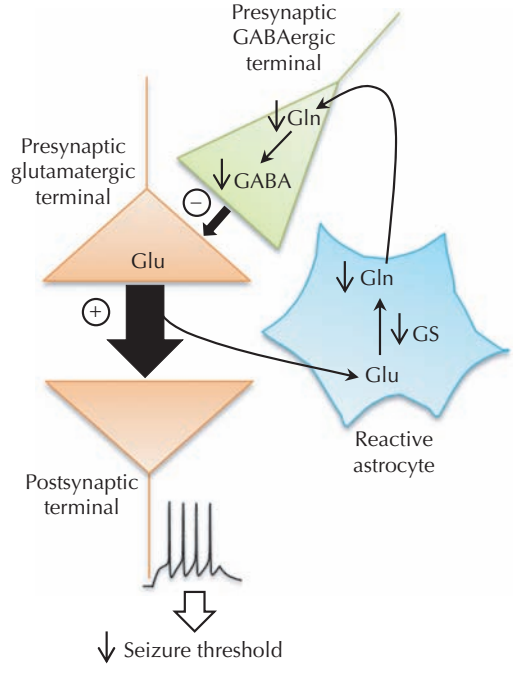

Figure 46–2. The activity change of glutamine synthetase (GS) in reactive astrocytes contributes to seizure development. Glutamate (Glu) released at the excitatory synapse is converted into glutamine (Gln) through activity of GS in astrocytes. Glutamine is used as a precursor for synthesis of GABA in GABAergic neurons. The loss of GS in reactive astrocytes leads to a decrease in Gln and GABA levels in GABAergic terminals. Consequently, presynaptic GABAergic inhibition is reduced, increasing presynaptic release of Glu. In turn, Glu enhances the excitability of postsynaptic neurons, leading to a reduction in the seizure threshold.

Early studies performed during epilepsy surgery demonstrated that the accumulation and impaired clearance of hippocampal glutamate in TLE were due to a slowing in the conversion of glutamate to glutamine,[113] suggesting a role for GS deficiency in epilepsy. Later, this observation was supported by two discoveries showing the loss of GS in clinical and experimental epilepsies. First, hippocampal tissue removed from patients with TLE during epilepsy surgery was characterized by downregulation of GS.[114] Second, GS was downregulated with elevated GFAP immunoreactivity during the chronic phase of epilepsy in an animal model

of TLE.[115] More recently, an animal model of chronic GS deficiency in the hippocampus has been developed to replicate the situation in human TLE.[116,117] In these studies, freely moving rats monitored with video and electroencephalography developed clusters of spontaneous recurrent seizures and neuropathological changes like those seen in human TLE after a continuous intrahippocampal infusion of the GS inhibitor methionine sulfoximine (MSO).

More importantly, recent studies suggest that downregulation of GS and the consequent reduction in the pool of the GABA precursor glutamine could partially deplete inhibitory synaptic terminals of GABA and impair GABAergic inhibition. In support of this notion, Liang et al.[118] showed that selective blockade of GS by MSO reduced the amplitude of inhibitory postsynaptic currents (IPSCs) in CA1 pyramidal neurons during repetitive stimulus trains. Interestingly, the MSO effect was prevented by the replenishment of glutamine in the bath perfusion.[118] Additionally, recent experiments performed with the same preparation showed no significant effects of MSO on glutamatergic transmission.[119] Together, these results suggest that the glial glutamate-glutamine cycle is the major contributor to synaptic GABA release and regulates inhibitory synaptic strength.

More recently, we tested the hypothesis that GS deficits may be contributing to TLE by reducing synaptic inhibition in the vicinity of reactive astrocytes.[120] First, in this study, a higher-titer viral transduction of astrocytes with enhanced green fluorescent protein (eGFP) via bilateral injections of adeno-associated virus into the mice hippocampus led to reactive astrocytosis. Reactive eGFP astrocytes showed high levels of expression of GFAP and vimentin, while nearby neurons and microglia were not altered. Second, consistent with the studies suggesting that reduced GS expression levels are associated with the development of astrocytosis,[111,114,115] a pronounced downregulation of GS associated with enhanced GFAP and vimentin expression was observed. Third, we hypothesized that the GS downregulation and, therefore, the reduced glutamine could generate a deficit in the inhibitory synaptic transmission in neurons located in the eGFP-positive areas. To test this hypothesis, we used brain slices to record evoked IPSCs (eIPSCs) and spontaneous miniature IPSCs (mIPSCs) in CA1 pyramidal neurons located in the eGFP-positive areas from mice treated with the adeno-associated virus. As expected, the amplitude of eIPSCs and the amplitude/frequency of mIPSCs were reduced while evoked excitatory postsynaptic currents (eEPSCs) were not altered, suggesting that only inhibitory synaptic transmission was impaired in CA1 pyramidal neurons proximal to reactive astrocytes. Furthermore, MSO had no effect on the amplitude of eIPSCs in CA1 neurons located in the vicinity of eGFP-positive reactive astrocytes, confirming that the evoked failure was due to GS deficiency. Interestingly, bath application of glutamine increased the amplitude of eIPSCs, providing further proof that eIPSC failure was mediated by neuronal glutamine starvation. These results suggest that reactive astrocytosis-induced GS downregulation leads to an interrupted neuronal glutamine supply, impaired neuronal production of GABA, and compromised inhibitory synaptic transmission (Fig. 46–2). Finally, to determine whether reactive astrocytosis was associated with network hyperexcitability, we used voltage-sensitive dye imaging techniques and stimulation of the temporoammonic pathway between the entorhinal cortex and CA1, a particular technique of stimulation known to constrain excitatory postsynaptic potentials (EPSPs) by triggering feedforward inhibition. In eGFP-positive hippocampal slices, the EPSPs propagated much further than in control slices from untreated animals. Furthermore, bath perfusion of eGFP-positive hippocampal slices with exogenous glutamine reduced the areas activated by the temporoammonic pathway stimulation. Thus, inhibitory deficits associated with reactive astrocytosis lead to hyperexcitability of the hippocampal network and this can be prevented by exogenously supplied glutamine. Altogether, these results suggest that the GS loss seen during reactive astrocytosis could contribute to elevated seizure susceptibility in TLE by reducing neuronal inhibition (Fig. 46–2). Consequently, protecting GS function might represent a promising therapeutic strategy to prevent seizures.

Reactive Astrocytosis and ADK Upregulation in Epilepsy

Under physiological conditions extra- and intracellular levels of adenosine are rapidly equilibrated via distinct families of nucleoside transporters.[121] Intracellularly, adenosine

is rapidly metabolized by phosphorylation to AMP via ADK,[121] the key enzyme of adenosine metabolism,[122] which, in adult brain, is predominantly expressed in astrocytes.[123] Thus, ADK is ideally located to control the astrocyte-based adenosine cycle by driving the influx of adenosine into the cell via bidirectional nucleoside transporters[122] (Fig. 46–3). Adenosine, in particular, plays a prominent role in seizure regulation and has been found to be elevated in patients following seizures, leading to the conclusion that adenosine released during a seizure mediates seizure arrest and postictal refractoriness.[124] The adenosine A1 receptor-mediated functions are largely responsible for the anti-convulsant and neuroprotective activity of adenosine.[96] Thus, binding of adenosine to A1 receptors that are highly expressed in the hippocampus leads to decreased neuronal excitability and synaptic transmission through postsynaptic membrane hyperpolarization and inhibition of presynaptic release, respectively[96] (Fig. 46–3). Consequently, changes in the homeostasis of the astrocyte-based adenosine cycle are to be expected in the epileptic brain.

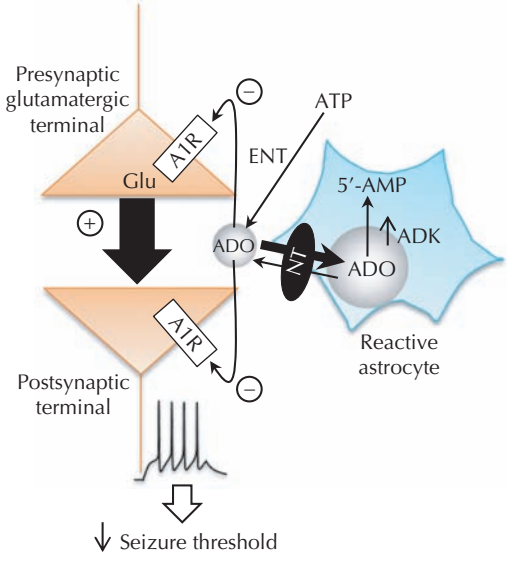

Figure 46–3. The activity change of adenosine kinase (ADK) in reactive astrocytes contributes to seizure development. Extracellular ATP is rapidly degraded into adenosine (ADO) by ectonucleotidases (ENT). Adenosine exerts both pre- and postsynaptic inhibitory effects via adenosine A1 receptors (A1R). The extra- and intracellular levels of ADO are equilibrated via nucleoside transporters (NT). The intracellular level of ADO depends on the activity of ADK in astrocytes that converts ADO into 5′-adenosine-monophosphate (5′-AMP). Overexpression of ADK in reactive astrocytes increases the influx of ADO into the cell, decreasing extracellular levels of ADO. Consequently, pre- and postsynaptic inhibitory effects of ADO are reduced, leading to a decrease in seizure threshold.

Recent studies from Boison's group have identified ADK as molecular link between reactive astrocytosis and neuronal dysfunction in epilepsy leading to the *ADK hypothesis of epileptogenesis.*[122] Thus, transgenic mice overexpressing ADK in the brain displayed a reduced tone of the endogenous anti-convulsant adenosine, leading to the emergence of spontaneous chronic seizures.[125] Furthermore, a direct association between the development of reactive astrocytosis and the upregulation of ADK has been shown.[125,126] Conversely, transgenic mice with a forebrain-selective reduction of ADK were resistant to seizure development.[126] Together, these studies indicate that reactive astrocytosis causes overexpression of ADK, which was shown to be sufficient to trigger seizures (Fig. 46–3). Thus, reconstitution or augmentation of adenosine and/or inhibition of ADK constitutes a pharmacological rationale for seizure suppression. In support of this notion, it has been demonstrated that intrahippocampal implants of ADK-deficient stem cell-derived neuronal precursors suppress kindling epileptogenesis, suggesting that any therapy leading to focal augmentation of the adenosine system has the potential to prevent epileptogenesis.[121,127,128]

ASTROCYTIC CA^{2+} SIGNALS, GLUTAMATE RELEASE, AND EPILEPSY

Several lines of evidence indicate that Ca^{2+} elevations in astrocytes leading to glutamate release and synchronization of neuronal firing (see above) could be involved in epilepsy. Thus, below, we discuss recent studies supporting the potential role for astrocytic Ca^{2+} signals and glutamate release in the generation of epileptiform activity.

Astrocytic Ca^{2+} Signals and Epilepsy

Under physiological conditions, Ca^{2+} signals in astrocytes arise to activation of mGluRs (see above). Interestingly, it has been shown that the protein expression levels of mGluRs were

increased in reactive astrocytes in animal models of epilepsy[129,130] and in hippocampal specimen from patients with TLE.[131] Furthermore, hippocampal cultured astrocytes derived from patients with TLE show increases in Ca^{2+} signals.[111] More recently, studies have shown that cortical epileptiform activity induced in vivo in anesthetized mice with bicuculline,[25] picrotoxin,[26] or the A-type K^+ channel blocker 4-aminopyridine[132] (4-AP) were associated with increases in Ca^{2+} signals in astrocytes, which could be suppressed by intraperitoneal injections of several anti-epileptic drugs including valproate, gabapentin, and phenytoin.[132] This positive correlation between increased astrocytic Ca^{2+} signaling and epileptiform activity onset suggests that these Ca^{2+} signals might contribute to seizure generation.

Astrocytic Glutamate Release and Epilepsy

Studies performed by Kang et al.[66] first demonstrated that Ca^{2+}-dependent release of glutamate from astrocytes might be involved in the generation of epileptiform discharges. In brain slices, the infusion of IP_3 through a patch-clamp pipette to increase Ca^{2+} signals into astrocytes was followed by the occurrence of slow, decayed transient inward currents (STCs) in nearby CA1 pyramidal neurons.[66] In current-clamp, STCs were able to depolarize the neuronal membrane and trigger firing of action potentials. This neuronal depolarization was reminiscent of the paroxysmal depolarization shift (PDS) underlying an interictal epileptiform event[133] that is known to be synchronized over many millimeters of epileptic brain.[134]

Later work from the same group supports the role of astrocytes in generating epilepsy.[132] Using 4-AP to induce interictal epileptiform activity in hippocampal slices, Tian et al.[132] showed that PDSs persisted in the presence of both TTX and different Ca^{2+} channel blockers that suppressed presynaptic release but were blocked by the ionotropic glutamate receptor antagonists CNQX and D-AP5. Thus, these results suggest that PDSs were triggered by release of glutamate from extrasynaptic sources. To determine whether Ca^{2+} signals in astrocytes are associated with PDSs, the authors used photolysis of caged Ca^{2+}. However, photolysis of caged Ca^{2+} in one astrocyte induced local PDS in the presence of TTX, suggesting that Ca^{2+} elevations in astrocytes lead to glutamate release, which targets nearby neurons to generate PDSs, the hallmark of epileptic activity.[133]

In contrast to studies from Nedergaard's group described above, our work[135] suggested that glutamate release from astrocytes was not necessary for the generation of epileptiform activity but rather that it could be modulatory. In this work, we induced both ictal and interictal epileptiform activity in hippocampal slices by removing Mg^{2+} (0 Mg^{2+}) in the presence of picrotoxin. The epileptiform activity thus generated triggered Ca^{2+} signals in astrocytes and increased the frequency of NMDA-receptor-mediated SICs that share certain properties with STCs. However, when slices were preincubated with D-AP5 to block NMDA-receptor-mediated SICs, treatment of the slices with 0 Mg^{2+} and picrotoxin was still able to trigger epileptiform activity, suggesting that glutamate release from astrocytes and SICs per se were not required to initiate epileptiform activity. Interestingly, D-AP5 reversibly reduced the duration of both ictal and interictal epileptiform events, suggesting that astrocytic glutamate, even though it is not necessary for the generation of epileptiform activity, might be important in determining the strength of epileptiform discharges.[135]

Faced with these two conflicting studies,[132,135] recent work by Gomez-Gonzalo et al.[65] attempted to determine the role of astrocytes in the generation of focal ictal discharges. First, the authors showed in enthorinal cortex (EC) slices from rats that while 0 Mg^{2+}/picrotoxin induced both ictal and interictal discharges, only ictal discharges were associated with Ca^{2+} elevations in astrocytes. Second, the frequency and duration of ictal discharges, as well as their associated astrocytic Ca^{2+} signals, were attenuated by mGluR5 and P_2Y receptor antagonists, whereas interictal discharges were unaffected, indicating that astrocytic Ca^{2+} elevations mediated by mGluR5 and P_2Y receptors do not have a role in the generation of interictal discharges. Furthermore, the selective stimulation of astrocytes with the peptide Thr-Phe-Leu-Leu-Arg-NH_2 (TFLLR-NH_2), known to induce astrocytic glutamate release and SICs via activation of the PAR-1 thrombin receptor,[68,136] was able to generate ictal discharges in the presence of 0 Mg^{2+}/picrotoxin, suggesting that

astrocytic glutamate release was sufficient to initiate ictal discharges in EC slices prone to generate epileptiform activity. Through a series of elegant experimental manipulations, Gomez-Gonzalo et al. were able to show that while neuronal activity is critical for the generation of ictal discharges, astrocytes can modulate the threshold for the generation of this epileptiform activity.[65]

In addition to its potential role in the generation of epileptiform activity, our recent study suggested that Ca^{2+}-dependent release of glutamate from astrocytes could also contribute to the typical delayed neuronal death observed after status epilepticus (SE).[137] Using two-photon in vivo microscopy and the pilocarpine model of epilepsy to induce SE in mice, we have shown that SE enhanced Ca^{2+} signals in astrocytes for 3 days and that this enhancement was associated with the period of delayed neuronal death.[137] More importantly, we found that post-SE administration of MPEP to block mGluR5 mediated Ca^{2+} signals in astrocytes (see above) and ifenprodil to selectively block NMDA-NR2B receptors mediated SICs (see above) provided significant neuronal protection.[137] Furthermore, we have shown that selective loading of Ca^{2+} chelators into astrocytes after SE also led to neuronal protection, suggesting neurotoxic roles for glutamatergic gliotransmission in epilepsy.[137] This notion is also supported by previous studies demonstrating that the activation of extrasynaptic NMDAR-NR2B receptors stimulates cyclic adenosine monophosphate response element binding protein (CREB) dephosphorylation and neuronal death.[138]

While controversial observations exist concerning the involvement of astrocytes in the generation of seizures, it is clear that astrocytic Ca^{2+} signals and astrocytic glutamate play an important role in the mechanisms of epilepsy. However, we now await the introduction of astrocyte-selective inhibitors to define the role of astrocytes in the process. Thus, gliotransmission should be considered as a potential therapeutic target in epilepsy.

CONCLUDING REMARKS

The extraordinary evolution of in vivo Ca^{2+} imaging techniques allowed us to appreciate the highly dynamic nature of Ca^{2+} signaling in astrocytes under physiological conditions. This Ca^{2+} excitability represents an original pathway for astrocytes to integrate and process the neuronal information in the brain. In response to neuronal activity, astrocytes release gliotransmitters to modulate both excitatory and inhibitory synaptic transmission, and consequently affect brain plasticity and mammalian behavior. It is striking to see how astrocytes react to epilepsy by changing their shape and functions. This glial reactivity leads to increases in neuronal excitability and consequently accelerates the evolution of this neuronal disorder. In addition to astrocytic reactivity, extensive experimental research suggests that astrocytic Ca^{2+} signaling and gliotransmitter release participate in the generation of seizures. However, data coming from this research are currently controversial, mainly due to the different experimental approaches and lack of selective pharmacological tools to block astrocytic Ca^{2+} signaling and gliotransmission. In the future, the development of transgenic animals bearing specific deficiencies interfering with astrocytic Ca^{2+} signals and gliotransmission, and the use of chronic models of epilepsy that more closely mimic the complex feature of seizures in epileptic patients, will represent new approaches to identify the role of astrocytes in the generation of seizures and rigorously evaluate the idea that these glial cells represent a target for developing new therapeutic strategies for epilepsy.

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The authors have no conflicts of interest to disclose.

REFERENCES

1. Halassa MM, Haydon PG. Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu Rev Physiol*. 2010;72:335–355.

2. Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev*. 2006;86(3):1009–1031.
3. Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N. Astroglial networks: a step further in neuroglial and gliovascular interactions. *Nat Rev Neurosci*. 2010;11(2):87–99.
4. Bushong EA, Martone ME, Jones YZ, Ellisman MH. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J Neurosci*. 2002;22(1):183–192.
5. Halassa MM, Fellin T, Takano H, Dong JH, Haydon PG. Synaptic islands defined by the territory of a single astrocyte. *J Neurosci*. 2007;27(24):6473–6477.
6. Haber M, Zhou L, Murai KK. Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. *J Neurosci*. 2006;26(35):8881–8891.
7. Hirrlinger J, Hulsmann S, Kirchhoff F. Astroglial processes show spontaneous motility at active synaptic terminals in situ. *Eur J Neurosci*. 2004;20(8):2235–2239.
8. Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, Knott GW. Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. *PLoS Biol*. 2006;4(11):e343.
9. Sontheimer H, Black JA, Waxman SG. Voltage-gated Na^+ channels in glia: properties and possible functions. *Trends Neurosci*. 1996;19(8):325–331.
10. Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ. Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science*. 1990;247(4941):470–473.
11. Guthrie PB, Knappenberger J, Segal M, Bennett MV, Charles AC, Kater SB. ATP released from astrocytes mediates glial calcium waves. *J Neurosci*. 1999;19(2):520–528.
12. Scemes E, Giaume C. Astrocyte calcium waves: what they are and what they do. *Glia*. 2006;54(7):716–725.
13. Iadecola C, Nedergaard M. Glial regulation of the cerebral microvasculature. *Nat Neurosci*. 2007;10(11):1369–1376.
14. Haydon PG. GLIA: listening and talking to the synapse. *Nat Rev Neurosci*. 2001;2(3):185–193.
15. Halassa MM, Fellin T, Haydon PG. The tripartite synapse: roles for gliotransmission in health and disease. *Trends Mol Med*. 2007;13(2):54–63.
16. Seifert G, Schilling K, Steinhauser C. Astrocyte dysfunction in neurological disorders: a molecular perspective. *Nat Rev Neurosci*. 2006;7(3):194–206.
17. Wetherington J, Serrano G, Dingledine R. Astrocytes in the epileptic brain. *Neuron*. 2008;58(2):168–178.
18. Minta A, Kao JP, Tsien RY. Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores. *J Biol Chem*. 1989;264(14):8171–8178.
19. Porter JT, McCarthy KD. Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. *J Neurosci*. 1996;16(16):5073–5081.
20. Verkhratsky A, Orkand RK, Kettenmann H. Glial calcium: homeostasis and signaling function. *Physiol Rev*. 1998;78(1):99–141.
21. Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci*. 2009;32(8):421–431.
22. Denk W, Strickler JH, Webb WW. Two-photon laser scanning fluorescence microscopy. *Science*. 1990;248(4951):73–76.
23. Helmchen F, Denk W. Deep tissue two-photon microscopy. *Nat Methods*. 2005;2(12):932–940.
24. Nimmerjahn A, Kirchhoff F, Kerr JN, Helmchen F. Sulforhodamine 101 as a specific marker of astroglia in the neocortex in vivo. *Nat Methods*. 2004;1(1):31–37.
25. Hirase H, Qian L, Bartho P, Buzsaki G. Calcium dynamics of cortical astrocytic networks in vivo. *PLoS Biol*. 2004;2(4):E96.
26. Gobel W, Helmchen F. In vivo calcium imaging of neural network function. *Physiology* (*Bethesda*). 2007;22:358–365.
27. Wang X, Lou N, Xu Q, Tian GF, Peng WG, Han X, Kang J, Takano T, Nedergaard M. Astrocytic Ca^{2+} signaling evoked by sensory stimulation in vivo. *Nat Neurosci*. 2006;9(6):816–823.
28. Winship IR, Plaa N, Murphy TH. Rapid astrocyte calcium signals correlate with neuronal activity and onset of the hemodynamic response in vivo. *J Neurosci*. 2007;27(23):6268–6272.
29. Petzold GC, Albeanu DF, Sato TF, Murthy VN. Coupling of neural activity to blood flow in olfactory glomeruli is mediated by astrocytic pathways. *Neuron*. 2008;58(6):897–910.
30. Schummers J, Yu H, Sur M. Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. *Science*. 2008;320(5883):1638–1643.
31. Dombeck DA, Khabbaz AN, Collman F, Adelman TL, Tank DW. Imaging large-scale neural activity with cellular resolution in awake, mobile mice. *Neuron*. 2007;56(1):43–57.
32. Nimmerjahn A, Mukamel EA, Schnitzer MJ. Motor behavior activates Bergmann glial networks. *Neuron*. 2009;62(3):400–412.
33. Chuquet J, Hollender L, Nimchinsky EA. High-resolution in vivo imaging of the neurovascular unit during spreading depression. *J Neurosci*. 2007;27(15):4036–4044.
34. Filosa JA, Bonev AD, Nelson MT. Calcium dynamics in cortical astrocytes and arterioles during neurovascular coupling. *Circ Res*. 2004;95(10):e73–e81.
35. Filosa JA, Bonev AD, Straub SV, Meredith AL, Wilkerson MK, Aldrich RW, Nelson MT. Local potassium signaling couples neuronal activity to vasodilation in the brain. *Nat Neurosci*. 2006;9(11):1397–1403.
36. Gordon GR, Choi HB, Rungta RL, Ellis-Davies GC, MacVicar BA. Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature*. 2008;456(7223):745–749.
37. Metea MR, Newman EA. Glial cells dilate and constrict blood vessels: a mechanism of neurovascular coupling. *J Neurosci*. 2006;26(11):2862–2870.
38. Mulligan SJ, MacVicar BA. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature*. 2004;431(7005):195–199.
39. Takano T, Tian GF, Peng W, Lou N, Libionka W, Han X, Nedergaard M. Astrocyte-mediated control of cerebral blood flow. *Nat Neurosci*. 2006;9(2):260–267.
40. Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, Carmignoto G. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci*. 2003;6(1):43–50.
41. Hamilton NB, Attwell D. Do astrocytes really exocytose neurotransmitters? *Nat Rev Neurosci*. 2010;11(4):227–238.

42. Perea G, Araque A. Glial calcium signaling and neuron–glia communication. *Cell Calcium*. 2005; 38(3–4):375–382.
43. Bezzi P, Gundersen V, Galbete JL, Seifert G, Steinhäuser C, Pilati E, Volterra A. Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. *Nat Neurosci*. 2004;7(6): 613–620.
44. Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, Domercq M, Matute C, Tonello F, Gundersen V, Volterra A. Glutamate exocytosis from astrocytes controls synaptic strength. *Nat Neurosci.* 2007;10(3):331–339.
45. Martineau M, Galli T, Baux G, Mothet JP. Confocal imaging and tracking of the exocytotic routes for D-serine-mediated gliotransmission. *Glia*. 2008;56(12): 1271–1284.
46. Montana V, Malarkey EB, Verderio C, Matteoli M, Parpura V. Vesicular transmitter release from astrocytes. *Glia*. 2006;54(7):700–715.
47. Jaiswal JK, Fix M, Takano T, Nedergaard M, Simon SM. Resolving vesicle fusion from lysis to monitor calcium-triggered lysosomal exocytosis in astrocytes. *Proc Natl Acad Sci USA*. 2007;104(35):14151–14156.
48. Li D, Ropert N, Koulakoff A, Giaume C, Oheim M. Lysosomes are the major vesicular compartment undergoing Ca^{2+}-regulated exocytosis from cortical astrocytes. *J Neurosci*. 2008;28(30):7648–7658.
49. Zhang Z, Chen G, Zhou W, Song A, Xu T, Luo Q, Wang W, Gu XS, Duan S. Regulated ATP release from astrocytes through lysosome exocytosis. *Nat Cell Biol*. 2007;9(8):945–953.
50. Montana V, Ni Y, Sunjara V, Hua X, Parpura V. Vesicular glutamate transporter-dependent glutamate release from astrocytes. *J Neurosci.* 2004;24(11): 2633–2642.
51. Zhang Q, Fukuda M, Van Bockstaele E, Pascual O, Haydon PG. Synaptotagmin IV regulates glial glutamate release. *Proc Natl Acad Sci USA*. 2004;101(25): 9441–9446.
52. Zhang Q, Pangrsic T, Kreft M, Krzan M, Li N, Sul JY, Halassa M, Van Bockstaele E, Zorec R, Haydon PG. Fusion-related release of glutamate from astrocytes. *J Biol Chem*. 2004;279(13):12724–12733.
53. Coco S, Calegari F, Pravettoni E, Pozzi D, Taverna E, Rosa P, Matteoli M, Verderio C. Storage and release of ATP from astrocytes in culture. *J Biol Chem*. 2003;278(2):1354–1362.
54. Mothet JP, Pollegioni L, Ouanounou G, Martineau M, Fossier P, Baux G. Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. *Proc Natl Acad Sci USA*. 2005;102(15):5606–5611.
55. Malarkey EB, Parpura V. Mechanisms of glutamate release from astrocytes. *Neurochem Int.* 2008;52(1–2):142–154.
56. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci*. 1999;22(5):208–215.
57. Fiacco TA, McCarthy KD. Intracellular astrocyte calcium waves in situ increase the frequency of spontaneous AMPA receptor currents in CA1 pyramidal neurons. *J Neurosci*. 2004;24(3):722–732.
58. Perea G, Araque A. Astrocytes potentiate transmitter release at single hippocampal synapses. *Science*. 2007;317(5841):1083–1086.
59. Pasti L, Volterra A, Pozzan T, Carmignoto G. Intracellular calcium oscillations in astrocytes: a highly plastic, bidirectional form of communication between neurons and astrocytes in situ. *J Neurosci*. 1997;17(20): 7817–7830.
60. Liu QS, Xu Q, Kang J, Nedergaard M. Astrocyte activation of presynaptic metabotropic glutamate receptors modulates hippocampal inhibitory synaptic transmission. *Neuron Glia Biol*. 2004;1(4):307–316.
61. Liu QS, Xu Q, Arcuino G, Kang J, Nedergaard M. Astrocyte-mediated activation of neuronal kainate receptors. *Proc Natl Acad Sci USA*. 2004;101(9): 3172–3177.
62. Parri HR, Gould TM, Crunelli V. Spontaneous astrocytic Ca^{2+} oscillations in situ drive NMDAR-mediated neuronal excitation. *Nat Neurosci*. 2001;4(8):803–812.
63. Angulo MC, Kozlov AS, Charpak S, Audinat E. Glutamate released from glial cells synchronizes neuronal activity in the hippocampus. *J Neurosci*. 2004; 24(31):6920–6927.
64. Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G. Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. *Neuron*. 2004;43(5):729–743.
65. Gómez-Gonzalo M, Losi G, Chiavegato A, Zonta M, Cammarota M, Brondi M, Vetri F, Uva L, Pozzan T, de Curtis M, Ratto GM, Carmignoto G. An excitatory loop with astrocytes contributes to drive neurons to seizure threshold. *PLoS Biol.* 2010;8(4):e1000352.
66. Kang N, Xu J, Xu Q, Nedergaard M, Kang J. Astrocytic glutamate release-induced transient depolarization and epileptiform discharges in hippocampal CA1 pyramidal neurons. *J Neurophysiol*. 2005;94(6):4121–4130.
67. Navarrete M, Araque A. Endocannabinoids mediate neuron–astrocyte communication. *Neuron*. 2008;57(6): 883–893.
68. Shigetomi E, Bowser DN, Sofroniew MV, Khakh BS. Two forms of astrocyte calcium excitability have distinct effects on NMDA receptor-mediated slow inward currents in pyramidal neurons. *J Neurosci*. 2008;28(26):6659–6663.
69. D'Ascenzo M, Fellin T, Terunuma M, Revilla-Sanchez R, Meaney DF, Auberson YP, Moss SJ, Haydon PG. mGluR5 stimulates gliotransmission in the nucleus accumbens. *Proc Natl Acad Sci USA*. 2007;104(6):1995–2000.
70. Perea G, Araque A. Properties of synaptically evoked astrocyte calcium signal reveal synaptic information processing by astrocytes. *J Neurosci*. 2005;25(9): 2192–2203.
71. Fellin T. Communication between neurons and astrocytes: relevance to the modulation of synaptic and network activity. *J Neurochem*. 2009;108(3):533–544.
72. Reyes-Haro D, Müller J, Boresch M, Pivneva T, Benedetti B, Scheller A, Nolte C, Kettenmann H. Neuron–astrocyte interactions in the medial nucleus of the trapezoid body. *J Gen Physiol*. 2010;135(6): 583–594.
73. Fiacco TA, Agulhon C, Taves SR, Petravicz J, Casper KB, Dong X, Chen J, McCarthy KD. Selective stimulation of astrocyte calcium in situ does not affect neuronal excitatory synaptic activity. *Neuron*. 2007;54(4):611–626.
74. Mothet JP, Parent AT, Wolosker H, Brady RO Jr, Linden DJ, Ferris CD, Rogawski MA, Snyder SH. D-Serine is an endogenous ligand for the glycine site

of the *N*-methyl-D-aspartate receptor. *Proc Natl Acad Sci USA*. 2000;97(9):4926–4931.
75. Schell MJ, Molliver ME, Snyder SH. D-Serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. *Proc Natl Acad Sci USA*. 1995;92(9):3948–3952.
76. Panatier A, Theodosis DT, Mothet JP, Touquet B, Pollegioni L, Poulain DA, Oliet SH. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell*. 2006;125(4):775–784.
77. Henneberger C, Papouin T, Oliet SH, Rusakov DA. Long-term potentiation depends on release of D-serine from astrocytes. *Nature*. 2010;463(7278):232–236.
78. Scemes E, Spray DC, Meda P. Connexins, pannexins, innexins: novel roles of "hemi-channels." *Pflugers Arch*. 2009;457(6):1207–1226.
79. Cotrina ML, Lin JH, López-García JC, Naus CC, Nedergaard M. ATP-mediated glia signaling. *J Neurosci*. 2000;20(8):2835–2844.
80. Cotrina ML, Lin JH, Alves-Rodrigues A, Liu S, Li J, Azmi-Ghadimi H, Kang J, Naus CC, Nedergaard M. Connexins regulate calcium signaling by controlling ATP release. *Proc Natl Acad Sci USA*. 1998;95(26): 15735–15740.
81. Koizumi S, Fujishita K, Tsuda M, Shigemoto-Mogami Y, Inoue K. Dynamic inhibition of excitatory synaptic transmission by astrocyte-derived ATP in hippocampal cultures. *Proc Natl Acad Sci USA*. 2003;100(19):11023–11028.
82. Anderson CM, Bergher JP, Swanson RA. ATP-induced ATP release from astrocytes. *J Neurochem*. 2004;88(1):246–256.
83. Darby M, Kuzmiski JB, Panenka W, Feighan D, MacVicar BA. ATP released from astrocytes during swelling activates chloride channels. *J Neurophysiol*. 2003;89(4):1870–1877.
84. Bal-Price A, Moneer Z, Brown GC. Nitric oxide induces rapid, calcium-dependent release of vesicular glutamate and ATP from cultured rat astrocytes. *Glia*. 2002;40(3):312–323.
85. MacVicar BA, Thompson RJ. Non-junction functions of pannexin-1 channels. *Trends Neurosci*. 2010;33(2): 93–102.
86. Suadicani SO, Brosnan CF, Scemes E. P2X7 receptors mediate ATP release and amplification of astrocytic intercellular Ca^{2+} signaling. *J Neurosci*. 2006;26(5): 1378–1385.
87. Maienschein V, Marxen M, Volknandt W, Zimmermann H. A plethora of presynaptic proteins associated with ATP-storing organelles in cultured astrocytes. *Glia*. 1999;26(3):233–244.
88. Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY, Takano H, Moss SJ, McCarthy K, Haydon PG. Astrocytic purinergic signaling coordinates synaptic networks. *Science*. 2005;310(5745):113–116.
89. Fellin T, Halassa MM, Terunuma M, Succol F, Takano H, Frank M, Moss SJ, Haydon PG. Endogenous nonneuronal modulators of synaptic transmission control cortical slow oscillations in vivo. *Proc Natl Acad Sci USA*. 2009;106(35):15037–15042.
90. Halassa MM, Florian C, Fellin T, Munoz JR, Lee SY, Abel T, Haydon PG, Frank MG. Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron*. 2009;61(2):213–219.
91. Sawada K, Echigo N, Juge N, Miyaji T, Otsuka M, Omote H, Yamamoto A, Moriyama Y. Identification of a vesicular nucleotide transporter. *Proc Natl Acad Sci USA*. 2008;105(15):5683–5686.
92. Gordon GR, Baimoukhametova DV, Hewitt SA, Rajapaksha WR, Fisher TE, Bains JS. Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. *Nat Neurosci*. 2005;8(8):1078–1086.
93. Gordon GR, Iremonger KJ, Kantevari S, Ellis-Davies GC, MacVicar BA, Bains JS. Astrocyte-mediated distributed plasticity at hypothalamic glutamate synapses. *Neuron*. 2009;64(3):391–403.
94. Zhang JM, Wang HK, Ye CQ, Ge W, Chen Y, Jiang ZL, Wu CP, Poo MM, Duan S. ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. *Neuron*. 2003;40(5): 971–982.
95. Zimmermann H, Braun N. Extracellular metabolism of nucleotides in the nervous system. *J Auton Pharmacol*. 1996;16(6):397–400.
96. Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. Adenosine and brain function. *Int Rev Neurobiol*. 2005;63:191–270.
97. Newman EA. Glial cell inhibition of neurons by release of ATP. *J Neurosci*. 2003;23(5):1659–1666.
98. Serrano A, Haddjeri N, Lacaille JC, Robitaille R. GABAergic network activation of glial cells underlies hippocampal heterosynaptic depression. *J Neurosci*. 2006;26(20):5370–5382.
99. Bjorklund O, Shang M, Tonazzini I, Dare E, Fredholm BB. Adenosine A1 and A3 receptors protect astrocytes from hypoxic damage. *Eur J Pharmacol*. 2008;596(1–3):6–13.
100. Martín ED, Fernández M, Perea G, Pascual O, Haydon PG, Araque A, Ceña V. Adenosine released by astrocytes contributes to hypoxia-induced modulation of synaptic transmission. *Glia*. 2007;55(1): 36–45.
101. Parkinson FE, Sinclair CJ, Othman T, Haughey NJ, Geiger JD. Differences between rat primary cortical neurons and astrocytes in purine release evoked by ischemic conditions. *Neuropharmacology*. 2002; 43(5):836–846.
102. Cohen-Gadol AA, Pan JW, Kim JH, Spencer DD, Hetherington HH. Mesial temporal lobe epilepsy: a proton magnetic resonance spectroscopy study and a histopathological analysis. *J Neurosurg*. 2004;101(4): 613–620.
103. Borges K, Gearing M, McDermott DL, Smith AB, Almonte AG, Wainer BH, Dingledine R. Neuronal and glial pathological changes during epileptogenesis in the mouse pilocarpine model. *Exp Neurol*. 2003;182(1):21–34.
104. Shapiro LA, Wang L, Ribak CE. Rapid astrocyte and microglial activation following pilocarpine-induced seizures in rats. *Epilepsia*. 2008;49(suppl 2):33–41.
105. Kron MM, Zhang H, Parent JM. The developmental stage of dentate granule cells dictates their contribution to seizure-induced plasticity. *J Neurosci*. 2010;30(6):2051–2059.
106. Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. *Glia*. 2005;50(4):427–434.
107. Wilhelmsson U, Li L, Pekna M, Berthold CH, Blom S, Eliasson C, Renner O, Bushong E, Ellisman M, Morgan TE, Pekny M. Absence of glial fibrillary acidic protein and vimentin prevents hypertrophy of astrocytic processes and improves post-traumatic regeneration. *J Neurosci*. 2004;24(21): 5016–5021.

108. Oberheim NA, Tian GF, Han X, Peng W, Takano T, Ransom B, Nedergaard M. Loss of astrocytic domain organization in the epileptic brain. *J Neurosci.* 2008;28(13):3264–3276.
109. Borges K, McDermott D, Irier H, Smith Y, Dingledine R. Degeneration and proliferation of astrocytes in the mouse dentate gyrus after pilocarpine-induced status epilepticus. *Exp Neurol*. 2006;201(2):416–427.
110. Martinez-Hernandez A, Bell KP, Norenberg MD. Glutamine synthetase: glial localization in brain. *Science*. 1977;195(4284):1356–1358.
111. Eid T, Williamson A, Lee TS, Petroff OA, de Lanerolle NC. Glutamate and astrocytes—key players in human mesial temporal lobe epilepsy? *Epilepsia.* 2008;49(suppl 2):42–52.
112. Hassel B, Dingledine R. Glutamate. In: Siegel GJ, Albers RW, Brady ST, Price DL, eds. *Basic Neurochemistry*. 7th ed. Burlington, MA: Elsevier; 2006: 267–290.
113. Petroff OA, Errante LD, Rothman DL, Kim JH, Spencer DD. Glutamate-glutamine cycling in the epileptic human hippocampus. *Epilepsia*. 2002;43(7): 703–710.
114. Eid T, Thomas MJ, Spencer DD, Rundén-Pran E, Lai JC, Malthankar GV, Kim JH, Danbolt NC, Ottersen OP, de Lanerolle NC. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet*. 2004;363(9402):28–37.
115. Hammer J, Alvestad S, Osen KK, Skare O, Sonnewald U, Ottersen OP. Expression of glutamine synthetase and glutamate dehydrogenase in the latent phase and chronic phase in the kainate model of temporal lobe epilepsy. *Glia.* 2008;56(8):856–868.
116. Eid T, Ghosh A, Wang Y, Beckström H, Zaveri HP, Lee TS, Lai JC, Malthankar-Phatak GH, de Lanerolle NC. Recurrent seizures and brain pathology after inhibition of glutamine synthetase in the hippocampus in rats. *Brain*. 2008;131(pt 8):2061–2070.
117. Wang Y, Zaveri HP, Lee TS, Eid T. The development of recurrent seizures after continuous intrahippocampal infusion of methionine sulfoximine in rats: a video-intracranial electroencephalographic study. *Exp Neurol*. 2009;220(2):293–302.
118. Liang SL, Carlson GC, Coulter DA. Dynamic regulation of synaptic GABA release by the glutamate-glutamine cycle in hippocampal area CA1. *J Neurosci*. 2006;26(33):8537–8548.
119. Kam K, Nicoll R. Excitatory synaptic transmission persists independently of the glutamate-glutamine cycle. *J Neurosci*. 2007;27(34):9192–9200.
120. Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ, Haydon PG, Coulter DA. Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nat Neurosci*. 2010;13(5):584–591.
121. Boison D. Adenosine augmentation therapies (AATs) for epilepsy: prospect of cell and gene therapies. *Epilepsy Res*. 2009;85(2–3):131–141.
122. Boison D. The adenosine kinase hypothesis of epileptogenesis. *Prog Neurobiol*. 2008;84(3):249–262.
123. Studer FE, Fedele DE, Marowsky A, Schwerdel C, Wernli K, Vogt K, Fritschy JM, Boison D. Shift of adenosine kinase expression from neurons to astrocytes during postnatal development suggests dual functionality of the enzyme. *Neuroscience.* 2006;142(1): 125–137.
124. During MJ, Spencer DD. Adenosine: a potential mediator of seizure arrest and postictal refractoriness. *Ann Neurol*. 1992;32(5):618–624.
125. Fedele DE, Gouder N, Güttinger M, Gabernet L, Scheurer L, Rülicke T, Crestani F, Boison D. Astrogliosis in epilepsy leads to overexpression of adenosine kinase, resulting in seizure aggravation. *Brain*. 2005;128(pt 10):2383–2395.
126. Li T, Ren G, Lusardi T, Wilz A, Lan JQ, Iwasato T, Itohara S, Simon RP, Boison D. Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. *J Clin Invest*. 2008;118(2): 571–582.
127. Boison D. Engineered adenosine-releasing cells for epilepsy therapy: human mesenchymal stem cells and human embryonic stem cells. *Neurotherapeutics*. 2009;6(2):278–283.
128. Boison D, Stewart KA. Therapeutic epilepsy research: from pharmacological rationale to focal adenosine augmentation. *Biochem Pharmacol*. 2009;78(12): 1428–1437.
129. Aronica E, van Vliet EA, Mayboroda OA, Troost D, da Silva FH, Gorter JA. Upregulation of metabotropic glutamate receptor subtypes mGluR3 and mGluR5 in reactive astrocytes in a rat model of mesial temporal lobe epilepsy. *Eur J Neurosci*. 2000;12(7): 2333–2344.
130. Steinhauser C, Seifert G. Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. *Eur J Pharmacol*. 2002;447(2–3):227–237.
131. Tang FR, Lee WL. Expression of the group II and III metabotropic glutamate receptors in the hippocampus of patients with mesial temporal lobe epilepsy. *J Neurocytol*. 2001;30(2):137–143.
132. Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M. An astrocytic basis of epilepsy. *Nat Med.* 2005;11(9):973–981.
133. Prince DA, Connors BW. Mechanisms of interictal epileptogenesis. *Adv Neurol*. 1986;44:275–299.
134. Korn SJ, Giacchino JL, Chamberlin NL, Dingledine R. Epileptiform burst activity induced by potassium in the hippocampus and its regulation by GABA-mediated inhibition. *J Neurophysiol*. 1987;57(1):325–340.
135. Fellin T, Gomez-Gonzalo M, Gobbo S, Carmignoto G, Haydon PG. Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices. *J Neurosci.* 2006;26(36): 9312–9322.
136. Lee CJ, Mannaioni G, Yuan H, Woo DH, Gingrich MB, Traynelis SF. Astrocytic control of synaptic NMDA receptors. *J Physiol*. 2007;581(pt 3): 1057–1081.
137. Ding S, Fellin T, Zhu Y, Lee SY, Auberson YP, Meaney DF, Coulter DA, Carmignoto G, Haydon PG. Enhanced astrocytic Ca^{2+} signals contribute to neuronal excitotoxicity after status epilepticus. *J Neurosci*. 2007;27(40):10674–10684.
138. Hardingham GE, Bading H. Coupling of extrasynaptic NMDA receptors to a CREB shut-off pathway is developmentally regulated. *Biochim Biophys Acta*. 2002;1600(1–2):148–153.

Chapter 47

Astrocyte Dysfunction in Epilepsy

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Recent work has identified glial cells, and astrocytes in particular, as active partners in neural information processing.[1] Application of advanced electrophysiological and Ca^{2+} imaging techniques revealed that astrocytes in acute brain slices or after fresh isolation from the tissue express a broad spectrum of functional ion channels and transmitter receptors similar to that of neurons.[2] The presence of ionotropic and metabotropic neurotransmitter receptors led to the conclusion that astrocytes are endowed with the machinery to sense and respond to neuronal activity. In 1994 two groups discovered that elevation of the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ in cultured astrocytes upon membrane receptor activation can induce glial release of glutamate.[3,4] This astonishing finding demonstrated for the first time that astrocytes sense neuronal activity and feed back to neurons to modulate central nervous system (CNS) signaling.[5] Later studies corroborated the view that astrocytes are direct communication partners of neurons and interact dynamically with synapses through uptake of neurotransmitters, receptor-mediated Ca^{2+} signaling, and subsequent gliotransmitter release. The intimate morphological and physiological interconnection between both cell types gave rise to the term *tripartite synapse*, which comprises not only pre- and postsynaptic elements but also the astrocytic process.[6,7]

According to a long-standing concept, astrocytes supply neurons with nutrition metabolites and oxygen. Fundamental new insight into this aspect of astrocyte function was gained through the discovery that astrocytes control cerebral blood flow, in an activity-dependent manner, by releasing vasoactive substances such as polyunsaturated fatty acids, adenosine, and prostaglandins. [8–10] In addition to the only recently discovered modulatory actions on brain signaling and circulation, astrocytes have been known for decades to serve homeostatic functions, including the clearance of neuronally released K^+ and glutamate from the extracellular space. Astrocytes are abundantly

coupled through gap junctions allowing them to redistribute elevated K^+ from sites of excessive neuronal activity to sites of lower extracellular K^+ concentration.

Despite the fact that the pathways enabling activation of these cells under physiological conditions are still ill-determined, evidence is emerging suggesting a critical role of astrocyte dysfunction in the pathogenesis of neurological disorders, including epilepsy.[11] Investigation of specimens from patients with pharmacoresistant mesial temporal lobe epilepsy (MTLE) and corresponding animal models of epilepsy revealed alterations in expression, subcellular localization, and function of astroglial K^+ and water channels, resulting in impaired K^+ buffering. Moreover, malfunction of glutamate transporters and the astrocytic glutamate-converting enzyme, glutamine synthetase (GS), as observed in epileptic tissue, suggested that astrocyte dysfunction is the cause of hyperexcitation, neurotoxicity, and the generation or spread of seizure activity. Accordingly, dysfunctional astrocytes should be considered promising targets for new therapeutic strategies. In this chapter, we will summarize current knowledge of astrocyte dysfunction in MTLE and discuss putative mechanisms underlying these alterations.

IMPAIRED K^+ BUFFERING IN TEMPORAL LOBE EPILEPSY

Loss of Inwardly Rectifying K^+ Channels in MTLE

Neuronal activity, propagation of action potentials, and synaptic activity after local depolarization lead to rapid fluctuations of the extracellular K^+ concentration $[K^+]_0$ because of the restricted volume of extracellular space.[12] If increases in $[K^+]_0$ remain uncorrected, the resting potential would become more positive and affect activation of transmembrane ion channels, receptors, und transporters. During neuronal hyperactivity in vivo, $[K^+]_0$ may increase from 3 mM to a ceiling level of 10–12 mM.[13] Such high $[K^+]_0$ levels can generate epileptiform activity in acute brain slices. Two different mechanisms are thought to balance $[K^+]_0$ during neuronal activity: K^+ uptake and spatial K^+ buffering (for review, see ref. 14). K^+ uptake, mediated by Na,K-ATPase (Na,K-adenosine triphosphatase) or Na-K-Cl cotransporters, is accompanied by cell swelling and local depolarization of astrocytes. Spatial K^+ buffering is driven by the glial syncytium membrane potential and the local K^+ equilibrium potential. This allows transfer of K^+ from regions of elevated $[K^+]_0$, through the syncytium, to regions of lower $[K^+]_0$. Spatial buffering depends on proper distribution and function of astrocytic K^+ channels, water channels, and gap junctions. In astrocytes, the inward-rectifying K^+ channel Kir4.1, which is activated by intracellular adenosine triphosphate (ATP), is thought to allow for K^+ influx at negative membrane potentials (for review, see refs. 15 and 16).

Because of their presumed role in K^+ homeostasis, the properties of astroglial Kir channels have been investigated in experimental and human epilepsy. Measurements of $[K^+]_0$ with ion-sensitive microelectrodes and patch-clamp studies suggested that impaired K^+ buffering in sclerotic human hippocampus resulted from altered Kir channel expression. Differences were observed in the effect of Ba^{2+} on stimulus-induced changes in $[K^+]_0$ in the CA1 region of hippocampal brain slices obtained from MTLE patients with hippocampal sclerosis (MTLE-HS) or without sclerosis (non-HS). In non-HS tissue, Ba^{2+} application significantly enhanced $[K^+]_0$, while this effect was not observed in HS specimens. Since Ba^{2+} is a blocker of Kir channels in astrocytes of the hippocampus,[17] this finding suggested impaired function of these channels in the sclerotic tissue.[18,19] The hypothesis could be confirmed with patch-clamp analyses demonstrating downregulation of Kir currents in the sclerotic human CA1 region of MTLE patients.[20,21] Accordingly, in MTLE-HS, impaired K^+ buffering and enhanced seizure susceptibility result from reduced expression of Kir channels. However, it is still unclear whether these changes are the cause or the consequence of the condition.

Fine mapping of a locus on mouse chromosome 1 identified *KCNJ10*, the gene encoding Kir4.1, as a candidate gene exhibiting a potentially important polymorphism with regard to fundamental aspects of seizure susceptibility.[22] Similarly, variations in *KCNJ10* in the human genome associate with multiple seizure phenotypes. Missense mutations of *KCNJ10* influence the risk of acquiring forms

of human epilepsy.[23] Mutations in the *KCNJ10* gene encoding Kir4.1 channels cause a multiorgan disorder in patients with clinical features of epilepsy, sensorineural deafness, ataxia, and electrolyte imbalance.[24,25] These patients suffer from generalized tonic-clonic seizures and focal seizures since childhood. Single nucleotide mutations in the *KCNJ10* gene cause missense or nonsense mutations on the protein level in the pore region, transmembrane helices, or the C terminus, the last resulting in deletion of a postsynaptic density, drosophila disc large tumor suppressor and zonula occludens-1 proteins (PDZ) binding domain and improper membrane localization of Kir4.1. These loss-of-function mutations of *KCNJ10* are homozygous or compound heterozygous. Experiments with heterologous expression systems demonstrated that the mutations indeed affect channel function and lead to reduced transmembrane currents.[24]

Genetic downregulation of Kir4.1, the main Kir channel subunit in astrocytes,[17,26–28] profoundly reduced the ability of astrocytes to remove glutamate and K^+ from the extracellular space, both in cell culture[29] and in vivo.[30] General knockout of Kir4.1 leads to early postnatal lethality, and mice with astrocytic deletion of the channel developed a pronounced behavioral phenotype, including seizures.[30,31] Thus, dysfunction of Kir4.1 is observed in different forms of epilepsy (Fig. 47–1).

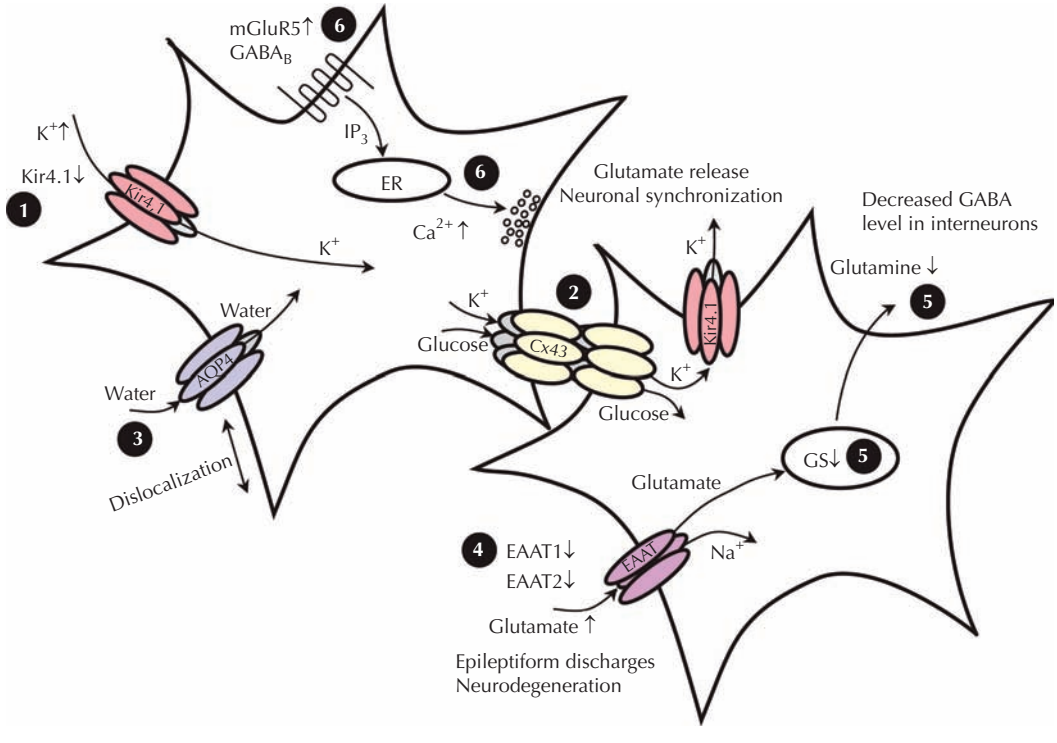

Figure 47–1. Epilepsy-associated alterations of functional properties in astrocytes. (1) Seizure activity leads to an increase in extracellular K^+ concentration. Downregulation of Kir channels was observed in astrocytes in human and experimental epilepsy. (2) Gap junctions mediate spatial redistribution of K^+. Genetic ablation of gap junction entails impaired K^+ buffering and hyperactivity. (3) Dislocation of water channels contributes to impaired K^+ buffering. (4) Astrocytes are primarily responsible for glutamate uptake. Reduction of the proteins excitatory amino acid transporters 1 and 2 (EAAT1 and EAAT2) is observed in human epileptic hippocampus. Elevated extracellular glutamate decreases the threshold for seizure induction. (5) Glutamate is converted into glutamine through glutamine synthetase (GS). In human epileptic hippocampus, loss of GS resulted in elevated extracellular glutamate levels. In experimental epilepsy, downregulation of GS was observed in the chronic phase. (6) Ca^{2+} elevations in astrocytes are mediated by metabotropic receptors. Activation of mGluR5 leads to increases in intracellular Ca^{2+}. The Ca^{2+} rise may oscillate and initiate Ca^{2+} wave propagation within the astrocyte network, activate Ca^{2+}-dependent ion channels, and induce glutamate release from astrocytes. Enhanced mGluR5 levels are observed in experimental and human epileptic tissue. ER, endoplasmic reticulum; IP_3, inositol (1,4,5)-triphosphate.

Interestingly, spinal cord injury-induced downregulation of Kir4.1 in the spinal cord can be partially rescued by administration of β-estradiol. Inhibition of estrogen receptors also reduces Kir4.1-mediated currents, suggesting that Kir4.1 channel expression depends on nuclear estrogen receptor signaling under physiological conditions.[32]

Gap Junctions and K^+ Buffering

A prerequisite for the operation of spatial K^+ buffering is the presence of Kir channels and connexins forming gap junctions.[14,33] According to this concept, K^+ entry into the astrocytic network occurs at sites of maximal extracellular K^+ accumulation, driven by the difference between the glial syncytium membrane potential and the local K^+ equilibrium potential. Since K^+ propagates through the glial network, at sites distant to elevated $[K^+]_0$ a driving force for K^+ efflux results because there local depolarization exceeds the K^+ equilibrium potential. Surprisingly, clearance and redistribution of K^+ are still preserved in the hippocampal stratum radiatum (but not in the lacunosum moleculare) of mice with coupling-deficient astrocytes, indicating that gap junction-independent mechanisms add to K^+ homeostasis in the brain (e.g., indirect coupling[34]; Fig. 47–2). Nevertheless, genetic deletion of astrocyte gap junctions leads to impaired K^+ buffering, spontaneous epileptiform activity, and a decreased threshold for eliciting seizure activity.[34]

Interplay between Kir Channels and AQP4 in K^+ Buffering

Ultrastructural analyses in rat demonstrated spatial overlap of Kir4.1 and the water channel aquaporin 4 (AQP4) in astroglial endfeet contacting the capillaries.[35,36] This finding gave rise to the hypothesis that K^+ clearance through Kir channels might depend critically on concomitant transmembrane flux of water in a given cell to dissipate osmotic imbalances due to K^+ redistribution. Subsequent functional work corroborated this idea by showing that in mice the clearance of extracellular K^+ is compromised if the number of perivascular AQP4 channels is decreased.[37] Similarly, impaired K^+

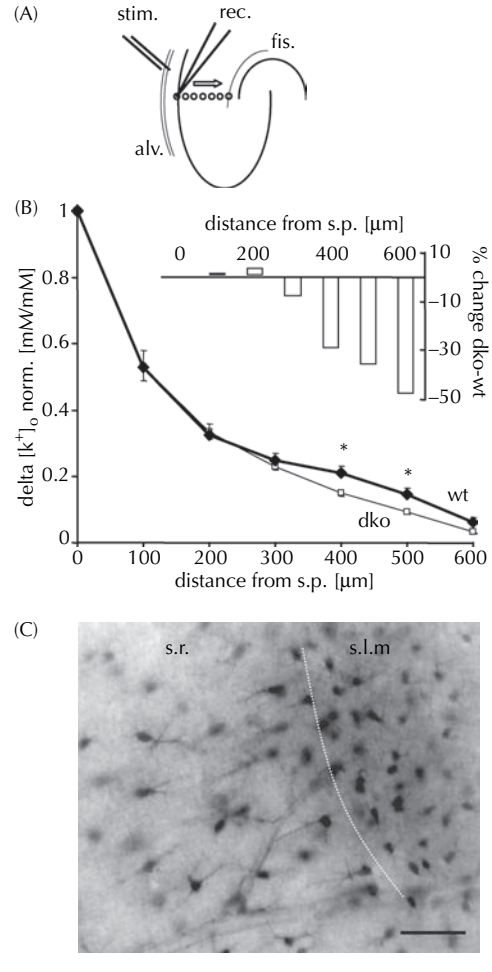

Figure 47–2. Impaired spatial buffering in the stratum lacunosum moleculare of mice with genetic deletion of C×43 and C×30 in astrocytes (dko mice). **A.** Experimental setup for the analysis of laminar profiles of changes in $[K^+]_0$. Stimulation was performed in the alveus (20 Hz, 100%), while the recording electrode was stepped from the stratum pyramidale to the hippocampal fissure (100 µm step size). **B.** Mean rises in $[K^+]_0$ (normalized to rise at the stratum pyramidale, s.p.) plotted against the distance from the stratum pyramidale (thin line, wild-type [wt]: $n = 8$ animals, 13 slices; thick line, dko, $n = 8$ animals, 15 slices). Normalized $[K^+]_0$ in dko mice reached lower levels at 400, 500, and 600 µm from the s.p. compared to the wt. See the inset for relative changes. *Changes differ significantly. **C.** The difference in astrocyte morphology and orientation in the stratum radiatum versus the stratum lacunosum moleculare, obtained after biocytin injection into a stratum radiatum astrocyte proximal to the stratum lacunosum moleculare. The white line indicates the boundary between stratum radiatum and stratum lacunosum moleculare. Note the small size and random orientation of cells in the stratum lacunosum moleculare. Scale bar: 50 µm. alv., alveus; fis., fissure; stim., stimulation electrode; rec. recording electrode; s.r. stratum radiatum; s.l.m., stratum lacunosum moleculare. From ref. 34.

buffering and prolonged seizure duration were observed in AQP4 knockout mice.[38] However, later work provided evidence against the concept of functional coupling of AQP4 and Kir4.1 channels.[39,40] Thus, there is a need to identify alternative mechanism(s) underlying the hyperactivity associated with AQP4 deficiency.

Epileptic rats show mislocalization of AQP4 in astrocytic endfeet contacting blood vessels in the hippocampus. Eight weeks after status epilepticus, immunohistochemistry revealed loss of AQP4 in vacuolized astrocytes of the hippocampus. Instead, AQP1 was found in astrocytes, a protein not expressed by these cells under physiological conditions. Nonvacuolized astrocytes still contained AQP4, even at higher levels, and in addition expressed AQP9.[41] In MTLE-HS patients, immunostaining indicated loss of AQP4 in vasculature-associated astrocyte endfeet compared with specimens from non-HS patients.[42] The decrease in perivascular AQP4 channels might be secondary, following disruption of the dystrophin complex that is essential for anchoring of AQP4 in the plasma membrane.[43] Together, these findings suggest that in MTLE-HS, dislocation of water channels in concert with decreased expression of Kir channels in astrocytes might underlie impaired K^+ buffering and increased seizure propensity (Fig. 47–1). The functional consequence of the upregulation of AQP subunits other than AQP4 in astrocytes of epileptic tissue remains unclear. Characterization of DNA variations in MTLE-HS patients with antecedent febrile seizures (MTLE-FS) identified single nucleotide polymorphisms (SNPs) in the *KCNJ10* gene, in the region between *KCNJ9* and *KCNJ10*, and in the *Aqp4* gene. The combination of *KCNJ10* and AQP4 gene variations was associated with MTLE-FS, supporting the hypothesis that impaired K^+ and water homeostasis is involved in the etiopathogenesis of MTLE.[44]

In addition to spatial buffering, transient K^+ accumulation can be counterbalanced by net K^+ uptake through Na,K-ATPase and the Na-K-Cl cotransporter NKCC1, at the cost of cell swelling due to concomitant water influx (reviewed in ref. 14). In rodent hippocampus, Na,K-ATPase was reported to have a potential role in maintaining low $[K^+]_0$ levels and to clear elevations in $[K^+]_0$ after epileptiform activity.[45,46] However, whether alterations in net K^+ uptake contribute to the increased $[K^+]_0$ levels seen in epileptic tissue has still to be determined.

AMBIGUOUS ROLE OF GAP JUNCTIONS IN EPILEPTOGENESIS

The abundant expression of gap junctions in astrocytes and their formation as a functional syncytium enables long-range intercellular exchange of ions, nutritional metabolites, amino acids, and nucleotides. The permeability of gap junctions is regulated by endogenous membrane receptors, second messengers, and pH (for review, see ref. 47). Thus, trafficking of nutritional metabolites such as glucose-6-phosphate and lactate through astrocytes is controlled by endogeneous compounds that are released by endothelial cells, astrocytes, and neurons in an activity-dependent manner.[48] In addition to the aforementioned homeostatic functions, astrocytic gap junctions may affect neuronal migration and proliferation. [49–51] Recent work has revealed that inhibition of gap junction coupling not only enhances glucose uptake, synthesis of nucleic acids, and proliferation of astrocytes.[52] Gap junctions formed by C×43 and C×30 also allow intercellular trafficking of glucose through the astrocytic network and deliver energetic metabolites from blood vessels to neurons to maintain synaptic transmission in the murine brain[53] (Fig. 47–1). Glucose uptake and trafficking was dependent on synaptic transmission: it was increased during epileptiform activity and, in turn, glucose delivery through the astrocytic network was needed to sustain epileptiform activity. Neuronal activity was sustained by the transport of nutrition metabolites through the astrocytic network even under conditions of transient limited substrate availability (see also ref. 51).

C×43 and C×30 are the main connexins forming gap junctions in astrocytes of the CNS[54] and, as discussed above, their cell type-specific deletion in mice led to the generation of spontaneous epileptiform activity and a decreased threshold for evoking seizure activity.[34] Disruption of the blood-brain barrier and albumin-dependent generation of epilepsy in rat is accompanied by a transient decrease of both connexin transcripts.[55,56] These findings

are in line with the long-standing concept that astrocyte gap junctions are essential for proper K^+ regulation[57] and help to counteract the generation of epileptiform activity. However, the opposite effect was observed in organotypic hippocampal slice cultures where long-term block of gap junctions through C×43 mimetic peptides attenuated spontaneous seizure-like events (but not evoked epileptiform responses[58]). The authors also observed that serum deprivation strongly reduced spontaneous recurrent network activity, and they assigned this effect to a neuroprotective role of gap junction communication. Hence, a decrease in gap junction permeability seems to exert opposite effects on excitability: a fast onset, pro-convulsive effect due to impaired K^+ redistribution but a delayed antiepileptic effect because of disruption of neuronal energy supply.

Despite these intriguing new insights into astrocyte function, the role of gap junctions in human epilepsy is still unresolved. Published data are not always consistent, indicating that (1) human epilepsy cannot be considered a uniform condition, (2) most of the currently available gap junction blockers do not distinguish between neuronal and glial gap junctions, and (3) these blockers usually have dramatic side effects.[59] Moreover, analysis of tissue samples is restricted to the chronic phase of the disorder and is likely to be affected by patients' long-lasting treatment with different antiepileptic drugs (AEDs), a problem inherent in experiments with neurosurgically resected specimens. Increased expression of C×43 protein was observed in low-grade tumors and reactive astrocytes of human epileptic cortical tissue surrounding tumors, although high-grade gliomas exhibited great variations in C×43.[60] Specimens from pharmacoresistant MTLE-HS patients showed strongly enhanced C×43 immunoreactivity and transcript levels.[61-,63] The authors speculate that upregulation of connexins might represent a compensatory response of astrocytes to cope with the enhanced K^+ release during seizure activity. However, in light of the aforementioned findings, enhanced coupling could also serve to fuel hyperactivity and thereby exacerbate generalized seizures. Importantly, it has to be emphasized that any functional evidence of enhanced gap junction coupling in human epilepsy is missing, which considerably limits conclusions that can be drawn from the above studies.

GLUTAMATE UPTAKE IN EPILEPSY

Glutamate Transporter in Astrocytes

The uptake of glutamate that helps to terminate the action of this neurotransmitter at CNS synapses is mediated mainly by transporters localized at the astrocytic membrane. The high efficiency of these glial transporters ensures the maintenance of low concentrations of extracellular glutamate to prevent excitotoxic cell death.[64,65] They are densely packed and keep the extracellular glutamate concentration in the nanomolar range, preventing significant receptor activation.[66–68] Fine tuning of extrasynaptic glutamate through glial glutamate transporters is important for proper synaptic function and plasticity.[69–72] Downregulation of glial glutamate transporters or metabolic inhibition of the glutamate-to-glutamine converting enzyme, GS, immediately causes extracellular accumulation of the transmitter, which, if it reaches micromolar concentrations, causes depolarization, compromised synaptic transmission, and induced neuronal death.[66,73–75] It is therefore not surprising that dysfunction of the astrocytic glutamate excitatory amino acid transporters 1 and 2 (EAAT1 and EAAT2) is observed under various pathological conditions, including epilepsy[11] (Fig. 47–1).

Excess of extracellular glutamate is found in human epileptogenic tissue and can induce recurrent seizures and neuronal cell death.[76,77] This may occur through the activation of neuronal and glial glutamate receptors. Indeed, a large body of evidence has shown that the activation of astrocytes by neuronal activity-derived glutamate is due mainly to activation of the metabotropic glutamate receptors (mGluRs) mGluR3 and mGluR5. Activation of these receptors affects cyclic adenosine monophosphate (cAMP) accumulation and leads to increases in intracellular Ca^{2+}, respectively. The Ca^{2+} rise may oscillate and initiate Ca^{2+} wave propagation within the astrocyte network, activate Ca^{2+}-dependent ion channels, and induce glutamate release from

astrocytes (cf. Chapter 52 in this volume). In epilepsy models, elevated protein levels for mGluR3, mGluR5, and mGluR8 have been found[59] (Fig. 47–1). High-resolution analysis of hippocampal specimens from TLE patients detected mGluR2/3, mGluR4, and mGluR8 in reactive astrocytes, suggesting involvement of these mGluRs in gliosis.[78] Enhanced levels of astroglial mGluR2/3 and mGluR5 were also observed in epileptic specimens from patients with focal cortical dysplasia.[79] Since their activation affects expression of EAAT1 and EAAT2[80] and elevates $[Ca^{2+}]_i$, astrocytic mGluRs might contribute to the generation of seizure foci.

Different reports exist about the regulation of glial glutamate transporters in patients presenting with pharmacoresistant MTLE. Employment of in situ hybridization and Western blot analysis in specimens from patients with HS[81] did not reveal changes in EAAT1 or EAAT2. In contrast, other groups reported downregulation of EAAT2 immunoreactivity in the CA1 region displaying profound neuronal loss in human HS.[82,83] It was found that EAAT1 was increased in the sclerotic CA2/3 region.[83] Later work showed downregulation of EAAT1 and EAAT2 in the CA1 region in HS and emphasized that it is still unclear whether this reduction is the cause or a consequence of the condition.[84] A critical analysis of immunohistochemical, Western blot, and mRNA data hinted at a redistribution, rather than a reduction, of glial glutamate transporter in human epilepsy, which was considered inadequate to account for the high glutamate concentrations during seizures.[85] The authors concluded that under these conditions, glutamate uptake is influenced by factors other than transporter protein levels, such as changes in the cells' metabolic state and downregulation of GS (see the next section).

Recent work reported that expression of EAAT2 is critically dependent on synaptic activity. In this study, EAAT2-mediated uptake was decreased after nerve fiber transection or neurodegeneration in a mouse model of amyotrophic lateral sclerosis (ALS).[86] Beta-lactam antibiotics increased glutamate uptake in primary human astrocytes through NFκB-mediated EAAT2 promoter activation.[87] Hence, the antibiotics might represent a therapeutic tool to counteract glutamate transporter dysfunction in neurological disorders such as ALS and epilepsy.[88]

Depolarization of astrocytes by inadequate K^+ buffering led to compromised functioning of the glial transporters.[29,30] In a rat model of cortical dysplasia, pharmacological inhibition of glial glutamate transporters in the lesion area led to opening of neuronal *N*-methyl-D-aspartate (NMDA) receptors, prolonged synaptic currents, and decreased the threshold for the induction of epileptiform activity.[89] This enhanced activity of NMDA receptors also triggered dephosphorylation of Kv2.1 K^+ channels, produced a negative shift of its voltage-dependent activation, and hence modulated excitability and neuronal plasticity in mice.[90]

Conversion of Glutamate to Glutamine by Astrocytes

For effective removal of excess extracellular glutamate, the transmitter must be converted by GS into the receptor-inactive substrate glutamine under consumption of ATP and ammonia. Increasing evidence indicates a loss of this astrocyte-specific enzyme in epilepsy. In MTLE-HS patients, loss of GS in the hippocampus was accompanied by elevated extracellular glutamate levels.[91–93] Infusion of ^{13}C-labeled glucose before resection of the hippocampus from MTLE-HS patients revealed an increased glutamate concentration, slowed glutamate-to-glutamine cycling, and decreased glutamine concentrations. Thus, failure of glutamate detoxification could account for continuing excitotoxicity and contribute to the pathogenesis.[94]

In experimental epilepsy, upregulation of GS and glial fibrillary acidic protein (GFAP) was observed in the latent phase, prior to recurrent seizure onset, while in the chronic phase GS was downregulated, with persistence of elevated GFAP immunoreactivity.[95] By contrast, glutamate dehydrogenase, another glutamate-degrading enzyme, remained unaltered in this rat model. Compatible with a potential causative role of GS loss in initiating epilepsy was the finding that pharmacological inhibition of GS produced recurrent seizure activity and rat brain pathology resembling MTLE-HS.[96] In the pentylenetetrazol epilepsy model, immunohistochemistry revealed unchanged GS protein levels. However, the enzyme underwent stress-induced nitration and partial inhibition

in severely affected hippocampal regions, which might result in locally altered glutamate and gamma-aminobutyric acid (GABA) metabolism.[97] Genetic inactivation of GS in mice leads to early embryonic lethality, while GS deletion on one allele increases the susceptibility to febrile seizures.[98]

Inhibition of GS in astrocytes and/or glutamine transporters in neurons reduced the amplitudes of evoked inhibitory postsynaptic currents (IPSCs) and GABA release from interneurons in the hippocampus (Fig. 47–1). Hence, in the rat, the glial glutamate-glutamine cycle is a major contributor to synaptic GABA release and regulates inhibitory synaptic strength,,[99] while inhibition of GS does not significantly affect glutamatergic transmission in the same species.[100] However, during periods of intense neuronal or epileptiform activity, a glial supply of glutamine and its transport into neurons are required.[101]

MTLE-HS is characterized by neuronal loss and reactive astrogliosis. In a model of selective astrogliosis, which leaves properties of neurons and microglia unaltered, deficient neuronal inhibition was observed in the hippocampus, while excitatory neurotransmission remained unchanged. Decreased inhibition resulted from impaired GS activity, compromised glutamine availability, and reduced GABA release from interneurons.[102] These astrogliosis-associated deficits generated hyperactivity, emphasizing the importance of proper GS function for inhibitory neurotransmission and prevention of seizures generation.

CONCLUDING REMARKS

The novel view of astrocytes as communication partners of neurons rather than "brain glue" has rekindled the question regarding the role of these cells in neurological disorders such as epilepsy. Indeed, an increasing body of evidence has documented astroglial dysfunction, and even dysregulation of astroglia-specific functions, in human and experimental epilepsy. This particularly concerns impaired uptake/conversion of glutamate and removal/redistribution of K^+, as observed in MTLE-HS. However, a number of key questions need to be addressed before a unifying picture can be proposed. For example, it is still unclear whether the reported glial alterations are a cause or a consequence of the condition. In addition, difficulties arise from the fact that the term *astrocyte* covers a heterogeneous group of cells, and this complicates comparison of individual studies. It is worthwhile, however, to emphasize that the molecular, functional, and structural characterization of astroglial heterogeneity is a rapidly evolving field that may soon lead to a better definition of astroglial subtypes. In a comprehensive approach that uses modern molecular genetics and in vivo models, we may now have the opportunity to clarify the specific roles of astroglia in epilepsy and to develop novel therapeutic approaches to fight this disorder.

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The authors declare no competing financial interests.

REFERENCES

1. Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev.* 2006;86:1009–1031.
2. Verkhratsky A, Steinhäuser C. Ion channels in glial cells. *Brain Res Rev.* 2000;32:380–412.
3. Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, Haydon PG. Glutamate-mediated astrocyte-neuron signalling. *Nature.* 1994;369:744–747.
4. Nedergaard M. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science.* 1994;263:1768–1771.
5. Pasti L, Volterra A, Pozzan T, Carmignoto G. Intracellular calcium oscillations in astrocytes: a highly plastic, bidirectional form of communication between neurons and astrocytes in situ. *J Neurosci.* 1997;17:7817–7830.

6. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci*. 1999;22:208–215.
7. Halassa MM, Fellin T, Haydon PG. The tripartite synapse: roles for gliotransmission in health and disease. *Trends Mol Med*. 2007;13:54–63.
8. Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, Carmignoto G. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci*. 2003;6: 43–50.
9. Mulligan SJ, MacVicar BA. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature*. 2004;431:195–199.
10. Koehler RC, Roman RJ, Harder DR. Astrocytes and the regulation of cerebral blood flow. *Trends Neurosci*. 2009;32:160–169.
11. Seifert G, Schilling K, Steinhäuser C. Astrocyte dysfunction in neurological disorders: a molecular perspective. *Nat Rev Neurosci*. 2006;7:194–206.
12. Nicholson C, Syková E. Extracellular space structure revealed by diffusion analysis. *Trends Neurosci*. 1998;21:207–215.
13. Heinemann U, Lux HD. Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of cat. *Brain Res*. 1977;120:231–249.
14. Kofuji P, Newman EA. Potassium buffering in the central nervous system. *Neuroscience*. 2004;129: 1045–1056.
15. Reimann F, Ashcroft FM. Inwardly rectifying potassium channels. *Curr Opin Cell Biol*. 1999;11:503–508.
16. Hibino H, Inanobe A, Furutani K, Murakami S, Findlay I, Kurachi Y. Inwardly rectifying potassium channels: their structure, function, and physiological roles. *Physiol Rev*. 2010;90:291–366.
17. Seifert G, Hüttmann K, Binder DK, Hartmann C, Wyczynski A, Neusch C, Steinhäuser C. Analysis of astroglial K^+ channel expression in the developing hippocampus reveals a predominant role of the Kir4.1 subunit. *J Neurosci.* 2009;29:7474–7488.
18. Kivi A, Lehmann TN, Kovacs R, Eilers A, Jauch R, Meencke HJ, Von Deimling A, Heinemann U, Gabriel S. Effects of barium on stimulus-induced rises of $[K^+]o$ in human epileptic non-sclerotic and sclerotic hippocampal area CA1. *Eur J Neurosci*. 2000;12: 2039–2048.
19. Jauch R, Windmuller O, Lehmann TN, Heinemann U, Gabriel S. Effects of barium, furosemide, ouabaine and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) on ionophoretically-induced changes in extracellular potassium concentration in hippocampal slices from rats and from patients with epilepsy. *Brain Res*. 2002;925:18–27.
20. Bordey A, Sontheimer H. Properties of human glial cells associated with epileptic seizure foci. *Epilepsy Res*. 1998;32:286–303.
21. Hinterkeuser S, Schröder W, Hager G, Seifert G, Blümcke I, Elger CE, Schramm J, Steinhäuser C. Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. *Eur J Neurosci*. 2000;12:2087–2096.
22. Ferraro TN, Golden GT, Smith GG, Martin JF, Lohoff FW, Gieringer TA, Zamboni D, Schwebel CL, Press DM, Kratzer SO, Zhao H, Berrettini WH, Buono RJ. Fine mapping of a seizure susceptibility locus on mouse Chromosome 1: nomination of Kcnj10 as a causative gene. *Mamm Genome*. 2004;15:239–251.
23. Buono RJ, Lohoff FW, Sander T, Sperling MR, O'Connor MJ, Dlugos DJ, Ryan SG, Golden GT, Zhao H, Scattergood TM, Berrettini WH, Ferraro TN. Association between variation in the human KCNJ10 potassium ion channel gene and seizure susceptibility. *Epilepsy Res*. 2004;58:175–183.
24. Bockenhauer D, Feather S, Stanescu HC, Bandulik S, Zdebik AA, Reichold M, Tobin J, Lieberer E, Sterner C, Landoure G, Arora R, Sirimanna T, Thompson D, Cross JH, van't HW, Al MO, Tullus K, Yeung S, Anikster Y, Klootwijk E, Hubank M, Dillon MJ, Heitzmann D, Arcos-Burgos M, Knepper MA, Dobbie A, Gahl WA, Warth R, Sheridan E, Kleta R. Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCNJ10 mutations. *N Engl J Med*. 2009;360: 1960–1970.
25. Scholl UI, Choi M, Liu T, Ramaekers VT, Hausler MG, Grimmer J, Tobe SW, Farhi A, Nelson-Williams C, Lifton RP. Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME syndrome) caused by mutations in KCNJ10. *Proc Natl Acad Sci USA*. 2009;106:5842–5847.
26. Olsen ML, Higashimori H, Campbell SL, Hablitz JJ, Sontheimer H. Functional expression of Kir4.1 channels in spinal cord astrocytes. *Glia*. 2006;53:516–528.
27. Neusch C, Papadopoulos N, Müller M, Maletzki I, Winter SM, Hirrlinger J, Handschuh M, Bähr M, Richter DW, Kirchhoff F, Hülsmann S. Lack of the Kir4.1 channel subunit abolishes K^+ buffering properties of astrocytes in the ventral respiratory group: impact on extracellular K^+ regulation. *J Neurophysiol*. 2006;95:1843–1852.
28. Tang X, Taniguchi K, Kofuji P. Heterogeneity of Kir4.1 channel expression in glia revealed by mouse transgenesis. *Glia.* 2009;57:1706–1715.
29. Kucheryavykh YV, Kucheryavykh LY, Nichols CG, Maldonado HM, Baksi K, Reichenbach A, Skatchkov SN, Eaton MJ. Downregulation of Kir4.1 inward rectifying potassium channel subunits by RNAi impairs potassium transfer and glutamate uptake by cultured cortical astrocytes. *Glia*. 2007;55:274–281.
30. Djukic B, Casper KB, Philpot BD, Chin LS, McCarthy KD. Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. *J Neurosci*. 2007;27: 11354–11365.
31. Kofuji P, Ceelen P, Zahs KR, Surbeck LW, Lester HA, Newman EA. Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: phenotypic impact in retina. *J Neurosci*. 2000;20: 5733–5740.
32. Olsen ML, Campbell SC, McFerrin MB, Floyd CL, Sontheimer H. Spinal cord injury causes a widespread, persistent loss of Kir4.1 and glutamate transporter 1: benefit of 17 beta-oestradiol treatment. *Brain*. 2010;133:1013–1025.
33. Walz W. Role of astrocytes in the clearance of excess extracellular potassium. *Neurochem Int.* 2000;36: 291–300.
34. Wallraff A, Köhling R, Heinemann U, Theis M, Willecke K, Steinhäuser C. The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. *J Neurosci*. 2006;26:5438–5447.

35. Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP. Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J Neurosci*. 1997;17:171–180.
36. Higashi K, Fujita A, Inanobe A, Tanemoto M, Doi K, Kubo T, Kurachi Y. An inwardly rectifying K^+ channel, Kir4.1, expressed in astrocytes surrounds synapses and blood vessels in brain. *Am J Physiol Cell Physiol*. 2001;281:C922–C931.
37. Amiry-Moghaddam M, Williamson A, Palomba M, Eid T, De Lanerolle NC, Nagelhus EA, Adams ME, Froehner SC, Agre P, Ottersen OP. Delayed K^+ clearance associated with aquaporin-4 mislocalization: phenotypic defects in brains of alpha-syntrophin-null mice. *Proc Natl Acad Sci USA*. 2003;100:13615–13620.
38. Binder DK, Yao X, Zador Z, Sick TJ, Verkman AS, Manley GT. Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. *Glia*. 2006;53:631–636.
39. Ruiz-Ederra J, Zhang H, Verkman AS. Evidence against functional interaction between aquaporin-4 water channels and Kir4.1 K^+ channels in retinal Muller cells. *J Biol Chem*. 2007;282:21866–21872.
40. Zhang H, Verkman AS. Aquaporin-4 independent Kir4.1 K^+ channel function in brain glial cells. *Mol Cell Neurosci*. 2008;37:1–10.
41. Kim JE, Ryu HJ, Yeo SI, Seo CH, Lee BC, Choi IG, Kim DS, Kang TC. Differential expressions of aquaporin subtypes in astroglia in the hippocampus of chronic epileptic rats. *Neuroscience*. 2009;163:781–789.
42. Eid T, Lee TS, Thomas MJ, Amiry-Moghaddam M, Bjornsen LP, Spencer DD, Agre P, Ottersen OP, De Lanerolle NC. Loss of perivascular aquaporin 4 may underlie deficient water and K^+ homeostasis in the human epileptogenic hippocampus. *Proc Natl Acad Sci USA*. 2005;102:1193–1198.
43. Amiry-Moghaddam M, Otsuka T, Hurn PD, Traystman RJ, Haug FM, Froehner SC, Adams ME, Neely JD, Agre P, Ottersen OP, Bhardwaj A. An alpha-syntrophin-dependent pool of AQP4 in astroglial end-feet confers bidirectional water flow between blood and brain. *Proc Natl Acad Sci USA*. 2003;100:2106–2111.
44. Heuser K, Nagelhus EA, Taubøll E, Indahl U, Berg PR, Lien S, Nakken S, Gjerstad L, Ottersen OP. Variants of the genes encoding AQP4 and Kir4.1 are associated with subgroups of patients with temporal lobe epilepsy. *Epilepsy Res*. 2010;88:55–64.
45. D'Ambrosio R, Gordon DS, Winn HR. Differential role of KIR channel and Na^+/K^+-pump in the regulation of extracellular K^+ in rat hippocampus. *J Neurophysiol*. 2002;87:87–102.
46. Xiong ZQ, Stringer FL. Sodium pump activity, not glial spatial buffering, clears potassium after epileptiform activity induced in the dentate gyrus. *J Neurophysiol*. 2000;83:1443–1451.
47. Rouach N, Avignone E, Meme W, Koulakoff A, Venance L, Blomstrand F, Giaume C. Gap junctions and connexin expression in the normal and pathological central nervous system. *Biol Cell*. 2002;94:457–475.
48. Giaume C, Tabernero A, Medina JM. Metabolic trafficking through astrocytic gap junctions. *Glia*. 1997;21:114–123.
49. Kunze A, Congreso MR, Hartmann C, Wallraff-Beck A, Hüttmann K, Bedner P, Requardt R, Seifert G, Redecker C, Willecke K, Hofmann A, Pfeifer A, Theis M, Steinhäuser C. Connexin expression by radial glia-like cells is required for neurogenesis in the adult dentate gyrus. *Proc Natl Acad Sci USA*. 2009;106:11336–11341.
50. Elias LA, Kriegstein AR. Gap junctions: multifaceted regulators of embryonic cortical development. *Trends Neurosci*. 2008;31:243–250.
51. Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N. Astroglial networks: a step further in neuroglial and gliovascular interactions. *Nat Rev Neurosci*. 2010;11:87–99.
52. Tabernero A, Medina JM, Giaume C. Glucose metabolism and proliferation in glia: role of astrocytic gap junctions. *J Neurochem*. 2006;99:1049–1061.
53. Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C. Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science*. 2008;322:1551–1555.
54. Nagy JI, Dudek FE, Rash JE. Update on connexins and gap junctions in neurons and glia in the mammalian nervous system. *Brain Res Brain Res Rev*. 2004;47:191–215.
55. Cacheaux LP, Ivens S, David Y, Lakhter AJ, Bar-Klain G, Shapira M, Heinemann U, Friedman A, Kaufer D. Transcriptome profiling reveals TGF-ß signaling involvement in epileptogenesis. *J Neurosci*. 2009;29:8927–8935.
56. David Y, Cacheaux LP, Ivens S, Lapilover E, Heinemann U, Kaufer D, Friedman A. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *J Neurosci*. 2009;29:10588–10599.
57. Orkand RK, Nicholls JG, Kuffler SW. Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J Neurophysiol*. 1966;29:788–806.
58. Samoilova M, Wentlandt K, Adamchik Y, Velumian AA, Carlen PL. Connexin 43 mimetic peptides inhibit spontaneous epileptiform activity in organotypic hippocampal slice cultures. *Exp Neurol*. 2008;210:762–775.
59. Steinhäuser C, Seifert G. Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. *Eur J Pharmacol*. 2002;447:227–237.
60. Aronica E, Gorter JA, Jansen GH, Leenstra S, Yankaya B, Troost D. Expression of connexin 43 and connexin 32 gap-junction proteins in epilepsy-associated brain tumors and in the perilesional epileptic cortex. *Acta Neuropathol (Berl)*. 2001;101:449–459.
61. Naus CCG, Bechberger JF, Paul DL. Gap junction gene expression in human seizure disorder. *Exp Neurol*. 1991;111:198–203.
62. Collignon F, Wetjen NM, Cohen-Gadol AA, Cascino GD, Parisi J, Meyer FB, Marsh WR, Roche P, Weigand SD. Altered expression of connexin subtypes in mesial temporal lobe epilepsy in humans. *J Neurosurg*. 2006;105:77–87.
63. Fonseca CG, Green CR, Nicholson LF. Upregulation in astrocytic connexin 43 gap junction levels may exacerbate generalized seizures in mesial temporal lobe epilepsy. *Brain Res*. 2002;929:105–116.
64. Danbolt NC. Glutamate uptake. *Prog Neurobiol*. 2001;65:1–105.
65. Choi DW. Excitotoxic cell death. *J Neurobiol*. 1992;23:1261–1276.

66. Herman MA, Jahr CE. Extracellular glutamate concentration in hippocampal slice. *J Neurosci.* 2007;27: 9736–9741.
67. Le Meur K, Galante M, Angulo MC, Audinat E. Tonic activation of NMDA receptors by ambient glutamate of non-synaptic origin in the rat hippocampus. *J Physiol*. 2007;580:373–383.
68. Cavelier P, Attwell D. Tonic release of glutamate by a DIDS-sensitive mechanism in rat hippocampal slices. *J Physiol*. 2005;564:397–410.
69. Arnth-Jensen N, Jabaudon D, Scanziani M. Cooperation between independent hippocampal synapses is controlled by glutamate uptake. *Nat Neurosci.* 2002;5: 325–331.
70. Huang H, Bordey A. Glial glutamate transporters limit spillover activation of presynaptic NMDA receptors and influence synaptic inhibition of Purkinje neurons. *J Neurosci*. 2004;24:5659–5669.
71. Filosa A, Paixao S, Honsek SD, Carmona MA, Becker L, Feddersen B, Gaitanos L, Rudhard Y, Schoepfer R, Klopstock T, Kullander K, Rose CR, Pasquale EB, Klein R. Neuron–glia communication via EphA4/ephrin-A3 modulates LTP through glial glutamate transport. *Nat Neurosci*. 2009;12:1285–1292.
72. Tzingounis AV, Wadiche JI. Glutamate transporters: confining runaway excitation by shaping synaptic transmission. *Nat Rev Neurosci*. 2007;8:935–947.
73. Sah P, Hestrin S, Nicoll RA. Tonic activation of NMDA receptors by ambient glutamate enhances excitability of neurons. *Science.* 1989;246:815–818.
74. Zorumski CF, Mennerick S, Que J. Modulation of excitatory synaptic transmission by low concentrations of glutamate in cultured rat hippocampal neurons. *J Physiol.* 1996;494:465–477.
75. Jabaudon D, Shimamoto K, Yasuda-Kamatani Y, Scanziani M, Gähwiler BH, Gerber U. Inhibition of uptake unmasks rapid extracellular turnover of glutamate of nonvesicular origin. *Proc Natl Acad Sci USA*. 1999;96:8733–8738.
76. Glass M, Dragunow M. Neurochemical and morphological changes associated with human epilepsy. *Brain Res Rev*. 1995;21:29–41.
77. During MJ, Spencer DD. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet*. 1993;341:1607–1610.
78. Tang FR, Lee WL. Expression of the group II and III metabotropic glutamate receptors in the hippocampus of patients with mesial temporal lobe epilepsy. *J Neurocytol*. 2001;30:137–143.
79. Aronica E, Gorter JA, Jansen GH, van Veelen CW, van Rijen PC, Ramkema M, Troost D. Expression and cell distribution of group I and group II metabotropic glutamate receptor subtypes in taylor-type focal cortical dysplasia. *Epilepsia*. 2003;44:785–795.
80. Aronica E, Gorter JA, Ijlst-Keizers H, Rozemuller AJ, Yankaya B, Leenstra S, Troost D. Expression and functional role of mGluR3 and mGluR5 in human astrocytes and glioma cells: opposite regulation of glutamate transporter proteins. *Eur J Neurosci*. 2003;17: 2106–2118.
81. Tessler S, Danbolt NC, Faull RLM, Storm-Mathisen J, Emson PC. Expression of the glutamate transporters in human temporal lobe epilepsy. *Neuroscience*. 1999;88:1083–1091.
82. Proper EA, Hoogland G, Kappen SM, Jansen GH, Rensen MG, Schrama LH, van Veelen CW, van Rijen PC, van Nieuwenhuizen O, Gispen WH, De Graan PN. Distribution of glutamate transporters in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. *Brain*. 2002;125:32–43.
83. Mathern GW, Mendoza D, Lozada A, Pretorius JK, Dehnes Y, Danbolt NC, Nelson N, Leite JP, Chimelli L. Hippocampal GABA and glutamate transporter immunoreactivity in patients with temporal lobe epilepsy. *Neurology*. 1999;52:453–472.
84. Sarac S, Afzal S, Broholm H, Madsen FF, Ploug T, Laursen H. Excitatory amino acid transporters EAAT-1 and EAAT-2 in temporal lobe and hippocampus in intractable temporal lobe epilepsy. *APMIS*. 2009;117:291–301.
85. Bjornsen LP, Eid T, Holmseth S, Danbolt NC, Spencer DD, De Lanerolle NC. Changes in glial glutamate transporters in human epileptogenic hippocampus: inadequate explanation for high extracellular glutamate during seizures. *Neurobiol Dis*. 2007;25: 319–330.
86. Yang Y, Gozen O, Watkins A, Lorenzini I, Lepore A, Gao Y, Vidensky S, Brennan J, Poulsen D, Won PJ, Li JN, Robinson MB, Rothstein JD. Presynaptic regulation of astroglial excitatory neurotransmitter transporter GLT1. *Neuron*. 2009;61:880–894.
87. Lee SG, Su ZZ, Emdad L, Gupta P, Sarkar D, Borjabad A, Volsky DJ, Fisher PB. Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes. *J Biol Chem*. 2008;283:13116–13123.
88. Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes HM, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature*. 2005;433:73–77.
89. Campbell SL, Hablitz JJ. Decreased glutamate transport enhances excitability in a rat model of cortical dysplasia. *Neurobiol Dis*. 2008;32:254–261.
90. Mulholland PJ, Carpenter-Hyland EP, Hearing MC, Becker HC, Woodward JJ, Chandler LJ. Glutamate transporters regulate extrasynaptic NMDA receptor modulation of Kv2.1 potassium channels. *J Neurosci.* 2008;28:8801–8809.
91. Eid T, Thomas MJ, Spencer DD, Runden-Pran E, Lai JC, Malthankar GV, Kim JH, Danbolt NC, Ottersen OP, De Lanerolle NC. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet.* 2004;363: 28–37.
92. van der Hel WS, Notenboom RG, Bos IW, van Rijen PC, van Veelen CW, De Graan PN. Reduced glutamine synthetase in hippocampal areas with neuron loss in temporal lobe epilepsy. *Neurology.* 2005;64: 326–333.
93. Eid T, Williamson A, Lee TS, Petroff OA, De Lanerolle NC. Glutamate and astrocytes— key players in human mesial temporal lobe epilepsy? *Epilepsia*. 2008;49(suppl 2):42–52.
94. Petroff OA, Errante LD, Rothman DL, Kim JH, Spencer DD. Glutamate-glutamine cycling in the epileptic human hippocampus. *Epilepsia*. 2002;43: 703–710.
95. Hammer J, Alvestad S, Osen KK, Skare O, Sonnewald U, Ottersen OP. Expression of glutamine

synthetase and glutamate dehydrogenase in the latent phase and chronic phase in the kainate model of temporal lobe epilepsy. *Glia*. 2008;56: 856–868.
96. Eid T, Ghosh A, Wang Y, Beckstrom H, Zaveri HP, Lee TS, Lai JC, Malthankar-Phatak GH, De Lanerolle NC. Recurrent seizures and brain pathology after inhibition of glutamine synthetase in the hippocampus in rats. *Brain*. 2008;131:2061–2070.
97. Bidmon HJ, Gorg B, Palomero-Gallagher N, Schleicher A, Haussinger D, Speckmann EJ, Zilles K. Glutamine synthetase becomes nitrated and its activity is reduced during repetitive seizure activity in the pentylentetrazole model of epilepsy. *Epilepsia*. 2008;49: 1733–1748.
98. van Gassen KL, van der Hel WS, Hakvoort TB, Lamers WH, De Graan PN. Haploinsufficiency of glutamine synthetase increases susceptibility to experimental febrile seizures. *Genes Brain Behav*. 2009;8:290–295.
99. Liang SL, Carlson GC, Coulter DA. Dynamic regulation of synaptic GABA release by the glutamate-glutamine cycle in hippocampal area CA1. *J Neurosci*. 2006;26:8537–8548.
100. Kam K, Nicoll R. Excitatory synaptic transmission persists independently of the glutamate-glutamine cycle. *J Neurosci*. 2007;27:9192–9200.
101. Tani H, Dulla CG, Huguenard JR, Reimer RJ. Glutamine is required for persistent epileptiform activity in the disinhibited neocortical brain slice. *J Neurosci*. 2010;30:1288–1300.
102. Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ, Haydon PG, Coulter DA. Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nat Neurosci*. 2010;13:584–591.

Chapter 48

Glia–Neuron Interactions in Ictogenesis and Epileptogenesis

Role of Inflammatory Mediators

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Over the past decade, an increasing number of observations have shown the existence of rapid regulatory and reciprocal cross-talk between neurons and glia during synaptic transmission.[1] In particular, neurotransmitters released from active synapses can stimulate receptors on glial cells, resulting in internal calcium elevation and activation of glia, which in turn can produce a neuromodulatory response by triggering gliotransmitter release.[2]

When a local inflammatory reaction is triggered in the brain following an injury, microglia and astrocytes become activated and start releasing a number of pro-inflammatory mediators that can alter profoundly the properties of glia via autocrine actions and perturb glia–neuron paracrine signaling.

Understanding which soluble mediators and what molecular mechanisms are crucially involved in these interactions is instrumental in demonstrating whether an imbalance in physiological glia–neuron communication may increase neuronal network excitability and reduce cell viability.[2,3] In support of this hypothesis, there is increasing evidence that pro-inflammatory cytokines released by

glia play prominent roles in hyperexcitability leading to seizure precipitation and recurrence, as well as in excitotoxic cell damage associated with seizures.[3,4] Moreover, recent evidence in rodent models of childhood infections and central nervous system (CNS) inflammation points to the role of specific cytokines in determining a chronic decreased seizure threshold, and long-term behavioral deficits, which mimic comorbities associated with epilepsy (see below).

Experimental data corroborate clinical evidence of activation of specific pro-inflammatory pathways in human epilepsies.[5–7]

This chapter reports clinical observations in drug-resistant epilepsies and experimental findings in adult and immature rodent models of seizures and epileptogenesis that causally link brain inflammation to the epileptic process. We discuss the role of specific inflammatory mediators of glia–neuron communication in the etiopathogenesis of seizures.

CLINICAL FINDINGS

Accumulating evidence indicates that activation of both innate and adaptive immune systems occurs in human epilepsy. The resulting inflammatory response, which chiefly involves resident brain cells such as glia and neurons, may contribute to the generation of seizures, neuronal damage, and cognitive impairment.[6,7]

Inflammatory and Immunological Biomarkers

Levels of some cytokines such as interleukin-6 (IL-6), IL-1β, and IL-1 receptor antagonist (ra) transiently increase in serum or plasma after various types of seizures.[8,9] There is also evidence of seizure-dependent increases in the cerebrospinal fluid (CSF) levels of these and other cytokines.[8,10–14] A recent study showed increased CSF levels of IL-6 and IL-1ra after tonic-clonic seizures or prolonged partial seizures, while IL-1β levels were decreased compared to those in control CSF.[14] This observation is at variance with previous reports showing an increase in IL-1β production, thus representing one example of the great variability observed when measuring cytokines in the blood or CSF of patients with epilepsy (for review see refs. 4 and 7). A major limitation of studies on CSF biomarkers (including inflammatory/immunological biomarkers) is the small sample size in distinct subpopulations of epilepsy patients. In addition, time of sampling, short half-life of cytokines, storage, and type of analysis may introduce additional variability in quantification. The challenge for future studies on CSF biomarkers in neurological disorders, including epilepsy, is to define the specific molecules that monitor the dynamics of pathological processes and to combine detection of these biomarkers with outcome data to improve diagnostic and prognostic accuracy.[15]

Numerous studies on plasma and CSF cytokine profiles after febrile seizures (FS) have the same limitations noted above. Accumulating data suggest that inflammation has an important role for both the development and long-term consequences of FS.[16–18] The involvement of an inflammatory response in FS development and/or recurrence has not yet led to advances in the treatment of FS. Clinical trials using nonsteroidal anti-inflammatory drugs did not prevent FS recurrence[19] and suggest that specific inflammatory pathways should be targeted as indicated by experimental studies (see the section "Experimental Models").

Genetic Studies

Several studies have examined the genetic association of IL-1β, IL-1α, and IL-1ra gene polymorphisms with temporal lobe epilepsy (TLE) and FS susceptibility. However, these studies did not have statistical power and hence provided contradictory and inconclusive results.[20,21] Thus, there is a need for collaborative collection of a large number of patients with pediatric and adult epilepsy that can provide the statistical power to associate inflammation and genetic susceptibility with seizures and epilepsy.[20]

Anti-inflammatory Drugs in Epilepsy

Although the role of inflammation in human epilepsy is still hypothetical, steroids and adrenocorticotropic hormone (ACTH), with

anti-inflammatory and immunomodulatory properties, as well as high doses of immunoglobulins, have been used to treat seizures in children with Rasmussen encephalitis (RE), West syndrome, and Lennox-Gastaut and Landau-Kleffner syndromes.[22] In addition, some antiepileptic drugs (AEDs), such as valproate and carbamazepine, also display anti-inflammatory actions.[23]

This evidence suggests that activation of immune/inflammatory processes in epilepsy is not a mere epiphenomenon of the underlying disease but might contribute to the pathophysiology of seizures.

Infectious and Autoimmune Diseases

Different common infectious or autoimmune diseases often present with seizures, or seizures develop during the course of the disease.[6,22] Neurotropic viruses, such as the herpes viruses, have been implicated in the development of seizures,[6,24] and recent studies detected human herpes viruses 6B in astrocytes of patients with hippocampal sclerosis and pregressive febrile status epilepticus.[25] An association between childhood immunizations (such as measles, mumps, rubella [MMR] vaccination) and an increased risk of FS has been suggested.[6,26]

Seizures arise in different autoimmune diseases affecting the CNS, such as systemic lupus erythematosus, stiff man syndrome, and Hashimoto's encephalopathy.[6,22,27] The detection of autoantibodies against neuronal proteins such as voltage-gated potassium channels, glutamic acid decarboxylase, and glutamate receptors in subpopulations of patients with epilepsy and seizure-related diseases supports the role of immune system dysfunction in certain forms of epilepsy.[28,29] Recently, attention has focused on the syndrome of noninfectious, nonparaneoplastic limbic encephalitis (LE) as a precipitating event in adult-onset TLE.[27,29]

Seizures have been recognized to occur in common immunologically mediated diseases such as multiple sclerosis (MS).[30] The mechanisms underlying the development of seizures in MS patients are still unclear.[30] A working hypothesis is that seizures may result from cortical demyelinating lesions (with or without inflammation). However, magnetic resonance imaging (MRI) evidence of cortical inflammation has been reported to be associated with epilepsy in MS.[31]

The prototype of inflammatory epileptic encephalopathy is represented by RE, a severe disease characterized by focal seizures and progressive deterioration of motor and cognitive functions.[32] Neuropathological features of RE include cortical inflammation restricted to one brain hemisphere, with microglial and astrocytic activation, T lymphocyte infiltration, and neuronal loss.[32,33] Pardo et al.[32] performed a comprehensive pathological evaluation of RE tissues and demonstrated that the cerebral cortex pathology within the affected hemisphere is multifocal and progressive. Different stages of inflammation coexist in the same patient. These observations are consistent with a progressive immune/inflammatory-mediated process involving both glia and lymphocytes and leading to neuronal damage.[32,34] The autoimmune nature of RE was suspected when autoantibodies against glutamate-receptor subunit 3 (GLUR3) were discovered.[35] However, these autoantibodies have also been detected in other epilepsy patients with severe early-onset disease and intractable seizures and are significantly associated with seizure frequency.[36] Although the etiology and pathogenesis of this severe inflammatory disease are still enigmatic, recent studies suggest as a key pathogenetic mechanism an antigen-driven major histocompatibility complex (MHC) class I restricted cytotoxic CD8+ T-cell-mediated attack of both neurons and astrocytes.[34,37] The therapeutic options for RE presently include surgery (hemispheric disconnection) to control seizures refractory to AEDs and long-term immunotherapy against structural and functional deterioration.[38,39]

Focal Epilepsy (Noninfectious, Nonautoimmune)

The occurrence of a complex and sustained inflammatory reaction, involving chiefly the activation of microglia and astrocytes and the related production of pro-inflammatory molecules, has been shown in the brain tissue of patients undergoing surgery for pharmacologically refractory focal epilepsy (Figure 48–1).

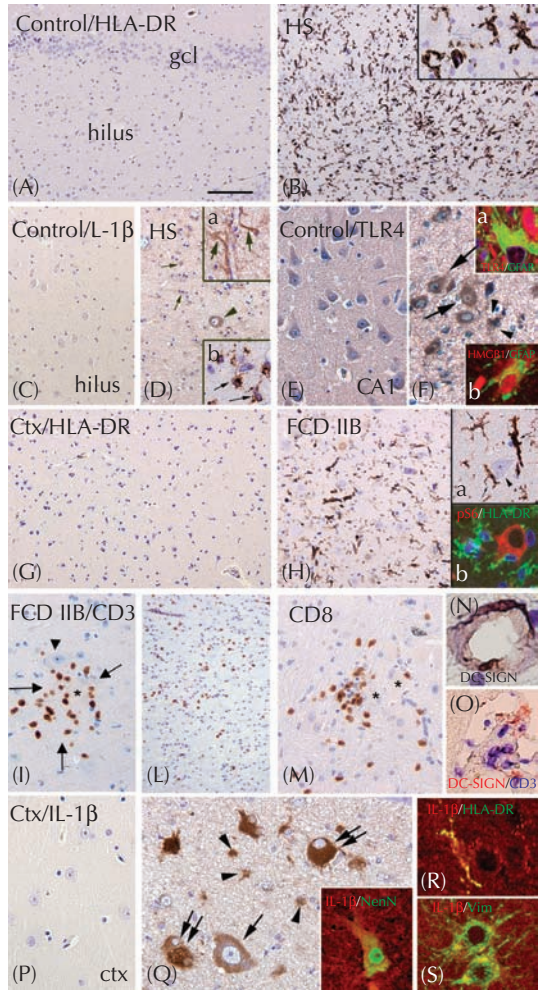

Figure 48–1. Inflammatory processes in chronic epilepsy patients: focal epilepsy.
Panels A–F: Hippocampal sclerosis (HS)[42]. **A,B**. human leukocyte antigen- (HLA-DR) immunoreactivity (IR) in the dentate region of control hippocampus (**A**; gcl: granule cell layer) and HS (**B**), showing strong, diffuse microglial activation in HS. **C,D**. Interleukin-1β IR in the hilar region of control hippocampus (**C**) and HS (**D**), showing HS expression in residual neurons (arrow) and reactive glial cells (arrows and inserts a and b; insert b shows IL-1β immunoreactivity in perivascular astrocytic endfeet). **E,F**. Toll like receptor (TLR4) IR in the CA1 region of control hippocampus (**E**) and HS (**F**), showing in HS expression in residual neurons (arrows) and reactive glial cells (arrowheads)[51]. Insert a in **F**: colocalization of TLR4 (red) with GFAP (green) in a reactive astrocyte. Insert b in **F**: High Mobility Group Box 1 (HMGB1; red) IR, showing both nuclear and cytoplasmic localization in astrocytes (GFAP, green) in HS.
Panels G–O: Focal cortical dysplasia (FCD) type IIB[65,67]. **G,H**. Human leukocyte antigen-DR IR in control cortex (**G**) and HS (**H**), showing strong, diffuse microglial activation within the dysplastic cortex; insert a in **H** shows activated microglial cells (arrows) surrounding a dysmorphic neuron (arrowhead); insert b in **H**: merged image showing HLA-DR-positive microglial cells (green) surrounding a phospho-S6 ribosomal protein (pS6)-positive balloon cell (red). **I,L**. An FCD specimen with CD3-positive cells in cortex (**I**) and white matter (**L**); arrows in **I** show lymphocytes surrounding a balloon cell (asterisk) and a dysmorphic neuron (arrowhead); **M**. CD8-positive T lymphocytes in FCD surrounding balloon cells (asterisks). **N–O**. An FCD specimen showing perivascular DC-SIGN (CD209)-positive cells. **O**. Perivascular distribution of DC-SIGN-positive (red) and CD3-positive (blue) cells.
Panels P–S: Interleukin-1β IR. **P,Q**. Interleukin-1β IR in control cortex (**P**) and in FCD (**Q**)[65,67]. Strong IL-1β IR was observed in large, dysplastic neurons (single arrow), in glial cells (single arrowheads), and in balloon cells (double arrowheads) within the dysplastic cortex (FCD; Q). Insert in Q: colocalization of IL-1β (red) with NeuN (green) in a dysmorphic neuron. **R**. colocalization of IL-1β (red) with HLA-DR (HLA; green) in a microglial cell. **S**. Colocalization of IL-1β (red) with vimentine (VIM; green) in astroglial cells.
Scale bar in **A**: **A-D**, **G-H**, **L**: 160 μm; **E-F, I, M, P-Q**: 80 μm. **N**: 20 μm. **O**: 40 μm; **Q-R**: 25 μm.

HIPPOCAMPAL SCLEROSIS

Hippocampal sclerosis (HS), also known as *Ammon's horn sclerosis*, is the most common neuropathological finding in patients undergoing surgery for intractable TLE. The minimal criteria for the diagnosis of HS are summarized in an International League Against Epilepsy (ILEA) Commission report[40] and include selective neuronal cell loss and gliosis in CA1 and endfolium. Astrogliosis is a major feature of HS. It can be confirmed by immunostaining for glial fibrillary acidic protein (GFAP), showing dense astrogliosis in the hilar region of the dentate gyrus as well as in cornu ammonis (CA) subfields, where prominent neuronal loss is observed (CA3, CA1). This often includes fibrillary gliosis, which supports the chronicity of the process. Microglial activation has also been shown within the hippocampus in HS cases.[41–44] In contrast to the prominent activation of glial cells, few cells of adaptive immunity (CD3/CD8-positive T lymphocytes, mainly associated with microvessels) have been detected in human TLE hippocampi.[42]

Crespel et al.[45] reported the activation of NFκB, a transcriptional factor activated by inflammatory molecules and responsible for transcriptional upregulation of various pro-inflammatory genes, in reactive astrocytes and surviving neurons in human HS specimens. A more recent histological study[42] demonstrated prominent and persistent activation of the IL-1β system, both in activated microglia and astrocytes and in neuronal cells, thus largely confirming the findings reported in chronic epileptic rats.[46]

The activation of inflammatory pathways in human TLE is supported by gene expression profile analysis[44] and includes the complement pathway, which was shown to be overexpressed by both reactive astrocytes and microglia/macrophages.[47] These observations suggest the existence of a reinforcing feedback between the pro-inflammatory cytokine system and the components of the complement cascade, which may be critical for the propagation of the inflammatory response in human TLE with HS.

A recent study demonstrated activation of the plasminogen system, as exemplified by increased expression of tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) in human HS.[48] This may critically influence both neuronal activity and the inflammatory response. Since IL-1β, complement components, and plasminogen activators have been reported to affect the permeability properties of the blood-brain barrier (BBB),[4] we might speculate that the leaky BBB that is observed in HS[49,50] might be partly sustained by the inflammatory actions of different molecules that surround the blood vessels.

Attention has recently focused on the role in seizure precipitation and recurrence of Toll-like receptor (TLR) signaling pathways[51] (see the section "Experimental Models" and ref. 51). Interestingly, prominent and persistent overexpression of TLR4 and its endogenous ligand, high mobility group box 1 (HMGB1), has been demonstrated in glia and neurons in human TLE within the hippocampus, with a cellular distribution that confirm the findings reported in chronic epileptic mice (Figure 48–2).[51]

FOCAL MALFORMATIONS OF CORTICAL DEVELOPMENT

Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem disorder resulting from a mutation in the *TSC1* or *TSC2* gene.[52] In addition to autism and cognitive disabilities, epilepsy is the most common neurological symptom of TSC. Epilepsy is present in 70%–80% of individuals with TSC (reviewed in ref. 52). The characteristic brain lesions of TSC are cortical tubers, subependymal nodules, and subependymal giant cell tumors.[53]

Activation of inflammatory pathways in cortical tubers was suggested initially by the presence of macrophages and alterations in the expression of tumor necrosis factor-α (TNF-α), NFκB, and cell adhesion molecules in these lesions.[54] A subsequent study demonstrated that prominent inflammatory changes (i.e., activation of the complement cascade and IL-1β signaling) observed in these lesions include both activated microglia and astrocytes, as well as the adaptive immune response.[55] In addition, focal changes in BBB permeability were detected in both cortical tubers and SEGA, as demonstrated by perivascular leakage of serum albumin, with uptake into astrocytes.[55] Gene expression analysis of cortical tubers showed that inflammatory genes and cell adhesion

molecules are highly expressed in tubers compared with autopsy controls.[48,56] Various potential mechanisms may underlie the presence of inflammatory cells in the TSC-associated lesions, and further investigation is required to ascertain the involvement of the mTOR pathway, influencing both brain parenchymal and adaptive immune responses.[57,58]

A recent study suggests that astrogliosis in tubers is a dynamic process, with progression of astrocytes from "reactive" to "gliotic."[59] In addition, apoptotic cell death is observed in cortical tubers.[54–56] Thus, prominent activation of the inflammatory cascade could also play a role in the dynamics of TSC lesions and might contribute to progressive neuronal and glial cell injury in TSC patients.

Finally, whether the presence of a prominent population of inflammatory cells may contribute to the behavioral disorders in TSC deserves further investigation. Immunological dysfunctions and microglial activation occurring early during development have been implicated in autism, which is common in TSC patients (for reviews, see ref. 60).

Focal Cortical Dysplasias

Focal cortical dysplasias (FCD) are sporadic architectural and cytoarchitectural malformations of the cerebral cortex that are recognized causes of chronic medically intractable epilepsy in children and young adults.[61–63] According to the current histopathological classification system, FCD have been classified as type I, characterized by cortical dyslamination, and type II, characterized by additional cytoarchitectural abnormalities (i.e., the presence of dysmorphic neurons and balloon cells).[62] Evidence of both necrotic and apoptotic cell death has been reported in pediatric FCD cases, suggesting that intractable epilepsy in childhood due to FCD could be associated with progressive cell injury.[64]

Activation of astrocytes and cells of the microglia/macrophage lineage has been described in FCD specimens from both pediatric and adult patients.[64–66] In a cohort of FCD patients, the density of activated human leukocyte antigen- (HLA-DR)-positive microglial cells (but not CD68-positive macrophages), and the level of expression of IL-1β in parenchymal glia and neurons, correlate positively with the duration of epilepsy, as well as with the frequency of seizures prior to surgical resection.[66,67] The number of HLA-DR-positive cells was significantly higher in FCD type II compared to type I despite the absence of significant differences in seizure frequency and duration.[65] Activation of complement and IL-1β signaling pathways and the chemokine monocyte chemoattractant protein-1 (chemokine (C-C motif) ligand 2, CCl2) in activated microglia, astrocytes, dysplastic neurons, and balloon cells were also more pronounced in FCD type II.[65] These results indicate that activation of inflammatory processes is not simply an effect of seizure activity, suggesting a role for the underlying neuropathology. mTOR activation observed within the cellular components of type II but not type I FCD could also contribute to the inflammatory response.

Some degree of activation of adaptive immunity has also been observed in FCD, and the presence of CD8+ T lymphocytes was greater in FCD type II specimens than in FCD type I.[65] In the same study, dendritic cells (antigen-presenting cells involved in the initiation of the adaptive immunity) were detected around intraparenchymal blood vessels only in FCD type II specimens.[65]

Increased levels of pro-inflammatory cytokines and chemokines have also been shown in pediatric FCD cases.[64] There is also evidence of activation of plasminogen,[65] toll-like receptors (TLR),[68] and vascular endotheliala growth factor (VEGF)-mediated signaling,[55] which, through different mechanisms, could contribute to glial activation and associated inflammatory reactions.

Glioneuronal Tumors

Glioneuronal tumors (GNTs), including ganglioglioma (GG) and dysembryoplastic neuroepithelial tumors (DNTs), are well-differentiated, slowly growing neuroepithelial tumors. They are rare tumors, representing approximately 1.3% of all brain tumors.[69] However, GG appear to constitute the most frequent tumor entity in young patients undergoing surgery for intractable epilepsy.[70]

Glioneuronal tumors are characterized by a mixture of dysplastic neurons and glial cells

and have been included in the group of developmental disorders characterized by abnormal cell proliferation.[71] Abundant populations of activated microglial cells and perivascular lymphocytes are common features of these tumors.[69,72] In both GG and DNT, a significant number of microglia/macrophages are observed within the tumor and in the peritumoral regions. The density of activated microglial cells, as well as the IL-1β levels in parenchymal cells, correlate with the duration of epilepsy, as well as with the frequency of seizures prior to surgical resection.[67,72] In GG, both gene expression and immunocytochemical studies[72,73] provide evidence of prominent activation of the inflammatory response, including the presence of perivascular T-lymphocyte and T-cell receptor signaling pathways within the tumor.[73] Upregulation of IL-1β and IL-1R1, as well as activation of the complement cascade and the Toll-like receptor pathway in glia and neurons, are consistent features of GG.[73]

EXPERIMENTAL MODELS

Experimental models of seizures and epilepsy were instrumental in investigating the causes of brain inflammation in human epilepsy, and in demonstrating that specific inflammatory pathways are crucially involved in seizure precipitation and recurrence.

Models of Seizures in Adult Animals

SEIZURES AND INFLAMMATION

A novel concept emerging from experimental findings is that seizure activity in adult mice or rats triggers the synthesis and release of various pro-inflammatory molecules in the brain. In particular, a pro-inflammatory response occurs during seizures provoked by chemoconvulsants or electrical stimulation in brain areas where seizures are generated and spread.[4] This response includes a rapid-onset rise in pro-inflammatory cytokines in glia and may also involve neurons. This phenomenon is accompanied by upregulation of TLR and their endogenous ligands, such as HMGB1,[51,74] followed by a cascade of downstream inflammatory events that include the activation of NFκB, chemokine production, complement system induction, and increased expression of adhesion molecules.[4,47,75–77] Upregulation of receptors for pro-inflammatory cytokines both in glia and neurons in seizure models suggests that both autocrine and paracrine actions take place, with possible different functional outcomes (for review, see ref. 4).

The astrocytic endfeet impinging on brain microvasculature and the endothelial cells of the BBB also produce inflammatory molecules during seizures, suggesting that brain inflammation is involved in the BBB damage described in experimental and human epileptogenic tissues (for reviews, see refs. 4, 7, and 78). Indeed, pro-inflammatory cytokines have been shown to cause the disassembling of the tight junctions, the production of nitric oxide (NO) and the activation of matrix metalloproteinases in endothelial cells.[3,4,78–80]

The cascade of inflammatory mediators in brain parenchyma, production of chemokines, and upregulation of adhesion molecules on endothelial cells may in principle recruit immunocompetent cells from the bloodstream, resulting in brain extravasation of macrophages, leukocytes, and lymphocytes. However, this peripheral inflammatory component is not prominent in animal models of TLE.[42,74] T and B cells, but not neurotrophils, were transiently detected in mouse brain between 24 and 72 h after generalized seizures induced by maximal electroshock test (MES).[81]

Importantly, seizures can induce cytokine expression independently on cell death, as clearly shown in nonlesional models of seizures.[17,81–83] By contrast, inflammation caused by seizures precedes cell loss in lesional models[42] and may contribute to it. The causal role of inflammation in cell loss is supported by findings in developmental models of seizures[84] and by the evidence that injections of inflammatory mediators can exacerbate apoptotic and excitotoxic cell death.[85,86]

ROLE OF INFLAMMATION IN SEIZURES

The injection of pro-inflammatory molecules such as IL-1β,[82,87] TLR agonists,[51] complement system components,[88] or specific prostaglandins[76,89] in rodent brain results in receptor-mediated pro-convulsant effects. In contrast, the intracerebral injection of specific antagonists of some of these pro-inflammatory

molecules, or interference with related intracellular signaling pathways, mediates powerful anti-convulsant or neuroprotective effects.[51,87,90–95] Transgenic mice with perturbed cytokine signaling show significant changes in seizure susceptibility or cell damage,[83,96–99] thus supporting the pharmacological evidence of a modulatory role of cytokines in neuronal excitability. IL-6 and TNF-α have either pro-convulsant or anti-convulsant effects, depending on the cytokine receptor subtype predominantly activated and/or the specific mechanism underlying epileptic activity initiation and spread (for review, see ref. 4). Similarly, cyclooxygenase-2 (COX-2) inhibition produces different outcomes on seizures, depending on the experimental models.[76] Pro-ictogenic or anti-convulsant effects are likely due to the types of prostaglandins produced in the various models of seizures. Thus, prostaglandin factor 2 (PGF2) has anti-convulsant properties,[92] while PGE2 is a pro-neurotoxic and pro-convulsant prostaglandin.[89,100]

Interleukin-1β has pro-convulsant activity in different models of acute seizures; accordingly, intracerebral application of IL-1ra or inhibition of IL-1β synthesis using caspase-1 inhibitors also provides anti-convulsant effects in models of AED-resistant seizures.[83,90,91,93,95] (for review, see ref. 4). HMGB1, an endogenous ligand of Toll-like and RAGE receptors with pro-inflammatory properties,[101] upon its release from neurons following injury or hyperexcitability, contributes to the precipitation and recurrence of seizures in mice.[51]

ROLE OF INFLAMMATION IN EPILEPTOGENESIS

Inflammatory responses induced by brain-damaging events such as neurotrauma, stroke, infection, FS, and status epilepticus are associated with acute symptomatic seizures and a high risk of epilepsy development.[102,103] In particular, immunohistochemical analysis of IL-1β and its receptor, IL-1R1, and complement factors showed that brain inflammation induced by status epilepticus persists during epileptogenesis and is still detectable in chronic epileptic tissue characterized by spontaneous recurrent seizures. These pro-inflammatory changes occur predominantly in activated microglia and astrocytes, although they also involve neurons and endothelial cells of the BBB.[42,47,51] COX2 shows a dual profile of induction since it is induced in neurons during status epilepticus, while it is significantly upregulated in astrocytes in epileptogenesis and during chronic seizures.[76,104,105] Microarray studies have shown that pro-inflammatory signals linked to the immune/inflammatory response are among the biological systems mostly upregulated during epileptogenesis.[106–108]

The evidence of lasting brain inflammation after various pro-epileptogenic injuries,[7,103,109] together with the established contribution of specific inflammatory mediators to the seizure threshold and epileptic activity, suggests that inflammation in the brain may have a role in the development of epilepsy. Pharmacological studies were therefore designed to interfere with specific pro-inflammatory pathways during epileptogenesis.

In this context, different COX-2 inhibitors were tested in the lithium-pilocarpine or electrical status epilepticus models, starting the treatments after status epilepticus and continuing drug administration for different lengths of time. Two studies, using celecoxib or parecoxib, showed a reduction in the percentage of epileptic rats and a decrease in spontaneous seizure frequency and duration, or reduced seizure severity, respectively. Reduced cell loss and milder microglia activation were also reported.[104,110] Conversely, no effects of COX-2 inhibitors on spontaneous seizure onset and severity or neuropathology were reported when SC8236 was used[111]; however, in this study, a longer duration of status epilepticus was allowed before treatment and drug administration during epileptogenesis was shorter.

Ravizza et al.[112] showed that blockade of IL-1β biosynthesis using systemic administration of a specific interleukin converting enzyme (ICE)/caspase-1 inhibitor prevents the acquisition of stage 5 seizures in the rapid kindling model of epileptogenesis without changing the afterdischarge duration. After drug withdrawal, electrical stimulation did not evoke generalized seizures; moreover, drug administration in fully kindled rats did not affect stage 5 convulsions. These results suggest an antiepileptogenic effect due to inhibition of IL-1β production in astrocytes. These results are supported by lack of IL-1β immunostaining in glia of treated rats compared to control kindled rats. In contrast, anti-convulsant effects of IL-1β were reported

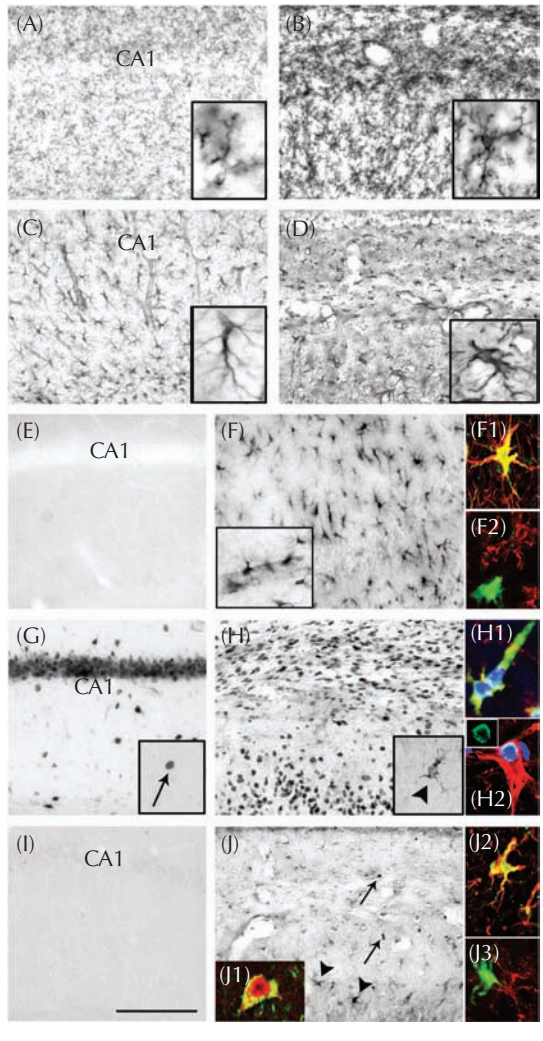

Figure 48–2. Inflammatory processes in the hippocampus of epileptic mice[51,95].
Panels A,D. Immunohistochemical evidence of gliosis: CD11b (**A,B**) and GFAP (**C,D**) staining in control (**A,C**) and epileptic mice (**B,D**). Insets report high magnification of immunopositive glia cells. In control mice, CD11b staining was observed in resting microglia cells (**A**), and GFAP immunoreactivity was observed in stellate-shaped astrocytes with thin processes denoting their resting state (**C**). In epileptic mice, CD11b denotes strongly immunopositive cells with a round shape and thick processes (**B**); GFAP immunostaining was increased in astrocytes exhibiting hypertrophic cell bodies and processes (**D**).
Panels E–F2. Interleukin-1β immunostaining control (**E**) and epileptic mice (**F–F2**). In control mice, IL-1β staining was absent (**E**), while in epileptic mice, it was increased in GFAP-positive parenchymal astrocytes (**F**; colocalization in **F1**: IL-1β green, GFAP red) and in perivascular astrocytes (inset in **F**). CD11b-positive microglia did not express IL-1β (**F2**: IL-1β green, CD11b red).
Panels G–H2. HMGB1 immunoreactivity in control (**H**) and epileptic mice (**H–H2**). In control mice HMGB1 was observed in the nuclei of pyramidal neurons, and in the nuclei of scattered cells in strata radiatum-lacunosum molecolare (**G** and its inset). Epileptic mice have an increased number of HMGB1-positive cells (**H**), and HMGB1 signal is also present in the cytoplasm of cells with glial morphology (inset in **H**). Colocalizations show that HMGB1 is present in the cytoplasm of CD11b-positive microglia-like cells (colocalization in **H1**: HMGB1 green, CD11b red, Hoecsht blue), and around nuclei in GFAP-positive astrocytes (colocalization in **H2**: HMGB1 green, GFAP red, Hoecsht blue; see also HMGB1 alone in the inset).
Panels I–J3. TLR4 signal in control (**I**) and epileptic mice (**J–J3**). TLR4 immunoreactivity is not detected in control mice (**I**), while in epileptic mice it is present in neurons (arrows in **J**; colocalization in **J1**: TLR4 green, NeuN red) and in GFAP-positive astrocytes (arrowheads in **J**; colocalization in **J2**: TLR4 green, GFAP red). CD11b-positive microglia do not express TLR4 (colocalization in **J3**: TLR4 green, CD11b red). Scale bar: **A–J**, 100 μm; insets in **A–J, F1, F2, H1, H2, J1–J3**, 20 μm.

in one study showing reduction of afterdischarge and stage 5 seizure duration in fully amygdala-kindled rats after intracerebroventricular (icv) cytokine injection.[113] Reduction of the kindling rate was also observed in the same study after repetitive daily icv injections of low doses of IL-1β. This cytokine, given by the icv route, might have caused increased levels of glucocorticoids in response to direct hypothalamic-pituitary-adrenal (HPA) axis activation, which in turn could be responsible for the protective effects in kindling.

The role of the leukocyte-endothelial cell adhesion mechanism in epileptogenesis was studied in lithium-pilocarpine-treated mice.[77] The underlying hypothesis is that leukocyte adhesion to brain microvessels would impair BBB permeability functions contributing to chronic hyperexcitability. Thus, leakage of serum albumin into brain parenchyma, and its subsequent astrocytic uptake, has been shown to decrease the K^+ buffering and glutamate reuptake capacity of glia leading to ionic imbalance and increased extracellular glutamate, which would favor the development of seizures.[114,115] The administration of specific antibodies against adhesion molecules after status epilepticus for 20 days resulted in a reduced frequency of spontaneous seizures, as assessed during antibody treatment, but no changes in their onset time or duration. Decreased neuropathology and preservation of exploratory behavior were observed in treated epileptic mice. Although these data suggest the potential involvement of leukocytes in epileptogenesis, one important caveat is that pilocarpine itself stimulates leukocytes via a primary peripheral inflammatory action. This effect provokes BBB leakage and ionic imbalance, and allows enough systemic pilocarpine to enter the brain to trigger seizures and epileptogenesis.[116] Therefore, it remains to be proven in additional experimental models whether this mechanism is generally operative in epilepsy.

T and B cells do not appear to play a significant role in epileptogenesis. This is suggested by two studies. One study showed that tacrolimus, an immunosuppressant drug blocking T-cell activation, did not modify the onset of spontaneous seizures, or their frequency and duration, when administered after electrically induced status epilepticus for 2 weeks.[117] The other study showed that mice lacking T and B cells develop status epilepticus and spontaneous seizures similarly to their wild-type controls.[118]

Models of Seizures in Immature Animals

Seizures occur more frequently early in life. Febrile seizures are the most frequent etiology of seizure in childhood. In humans, initial precipitating injuries, including FS during childhood, are risk factors for the development of epilepsy.[119] Animal models have been used to elucidate the underlying mechanisms of seizure occurrence and epileptogenesis in immature brain, and inflammation has emerged as a possible major contributor.

If inflammation facilitates seizure occurrence in mature brain in almost all models, it affects seizures differently in immature brain, depending on the trigger of seizures.

Interleukin-1β lowers the core temperature threshold that results in seizures in a postnatal (PN) day 14 mouse model of febrile convulsions, acting on IL-1R1.[16] Similarly, using a subconvulsive dose of kainate in PN14 rats, Heida and Pittman[120] showed that lipopolysaccharide (LPS) exerts pro-convulsant effects when the animals are febrile by favoring seizure precipitation in 50% of rats. At the onset of seizures, IL-1β was significantly increased in the hippocampus only in rats experiencing seizures after administration of LPS and kainate. When IL-1β was given icv in LPS-treated febrile rats, the percentage of animal seizing after a subconvulsant dose of kainate was significantly increased and the onset time to seizures was reduced. The opposite was found after icv injection of IL-1ra. These studies show that FS may be caused by excessive amounts of IL-1β in the hippocampus.

In contrast, LPS at low doses that do not increase the core temperature did not change acute susceptibility to short hyperthermic seizures in both PN11 and PN16 rats.[121] However, these animals developed a decreased threshold to pentylentetrazol (PTZ) as adults. A similar nonfebrile dose of LPS did not exacerbate lithium-pilocarpine-induced status epilepticus at PN7 and PN14. On the contrary, it induced

a delay in the onset of status epilepticus. However, these rats developed increased seizure-induced hippocampal damage.[122] Nonfebrile doses of LPS have been reported to decrease the seizure threshold in adult mice exposed to PTZ[123] and to accelerate the onset of seizures in lithium-pilocarpine-treated adult rats (Auvin et al., unpublished data).

It appears from these findings that relatively low doses of LPS in immature rats, which do not cause an increase in core temperature, do not alter the acute susceptibility to seizures, although they increase the seizure-induced cell loss and the long-term predisposition to seizures. Moreover, the effects of LPS in immature and adult animals clearly differ since nonfebrile doses exacerbate seizures in adults. The mechanisms underlying these effects, and the role played by fever and brain inflammation in determining the short- and long-term changes in seizure threshold, remain to be to be elucidated. Interactions between LPS-induced cytokine production and activation of the HPA axis, with consequent production of anti-inflammatory glucocorticoids, may be important determinants of the outcomes since both phenomena are developmentally regulated and may be differently affected by the experimental setting adopted to induce seizures.

Inflammation and Seizure-Induced Cell Injury in Immature Brain

The relationship between seizures and neuronal injury in rodents is specific to the stage of development and the model employed to precipitate seizures. The younger the animal, the lower the level of seizure-induced cell injury.[124,125] The key determinants of the age-dependent occurrence of seizure-induced injury are still incompletely understood. Recent data point to the possible involvement of inflammatory mediators, as exemplified below.

When PN9 to PN21 rats are exposed to status epilepticus induced by kainate, both cytokine expression and glia activation occur from the second postnatal week on; this temporal pattern of seizure-induced inflammation closely overlaps with that of seizure-induced cell injury. Moreover, brain inflammation precedes evidence of cell loss, suggesting that pro-inflammatory cytokines may contribute to its occurrence.[84] A causal link between inflammation and cell death in immature brain is further supported by the evidence that LPS given prior to lithium-pilocarpine-induced status epilepticus increases cell injury in hippocampus at PN7 and PN14.[122] This effect occurs in the absence of changes in body temperature or in the duration of status epilepticus.[122]

These studies suggest that inflammation during postnatal development may enhance cell injury following prolonged seizures, highlighting a possible contribution to the development of epileptogenesis.

Long-Term Consequences of Inflammation for Immature Brain

Systemic or CNS inflammation *by itself* during a critical postnatal period is able to induce long-term changes in neuronal excitability and alterations in physiological behaviors.

When LPS was given to rats on PN7 or PN14, but not before (PN1) or afterward (PN20), seizure susceptibility was increased when these rats become adults, as assessed using PTZ, lithium-pilocarpine, and kainate. Brain inflammation induced in PN14 mice by icv injection of polyinosinic:polycytidylic acid (Poly I:C), a TLR3 agonist that mimics viral infections, was also responsible for the increased seizure susceptibility in adulthood, as shown using PTZ and lithium-pilocarpine.

A deficit in contextual fear conditioning memory was reported in both experimental settings, while retention of spatial memory was affected only by the LPS treatment.

The LPS study points out the involvement of TNF-α and activated astrocytes[126] in the long-term consequences of brain inflammation, while the Poly I:C study highlights the possible involvement of IL-1β and activated microglia.[127] This suggests that different inflammatory mechanisms may be activated, depending on the first trigger. Interestingly, in both models of inflammation, long-term changes in hippocampal levels of several glutamate receptor subunits were reported,[127,128] revealing a pathophysiological relationship between brain cytokines, glutamate receptors, behavior, and seizures.

Inflammation in immature brain also seems to act as a disease modifier when it is *coupled*

to a second hit. Systemic injection of low and nonfebrile dose of LPS in PN14 rats before lithium-pilocarpine did not change the severity of acute status epilepticus but resulted in more severe (stages 3–4) spontaneous seizures in adulthood.[121] Administration of LPS did not change the number of rats that became epileptic or the frequency and duration of spontaneous seizures. No significant changes in cell number in the CA1 sector was observed in LPS-pretreated rats, although in some animals Fluoro-Jade-positive cells were detected, suggesting ongoing neurodegeneration, which was absent in rats not preexposed to LPS. A more intense reactive gliosis was also found in CA1 in rats pretreated with LPS.[129]

It was found that LPS enhanced rapid kindling progression in P14 rats and increased hippocampal excitability after kindling completion. These effects were prevented by IL-1ra, indicating the involvement of IL-1β in the mechanisms of hyperexcitability.[129,130] The possibility that brain inflammation contributes to epileptogenesis, is supported by the work of Marcon et al.[131] By inducing status epilepticus in PN9 and PN21 rats, these authors showed long-lasting brain inflammation and vascular changes, including BBB damage and angiogenesis, in PN21 rats but not in PN9 rats. Notably, P21 rats, but not P9 rats, showed a propensity to develop epilepsy after status epilepticus.

Mechanisms of Hyperexcitability

Emerging evidence has shown that nonconventional intracellular signaling pathways are activated by pro-inflammatory mediators in the epileptogenic tissue in addition to the classical induction of NFκB-mediated gene transcription described during peripheral inflammation. These novel mechanisms are likely to contribute to the neuronal hyperexcitability underlying seizures and to mediate at least part of the inflammation-related glia–neuron interactions. For example, cytokines can modify the function of glutamate and GABA receptors by altering receptor trafficking and their subunit assembly at neuronal membranes. Cytokines can also modulate glutamate receptor-mediated calcium influx in neurons by promoting alpha-amino-3-hydroxy-5-methyl-4-isoxalzolepropionate (AMPA)-GLUR2 and *N*-methyl-D-aspartate (NMDA-NR2B) receptor subunit phosphorylation via PI3K or Src kinases, respectively.[132] Recently, activation of IL-1R/Toll-like receptor signaling in neurons, either by IL-1β or by HMGB1, has been shown to play a pivotal role in seizure precipitation and recurrence via rapid Src kinase-catalyzed phosphorylation of NMDA-NR2B receptors.[51,87]

Cytokines and prostaglandins can also directly alter voltage-gated ion channel function.[132] In particular, somatic and dendritic membrane excitability was significantly reduced in CA1 pyramidal neurons using a selective COX-2 inhibitor, and PGE2 produced increased firing and excitatory postsynaptic potentials, most likely by reducing potassium currents in CA1 neurons.[133,134]

Activation of the complement system and membrane attack complex (MAC) assembly in erythrocyte membranes leads to the formation of channel conductances, resulting in Ca^{2+} and Na^{+} influx and K^{+} efflux, with the net effect of depolarizing the membrane potential.[135] If this mechanism is also operative in neurons, it may explain why MAC assembly in the hippocampus provokes seizures.

In addition, cytokines and prostaglandins inhibit glutamate reuptake by astrocytes[136,137] and enhance its astrocytic release,[138] thus resulting in increased extracellular glutamate concentration. In this regard, astrocytic glutamate release appears to contribute significantly to seizure-like events.[139,140]

Finally, inflammatory mediators can also increase vascular permeability and angiogenesis (for review, see refs. 3 and 141); their overexpression in perivascular astrocytes and endothelial cells in epilepsy may affect BBB permeability and promote excitability in surrounding neurons.[4,78]

CONCLUSIONS

Clinical and experimental evidence substantiates the role of brain inflammation in the etiopathogenesis of seizures. Clinical studies show that brain inflammation is a common substrate in epilepsies of different etiologies.

Experimental studies show that recurrent seizures can trigger and perpetuate brain inflammation even in the absence of cell loss or other concomitant or preexisting neuropathology.

Long-term inflammation, in turn, promotes chronic hyperexcitability, is detrimental to neuronal survival, induces behavioral dysfunctions, and may contribute to the maladaptive plasticity underlying epileptogenesis.

Pharmacological studies in models of seizures and epilepsy, including models of drug-resistant epilepsy, demonstrate that interfering with specific pro-inflammatory pathways can effectively reduce seizures.

These findings therefore envision novel therapies for epilepsy by targeting specific pro-inflammatory pathways. This approach has two advantages: the possibility of using drugs already available in clinical practice for septic shock or autoimmune diseases and the likelihood of interfering with a mechanism involved in the pathophysiology of seizures. This approach may therefore provides curative rather than merely symptomatic treatment.[142]

DISCLOSURE STATEMENT

The authors declare no conflicts of interest related to the material reported and discussed in this chapter.

REFERENCES

1. Bezzi P, Domercq M, Vesce S, Volterra A. Neuron-astrocyte cross-talk during synaptic transmission: physiological and neuropathological implications. *Prog Brain Res.* 2001;132:255–265.
2. Araque A. Astrocytes process synaptic information. *Neuron Glia Biol.* 2008;4(1):3–10.
3. Allan SM, Tyrrell PJ, Rothwell NJ. Interleukin-1 and neuronal injury. *Nat Rev Immunol.* 2005;5(8): 629–640.
4. Vezzani A, Balosso S, Ravizza T. The role of cytokines in the pathophysiology of epilepsy. *Brain Behav Immun.* 2008;22(6):797–803.
5. Vezzani A, Baram TZ. New roles for interleukin-1beta in the mechanism of epilepsy. *Epilepsy Curr.* 2007;7(2):45–50.
6. Choi J, Koh S. Role of brain inflammation in epileptogenesis. *Yonsei Med J.* 2008;49(1):1–18.
7. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol.* 2011;7(1): 31–40.
8. Peltola J, Palmio J, Korhonen L, et al. Interleukin-6 and interleukin-1 receptor antagonist in cerebrospinal fluid from patients with recent tonic-clonic seizures. *Epilepsy Res.* 2000;41(3):205–211.
9. Lehtimaki K, Keranen T, Huuhka M, et al. Increase in plasma proinflammatory cytokines after electroconvulsive therapy in patients with depressive disorder. *J ECT.* 2008;24(1):88–91.
10. Virta M, Hurme M, Helminen M. Increased plasma levels of pro- and anti-inflammatory cytokines in patients with febrile seizures. *Epilepsia.* 2002;43(8): 920–923.
11. Haspolat S, Mihçi E, Coşkun M, Gümüslü S, Ozben T, Yegin O. Interleukin-1beta, tumor necrosis factor-alpha, and nitrite levels in febrile seizures. *J Child Neurol.* 2005;17(10):749–751.
12. Ichiyama T, Suenaga N, Kajimoto M, et al. Serum and CSF levels of cytokines in acute encephalopathy following prolonged febrile seizures. *Brain Dev.* 2008;30(1):47–52.
13. Lehtimaki KA, Keranen T, Huhtala H, et al. Regulation of IL-6 system in cerebrospinal fluid and serum compartments by seizures: the effect of seizure type and duration. *J Neuroimmunol.* 2004;152(1–2):121–125.
14. Lehtimaki KA, Keranen T, Palmio J, Peltola J. Levels of IL-1beta and IL-1ra in cerebrospinal fluid of human patients after single and prolonged seizures. *Neuroimmunomodulation.* 2010;17(1):19–22.
15. Petzold A. CSF biomarkers for improved prognostic accuracy in acute CNS disease. *Neurol Res.* 2007;29(7): 691–708.
16. Dubé C, Vezzani A, Behrens M, Bartfai T, Baram TZ. Interleukin-1beta contributes to the generation of experimental febrile seizures. *Ann Neurol.* 2005;57(1): 152–155.
17. Dubé CM, Ravizza T, Hamamura M, et al. Epileptogenesis provoked by prolonged experimental febrile seizures: mechanisms and biomarkers. *J Neurosci.* 2010;30(22):7484–7494.
18. Dubé CM, Brewster AL, Richichi C, Zha Q, Baram TZ. Fever, febrile seizures and epilepsy. *Trends Neurosci.* 2007;30(10):490–496.
19. Strengell T, Uhari M, Tarkka R, et al. Antipyretic agents for preventing recurrences of febrile seizures: randomized controlled trial. *Arch Pediatr Adolesc Med.* 2009;163(9):799–804.
20. Tan NC, Mulley JC, Berkovic SF. Genetic association studies in epilepsy: "the truth is out there." *Epilepsia.* 2004;45(11):1429–1442.
21. Ozkara C, Uzan M, Tanriverdi T, et al. Lack of association between IL-1beta/alpha gene polymorphisms and temporal lobe epilepsy with hippocampal sclerosis. *Seizure.* 2006;15(5):288–291.
22. Palace J, Lang B. Epilepsy: an autoimmune disease? *J Neurol Neurosurg Psychiatry.* 2000;69(6):711–714.
23. Mlodzikowska-Albrecht J, Steinborn B, Zarowski M. Cytokines, epilepsy and epileptic drugs—is there a mutual influence? *Pharmacol Rep.* 2007;59(2): 129–138.
24. Misra UK, Tan CT, Kalita J. Viral encephalitis and epilepsy. *Epilepsia.* 2008;49(suppl 6):13–18.
25. Theodore WH, Epstein L, Gaillard WD, Shinnar S, Wainwright MS, Jacobson S. Human herpes virus 6B: a possible role in epilepsy? *Epilepsia.* 2008;49(11): 1828–1837.
26. Vestergaard M, Hviid A, Madsen KM, et al. MMR vaccination and febrile seizures: evaluation of susceptible subgroups and long-term prognosis. *JAMA.* 2004;292(3):351–357.
27. Bien CG, Urbach H, Schramm J, et al. Limbic encephalitis as a precipitating event in adult-onset temporal lobe epilepsy. *Neurology.* 2007;69(12):1236–1244.

28. McKnight K, Jiang Y, Hart Y, et al. Serum antibodies in epilepsy and seizure-associated disorders. *Neurology.* 2005;65(11):1730–1736.
29. Niehusmann P, Dalmau J, Rudlowski C, et al. Diagnostic value of *N*-methyl-D-aspartate receptor antibodies in women with new-onset epilepsy. *Arch Neurol.* 2009;66(4):458–464.
30. Kelley BJ, Rodriguez M. Seizures in patients with multiple sclerosis: epidemiology, pathophysiology and management. *CNS Drugs.* 2009;23(10):805–815.
31. Calabrese M, De Stefano N, Atzori M, et al. Extensive cortical inflammation is associated with epilepsy in multiple sclerosis. *J Neurol.* 2008;255(4):581–586.
32. Pardo CA, Vining EP, Guo L, Skolasky RL, Carson BS, Freeman JM. The pathology of Rasmussen syndrome: stages of cortical involvement and neuropathological studies in 45 hemispherectomies. *Epilepsia.* 2004; 45(5):516–526.
33. Wirenfeldt M, Clare R, Tung S, Bottini A, Mathern GW, Vinters HV. Increased activation of Iba1+ microglia in pediatric epilepsy patients with Rasmussen's encephalitis compared with cortical dysplasia and tuberous sclerosis complex. *Neurobiol Dis.* 2009;34(3): 432–440.
34. Bien CG, Bauer J, Deckwerth TL, et al. Destruction of neurons by cytotoxic T cells: a new pathogenic mechanism in Rasmussen's encephalitis. *Ann Neurol.* 2002; 51(3):311–318.
35. Rogers SW, Andrews PI, Gahring LC, et al. Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis. *Science.* 1994;265(5172):648–651.
36. Mantegazza R, Bernasconi P, Baggi F, et al. Antibodies against GluR3 peptides are not specific for Rasmussen's encephalitis but are also present in epilepsy patients with severe, early onset disease and intractable seizures. *J Neuroimmunol.* 2002;131(1–2):179–185.
37. Schwab N, Bien CG, Waschbisch A, et al. CD8+ T-cell clones dominate brain infiltrates in Rasmussen encephalitis and persist in the periphery. *Brain.* 2009; 132(pt 5):1236–1246.
38. Bien CG, Schramm J. Treatment of Rasmussen encephalitis half a century after its initial description: promising prospects and a dilemma. *Epilepsy Res.* 2009;86(2–3):101–112.
39. Thilo B, Stingele R, Knudsen K, et al. A case of Rasmussen encephalitis treated with rituximab. *Nat Rev Neurosci.* 2009;5(8):458–462.
40. Wieser HG. ILAE Commission Report. Mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia.* 2004;45(6):695–714.
41. Sheng JG, Boop FA, Mrak RE, Griffin WS. Increased neuronal beta-amyloid precursor protein expression in human temporal lobe epilepsy: association with interleukin-1 alpha immunoreactivity. *J Neurochem.* 1994;63(5):1872–1879.
42. Ravizza T, Gagliardi B, Noé F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. *Neurobiol Dis.* 2008;29(1):142–160.
43. Beach TG, Woodhurst WB, MacDonald DB, Jones MW. Reactive microglia in hippocampal sclerosis associated with human temporal lobe epilepsy. *Neurosci Lett.* 1995;191(1–2):27–30.
44. Aronica E, Gorter JA. Gene expression profile in temporal lobe epilepsy. *Neuroscientist.* 2007;13(2):100–108.
45. Crespel A, Coubes P, Rousset MC, et al. Inflammatory reactions in human medial temporal lobe epilepsy with hippocampal sclerosis. *Brain Res.* 2002;952(2): 159–169.
46. Ravizza T, Vezzani A. Status epilepticus induces time-dependent neuronal and astrocytic expression of interleukin-1 receptor type I in the rat limbic system. *Neuroscience.* 2006;137(1):301–308.
47. Aronica E, Boer K, van Vliet EA, et al. Complement activation in experimental and human temporal lobe epilepsy. *Neurobiol Dis.* 2007;26(3):497–511.
48. Iyer AM, Zurolo E, Boer K, et al. Tissue plasminogen activator and urokinase plasminogen activator in human epileptogenic pathologies. *Neuroscience.* 2010;19(3):929–945.
49. Rigau V, Morin M, Rousset MC, et al. Angiogenesis is associated with blood-brain barrier permeability in temporal lobe epilepsy. *Brain.* 2007;130(pt 7): 1942–1956.
50. van Vliet EA, da Costa Araujo S, Redeker S, van Schaik R, Aronica E, Gorter JA. Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. *Brain.* 2007;130(2):521–534.
51. Maroso M, Balosso S, Ravizza T, et al. Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. *Nat Med.* 2010;16(4):413–419.
52. Orlova KA, Crino PB. The tuberous sclerosis complex. *Ann NY Acad Sci.* 2010;1184:87–105.
53. Mizuguchi M, Takashima S. Neuropathology of tuberous sclerosis. *Brain Dev.* 2001;23(7):508–515.
54. Maldonado M, Baybis M, Newman D, et al. Expression of ICAM-1, TNF-alpha, NF kappa B, and MAP kinase in tubers of the tuberous sclerosis complex. *Neurobiol Dis.* 2003;14(2):279–290.
55. Boer K, Jansen F, Nellist M, et al. Inflammatory processes in cortical tubers and subependymal giant cell tumors of tuberous sclerosis complex. *Epilepsy Res.* 2008;78(1):7–21.
56. Boer K, Crino PB, Gorter JA, et al. Gene expression analysis of tuberous sclerosis complex cortical tubers reveals increased expression of adhesion and inflammatory factors *Brain Pathol.* 2010;20:704–719.
57. Weichhart T, Saemann MD. The multiple facets of mTOR in immunity. *Trends Immunol.* 2009;30(5): 218–226.
58. Wong M. Mechanisms of epileptogenesis in tuberous sclerosis complex and related malformations of cortical development with abnormal glioneuronal proliferation. *Epilepsia.* 2008;49(1):8–21.
59. Sosunov AA, Wu X, Weiner HL, et al. Tuberous sclerosis: a primary pathology of astrocytes? *Epilepsia.* 2008;49(suppl 2):53–62.
60. Chew LJ, Takanohashi A, Bell M. Microglia and inflammation: impact on developmental brain injuries. *Ment Retard Dev Disabil Res Rev.* 2006;12(2): 105–112.
61. Thom M. Recent advances in the neuropathology of focal lesions in epilepsy. *Expert Rev Neurother.* 2004;4(6):973–984.
62. Blumcke I. Neuropathology of focal epilepsies: a critical review. *Epilepsy Behav.* 2009;15(1):34–39.
63. Najm IM, Tilelli CQ, Oghlakian R. Pathophysiological mechanisms of focal cortical dysplasia: a critical review of human tissue studies and animal models. *Epilepsia.* 2007;48(suppl 2):21–32.

64. Choi J, Nordli DR Jr, Alden TD, et al. Cellular injury and neuroinflammation in children with chronic intractable epilepsy. *J Neuroinflamm.* 2009;6:38–52.
65. Iyer A, Zurolo E, Spliet WG, et al. Evaluation of the innate and adaptive immunity in type I and type II focal cortical dysplasias. *Epilepsia.* 2010;51(9):1763–1773.
66. Boer K, Spliet WG, van Rijen PC, Redeker S, Troost D, Aronica E. Evidence of activated microglia in focal cortical dysplasia. *J Neuroimmunol.* 2006;173(1–2): 188–195.
67. Ravizza T, Boer K, Redeker S, et al. The IL-1beta system in epilepsy-associated malformations of cortical development. *Neurobiol Dis.* 2006;24(1):128–143.
68. Zurolo E, Iyer A, Maroso M, et al. Activation of TLR, RAGE and HMGB1 signaling in malformations of cortical development. *Brain*. 2011;134(Pt 4):1015–1032.
69. Louis DN, Ohgaki H, Wiestler OD, Cavanee WK. *WHO Classification of Tumours of the Central Nervous System. Acta Neuropathol*. 2007;114(2):97–109.
70. Blumcke I, Wiestler OD. Gangliogliomas: an intriguing tumor entity associated with focal epilepsies. *J Neuropathol Exp Neurol.* 2002;61(7):575–584.
71. Barkovich AJ, Kuzniecky RI, Jackson GD, Guerrini R, Dobyns WB. A developmental and genetic classification for malformations of cortical development. *Neurology.* 2005;65(12):1873–1887.
72. Aronica E, Gorter JA, Redeker S, et al. Distribution, characterization and clinical significance of microglia in glioneuronal tumours from patients with chronic intractable epilepsy. *Neuropathol Appl Neurobiol.* 2005;31(3):280–291.
73. Aronica E, Boer K, Becker A, et al. Gene expression profile analysis of epilepsy-associated gangliogliomas. *Neuroscience.* 2008;151(1):272–292.
74. Turrin NP, Rivest S. Innate immune reaction in response to seizures: implications for the neuropathology associated with epilepsy. *Neurobiol Dis.* 2004;16(2):321–334.
75. Librizzi L, Regondi MC, Pastori C, Frigerio S, Frassoni C, de Curtis M. Expression of adhesion factors induced by epileptiform activity in the endothelium of the isolated guinea pig brain in vitro. *Epilepsia.* 2007;48(4):743–751.
76. Kulkarni SK, Dhir A. Cyclooxygenase in epilepsy: from perception to application. *Drugs Today* (*Barc*). 2009;45(2):135–154.
77. Fabene PF, Mora GN, Martinello M, et al. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. *Nat Med.* 2008;14(12):1377–1383.
78. Oby E, Janigro D. The blood-brain barrier and epilepsy. *Epilepsia.* 2006;47(11):1761–1774.
79. Ferrari CC, Depino AM, Prada F, et al. Reversible demyelination, blood-brain barrier breakdown, and pronounced neutrophil recruitment induced by chronic IL-1 expression in the brain. *Am J Pathol.* 2004;165(5):1827–1837.
80. Candelario-Jalil E, Taheri S, Yang Y, et al. Cyclooxygenase inhibition limits blood-brain barrier disruption following intracerebral injection of tumor necrosis factor-alpha in the rat. *J Pharmacol Exp Ther.* 2007;323(2):488–498.
81. Silverberg J, Ginsburg D, Orman R, Amassian V, Durkin HG, Stewart M. Lymphocyte infiltration of neocortex and hippocampus after a single brief seizure in mice. *Brain Behav Immun.* 2010;24(2):263–272.
82. Vezzani A, Conti M, De Luigi A, et al. Interleukin-1beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: functional evidence for enhancement of electrographic seizures. *J Neurosci.* 1999;19(12):5054–5065.
83. Vezzani A, Moneta D, Conti M, et al. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. *Proc Natl Acad Sci USA.* 2000;97(21):11534–11539.
84. Rizzi M, Perego C, Aliprandi M, et al. Glia activation and cytokine increase in rat hippocampus by kainic acid-induced status epilepticus during postnatal development. *Neurobiol Dis.* 2003;14(3):494–503.
85. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. *Nat Rev Neurosci.* 2001;2(10):734–744.
86. Bernardino L, Xapelli S, Silva AP, et al. Modulator effects of interleukin 1beta and tumor necrosis factor-alpha on AMPA-induced excitotoxicity in mouse organotypic hippocampal slice cultures. *J Neurosci.* 2005;25(29):6734–6744.
87. Balosso S, Maroso M, Sanchez-Alavez M, et al. A novel non-transcriptional pathway mediates the proconvulsive effects of interleukin-1beta. *Brain.* 2008;131(pt 12): 3256–3265.
88. Xiong ZQ, Qian W, Suzuki K, McNamara JO. Formation of complement membrane attack complex in mammalian cerebral cortex evokes seizures and neurodegeneration. *J Neurosci.* 2003;23(3):955–960.
89. Oliveira MS, Furian AF, Royes LF, et al. Cyclooxygenase-2/PGE2 pathway facilitates pentylenetetrazol-induced seizures. *Epilepsy Res.* 2008;79(1): 14–21.
90. De Simoni MG, Perego C, Ravizza T, et al. Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. *Eur J Neurosci.* 2000;12(7):2623–2633.
91. Vezzani A, Moneta D, Richichi C, et al. Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis. *Epilepsia.* 2002;43(suppl 5):30–35.
92. Kim HJ, Chung JI, Lee SH, Jung YS, Moon CH, Baik EJ. Involvement of endogenous prostaglandin F2alpha on kainic acid-induced seizure activity through FP receptor: the mechanism of proconvulsant effects of COX-2 inhibitors. *Brain Res.* 2008;1193:153–161.
93. Marchi N, Fan Q, Ghosh C, et al. Antagonism of peripheral inflammation reduces the severity of status epilepticus. *Neurobiol Dis.* 2009;33(2):171–181.
94. Spigolon G, Veronesi C, Bonny C, Vercelli A. c-Jun N-terminal kinase signaling pathway in excitotoxic cell death following kainic acid-induced status epilepticus. *Eur J Neurosci.* 2010;31(7):1261–1272.
95. Maroso M, Balosso S, Ravizza T, et al. ICE/Caspase 1 inhibition reduces acute seizures and drug resistant chronic epileptic activity in mice *Neurotherapeutics.* 2011;8(2):304–15..
96. Samland H, Huitron-Resendiz S, Masliah E, Criado J, Henriksen SJ, Campbell IL. Profound increase in sensitivity to glutamatergic- but not cholinergic agonist-induced seizures in transgenic mice with astrocyte production of IL-6. *J Neurosci Res.* 2003;73(2): 176–187.
97. De Sarro G, Ibbadu GF, Marra R, et al. Seizure susceptibility to various convulsant stimuli in dystrophin-deficient mdx mice. *Neurosci Res.* 2004;50(1):37–44.

98. De Luca G, Di Giorgio RM, Macaione S, et al. Susceptibility to audiogenic seizure and neurotransmitter amino acid levels in different brain areas of IL-6-deficient mice. *Pharmacol Biochem Behav.* 2004;78(1):75–81.
99. Lu MO, Zhang XM, Mix E, et al. TNF-alpha receptor 1 deficiency enhances kainic acid-induced hippocampal injury in mice. *J Neurosci Res.* 2008;15;86(7): 1608–1614.;
100. Takemiya T, Maehara M, Matsumura K, Yasuda S, Sugiura H, Yamagata K. Prostaglandin E2 produced by late induced COX-2 stimulates hippocampal neuron loss after seizure in the CA3 region. *Neurosci Res.* 2006;56(1):103–110.
101. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol.* 2007; 81(1):1–5.
102. Pitkanen A, Sutula TP. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. *Lancet Neurol.* 2002;1(3): 173–181.
103. Bartfai T, Sanchez-Alavez M, Andell-Jonsson S, et al. Interleukin-1 system in CNS stress: seizures, fever, and neurotrauma. *Ann NY Acad Sci.* 2007;1113:173–177.
104. Jung KH, Chu K, Lee ST, et al. Cyclooxygenase-2 inhibitor, celecoxib, inhibits the altered hippocampal neurogenesis with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. *Neurobiol Dis.* 2006;23(2):237–246.
105. Lee B, Dziema H, Lee KH, Choi YS, Obrietan K. CRE-mediated transcription and COX-2 expression in the pilocarpine model of status epilepticus. *Neurobiol Dis.* 2007;25(1):80–91.
106. Lukasiuk K, Pitkanen A. Large-scale analysis of gene expression in epilepsy research: is synthesis already possible? *Neurochem Res.* 2004;29(6):1169–1178.
107. Gorter JA, van Vliet EA, Aronica E, et al. Potential new antiepileptogenic targets indicated by microarray analysis in a rat model for temporal lobe epilepsy. *J Neurosci.* 2006;26(43):11083–11110.
108. Majores M, Eils J, Wiestler OD, Becker AJ. Molecular profiling of temporal lobe epilepsy: comparison of data from human tissue samples and animal models. *Epilepsy Res.* 2004;60(2–3):173–178.
109. Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. *Epilepsia.* 2005; 46(11):1724–1743.
110. Polascheck N, Bankstahl M, Loscher W. The COX-2 inhibitor parecoxib is neuroprotective but not antiepileptogenic in the pilocarpine model of temporal lobe epilepsy. *Exp Neurol.* 2010;224(1):219–233.
111. Holtman L, van Vliet EA, van Schaik R, Queiroz CM, Aronica E, Gorter JA. Effects of SC58236, a selective COX-2 inhibitor, on epileptogenesis and spontaneous seizures in a rat model for temporal lobe epilepsy. *Epilepsy Res.* 2009;84(1):56–66.
112. Ravizza T, Noé F, Zardoni D, Vaghi V, Sifringer M, Vezzani A. Interleukin converting enzyme inhibition impairs kindling epileptogenesis in rats by blocking astrocytic IL-1beta production. *Neurobiol Dis.* 2008;31(3):327–333.
113. Sayyah M, Beheshti S, Shokrgozar MA, et al. Antiepileptogenic and anticonvulsant activity of interleukin-1 beta in amygdala-kindled rats. *Exp Neurol.* 2005;191(1):145–153.
114. Seiffert E, Dreier JP, Ivens S, et al. Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex. *J Neurosci.* 2004;24(36): 7829–7836.
115. David Y, Cacheaux LP, Ivens S, et al. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *J Neurosci.* 2009;29(34):10588–10599.
116. Vezzani A, Janigro D. Leukocyte-endothelial adhesion mechanisms in epilepsy: cheers and jeers. *Epilepsy Curr.* 2009;9(4):118–121.
117. Lukasiuk K, Sliwa A. FK506 aggravates development and severity of disease in the rat model of temporal lobe epilepsy. *The 8th European Congress on Epileptology, Berlin.* 2008;Abstract number Y175:102.
118. Zardoni D, Maroso M, Balosso S, Ravizza T, Noé F, Vezzani A. The role of T- and B-cells in seizures and epilepsy. *The 9th European Congress on Epileptology.* 2010;Rhodes(June 27–July 1).
119. Mathern GW, Pretorius JK, Babb TL. Influence of the type of initial precipitating injury and at what age it occurs on course and outcome in patients with temporal lobe seizures. *J Neurosurg.* 1995;82(2):220–227.
120. Heida JG, Pittman QJ. Causal links between brain cytokines and experimental febrile convulsions in the rat. *Epilepsia.* 2005;46(12):1906–1913.
121. Auvin S, Porta N, Nehlig A, Lecointe C, Vallee L, Bordet R. Inflammation in rat pups subjected to short hyperthermic seizures enhances brain long-term excitability. *Epilepsy Res.* 2009;86(2–3):124–130.
122. Auvin S, Shin D, Mazarati A, Nakagawa J, Miyamoto J, Sankar R. Inflammation exacerbates seizure-induced injury in the immature brain. *Epilepsia.* 2007; 48(suppl 5):27–34.
123. Sayyah M, Javad-Pour M, Ghazi-Khansari M. The bacterial endotoxin lipopolysaccharide enhances seizure susceptibility in mice: involvement of proinflammatory factors: nitric oxide and prostaglandins. *Neuroscience.* 2003;122(4):1073–1080.
124. Haas KZ, Sperber EF, Opanashuk LA, Stanton PK, Moshe SL. Resistance of immature hippocampus to morphologic and physiologic alterations following status epilepticus or kindling. *Hippocampus.* 2001;11(6):615–625.
125. Sankar R, Shin DH, Liu H, Mazarati A, Pereira de Vasconcelos A, Wasterlain CG. Patterns of status epilepticus-induced neuronal injury during development and long-term consequences. *J Neurosci.* 1998;18(20):8382–8393.
126. Galic MA, Riazi K, Heida JG, et al. Postnatal inflammation increases seizure susceptibility in adult rats. *J Neurosci.* 2008;28(27):6904–6913.
127. Galic MA, Riazi K, Henderson AK, Tsutsui S, Pittman QJ. Viral-like brain inflammation during development causes increased seizure susceptibility in adult rats. *Neurobiol Dis.* 2009;36(2):343–351.
128. Harré EM, Galic MA, Mouihate A, Noorbakhsh F, Pittman QJ. Neonatal inflammation produces selective behavioural deficits and alters *N*-methyl-D-aspartate receptor subunit mRNA in the adult rat brain. *Eur J Neurosci.* 2008;27(3):644–653.
129. Auvin S, Mazarati A, Shin D. Inflammation enhances epileptogenesis in immature rat brain. *Neurobiol Dis.* 2010;40(1):303–310.

130. Auvin S, Shin D, Mazarati A, Sankar R. Inflammation induced by LPS enhances epileptogenesis in immature rat and may be partially reversed by IL1RA. *Epilepsia.* 2010;51(suppl 3):34–38.
131. Marcon J, Gagliardi B, Balosso S, et al. Age-dependent vascular changes induced by status epilepticus in rat forebrain: implications for epileptogenesis. *Neurobiol Dis.* 2009;34(1):121–132.
132. Viviani B, Gardoni F, Marinovich M. Cytokines and neuronal ion channels in health and disease. *Int Rev Neurobiol.* 2007;82:247–263.
133. Chen C, Bazan NG. Lipid signaling: sleep, synaptic plasticity, and neuroprotection. *Prostaglandins Other Lipid Mediat.* 2005;77(1–4):65–76.
134. Slanina KA, Schweitzer P. Inhibition of cyclooxygenase-2 elicits a CB1-mediated decrease of excitatory transmission in rat CA1 hippocampus. *Neuropharmacology.* 2005;49(5):653–659.
135. Wiedmer T, Sims PJ. Effect of complement proteins C5b-9 on blood platelets. Evidence for reversible depolarization of membrane potential. *J Biol Chem.* 1985;260(13):8014–8019.
136. Casamenti F, Prosperi C, Scali C, et al. Interleukin-1beta activates forebrain glial cells and increases nitric oxide production and cortical glutamate and GABA release in vivo: implications for Alzheimer's disease. *Neuroscience.* 1999;91(3):831–842.
137. Hu S, Sheng WS, Ehrlich LC, Peterson PK, Chao CC. Cytokine effects on glutamate uptake by human astrocytes. *Neuroimmunomodulation.* 2000;7(3):153–159.
138. Bezzi P, Domercq M, Brambilla L, et al. CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity. *Nat Neurosci.* 2001;4(7):702–710.
139. Fellin T, Gomez-Gonzalo M, Gobbo S, Carmignoto G, Haydon PG. Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices. *J Neurosci.* 2006;26(36): 9312–9322.
140. Tian GF, Azmi H, Takano T, et al. An astrocytic basis of epilepsy. *Nat Med.* 2005;11(9):973–981.
141. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. *Br J Pharmacol.* 2006;147(suppl 1):S232–S240.
142. Vezzani A, Balosso S, Maroso M, Zardoni D, Noé F, Ravizza T. ICE/caspase 1 inhibitors and IL-1beta receptor antagonists as potential therapeutics in epilepsy. *Curr Opin Investig Drugs.* 2010;11(1): 43–50.

Chapter 49

Glia–Neuron Interactions

Neurosteroids and Epileptogenesis

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BACKGROUND

The relationship between glial and neuronal cells has become a central topic in research aimed at establishing therapeutic strategies to restore brain functions after damage.[1–4] A brain lesion may be rather selective, involving only neuronal cells, as occurs in neurodegenerative diseases (e.g., Parkinson's disease), or it can affect all cell types present in the tissue, as in the case of pannecrosis found after cerebral ischemia or traumatic brain injury. In the latter case, regeneration and repair depend on the specific properties of the cell type that has to be replaced.

Regeneration is limited for neurons, whereas glial cells can proliferate and invade the damaged brain area that needs to be restored. These properties of glial cells lead to formation of a glial scar, consisting of astrocytes, microglial cells, macrophages, and extracellular matrix, which replace the damaged nervous tissue; a classical example of a glial scar is found in the CA1 region of patients affected by mesial temporal lobe epilepsy. Figure 49–1 illustrates reactive astrocytes and microglial cells, identified by their markers, glial fibrillary acidic protein (GFAP) and heme oxygenase-1 (HO-1), respectively, which can be found 1 week after exposure to pilocarpine-induced status epilepticus (SE) lasting for 30 min (cf. ref. 5). Evolution of the lesion into a scar has long been interpreted as the most important factor dampening the capacity to restore the normal

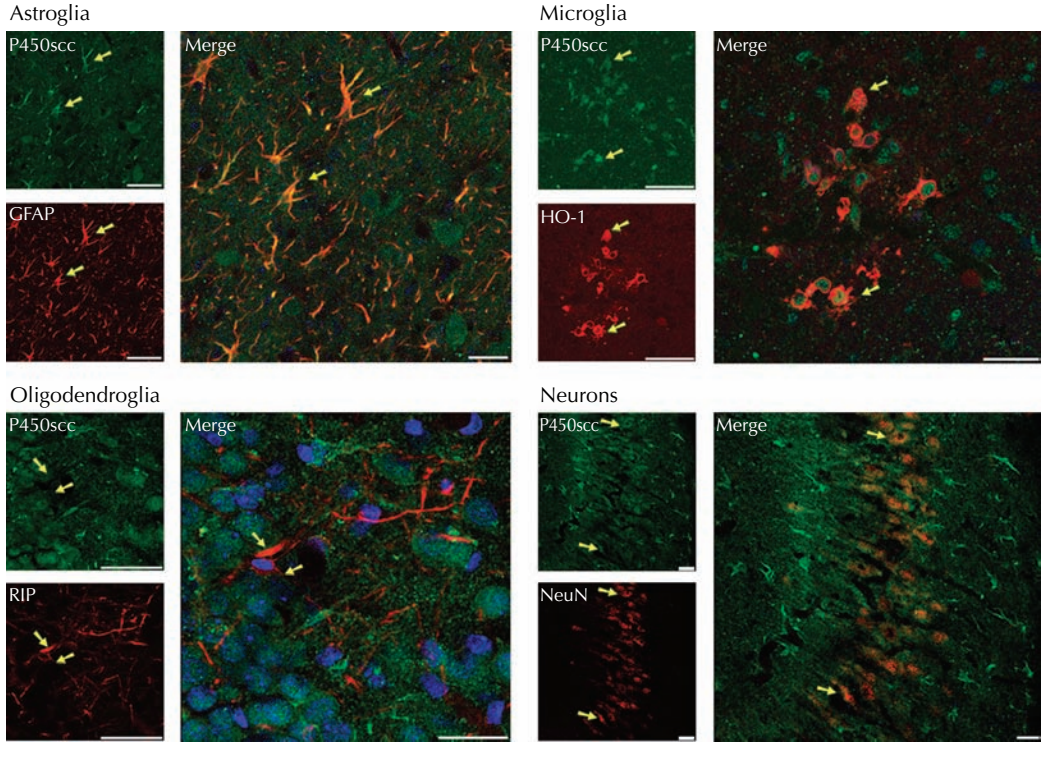

Figure 49–1. Identification of cell types involved in neurosteroid synthesis in the CA3 region of pilocarpine-treated rats. Microphotographs illustrating the colocalization of the cytochrome P450scc with markers of astrocytes (identified with GFAP), microglial cells (HO-1, expressed in the activated microglia), oligodendrocytes (RIP, which identifies the enzyme 2′,3′-cyclic nucleotide 3′-phosphodiesterase), and neurons (neuron-specific nuclear protein [NeuN]) in rats exposed to pilocarpine-induced SE lasting for 30 min 1 week after the treatment. Arrows point to examples of cells costained with P450scc and the respective cell-specific marker. Note that especially reactive astrocytes and, secondarily, neurons express the highest levels of P450scc immunoreactivity. The vast majority of HO-1-positive microglial cells also appear to present P450scc staining. At variance, RIP-positive oligodendrocytes present very low levels of P450scc immunoreactivity. Scale bars, 25 μm.

function of neuronal networks.[3] Although this process can be modulated by a variety of molecules synthesized in response to damage by the different cell types involved, astrocytes and neurons have in common the ability to secrete neurosteroids, which are able to deeply influence the outcome of a lesion.[6]

In contrast to neuroglia (astrocytes, ependymal cells, and oligodendrocytes), microglial cells are mesodermal in origin and serve as the central nervous system's innate immune apparatus.[4] Resting microglial cells, as they are found in normal conditions, are resident but not functionally silent elements, as they make transient but regularly recurrent contacts with synapses.[7] Following an insult, microglial cells become reactive by expanding their ramified morphology and assuming an ameboid structure that enables them to migrate. It is highly controversial whether only resident or also circulating precursor cells may be transformed into reactive microglia.[8,9] In any case, resident microglial cells have the potential to develop into mature macrophages, acting as phagocytes to remove dying cells and as regulators of the inflammatory reaction by releasing cytokines.[4] Although there is evidence of a neurotoxic role for reactive microglia,[10] a neuroprotective effect of microglial cells has been found in different models of neurodegeneration or acute lesions.[4]

Following the development of a lesion, astrocytes, which are the main glial cell type involved in assisting neuronal transmission in normal conditions,[11] transform into reactive cells by developing hypertrophic cytoplasmic processes and somas and by overproducing intermediate filaments (GFAP, vimentin, and

nestin), trophic factors (ciliary neurotrophic factor, basic fibroblast growth factor, and nerve growth factor), or pro-inflammatory (interleukins [ILs], prostaglandins) and neurotoxic (protein S100β in its reduced state, nitric oxide) molecules.[2] As in the case of microglial cells, the role of astrocytes in the recovery from a lesion is controversial, but lines of evidence suggest that these glial cells contribute to preserve spared neurons and keep them from dying.[3] A further intriguing aspect of astrocyte reactivity is that gliosis can be directly elicited by neuronal overactivation, such as that occurring during brief seizures in the absence of overt neuronal damage.[12] At variance, spreading depression was also demonstrated to produce upregulation of GFAP synthesis.[13] This evidence suggests that neuronal damage is not necessary to activate a glial response and that astrocytes are indeed responsive to perturbations in neuronal network functioning.

MULTIFACETED GLIAL MODULATION OF NEURONAL EXCITABILITY

One of the recent major achievements of neuroscience is the recognition of the role played by astrocytes in modulating neuronal transmission.[10,14–16] Transmitters released by glial cells (*gliotransmitters*), such as adenosine or adenosine triphosphate[17] and D-serine,[18] have been proved to modulate synaptic plasticity in the hippocampus, whereas glia-derived gamma-aminobutyric acid (GABA) and glutamate are both able to influence the properties of postsynaptic currents (cf. ref. 11). For instance, slow inward currents are generated by hippocampal neurons in response to glutamate released by astrocytes, a recently identified mechanism of neuronal synchronization that is particularly evident in the juvenile rodent brain.[19–21]

In the epileptic brain, decreased astrocytic expression of glutamine synthetase may favor extracellular glutamate accumulation by altering the glutamate-GABA metabolic cycle.[22] Notably, ictogenesis is observed in mice when the astrocytic glutamate uptake function is impaired by genetic ablation of the glutamate transporter 1/excitatory amino acid transporter 2 (GTL-1/EAAT2).[23] Nonetheless, glial cells may also prolong epileptogenesis by releasing neurosteroids,[24,25] which are potent modulators of GABAergic transmission.[26] Thus, astroglial cell function could likely have important consequences for the ability of neurons to synchronize and generate epileptic activity (cf. ref. 27).

The impact of these diverse glial functions on neuronal synchronization is expected to be more pronounced in the presence of brain damage, when glial cells are activated to aid in recovery from a lesion. In this situation, the astrocytic release of trophic factors[3] and cytokines,[28] or of regenerative molecules such as neurosteroids,[6,29] may deeply alter the modulatory effects of glial cells on neuronal transmission.

Astrocyte–Neuron Interactions Leading to Synchronization in Undamaged Tissue

Astrocytes play an important role in neuronal synchronization in undamaged tissue; thus, they can potentially participate in generating seizures in the presence of ictogenic stimuli. Accordingly, glutamate is released by astrocytes in response to calcium oscillations in the hippocampus, thereby acting as a gliotransmitter contributing to synchronous neuronal firing.[20]

In vitro experiments have demonstrated that the frequency of calcium oscillations significantly increases in astrocytes during epileptiform activity, a phenomenon that is sensitive to antiepileptic drugs.[21] More recently, experiments performed in the rat entorhinal cortex in vitro and in the guinea pig whole brain preparation[30] have demonstrated that calcium elevation in astrocytes correlates with the initiation and maintenance of ictal-like activity, which is further enhanced by stimulation of astrocytic calcium signaling. In contrast, interictal-like discharges are not correlated to or influenced by changes in astrocytic calcium elevations.

This evidence further supports the view that glia and neurons are reciprocally engaged in a loop of recurrent excitation, which, in turn, may initiate and sustain ictal-like synchronization. It is important to stress that astrocytes can synthesize dehydroepiandrosterone[31] and its sulfated form, which, along with other neurosteroids, such as sulfated pregnenolone and

pregnanolone, can potently modulate glutamatergic transmission.[32–34] Therefore, it would be relevant to understand if and how these glia-derived neurosteroids may influence the astrocyte-neuron excitatory loop contributing to ictogenesis.

Astrocyte–Neuron Interactions Leading to Synchronization in Damaged Tissue

The role of glial cells in regulating neuronal synchronous activation leading to ictogenesis may be more pronounced in damaged tissue, where gliotic processes are triggered to aid in brain repair. In light of this view, neuronal hyperexcitability seen in postlesional epilepsies, which follow a brain damage, may represent a paradoxical consequence of reactive gliosis and the resulting glial scar. As shown in a recent study,[35] focal increase in reactive astrocytes in CA1 leads to impairment of inhibitory synaptic currents, which is paralleled by a relative increase of synaptic excitability consequent to impairment of the astrocytic glutamate-glutamine cycle.

Whereas the functional consequences of the glial scar are still a matter of controversy,[3] there is evidence suggesting that astrocytes can provide protection from secondary neurodegeneration.[36] Accordingly, by selectively ablating newly formed astrocytes in transgenic mice, it has been shown that neuronal cell loss is significantly increased a week after a moderate traumatic brain injury.[37] Thus, therapeutic strategies aimed at activating astrocytes may be beneficial to the progression of epileptogenic brain lesions.[1,2,38]

Various mechanisms are altered because of the astroglial reaction to tissue damage, and they have been suggested to be involved in promoting neuronal synchronization.[27,39] First, astrocytes are key players in buffering increased extracellular potassium, but this function is disrupted in the presence of blood-brain barrier leakage; accordingly, active transport of extravasated albumin into astrocytes induces the release of transforming growth factor β (TGF-β), which, by an autocrine mechanism, impairs potassium transport in astroglial cells.[39,40] Notably, potassium homeostasis can be further dysregulated by downregulation of channels involved in mediating water flow from the interstitium to blood vessels, especially aquaporin 4, as seen in the astrocytes of patients affected by mesial temporal lobe epilepsy.[41] Second, astrocytes are normally organized in nonoverlapping domains that are exclusive for each astroglial cell,[10] but this segregation is partially lost in epileptic mice: this change appears to be specifically related to seizure activity, and it is accompanied by morphological modifications in neuronal shape consisting of hypertrophy of apical dendrites and increased spine density.[42] Third, astrocytes are actively involved in metabolizing the endogenous anti-convulsant adenosine by the enzyme adenosine kinase (ADK); consistently, in ADK-deficient mice, the reactive gliosis consequent to kainate-induced neuronal damage is not accompanied by development of spontaneous recurring seizures.[43] Fourth, astrocytes activated by brain damage produce and secrete pro-inflammatory molecules, such as IL-1β, which displays pro-convulsive effects.[44] Although it is unclear whether these mechanisms can be affected by neurosteroids, some studies have shown that neurosteroids such as progesterone and allopregnanolone can modulate astroglial reactivity[45] and aquaporine 4 expression[46] and can interfere with inflammatory processes.[47]

Microglia–Neuron Interactions

Inflammation is generally regarded as pro-convulsive. Not only central but also peripheral inflammation (e.g., in the gut) has been shown to decrease the threshold to pentylenetetrazole-induced seizures, probably by increasing microglial cell activation and tumor necrosis factor-α (TNF-α) release in the hippocampus.[48] Microglial cells are activated within 15 min of a brain insult and synthesize IL-1β, which, in turn, is required to activate astrocytes.[49] Other cytokines and mediators released by activated microglial cells, such as IL-6 and the vascular endothelial growth factor (VEGF), have been found to be increased in the brain or plasma of animals exposed to seizures and in patients affected by epilepsy.[50] At least in the case of IL-1β, a well- defined molecular mechanism linking this glial cytokine with increased neuronal excitability has been identified, and it involves the activation of NR2B-containing *N*-methyl-D-aspartate (NMDA) receptors.[44] Although

there is no direct evidence that neurosteroids interfere with the pro-epileptogenic effects of microglial cell activation, a few studies suggest that progesterone and allopregnanolone may interact with the complement cascade to decrease inflammation[51] or to reduce the peaks of IL-1β and TNF-α induction after traumatic brain injury.[47]

NEUROSTEROIDS AND EPILEPTOGENESIS

At the beginning of the 1980s, Baulieu and colleagues found that brain levels of the steroid dehydroepiandrosterone sulfate were independent of peripheral synthesis, and that they were clearly increased by stress in castrated and adrenalectomized rats.[52] This was the first demonstration of local steroid synthesis in neural tissue and it opened the door to the concept of *neurosteroids,* that is, molecules that are directly synthesized from cholesterol in the brain. Later, it was found that the cytochrome P450 cholesterol side chain cleavage (P450scc) enzyme—which is involved in transforming cholesterol into the steroid precursor pregnenolone (Table 49–1)—is localized mainly in myelinated fibers of the white matter, (i.e., in oligodendrocytes), with the exception of scattered neuronal cells that were found to express P450scc in some limbic structures.[55] Further investigation has demonstrated that the P450scc enzyme can be found in neurons of different brain regions, including the cerebellar Purkinje cells[63] and hippocampal pyramidal cells,[56] as well as in astrocytes.[31]

We have recently reported that P450scc immunoreactivity colocalizes with HO-1, which is also expressed in reactive microglial cells[54] (Fig. 49–1). Interestingly, increased neurosteroid synthesis was found to accompany astrocytosis in the brain of postnatal myelin mutant jimpy mice,[64] suggesting that, contrary to what is found in normal neural tissue,[56] reactive astrocytes represent an important source of neurosteroids in pathological conditions.

P450scc and Epileptogenesis

Based on the localization of enzymes involved in neurosteroid synthesis in a multiplicity of brain cells (Table 49–1), and on the fact that P450scc expression can be changed by varying the glial cell's metabolic state, we surmised that neurosteroid levels could be altered in epilepsies associated with brain damage. In this context, neurosteroid levels could be downregulated because of a substantial loss of synthesizing cells due to damage or, alternatively, upregulated as a consequence of glial cell reactivity. To assess how these changes could affect epileptogenesis, we have recently characterized P450scc immunoreactivity after pilocarpine-induced SE in adult rats.[24,54] In this model, induction of both excitotoxic and vascular lesions is dependent on the duration of SE and presents with a regional specificity:[5] in the medial entorhinal cortex, layer III neurons are largely lost because of sustained glutamate release,[65] whereas a highly predictable ischemic-like lesion (pannecrosis) develops in the stratum lacunosum-moleculare of CA3 (Fig. 49–2), causing disappearance of myelinated fibers and astrocytes (cf. ref. 5).

According to quantification of P450scc immunoreactivity, the induction of this enzyme exhibits an increasing gradient from parahippocampal to hippocampal regions that is also dependent on SE duration (Fig. 49–3). Interestingly, the most significant increase in P450scc immunoreactivity in the hippocampus is found both in neurons and in glial cells in the surroundings of the ischemic lesion identified in CA3 stratum lacunosum-moleculare[24] (Fig. 49–2). However, the neuron-specific changes are limited to the first week after SE, whereas those in glial cells are long-lasting and approximately equivalent to the latent period, that is, the time lag preceding the appearance of spontaneous recurrent seizures following SE. The prevalence of P450scc induction in hippocampal astrocytes can be clearly appreciated in experiments of colocalization with cell-specific markers. In this way, we unambiguously identified the different P450scc-positive cell types in tissue obtained 1 week after pilocarpine-induced SE (Fig. 49–1). It should be emphasized that the time course of increased expression of glial P450scc does not parallel that of astrocyte reactivity since GFAP immunoreactivity is still remarkably intense even 3 weeks after SE, when P450scc immunoreactivity is already largely reduced (Fig. 49–2). This phenomenon may explain why gliosis, which is always present in postlesional epilepsies, does

Table 49–1 **Cellular Localization of Enzymes Involved in the Synthesis of Neurosteroids Able to Modulate $GABA_A$ Receptor Currents in the Rat Brain: Allopregnanolone (3α,5α-Tetrahydroprogesterone) and Allodeoxycorticosterone (3α,5α-Tetrahydrodeoxycorticosterone)**

Reaction	Cholesterol ↓ Pregnenolone	Pregnenolone ↓ Progesterone	Progesterone ↓ Deoxycorticosterone	Progesterone/ Deoxycorticosterone ↓ 5α-Dihydroprogesterone/ 5α-Dihydrodeoxycorticosterone	5α-Dihydroprogesterone/ 5α-Dihydrodeoxycorticosterone ↓ 3α,5α-Tetrahydroprogesterone/ 3α,5α-Tetrahydrodeoxycorticosterone
Enzyme	P450scc	3β-Hydroxysteroid dehydrogenase Δ^5-Δ^4-isomerase	21-Hydroxylase	5α-Reductase	3α-Hydroxysteroid dehydrogenase
Cell	A,[53] AA,[54] AM,[54] N,[53,55,56] O[53,55,57]	A,[31] N,[58] O[31]	ND[59]	A,[60] N,[61] O[60]	A,[60] N,[61] O[60]
Blocker	Aminoglutethimide[62]	Trilostane[62]	Quinidine[59]	Finasteride, SKF 105111[62]	Indomethacin, Provera[62]

Abbreviations: A, astrocytes; AA, activated astrocytes; AM, activated microglial cells; N, neurons; ND, not determined; O, oligodendrocytes; P450scc, P450 cholesterol side chain cleavage enzyme.

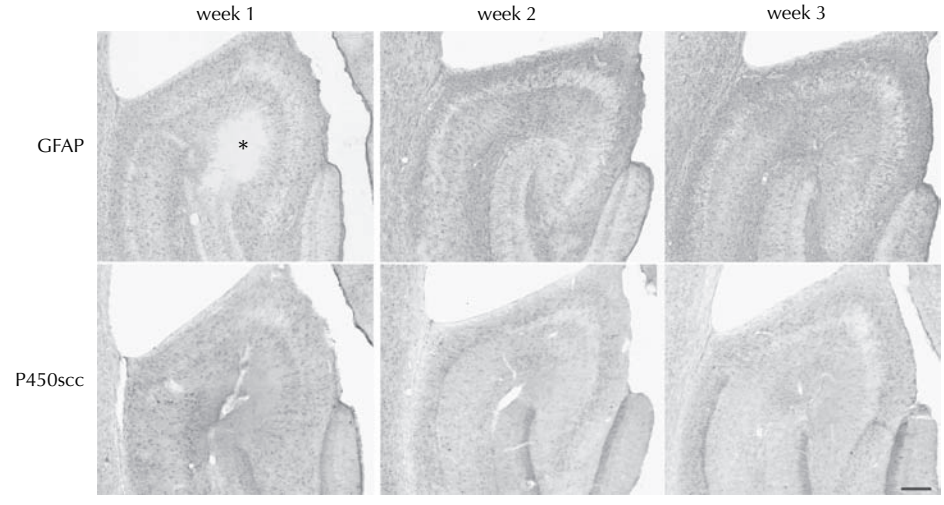

Figure 49–2. Time course of astrocyte reactivity following SE lasting for 30 min. Changes in GFAP and P450scc enzyme immunoreactivity in rats exposed to pilocarpine-induced SE lasting for 30 min 1, 2, and 3 weeks after the treatment. Note that the GFAP staining is increased 2 weeks compared to 1 week after SE, and it is still maintained 3 weeks after SE. Conversely, the P450scc staining decreases progressively from 1 to 3 weeks after SE, suggesting a very different time course in the upregulation of these astroglial proteins. The asterisk points to the ischemic-like lesion in CA3 stratum lacunosum-moleculare (cf. ref. 5), around which the glial reaction takes place. Scale bar, 200 μm.

not control (and indeed contributes to) ictogenesis in the epileptic focus.

During the first weeks after SE, the extent of P450scc induction in hippocampal astrocytes appears to have major consequences for epileptogenesis. By varying the duration of the initial SE induced by pilocarpine, we discovered that the length of the latent period progressively decreases as pharmacological cessation of SE by diazepam administration is anticipated

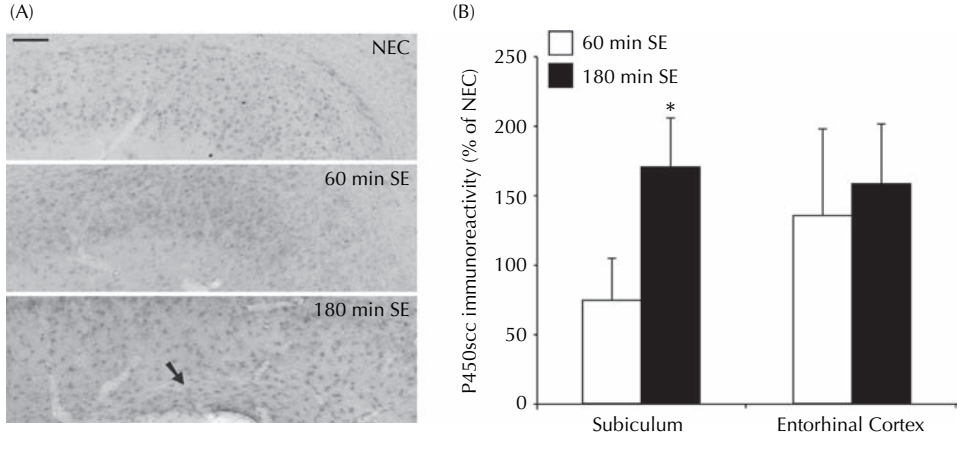

Figure 49–3. Changes in P450scc immunoreactivity in the subiculum and entorhinal cortex of pilocarpine-treated rats. **A.** In the subiculum, P450scc immunoreactivity is found in neurons in the pyramidal cell layer of nonepileptic control (NEC) rats. This distribution is unchanged after exposure to 60 min of SE 1 week after pilocarpine treatment. In contrast, in tissue obtained from animals exposed to 180 min of SE, P450scc immunopositive cells are found in all of the subicular layers. These cells also appear to be represented by glial cells (arrow). **B.** Quantification of P450scc immunoreactivity demonstrated a significant increase ($^*p < .05$, analysis of variance followed by Tukey's test; data are expressed as mean ± SEM) in the group exposed to 180 min of SE compared to NEC rats. An increase in P450scc immunoreactivity was also seen in the entorhinal cortex of both groups of pilocarpine-treated rats, but it was not significantly different from NEC values. Scale bar, 100 μm.

from 180 to 90, 60, and 30 min.[24,54,66] Notably, this correlation between SE duration and the duration of the latent period was also found to be paralleled by P450scc induction. It should also be emphasized that young, 3-week-old rats exposed to short-duration (60 min) SE present with a more pronounced induction of P450scc than that seen in adult animals,[66] and they rarely present with stage 5 seizures[67] during the chronic epileptic period.[5] However, the ischemic-like lesion seen in CA3 stratum lacunosum-moleculare of adult animals infrequently develops in young pilocarpine-treated rats,[5] suggesting that the more pronounced induction of P450scc synthesis in this group is probably due to the age-related hyperreactivity of astrocytes to brain damage, as described in young rats exposed to kainate[68] or to mechanical injury.[69]

5α-Reduced Neurosteroids and Epileptogenesis

Increased P450scc synthesis after SE is suggestive but not predictive of increased neurosteroid levels, since it has been established that the cholesterol transport machinery in mitochondria represents the real rate-limiting event in neurosteroid synthesis.[70,71] Therefore, we further challenged the role of neurosteroids in delaying seizure onset in the pilocarpine model by treating rats exposed to SE with finasteride (100 mg/kg), a specific and irreversible inhibitor of 5α-reductase (Table 49–1). This procedure anticipated the onset of spontaneous recurrent seizures in pilocarpine-treated rats,[24] a phenomenon that was probably related to decreased synthesis of neurosteroids modulating $GABA_A$ receptors. In fact, at 10 mg/kg, finasteride was shown to acutely deplete allopregnanolone without affecting 3α-dihydroprogesterone, progesterone, or deoxycorticosterone.[72] When repeatedly administered at a dose of 25 mg/kg, finasteride significantly decreases both allopregnanolone and allotetrahydrodeoxycorticosterone brain levels.[73] However, whether chronic treatment with finasteride could affect brain concentrations of other neurosteroids in the long term is still a matter of debate (see ref. 74).

In order to exclude the possibility that modulation of epileptogenesis by finasteride could be explained by other variables, we have tested its effect in different paradigms of SE duration and addressed whether the effect of 5α-reductase inhibition correlates with the extent of P450scc induction. Consistent with the correlation between SE duration, P450scc induction, and the duration of the latent period, finasteride was able to anticipate the appearance of stage 5 seizures in rats experiencing at least 180 min of SE, whereas it was ineffective in rats exposed to 90 min or less of continuous pilocarpine-induced seizures.[54,66] In addition, we compared the effects of finasteride administration on adult (8-week-old) and young (3-week-old) rats exposed to 60 min of SE and found that finasteride was ineffective in altering the duration of the latent period in adult rats, which presented with spontaneous recurrent seizures as early as 7 days after SE, as it occurs in rats with an insufficient induction of P450scc.[24] Consistently, administration of finasteride to young rats anticipated seizure manifestation in approximately 50% of the animals during the first 7 days following SE, when P450scc levels are increased. Then finasteride was not able to disclose other stage 5 seizures in the further treatment days,[66] when P450scc reached its peak level (Fig. 49–4). Notably, these experimental groups clearly differed in the extent and duration of P450scc induction in the CA3 hippocampal subfield (Fig. 49–4). Therefore, these findings suggest that neurosteroid synthesis is related to the extent of P450scc induction in glial cells following SE and that neurosteroids influence epileptogenesis.

Neurosteroids, Epileptogenesis, and Inhibition

Neurosteroids may decrease neuronal excitability by enhancing $GABA_A$-mediated inhibition.[26,75] Allopregnanolone and allotetrahydrodeoxycorticosterone, two major products of neurosteroid synthesis[25] (cf. Table 49–1), interact with $GABA_A$ receptors at nanomolar concentrations as positive allosteric modulators, whereas at micromolar concentrations they can directly open $GABA_A$ receptor channels.[26,76] Of particular interest are extrasynaptic $GABA_A$ receptors, since they appear to be the preferential target of neurosteroids (cf. ref. 75). In contrast to synaptic $GABA_A$ receptors, which generate "phasic" inhibitory postsynaptic currents (IPSCs), extrasynaptic $GABA_A$

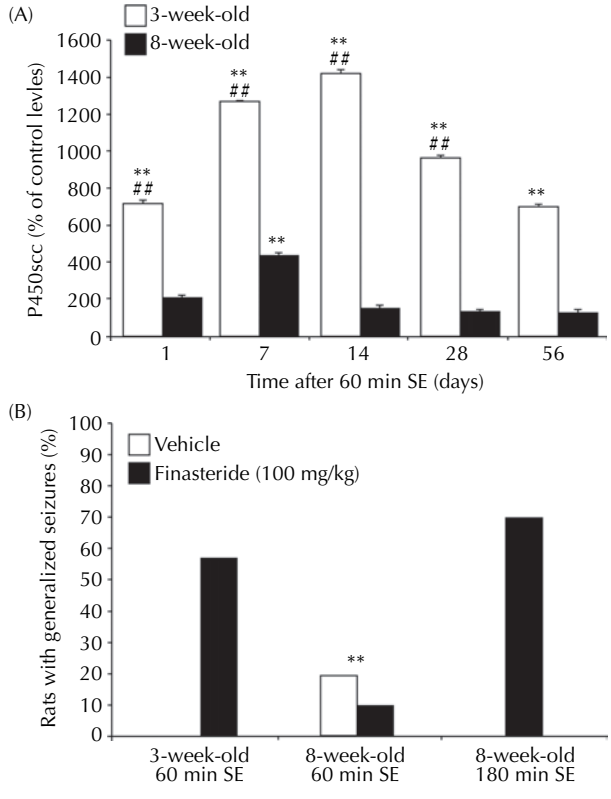

Figure 49–4. Effects of changes in neurosteroid metabolism on epileptogenesis. **A.** Time course of the changes in P450scc immunoreactivity in rats exposed to pilocarpine-induced SE lasting for 60 min, at different ages (3 and 8 weeks), and studied 7, 14, 28, and 56 days after the treatment. Note that P450scc immunoreactivity is higher in young rats at any time interval. $^{**}p < .01$ versus control values; $^{\#\#}p < .01$ versus 8-week-old rats, analysis of variance followed by the Games-Howell test; data are expressed as mean ± SEM. In **B**, the percentages of rats presenting with generalized seizures (stage 5 of Racine's scale[67]) are shown in groups with different levels of P450scc induction (cf. refs. 54 and 66 and panel **A**); note that these values refer to generalized seizures manifest during the first 7 days of treatment with the neurosteroid synthesis inhibitor finasteride (100 mg/kg/day, starting on the 4th day after SE) or the vehicle (cf. ref. 5). Stage 5 seizures were not observed in the groups of 3-week-old rats exposed to 60 min of SE or 8-week-old rats exposed to 180 min of SE, in which we found high levels of P450scc immunoreactivity (cf. refs. 54 and 66). Interestingly, finasteride treatment revealed stage 5 seizures in the majority of rats in these groups. In contrast, in 8-week-old rats exposed to 60 min of SE, in which P450scc was scarcely induced, generalized seizures were observed in vehicle-treated animals during the first 10 days after SE. Finasteride was not able to alter this finding. $^{**}p < .01$, chi-square test.

receptors maintain a constant inhibitory tone by generating a "tonic" (always-on) current. They are characterized by lack of the gamma subunit, consist of alpha, beta and delta subunits assemblies, and differ from those mediating inhibitory synaptic activity in that they are insensitive to benzodiazepines; moreover, they have a higher affinity for GABA and exhibit minimal or no desensitization.[77,78] The presence of the delta subunit in the receptor assembly contributes to the high sensitivity of extrasynaptic $GABA_A$ receptors to neurosteroids.[79]

An increased synthesis of allopregnanolone and allotetrahydrodeoxycorticosterone may parallel the induction of P450scc following pilocarpine-induced SE,[54] leading to the question of whether the ability of endogenous neurosteroids to delay the manifestation of spontaneous recurrent seizures after an epileptogenic insult[24] may stem from their interaction with extrasynaptic $GABA_A$ receptors, and thus from the enhancement of tonic inhibition. We addressed this question in subicular neurons, since this parahippocampal structure is particularly relevant to temporal lobe epileptogenesis. In fact, the subiculum gates hippocampal output activity[80] and reciprocally interacts with the entorhinal cortex to sustain

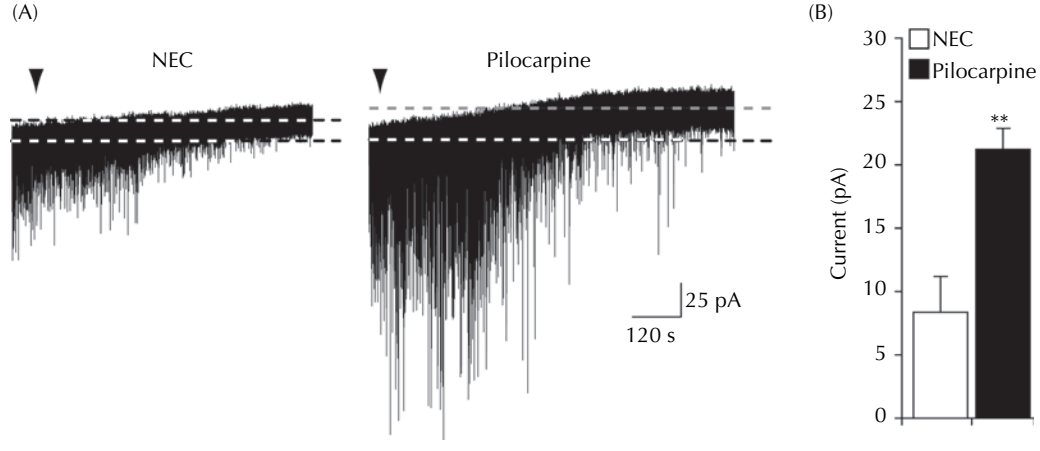

Figure 49–5. Early enhancement of tonic inhibition of subicular neurons following pilocarpine-induced SE lasting for 2 h. **A.** Representative whole-cell voltage-clamp recordings obtained from subicular neurons of NEC and pilocarpine-treated rat tissue 4 days after induction of SE lasting for 2 h. Bath application of the $GABA_A$ receptor blocker picrotoxin (PTX, 100 μM, arrowheads) reveals the presence of a tonic current, which is larger in the subicular neuron exposed to SE. Recordings were performed at $V_m = -70$ mV, in the symmetric chloride condition, and at room temperature (~21°C). Measurements of the holding current before and after application of PTX were taken at the levels indicated by the dashed lines. **B.** Tonic inhibition is significantly increased in subicular neurons during the latent period (NEC: 9.06 ± 3.02 pA; pilocarpine: 22.97 ± 1.78 pA; $n = 7$ and 5, respectively; $^{**}p < .01$, unpaired Student's t-test; data are expressed as mean ± SEM).

ictogenesis in pilocarpine-treated epileptic tissue.[81] In addition, P450scc induction is significantly increased in the subiculum following pilocarpine-induced SE (cf. Fig. 49–3). Remarkably, preliminary findings obtained in our laboratory[66] indicate that tonic inhibition is increased in subicular neurons 3–5 days after pilocarpine-induced SE lasting for 2 h (Fig. 49–5). This phenomenon may represent an early compensatory mechanism to dampen the spread of excitation from the hippocampus to parahippocampal regions following an epileptogenic insult. It remains, however, to be established if this phenomenon is consequent to the increased expression of P450scc and/or of $GABA_A$ receptor subunits mediating the tonic current,[77,78] as well as its temporal profile until the establishment of the chronic epileptic condition.

CONCLUSIONS

Overall, the evidence reviewed in this chapter indicates a complex interaction between glia and neurons in postlesional epilepsies, depicting a scenario similar to that found when analyzing the role of glial cells in the recovery from brain damage. Indeed, astrocytes may exert both pro-epileptogenic and anti-epileptogenic roles through (1) indirect modulation of neuronal function via release of inflammatory cytokines and neurosteroids, or by altering neurotransmitter release, and (2) by directly influencing neuronal activity via release of gliotransmitters.

Neurosteroids seem to play an important role in regulating epileptogenesis. As such, neuroactive steroidal compounds, which are synthetic analogues of endogenous neurosteroids, are currently attracting much attention among the potential antiepileptic drugs under investigation, thanks to their improved pharmacokinetics. However, depending on their chemical composition, these compounds can also increase neuronal excitability by inhibiting GABAergic signaling or enhancing glutamatergic transmission, leading to the need for carefully addressing their potential use for epilepsy treatment. Moreover, the synthesis and release of endogenous neurosteroids appear to be time-dependent and influenced by several factors, among which the most important is the glial response to damage; accordingly, the degree of tissue injury determines the extent of P450scc induction.

Clarifying these aspects may help shed more light on how an epileptogenic insult may trigger physiological processes of brain repair, which

should be beneficial but may lead paradoxically to the development of a chronic epileptic condition. For this purpose, the most important goal for future research may be to dissociate the increase in neurosteroid synthesis by astrocytes (or other possible sources) from insult to nervous tissue.

DISCLOSURE STATEMENT

The authors have not declared any conflicts of interest.

REFERENCES

1. Liberto CM, Albrecht PJ, Herx LM, Yong VW, Levison SW. Pro-regenerative properties of cytokine-activated astrocytes. *J Neurochem*. 2004;89:1092–1100.
2. Escartin C, Bonvento G. Targeted activation of astrocytes: a potential neuroprotective strategy. *Mol Neurobiol*. 2008;38:231–241.
3. Rolls A, Shechter R, Schwartz M. The bright side of the glial scar in CNS repair. *Nat Rev Neurosci*. 2009;10: 235–241.
4. Graeber MB, Streit WJ. Microglia: biology and pathology. *Acta Neuropathol*. 2010;119:89–105.
5. Biagini G, Baldelli E, Longo D, Contri MB, Guerrini U, Sironi L, Gelosa P, Zini I, Ragsdale DS, Avoli M. Proepileptic influence of a focal vascular lesion affecting entorhinal cortex–CA3 connections after status epilepticus. *J Neuropathol Exp Neurol*. 2008;67:687–701.
6. Charalampopoulos I, Remboutsika E, Margioris AN, Gravanis A. Neurosteroids as modulators of neurogenesis and neuronal survival. *Trends Endocrinol Metab.* 2008;19:300–307.
7. Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J Neurosci*. 2009;29:3974–3980.
8. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci*. 2007;10:1538–1543.
9. Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, Heikenwalder M, Brück W, Priller J, Prinz M. Microglia in the adult brain arise from Ly-6C^{hi}CCR2^{+} monocytes only under defined host conditions. *Nat Neurosci*. 2007;10:1544–1553.
10. Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci*. 2005;6:626–640.
11. Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci*. 2009;32:421–431.
12. Dwork AJ, Arango V, Underwood M, Ilievski B, Rosoklija G, Sackeim HA, Lisanby SH. Absence of histological lesions in primate models of ECT and magnetic seizure therapy. *Am J Psychiatry*. 2004;161: 576–578.
13. Kraig RP, Dong LM, Thisted R, Jaeger CB. Spreading depression increases immunohistochemical staining of glial fibrillary acidic protein. *J Neurosci*. 1991;11:2187–2198.
14. Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev*. 2006;86:1009–1031.
15. Santello M, Volterra A. Synaptic modulation by astrocytes via Ca^{2+}-dependent glutamate release. *Neuroscience*. 2009;158:253–259.
16. Halassa MM, Haydon PG. Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu Rev Physiol*. 2010;72:335–355.
17. Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY, Takano H, Moss SJ, McCarthy K, Haydon PG. Astrocytic purinergic signaling coordinates synaptic networks. *Science*. 2005;310: 113–116.
18. Yang Y, Ge W, Chen Y, Zhang Z, Shen W, Wu C, Poo M, Duan S. Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. *Proc Natl Acad Sci USA.* 2003;100:15194–15199.
19. Angulo MC, Kozlov AS, Charpak S, Audinat E. Glutamate released from glial cells synchronizes neuronal activity in the hippocampus. *J Neurosci*. 2004;24:6920–6927.
20. Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G. Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. *Neuron*. 2004;43:729–743.
21. Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M. An astrocytic basis of epilepsy. *Nat Med*. 2005;11:973–981.
22. Eid T, Williamson A, Lee TS, Petroff OA, de Lanerolle NC. Glutamate and astrocytes—key players in human mesial temporal lobe epilepsy? *Epilepsia*. 2008; 49(suppl 2):42–52.
23. Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science.* 1997;276:1699–1702.
24. Biagini G, Baldelli E, Longo D, Pradelli L, Zini I, Rogawski MA, Avoli M. Endogenous neurosteroids modulate epileptogenesis in a model of temporal lobe epilepsy. *Exp Neurol.* 2006;201:519–524.
25. Mellon SH, Griffin LD. Neurosteroids: biochemistry and clinical significance. *Trends Endocrinol Metab.* 2002;13:35–43.
26. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the $GABA_A$ receptor. *Nat Rev Neurosci.* 2005;6:565–575.
27. Wetherington J, Serrano G, Dingledine R. Astrocytes in the epileptic brain. *Neuron*. 2008;58:168–167.
28. Seth P, Koul N. Astrocyte, the star avatar: redefined. *J Biosci.* 2008;33:405–421.
29. Wang JM, Liu L, Irwin RW, Chen S, Brinton RD. Regenerative potential of allopregnanolone. *Brain Res Rev*. 2008;57:398–409.
30. Gómez-Gonzalo M, Losi G, Chiavegato A, Zonta M, Cammarota M, Brondi M, Vetri F, Uva L, Pozzan T, de Curtis M, Ratto GM, Carmignoto G. An excitatory loop with astrocytes contributes to drive neurons to seizure threshold. *PLoS Biol*. 2010;8:e1000352.

31. Zwain IH, Yen SS. Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. *Endocrinology.* 1999;140:3843–3852.
32. Sedlácek M, Korínek M, Petrovic M, Cais O, Adamusová E, Chodounská H, Vyklický L Jr. Neurosteroid modulation of ionotropic glutamate receptors and excitatory synaptic transmission. *Physiol Res*. 2008;57(suppl 3):S49–S57.
33. Kussius CL, Kaur N, Popescu GK. Pregnanolone sulfate promotes desensitization of activated NMDA receptors. *J Neurosci*. 2009;29:6819–6827.
34. Zheng P. Neuroactive steroid regulation of neurotransmitter release in the CNS: action, mechanism and possible significance. *Prog Neurobiol*. 2009;89:134–152.
35. Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ, Haydon PG, Coulter DA. Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nat Neurosci.* 2010;13:584–591.
36. Cui W, Allen ND, Skynner M, Gusterson B, Clark AJ. Inducible ablation of astrocytes shows that these cells are required for neuronal survival in the adult brain. *Glia*. 2001;34:272–282.
37. Myer DJ, Gurkoff GG, Lee SM, Hovda DA, Sofroniew MV. Essential protective roles of reactive astrocytes in traumatic brain injury. *Brain*. 2006;129: 2761–2772.
38. Biagini G, Frasoldati A, Fuxe K, Agnati LF. The concept of astrocyte-kinetic drug in the treatment of neurodegenerative diseases: evidence for L-deprenyl-induced activation of reactive astrocytes. *Neurochem Int*. 1994;25:17–22.
39. David Y, Cacheaux LP, Ivens S, Lapilover E, Heinemann U, Kaufer D, Friedman A. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *J Neurosci*. 2009;29:10588–10599.
40. Cacheaux LP, Ivens S, David Y, Lakhter AJ, Bar-Klein G, Shapira M, Heinemann U, Friedman A, Kaufer D. Transcriptome profiling reveals TGF-beta signaling involvement in epileptogenesis. *J Neurosci*. 2009;29:8927–8935.
41. Eid T, Lee TS, Thomas MJ, Amiry-Moghaddam M, Bjørnsen LP, Spencer DD, Agre P, Ottersen OP, de Lanerolle NC. Loss of perivascular aquaporin 4 may underlie deficient water and K^+ homeostasis in the human epileptogenic hippocampus. *Proc Natl Acad Sci USA*. 2005;102:1193–1198.
42. Oberheim NA, Tian GF, Han X, Peng W, Takano T, Ransom B, Nedergaard M. Loss of astrocytic domain organization in the epileptic brain. *J Neurosci*. 2008;28: 3264–3276.
43. Li T, Ren G, Lusardi T, Wilz A, Lan JQ, Iwasato T, Itohara S, Simon RP, Boison D. Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. *J Clin Invest*. 2008;118:571–582.
44. Balosso S, Maroso M, Sanchez-Alavez M, Ravizza T, Frasca A, Bartfai T, Vezzani A. A novel non-transcriptional pathway mediates the proconvulsive effects of interleukin-1β. *Brain*. 2008;131:3256–3265.
45. Kruse MS, Rey M, Barutta J, Coirini H. Allopregnanolone effects on astrogliosis induced by hypoxia in organotypic cultures of striatum, hippocampus, and neocortex. *Brain Res*. 2009;1303:1–7.
46. Guo Q, Sayeed I, Baronne LM, Hoffman SW, Guennoun R, Stein DG. Progesterone administration modulates AQP4 expression and edema after traumatic brain injury in male rats. *Exp Neurol*. 2006;198: 469–478.
47. He J, Evans CO, Hoffman SW, Oyesiku NM, Stein DG. Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. *Exp Neurol*. 2004;189:404–412.
48. Riazi K, Galic MA, Kuzmiski JB, Ho W, Sharkey KA, Pittman QJ. Microglial activation and TNFα production mediate altered CNS excitability following peripheral inflammation. *Proc Natl Acad Sci USA*. 2008;105: 17151–17156.
49. Herx LM, Yong VW. Interleukin-1β is required for the early evolution of reactive astrogliosis following CNS lesion. *J Neuropathol Exp Neurol*. 2001;60:961–971.
50. Rao RS, Prakash A, Medhi B. Role of different cytokines and seizure susceptibility: a new dimension towards epilepsy research. *Indian J Exp Biol*. 2009;47: 625–634.
51. Vanlandingham JW, Cekic M, Cutler S, Hoffman SW, Stein DG. Neurosteroids reduce inflammation after TBI through CD55 induction. *Neurosci Lett*. 2007; 425:94–98.
52. Corpéchot C, Robel P, Axelson M, Sjövall J, Baulieu EE. Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. *Proc Natl Acad Sci USA*. 1981;78:4704–4707.
53. Mellon SH, Deschepper CF. Neurosteroid biosynthesis: genes for adrenal steroidogenic enzymes are expressed in the brain. *Brain Res*. 1993;629:283–292.
54. Biagini G, Longo D, Baldelli E, Zoli M, Rogawski MA, Bertazzoni G, Avoli M. Neurosteroids and epileptogenesis in the pilocarpine model: evidence for a relationship between P450scc induction and length of the latent period. *Epilepsia.* 2009;50(suppl 1): 53–58.
55. Le Goascogne C, Robel P, Gouézou M, Sananès N, Baulieu EE, Waterman M. Neurosteroids: cytochrome $P\text{-}450_{scc}$ in rat brain. *Science.* 1987;237:1212–1215.
56. Shibuya K, Takata N, Hojo Y, Furukawa A, Yasumatsu N, Kimoto T, Enami T, Suzuki K, Tanabe N, Ishii H, Mukai H, Takahashi T, Hattori TA, Kawato S. Hippocampal cytochrome P450s synthesize brain neurosteroids which are paracrine neuromodulators of synaptic signal transduction. *Biochim Biophys Acta*. 2003;1619:301–316.
57. Iwahashi K, Ozaki HS, Tsubaki M, Ohnishi J, Takeuchi Y, Ichikawa Y. Studies of the immunohistochemical and biochemical localization of the cytochrome P-450scc-linked monooxygenase system in the adult rat brain. *Biochim Biophys Acta*. 1990;1035: 182–189.
58. Furukawa A, Miyatake A, Ohnishi T, Ichikawa Y. Steroidogenic acute regulatory protein (StAR) transcripts constitutively expressed in the adult rat central nervous system: colocalization of StAR, cytochrome $P\text{-}450_{SCC}$ (CYP XIA1), and 3β-hydroxysteroid dehydrogenase in the rat brain. *J Neurochem.* 1998;71: 2231–2238.
59. Kishimoto W, Hiroi T, Shiraishi M, Osada M, Imaoka S, Kominami S, Igarashi T, Funae Y. Cytochrome P450 2D catalyze steroid 21-hydroxylation in the brain. *Endocrinology.* 2004;145:699–705.
60. Melcangi RC, Celotti F, Castano P, Martini L. Differential localization of the 5 alpha-reductase and the 3 alpha-hydroxysteroid dehydrogenase in neuronal and glial cultures. *Endocrinology*. 1993;132: 1252–1259.

61. Agís-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, Guidotti A. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *Proc Natl Acad Sci USA*. 2006;103: 14602–14607.
62. Belelli D, Herd MB, Mitchell EA, Peden DR, Vardy AW, Gentet L, Lambert JJ. Neuroactive steroids and inhibitory neurotransmission: mechanisms of action and physiological relevance. *Neuroscience.* 2006;138:821–829.
63. Ukena K, Usui M, Kohchi C, Tsutsui K. Cytochrome P450 side-chain cleavage enzyme in the cerebellar Purkinje neuron and its neonatal change in rats. *Endocrinology.* 1998;139:137–147.
64. Le Goascogne C, Eychenne B, Tonon MC, Lachapelle F, Baumann N, Robel P. Neurosteroid progesterone is up-regulated in the brain of jimpy and shiverer mice. *Glia.* 2000;29:14–24.
65. Eid T, Du F, Schwarcz R. Ibotenate injections into the pre- and parasubiculum provide partial protection against kainate-induced epileptic damage in layer III of rat entorhinal cortex. *Epilepsia.* 2001;42: 817–824.
66. Biagini G, Panuccio G, Avoli M. Neurosteroids and epilepsy. *Curr Opin Neurol*. 2010;23:170–176.
67. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol*. 1972;32:281–294.
68. Abdel-Rahman A, Rao MS, Shetty AK. Nestin expression in hippocampal astrocytes after injury depends on the age of the hippocampus. *Glia*. 2004;47: 299–313.
69. Vega-Avelaira D, Moss A, Fitzgerald M. Age-related changes in the spinal cord microglial and astrocytic response profile to nerve injury. *Brain Behav Immun*. 2007;21:617–623.
70. Sierra A, Lavaque E, Perez-Martin M, Azcoitia I, Hales DB, Garcia-Segura LM. Steroidogenic acute regulatory protein in the rat brain: cellular distribution, developmental regulation and overexpression after injury. *Eur J Neurosci*. 2003;18:1458–1467.
71. Sierra A. Neurosteroids: the StAR protein in the brain. *J Neuroendocrinol*. 2004;16:787–793.
72. Mukai Y, Higashi T, Nagura Y, Shimada K. Studies on neurosteroids XXV. Influence of a 5α-reductase inhibitor, finasteride, on rat brain neurosteroid levels and metabolism. *Biol Pharm Bull*. 2008;31:1646–1650.
73. Concas A, Mostallino MC, Porcu P, Follesa P, Barbaccia ML, Trabucchi M, Purdy RH, Grisenti P, Biggio G. Role of brain allopregnanolone in the plasticity of γ-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. *Proc Natl Acad Sci USA*. 1998;95:13284–13289.
74. Ford MM, Nickel JD, Finn DA. Treatment with and withdrawal from finasteride alter ethanol intake patterns in male C57BL/6J mice: potential role of endogenous neurosteroids? *Alcohol.* 2005;37:23–33.
75. Lambert JJ, Cooper MA, Simmons RD, Weir CJ, Belelli D. Neurosteroids: endogenous allosteric modulators of $GABA_A$ receptors. *Psychoneuroendocrinology.* 2009;34(suppl 1):S48–S58.
76. Puia G, Santi MR, Vicini S, Pritchett DB, Purdy RH, Paul SM, Seeburg PH, Costa E. Neurosteroids act on recombinant human $GABA_A$ receptors. *Neuron*. 1990;4:759–765.
77. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. *Nat Rev Neurosci*. 2005;6:215–229.
78. Glykys J, Mann EO, Mody I. Which $GABA_A$ receptor subunits are necessary for tonic inhibition in the hippocampus? *J Neurosci*. 2008;28:1421–1426.
79. Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen RW, Homanics GE. Attenuated sensitivity to neuroactive steroids in γ-aminobutyrate type A receptor delta subunit knockout mice. *Proc Natl Acad Sci USA*. 1999;96:12905–12910.
80. Benini R, Avoli M. Rat subicular networks gate hippocampal output activity in an in vitro model of limbic seizures. *J Physiol*. 2005;566:885–900.
81. Panuccio G, D'Antuono M, de Guzman P, De Lannoy L, Biagini G, Avoli M. In vitro ictogenesis and parahippocampal networks in a rodent model of temporal lobe epilepsy. *Neurobiol Dis*. 2010;39:372–380.

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SECTION 4

Epilepsy Genes and Development

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Chapter 50

Genetic Epidemiology and Gene Discovery in Epilepsy

Ruth Ottman
Neil Risch

Over the last two decades, more than 20 genes with a major effect on the risk for human epilepsy have been identified, providing important clues to pathogenic mechanisms and enabling some patients to discover the cause of their disorder.[1] However, the genes identified so far affect risk in a very small proportion of patients—primarily those from families consistent with Mendelian modes of inheritance. Most epilepsies occur in the absence of a significant family history, and identifying and characterizing the genetic mechanisms in these *complex epilepsies* is a major challenge for the next decade.[2] Here we discuss the meaning of *complex inheritance* as it applies to epilepsy, findings from current research, and approaches likely to be advantageous for gene identification in these forms of epilepsy.

EPILEPSY AS A COMPLEX DISEASE

Epilepsy is familial and genetically influenced: risk is increased two- to fourfold in the first-degree relatives of people with epilepsy of unknown cause (either idiopathic generalized epilepsy [IGE] or nonlesional focal epilepsy [NFE])[3–5]; twin studies consistently show higher concordance in monozygotic than dizygotic pairs[6–9]; and a large number of genes with a major effect on susceptibility has already been identified.[10–12] Yet, most people with epilepsy have no affected relatives. For example, in the Epilepsy Family Study of Columbia University (EFSCU),[4,5,13] we collected family history information on 1957 people with epilepsy ascertained from voluntary organizations without regard to their family histories. The proportion of subjects with a positive family history (one or more first-degree relative with epilepsy) was 15% in those with IGE and 12% in those with NFE. Most of those with a family history had just one affected relative, and very few families appeared consistent with a Mendelian model.[14]

Several mechanisms could account for the apparent paradox that a disorder with a genetic component usually occurs sporadically (i.e., in

the absence of a family history). First, as with many common disorders, inheritance in the majority of cases is likely to be multifactorial, with most of the genetic influence consisting of complex disease genes—that is, genetic variants that individually have a small or modest effect and act in concert with each other and with (as yet unspecified) environmental factors in their influence on epileptogenesis. In this model, relatives will be affected only if they inherit sufficient pathogenic variants at multiple disease loci and/or multiple nongenetic risk factors. The allele frequency distribution of these contributing loci can range from common to rare, with penetrances from low to moderate. We say *moderate* because genes with high penetrance are considered Mendelian and tend to lead to strong segregation patterns in families. The distribution and types of genetic variants underlying complex epilepsies are unknown, but have an important influence on optimal study designs for gene identification, as we discuss below.

While family studies provide the basis for examining genes that are inherited, some cases may also have a genetic contribution but not due to inheritance. These result from de novo germline mutations and somatic mutations occurring in critical brain regions. The importance of de novo mutations is already well documented. They are extremely important in Dravet syndrome, where more than 70% of patients have *SCN1A* mutations and more than 95% of these arise de novo.[15,16] Such de novo mutations most often account for a high proportion of severe early-onset forms of epilepsy in which affected subjects seldom reproduce (and hence mutations are not generally inherited). However, rare de novo mutations have also been identified in genes identified in Mendelian epilepsies, in some isolated cases phenotypically similar to those in the families in which the genes were found.[17–22] Also, copy number variants (CNVs) have recently been associated with complex epilepsies and many of these occur de novo, although associated risks appear to be moderate rather than Mendelian.[23]

As is true for many complex diseases, there is no clear dividing line between Mendelian and complex epilepsy genes. Many genes likely influence risk for epilepsy, with wide variation in the magnitude of their effects. As mentioned above, genes with larger effects (higher penetrance) produce Mendelian patterns of inheritance, whereas those with smaller effects (lower penetrance) produce complex patterns. The genes identified so far have generally fallen in the high range of this continuum, which is why they were easiest to identify. However, even for many of these genes, penetrance is incomplete, suggesting that other genes or environmental factors influence their effects. Also, similar pathogenic mechanisms may be involved in Mendelian and complex epilepsies, and the discovery that most of the Mendelian genes identified so far have encoded voltage-gated or ligand-gated ion channels points to this class of genes as prime candidates for the genetically complex epilepsies.

Complexities in Phenotype Definition: Lessons from Mendelian Epilepsies

Evidence from Mendelian epilepsy syndromes provides important information about complexity in the relationship of genotype to phenotype that is likely to be relevant for non-Mendelian epilepsies. First, genetic heterogeneity is extensive and occurs at multiple levels. At the highest level, different genetic mechanisms—Mendelian, genetically complex, or even acquired—can cause the same syndrome, making it very difficult, if not impossible, to classify syndromes according to genetic mechanisms in any consistent way.

For example, mutations in the leucine-rich, glioma inactivated 1 gene (*LGI1*) are found in approximately 50% of families with autosomal dominant partial epilepsy with auditory symptoms (ADPEAF), defined as families containing two or more individuals with temporal lobe epilepsy with ictal auditory symptoms or receptive aphasia.[24] However, very few patients with the clinical features that define this syndrome come from families with autosomal dominant inheritance; the vast majority are sporadic, and de novo mutations in *LGI1* are found in <2% of these sporadic cases.[19,20,25–27] The same phenotype is observed in other patients with lesional epilepsies.[28] Thus, this syndrome is due to dominant mutations in *LGI1* (or other unidentified genes) in a few rare cases and genetically complex or acquired in most.

The same principle applies to the IGEs. Recurrence risk is higher in siblings than in most other forms of epilepsy; in a recent population-based study, the risk of epilepsy to age 40 was 7.6% in the siblings of incident IGE patients compared with 4.6% for siblings of all incident patients.[29] This higher recurrence risk is consistent with a range of different underlying genetic and nongenetic models. Mutations in *GABRA1* or *EFHC1* have been identified in some unusual families with a very high incidence of juvenile myoclonic epilepsy,[30,31] and other monogenic forms of IGE may yet be discovered. However, like other forms of epilepsy, most IGEs are presumably genetically complex, caused by a mixture of genetic and nongenetic factors. In three major twin studies that examined IGEs specifically, concordance rates in monozygotic twins ranged from 65% to 80% and in dizygotic twins from 0% to 33%.[6,7,9]

The International League Against Epilepsy Commission on Classification and Terminology recently recommended that the name *genetic generalized epilepsy* be used instead of *idiopathic generalized epilepsy* for these disorders.[32] Although the Commission explicitly stated that the use of this term "does not exclude the possibility that environmental factors (outside the individual) may contribute to the expression of disease," we believe it is misleading to incorporate the term *genetic* into the name of a disorder in the absence of Mendelian inheritance patterns in families or molecular information about the specific genes involved. Use of this term implies that IGE is exclusively genetic (which is obviously incorrect based on the evidence from monozygotic twins) and suggests that genetic forms of generalized epilepsy can be distinguished clinically from nongenetic forms.

At the second level of genetic heterogeneity, within epilepsies with clear-cut Mendelian inheritance, mutations in different genes (often encoding different subunits of the same receptor) have been found to cause the same syndrome in different families (locus heterogeneity).[2,11] At the third level, in families with a mutation in the same gene, the specific molecular change usually varies among families (allelic heterogeneity).

A second complication is variable expressivity: mutations in a single gene can produce different epilepsy phenotypes in different individuals. The best example of this is genetic epilepsy with febrile seizures plus (GEFS+), in which the phenotypes within a family with a single mutation in *SCN1A* can range from typical febrile seizures to febrile seizures plus (i.e., febrile seizures persisting beyond age 6 or accompanied by afebrile generalized tonic seizures), IGEs, temporal lobe epilepsy, myoclonic-astatic epilepsy, or Dravet syndrome.[33,34] As with complex inheritance, this variability is likely to result from the modifying effects of other genes or environmental factors.

GENE IDENTIFICATION IN THE COMPLEX EPILEPSIES

Positional Cloning

The armamentarium of methods for gene identification in complex disorders includes an array of approaches (Table 50–1). Almost all of the genes identified so far in the epilepsies were found using positional cloning: linkage analysis followed by sequencing of the genes in the chromosomal regions with evidence for cosegregation with disease in families. This is the optimal approach for Mendelian disorders, with a long history of success for a wide range of diseases. However, it has limited power with complex disorders, in which the effect of the gene sought through linkage may be too low to be detected by this approach.[35] Also, practical problems complicate the design of linkage studies in complex diseases, such as the scarcity of families containing multiple affected individuals and uncertainty about how to define the phenotype and about what mode of inheritance should be assumed in the analysis.

Substantial efforts have focused on gene mapping in the IGEs, but few findings have been replicated, probably because of a combination of phenotypic variability, genetic heterogeneity, and low statistical power. Linkage disequilibrium mapping at two linkage regions on chromosomes 6p21.3 and 18q21.1 suggested two potential susceptibility genes (*BRD2*, *ME2*) predisposing to common IGE subtypes,[36,37] but some studies have not confirmed these findings[38–42] and causative mutations have not yet been identified in these genes.

Table 50–1 **Methods for Gene Identification in Genetically Complex Epilepsies**

Method	Basic Strategy	Advantages and Disadvantages	Application to Epilepsy
Positional cloning	(1) Localize a disease gene to a small chromosomal region by evaluating cosegregation of genetic markers with disease within families; (2) sequence genes in the linkage region to identify the mutation	Excellent for identification of Mendelian genes; low power for complex disease genes	Most previously discovered genes found with this approach
Association study	Evaluate difference in allele frequency between affected and unaffected individuals	Better power than positional cloning for genes with low penetrance; susceptible to population stratification; not applicable to rare variants	
Candidate genes	Restricted to variants within genes hypothesized to affect the disorder	Good if biology is known, but pathogenic variants and genes may be missed by restriction to candidates	Large number of studies but few replicated findings
Genome-wide SNPs	SNP evaluated genome-wide to detect either causal variants or variants in linkage disequilibrium with causal variants	No gene assumptions; powerful for common moderate-risk alleles but not for rare high-risk variants	One negative published study to date; others currently in progress
Copy number variants	SNP typing or massively parallel sequencing used to identify small deletions or duplications with increased frequency in affected individuals	Increased chance of functional significance; genome-wide detection strategy	Several new findings, but associated risks remain to be determined
Massively parallel sequencing	Next-generation high-throughput sequencing carried out genome-wide to detect disease-associated variants	Applicable to rare variants with high risk; large numbers of variants detected; methods for statistical analysis under development	
Whole genome	Covers the full genomic sequence	Still relatively expensive (but cost dropping)	Studies underway
Whole exome	Restricted to protein-coding sequences	Much less expensive and focused on genomic regions of high functionality but may miss some important variants	Studies underway

Allelic Association Studies

Allelic association studies are aimed at detecting genetic variants (usually single nucleotide polymorphisms [SNPs]) that are more common in people with epilepsy than in unaffected persons from the same population. They have greater statistical power for the detection of genes with small effect[35] and do not require families with multiple affected individuals. While most studies have been based on a case-control design, affected individuals with parents (triads) have also been used for early-onset disorders, testing for transmission disequilibrium to the affected child.[43] In the design and interpretation of case-control studies (but not triads), the potential for confounding due to population stratification must be considered. It arises when the cases and controls in a study have different genetic ancestries, and the ancestral groups differ in their allelic distributions, so that cases and controls differ in the frequency of a SNP for reasons unrelated to the disease. While triads require no formal correction for confounding due to stratification, for case-control studies several methods have been developed to control for this potential confounding, including genomic control,[44] structured association tests,[45] and, more recently, the use of principal components analysis to define the major sources of population structure and then adjust for them in a logistic regression analysis (for discrete outcomes) or linear regression (for continuous outcomes).[46] The large number of SNPs typically genotyped in a genome-wide association (GWA) study makes these approaches very powerful for detecting and adjusting for population structure. Once population stratification has been taken into account, a significantly increased frequency of a variant in people with epilepsy would suggest that it either directly affects risk or is in linkage disequilibrium with a nearby causal variant.

Most of the association studies carried out in epilepsy so far have been relatively small, candidate gene-based studies, and few findings have been confirmed. Many of the published studies have had methodological limitations such as small sample size, lack of control for potential population stratification, and failure to adjust for multiple statistical tests.[47] One large multisite study of common variations in 279 candidate genes failed to identify clear associations across multiple sites.[48] However, the association findings are more convincing for some candidate genes, such as the calcium channel subunit gene *CACNA1H*.[49–51]

Genome-wide association studies have yielded confirmed associations of common variants with a wide range of complex diseases,[52] although in most cases the associated variants explain little of the heritability of the diseases in which they have been found.[53,54] The epilepsy field has lagged behind others in the investigation of common genetic variation. Only one GWA study has been published so far and that study, which included nearly 4000 European patients with a wide array of nonlesional and lesional focal epilepsies, was essentially negative; none of the variants examined reached genome-wide significance.[55] Power was sufficient to exclude variants with an odds ratio of 1.3 or greater, and thus the results argue against common genetic effects of this magnitude that are shared across different (and likely heterogeneous) forms of focal epilepsy in European populations. Although sample sizes of specific subgroups (e.g., nonlesional as opposed to lesional focal epilepsies, in which family studies show a stronger genetic influence[4]) will be smaller, leading to potentially reduced power, the effect sizes are likely to be larger in these more homogeneous subgroups, and so this type of analysis is still worthwhile to pursue. Genome-wide association studies are underway in other forms of epilepsy, such as the IGEs, and their success in identifying common variants that influence susceptibility remains to be seen.

However, studies of structural variation have made progress in identifying rare genomic variants that contribute to the risk for genetically complex epilepsies.[23] In several studies, approximately 1% of individuals with IGE were found to carry a rare microdeletion at chromosome 15q13.3 that had previously been identified in individuals with intellectual disability, schizophrenia, and autism.[56–58] Five additional candidate microdeletions that had also been found in other neuropsychiatric disorders, at chromosomes 1q21.1, 15q11.2, 16p11.2, 16p13.11, and 22q11.2, were observed collectively in 1.8% of IGE cases, with a significant excess of the 15q11.2 and 16p13.11 variants.[58] A genome-wide analysis

of CNVs in a wide range of epilepsies (not only IGE) found an excess of large deletions (>100 kb) at chromosome 16p13.11 in cases with highly variable phenotypes.[59] Another genome-wide study of patients with a range of idiopathic epilepsy types found deletions at chromosome 15q11.2, 15q13.3, or 16p13.11 in approximately 3% of patients.[60] In studies with available family data, a substantial proportion of these microdeletions have been found to be de novo, and among those that are inherited, cosegregation with epilepsy in families appears to be inconsistent, with some affected family members failing to carry the variant present in the proband.[56–58] This lack of cosegregation with disease in families, despite very high estimated odds ratios for the variants (e.g., an estimated odds ratio of 68 for the 15q13.3 microdeletion),[57] is paradoxical and requires explanation. The associations of these microdeletions with a wide range of neuropsychiatric disorders, including intellectual disability, autism, schizophrenia, and epilepsy, raise intriguing questions about shared pathogenic mechanisms for these disorders.

Massively Parallel Sequencing

Although the findings from GWA studies are not yet available for most forms of epilepsy, evidence from other complex disorders suggests that the individual contribution of common variants to the overall genetic component of the epilepsies is likely to be small. On the other hand, the findings from studies of structural variation suggest that rare genetic variants play an important role in the genetic architecture of the epilepsies. The potential for GWA studies to identify rare variants (i.e., less than 2% in frequency) is limited because most are not represented, either directly or indirectly (through high linkage disequilibrium), on the panel of SNP genotypes investigated. However, rare variants can now be investigated using massively parallel sequencing approaches, so-called *next-generation sequencing* (NGS), applied either to whole genomes or to protein-coding regions (whole exome sequencing) at costs that are not prohibitive (at least on a small scale, to date).[61] Several recent studies using this approach have succeeded in identifying genes for Mendelian disorders, using very small numbers of patients or families.[62] Most of these studies focused on extremely rare Mendelian disorders with high penetrance—an optimal situation for disease gene detection (just as it is for linkage analysis and positional cloning). However, application of NGS is expanding rapidly to include disorders with more complex features such as locus heterogeneity and uncertainty about phenotype definition.[63,64] We are currently conducting a study of whole genome sequencing in multiplex epilepsy families,[65] and another major collaborative NGS study in the epilepsies has recently begun.

One of the challenges in this field concerns the development of study designs and statistical approaches for analysis of the large number of variants identified through NGS.[66] The strategy used in our current study of multiplex epilepsy families is to sequence two affected individuals in each family, selected to be as distantly related as possible, and "connected" by family branches that also contain affected individuals. The underlying assumption is that families containing multiple affected individuals are likely to harbor genomic variants with a strong risk-raising effect, and affected individuals in the same family are likely to carry the same pathogenic variants. Since distant relatives are unlikely to share rare variants by chance, the sharing of variants between the sequenced family members can be used as a "filter" to reduce the number of potentially causative variants for further analysis. Restriction to distant relatives connected by family branches containing other affected individuals protects against the possibility that the two relatives have different genetic causes of their epilepsy.

So far, we have completed whole genome sequencing to an average coverage of 38× in two affected individuals from each of nine families with various forms of nonacquired epilepsy (average 6.2 affected per family).[65] The total number of variants averaged about 4.4 million per individual, and restriction to rare, shared, potentially functional (i.e., missense, protein truncating, or splice site-disrupting) variants reduced the number to an average of 108 per family. Our planned follow-up studies include genotyping in other family members to evaluate cosegregation with epilepsy and case-control analyses in larger cohorts.

Cosegregation with disease is the hallmark of rare high-penetrance variants, so multiplex

families such as these are indispensable for validating any such findings. However, linkage analysis in these same pedigrees did not produce convincing evidence for any particular chromosomal region(s), and so if rare, high-risk variants are involved, there must be extreme locus heterogeneity to account for the negative linkage results. Rare variants with moderate to low penetrance will be more difficult to identify and validate, as these will not segregate in families, similar to common, low-penetrance variants.

PHENOTYPE DEFINITION

One of the most challenging issues for genetic research on epilepsy is the extreme clinical and etiological heterogeneity of the disorder. The epilepsies, defined broadly as recurrent unprovoked seizures, include a wide array of different syndromes presumed to have different pathogenic mechanisms. However, the extent to which the different clinical entities also differ with respect to their genetic contributions remains unclear, and hence it is uncertain which features should be used to separate the epilepsies into subgroups likely to share susceptibility genes. This lack of clear information about the relationship of phenotype to genotype dramatically impedes efforts at gene discovery because it results in samples with uncontrolled heterogeneity and reduced statistical power.[67]

Here we review two types of studies that have been used to advantage to elucidate shared and distinct genetic influences on different clinically defined subsets of epilepsy. Each of these approaches can also be used to study the possibility of shared genetic susceptibility to epilepsy and other disorders, such as migraine or depression.

Familial Aggregation Studies

Familial aggregation studies are epidemiological designs that examine familial risks in a population context. A sample of probands with epilepsy is ascertained and divided into subsets based on syndrome or other clinical features (age at onset, seizure type, etc.). Then in the first stage of analysis, the risk of all types of epilepsy is examined in the relatives of different subgroups of probands and compared with the risk in the population or in the relatives of unaffected controls. The results of this analysis provide information about the relative genetic contribution to different types of epilepsy and guidance about which probands are likely to be most informative for molecular genetic studies. They also provide extremely useful information for genetic counseling.[29,68,69] Family studies have provided evidence for several important predictors of familial risk. First, probably the most consistent observation in the genetic epidemiology of the epilepsies is the *maternal effect*: the risk for epilepsy is approximately twice as high in the offspring of affected women as in the offspring of affected men.[70–72] Although this phenomenon remains unexplained, several possible causes have been excluded, such as effects on offspring epilepsy risk of intrauterine exposure to antiepileptic medications or maternal seizures or X-linked genetic models (since the risks are similar in male and female offspring).[70,71] Second, the risk of epilepsy is increased in the relatives of probands with epilepsy of unknown cause (either IGE or NFE), but not in the relatives of probands with symptomatic epilepsy (defined as epilepsy associated with structural or metabolic insults to the central nervous system, such as severe head trauma, stroke, brain tumor, etc.).[4] This suggests either that the genetic contributions are minimal in symptomatic epilepsies or that the genes involved raise susceptibility only in the presence of the (uncommon) risk factors and thus the majority of relatives, in whom the risk factors are absent, do not have an increased risk. Third, the increased familial risk is greatest when the relatives are younger than 35 years and also if the proband has onset before age 35.[4] Fourth, recurrence risk patterns differ, depending on the relationship to the proband and the proband's epilepsy type (IGE vs. NFE). In data from EFSCU, risks in parents and siblings were greater if the proband had IGE versus NFE.[5,73] However, this pattern was not observed in offspring, either in EFSCU[5,73] or in a population-based study in Rochester, Minnesota.[74]

In the second stage of analysis, the risk for specific clinically defined subtypes of epilepsy

(or other disorders) is examined in the relatives of specific subgroups of probands. If different genes influence the risk for different types of epilepsy (distinct genetic effects), risk in the relatives should be increased only for the same type of epilepsy as in the proband. In contrast, if the same genes influence the risk for different types of epilepsy (shared genetic effects), the increased risk in the relatives will not be restricted to the same type as in the proband. In two previous family studies, the risk for NFE was significantly increased in the relatives of probands with IGE, providing evidence for shared genetic influences on IGE and NFE.[73,74]

Family Concordance Studies

Hypotheses about shared and distinct genetic influences on different clinically defined subsets of epilepsy can also be tested using family concordance studies.[75–77] As opposed to familial aggregation studies, in which a systematic sampling scheme is used to ascertain probands, these studies assess the concordance of epilepsy types (syndromes, seizure types, or subsets defined by other clinical features) in sets of families containing multiple affected individuals, which are often ascertained unsystematically. The rationale for family concordance analysis is that if some of the genetic influences on different epilepsy types are distinct, families will tend to be concordant, that is, the proportion of families in which all affected individuals have the same type of epilepsy will exceed that expected by chance. The expected proportion of concordant families is assessed using a permutation approach, taking into account the overall proportion of individuals with a given clinical feature in the dataset, the number of affected individuals in each family, and the proband's epilepsy type.[75] The results of research using this approach have provided evidence for distinct genetic influences on IGE and NFE[76] and, within the IGEs, for distinct genetic influences on myoclonic and absence seizures.[77–79]

With regard to the shared and distinct genetic influences on IGE and NFE, the results of familial aggregation studies and family concordance studies appear to differ, but this may be an artifact of study design. In familial aggregation studies, the null hypothesis is that all genetic effects are distinct. This hypothesis is rejected (leading to the conclusion that some genetic effects are shared) when a significant increase in risk in relatives is observed for an epilepsy type different from that in the proband. In family concordance studies, the null hypothesis is exactly the opposite: that all genetic effects are shared. This hypothesis is rejected (leading to the conclusion that some genetic effects are distinct) when families show excess concordance for a specific epilepsy type. Taken together, the results of these two study designs indicate that there are both shared and distinct genetic influences on generalized and focal epilepsies, as has already been observed, for example, in families with GEFS+.

RECOMMENDATIONS FOR FUTURE STUDIES

What approaches are most promising for future studies of the genetic contributions to the complex epilepsies? First, efforts to identify, clinically characterize, and carry out positional cloning studies in families with Mendelian epilepsies should continue. Although Mendelian epilepsy syndromes are rare, identification of the genes that cause them can provide extremely important information about basic epileptogenic mechanisms and suggest candidate genes to be investigated in complex epilepsies. Genome-wide association studies already underway should soon provide evidence about the importance of common variants in the epilepsies. Current evidence suggests that additional rare variants are likely to contribute to genetic susceptibility in epilepsy, and NGS has the greatest promise for identifying them. However, the failure to identify additional loci harboring high-risk variants through linkage analysis, in numerous multiplex families, suggests either a limited role for additional high-penetrance mutations or extreme locus heterogeneity. Collaborative efforts, rigorous phenotyping, and study designs that allow analysis of subgroups predicted to share susceptibility genes are crucial to maximize the likelihood of success.

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REFERENCES

1. Ottman R, Hirose S, Jain S, Lerche H, Lopes-Cendes I, Noebels JL, Serratosa J, Zara F, Scheffer IE. Genetic testing in the epilepsies—report of the ILAE Genetics Commission. *Epilepsia*. 2010;51:655–670.
2. Ottman R. Analysis of genetically complex epilepsies. *Epilepsia*. 2005;46(suppl 10):7–14.
3. Annegers JF, Hauser WA, Anderson VE, Kurland LT. The risks of seizure disorders among relatives of patients with childhood onset epilepsy. *Neurology*. 1982;32: 174–179.
4. Ottman R, Annegers JF, Risch N, Hauser WA, Susser M. Relations of genetic and environmental factors in the etiology of epilepsy. *Ann Neurol*. 1996;39:442–449.
5. Ottman R, Lee JH, Risch N, Hauser WA, Susser M. Clinical indicators of genetic susceptibility to epilepsy. *Epilepsia*. 1996;37:353–361.
6. Vadlamudi L, Andermann E, Lombroso CT, Schachter SC, Milne RL, Hopper JL, Andermann F, Berkovic SF. Epilepsy in twins: insights from unique historical data of William Lennox. *Neurology*. 2004;62: 1127–1133.
7. Berkovic SF, Howell RA, Hay DA, Hopper JL. Epilepsies in twins: genetics of the major epilepsy syndromes. *Ann Neurol*. 1998;43:435–445.
8. Corey LA, Berg K, Pellock JM, Solaas MH, Nance WE, DeLorenzo RJ. The occurrence of epilepsy and febrile seizures in Virginian and Norwegian twins. *Neurology*. 1991;41:1433–1436.
9. Kjeldsen MJ, Corey LA, Christensen K, Friis ML. Epileptic seizures and syndromes in twins: the importance of genetic factors. *Epilepsy Res*. 2003;55: 137–146.
10. Reid CA, Berkovic SF, Petrou S. Mechanisms of human inherited epilepsies. *Prog Neurobiol*. 2009;87: 41–57.
11. Ottman R, Hirose S, Jain S, Lerche H, Lopes-Cendes I, Noebels JL, Serratosa J, Zara F, Scheffer IE. Genetic testing in the epilepsies—Report of the ILAE Genetics Commission. *Epilepsia*. 2010;51:655–670.
12. Helbig I, Scheffer IE, Mulley JC, Berkovic SF. Navigating the channels and beyond: unravelling the genetics of the epilepsies. *Lancet Neurol*. 2008;7: 231–245.
13. Ottman R, Susser M. Data collection strategies in genetic epidemiology: The Epilepsy Family Study of Columbia University. *J Clin Epidemiol*. 1992;45: 721–727.
14. Ottman R, Hauser WA, Barker-Cummings C, Lee JH, Risch N. Segregation analysis of cryptogenic epilepsy and an empirical test of the validity of the results. *Am J Hum Genet*. 1997;60:667–675.
15. Mulley JC, Scheffer IE, Petrou S, Dibbens LM, Berkovic SF, Harkin LA. SCN1A mutations and epilepsy. *Hum Mutat*. 2005;25:535–542.
16. Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, Sadleir LG, Andermann E, Gill D, Farrell K, Connolly M, Stanley T, Harbord M, Andermann F, Wang J, Batish SD, Jones JG, Seltzer WK, Gardner A, Sutherland G, Berkovic SF, Mulley JC, Scheffer IE. The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain*. 2007;130: 843–852.
17. Phillips HA, Marini C, Scheffer IE, Sutherland GR, Mulley JC, Berkovic SF. A de novo mutation in sporadic nocturnal frontal lobe epilepsy. *Ann Neurol*. 2000;48:264–267.
18. Bertrand D, Elmslie F, Hughes E, Trounce J, Sander T, Bertrand S, Steinlein OK. The CHRNB2 mutation I312M is associated with epilepsy and distinct memory deficits. *Neurobiol Dis*. 2005;20:799–804.
19. Bisulli F, Tinuper P, Scudellaro E, Naldi I, Bagattin A, Avoni P, Michelucci R, Nobile C. A de novo LGI1 mutation in sporadic partial epilepsy with auditory features. *Ann Neurol*. 2004;56:455–456.
20. Michelucci R, Mecarelli O, Bovo G, Bisulli F, Testoni S, Striano P, Striano S, Tinuper P, Nobile C. A de novo LGI1 mutation causing idiopathic partial epilepsy with telephone-induced seizures. *Neurology*. 2007;68:2150–2151.
21. Ishii A, Fukuma G, Uehara A, Miyajima T, Makita Y, Hamachi A, Yasukochi M, Inoue T, Yasumoto S, Okada M, Kaneko S, Mitsudome A, Hirose S. A de novo KCNQ2 mutation detected in non-familial benign neonatal convulsions. *Brain Dev*. 2009;31:27–33.
22. Claes LR, Ceulemans B, Audenaert D, Deprez L, Jansen A, Hasaerts D, Weckx S, Claeys KG, Del-Favero J, Van Broeckhoven C, De Jonghe P. De novo KCNQ2 mutations in patients with benign neonatal seizures. *Neurology*. 2004;63:2155–2158.
23. Scheffer IE, Berkovic SF. Copy number variants—an unexpected risk factor for the idiopathic generalized epilepsies. *Brain*. 2010;133:7–8.
24. Ottman R, Winawer MR, Kalachikov S, Barker-Cummings C, Gilliam TC, Pedley TA, Hauser WA. LGI1 mutations in autosomal dominant partial epilepsy with auditory features. Neurology. 2004;62: 1120–1126.
25. Bisulli F, Tinuper P, Avoni P, Striano P, Striano S, d'Orsi G, Vignatelli L, Bagattin A, Scudellaro E, Florindo I, Nobile C, Tassinari CA, Baruzzi A, Michelucci R. Idiopathic partial epilepsy with auditory features (IPEAF): a clinical and genetic study of 53 sporadic cases. *Brain*. 2004;127:1343–1352.
26. Flex E, Pizzuti A, Di Bonaventura C, Douzgou S, Egeo G, Fattouch J, Manfredi M, Dallapiccola B, Giallonardo AT. LGI1 gene mutation screening in sporadic partial epilepsy with auditory features. *J Neurol*. 2005;252:62–66.
27. Michelucci R, Pasini E, Nobile C. Lateral temporal lobe epilepsies: clinical and genetic features. *Epilepsia*. 2009;50(suppl 5):52–54.

28. Florindo I, Bisulli F, Pittau F, Naldi I, Striano P, Striano S, Michelucci R, Testoni S, Baruzzi A, Tinuper P. Lateralizing value of the auditory aura in partial seizures. *Epilepsia*. 2006;47(suppl 5):68–72.
29. Peljto A, Barker-Cummings C, Leibson CL, Vasoli VM, Hauser WA, Buchhalter J, Ottman R. Estimates of familial risk for genetic counseling in the epilepsies. Abstract 3.304. American Epilepsy Society Annual Meeting, 2010; www.aes.org.
30. Cossette P, Liu L, Brisebois K, Dong H, Lortie A, Vanasse M, Saint-Hilaire JM, Carmant L, Verner A, Lu WY, Wang YT, Rouleau GA. Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nat Genet*. 2002;31:184–189.
31. Suzuki T, Delgado-Escueta AV, Aguan K, Alonso ME, Shi J, Hara Y, Nishida M, Numata T, Medina MT, Takeuchi T, Morita R, Bai D, Ganesh S, Sugimoto Y, Inazawa J, Bailey JN, Ochoa A, Jara-Prado A, Rasmussen A, Ramos-Peek J, Cordova S, Rubio-Donnadieu F, Inoue Y, Osawa M, Kaneko S, Oguni H, Mori Y, Yamakawa K. Mutations in EFHC1 cause juvenile myoclonic epilepsy. *Nat Genet*. 2004;36: 842–849.
32. Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Moshe SL, Nordli D, Plouin P, Scheffer IE. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia*. 2010;51:676–685.
33. Singh R, Scheffer IE, Crossland K, Berkovic SF. Generalized epilepsy with febrile seizures plus: a common childhood-onset genetic epilepsy syndrome. *Ann Neurol*. 1999;45:75–81.
34. Mulley JC, Scheffer IE, Petrou S, Berkovic SF. Channelopathies as a genetic cause of epilepsy. *Curr Opin Neurol*. 2003;16:171–176.
35. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science*. 1996;273: 1516–1517.
36. Pal DK, Evgrafov OV, Tabares P, Zhang F, Durner M, Greenberg DA. BRD2 (RING3) is a probable major susceptibility gene for common juvenile myoclonic epilepsy. *Am J Hum Genet*. 2003;73:261–270.
37. Greenberg DA, Cayanis E, Strug L, Marathe S, Durner M, Pal DK, Alvin GB, Klotz I, Dicker E, Shinnar S, Bromfield EB, Resor S, Cohen J, Moshe SL, Harden C, Kang H. Malic enzyme 2 may underlie susceptibility to adolescent-onset idiopathic generalized epilepsy. *Am J Hum Genet*. 2005;76:139–146.
38. Lorenz S, Taylor KP, Gehrmann A, Becker T, Muhle H, Gresch M, Tauer U, Sander T, Stephani U. Association of BRD2 polymorphisms with photoparoxysmal response. *Neurosci Lett*. 2006;400:135–139.
39. Cavalleri GL, Walley NM, Soranzo N, Mulley J, Doherty CP, Kapoor A, Depondt C, Lynch JM, Scheffer IE, Heils A, Gehrmann A, Kinirons P, Gandhi S, Satishchandra P, Wood NW, Anand A, Sander T, Berkovic SF, Delanty N, Goldstein DB, Sisodiya SM. A multicenter study of BRD2 as a risk factor for juvenile myoclonic epilepsy. *Epilepsia*. 2007;48:706–712.
40. de Kovel CG, Pinto D, de Haan GJ, Kasteleijn-Nolst Trenite DG, Lindhout D, Koeleman BP. Association analysis of BRD2 (RING3) and epilepsy in a Dutch population. *Epilepsia*. 2007;48:2191–2192.
41. Layouni S, Buresi C, Thomas P, Malafosse A, Dogui M. BRD2 and TAP-1 genes and juvenile myoclonic epilepsy. *Neurol Sci*. 2010;31:53–56.
42. Lenzen KP, Heils A, Lorenz S, Hempelmann A, Sander T. Association analysis of malic enzyme 2 gene polymorphisms with idiopathic generalized epilepsy. *Epilepsia*. 2005;46:1637–1641.
43. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet*. 1993;52:506–516.
44. Devlin B, Roeder K, Wasserman L. Genomic control, a new approach to genetic-based association studies. *Theor Popul Biol*. 2001;60:155–166.
45. Pritchard JK, Donnelly P. Case-control studies of association in structured or admixed populations. *Theor Popul Biol*. 2001;60:227–237.
46. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38:904–909.
47. Tan NC, Mulley JC, Berkovic SF. Genetic association studies in epilepsy: "the truth is out there." *Epilepsia*. 2004;45:1429–1442.
48. Cavalleri GL, Weale ME, Shianna KV, Singh R, Lynch JM, Grinton B, Szoeke C, Murphy K, Kinirons P, O'Rourke D, Ge D, Depondt C, Claeys KG, Pandolfo M, Gumbs C, Walley N, McNamara J, Mulley JC, Linney KN, Sheffield LJ, Radtke RA, Tate SK, Chissoe SL, Gibson RA, Hosford D, Stanton A, Graves TD, Hanna MG, Eriksson K, Kantanen AM, Kalviainen R, O'Brien TJ, Sander JW, Duncan JS, Scheffer IE, Berkovic SF, Wood NW, Doherty CP, Delanty N, Sisodiya SM, Goldstein DB. Multicentre search for genetic susceptibility loci in sporadic epilepsy syndrome and seizure types: a case-control study. *Lancet Neurol*. 2007;6:970–980.
49. Chen Y, Lu J, Pan H, Zhang Y, Wu H, Xu K, Liu X, Jiang Y, Bao X, Yao Z, Ding K, Lo WH, Qiang B, Chan P, Shen Y, Wu X. Association between genetic variation of CACNA1H and childhood absence epilepsy. *Ann Neurol*. 2003;54:239–243.
50. Heron SE, Phillips HA, Mulley JC, Mazarib A, Neufeld MY, Berkovic SF, Scheffer IE. Genetic variation of CACNA1H in idiopathic generalized epilepsy. *Ann Neurol*. 2004;55:595–596.
51. Heron SE, Khosravani H, Varela D, Bladen C, Williams TC, Newman MR, Scheffer IE, Berkovic SF, Mulley JC, Zamponi GW. Extended spectrum of idiopathic generalized epilepsies associated with CACNA1H functional variants. *Ann Neurol*. 2007;62:560–568.
52. McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JP, Hirschhorn JN. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet*. 2008;9:356–369.
53. Goldstein DB. Common genetic variation and human traits. *N Engl J Med*. 2009;360:1696–1698.
54. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarroll SA, Visscher PM. Finding the missing

heritability of complex diseases. *Nature*. 2009;461: 747–753.
55. Kasperaviciute D, Catarino CB, Heinzen EL, Depondt C, Cavalleri GL, Caboclo LO, Tate SK, Jamnadas-Khoda J, Chinthapalli K, Clayton LM, Shianna KV, Radtke RA, Mikati MA, Gallentine WB, Husain AM, Alhusaini S, Leppert D, Middleton LT, Gibson RA, Johnson MR, Matthews PM, Hosford D, Heuser K, Amos L, Ortega M, Zumsteg D, Wieser HG, Steinhoff BJ, Kramer G, Hansen J, Dorn T, Kantanen AM, Gjerstad L, Peuralinna T, Hernandez DG, Eriksson KJ, Kalviainen RK, Doherty CP, Wood NW, Pandolfo M, Duncan JS, Sander JW, Delanty N, Goldstein DB, Sisodiya SM. Common genetic variation and susceptibility to partial epilepsies: a genome-wide association study. *Brain*. 2010;133:2136–2147.
56. Helbig I, Mefford HC, Sharp AJ, Guipponi M, Fichera M, Franke A, Muhle H, de Kovel C, Baker C, von Spiczak S, Kron KL, Steinich I, Kleefuss-Lie AA, Leu C, Gaus V, Schmitz B, Klein KM, Reif PS, Rosenow F, Weber Y, Lerche H, Zimprich F, Urak L, Fuchs K, Feucht M, Genton P, Thomas P, Visscher F, de Haan GJ, Moller RS, Hjalgrim H, Luciano D, Wittig M, Nothnagel M, Elger CE, Nurnberg P, Romano C, Malafosse A, Koeleman BP, Lindhout D, Stephani U, Schreiber S, Eichler EE, Sander T. 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. *Nat Genet*. 2009;41:160–162.
57. Dibbens LM, Mullen S, Helbig I, Mefford HC, Bayly MA, Bellows S, Leu C, Trucks H, Obermeier T, Wittig M, Franke A, Caglayan H, Yapici Z, Sander T, Eichler EE, Scheffer IE, Mulley JC, Berkovic SF. Familial and sporadic 15q13.3 microdeletions in idiopathic generalized epilepsy: precedent for disorders with complex inheritance. *Hum Mol Genet*. 2009;18: 3626–3631.
58. de Kovel CG, Trucks H, Helbig I, Mefford HC, Baker C, Leu C, Kluck C, Muhle H, von Spiczak S, Ostertag P, Obermeier T, Kleefuss-Lie AA, Hallmann K, Steffens M, Gaus V, Klein KM, Hamer HM, Rosenow F, Brilstra EH, Trenite DK, Swinkels ME, Weber YG, Unterberger I, Zimprich F, Urak L, Feucht M, Fuchs K, Moller RS, Hjalgrim H, De Jonghe P, Suls A, Ruckert IM, Wichmann HE, Franke A, Schreiber S, Nurnberg P, Elger CE, Lerche H, Stephani U, Koeleman BP, Lindhout D, Eichler EE, Sander T. Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies. *Brain*. 2010;133:23–32.
59. Heinzen EL, Radtke RA, Urban TJ, Cavalleri GL, Depondt C, Need AC, Walley NM, Nicoletti P, Ge D, Catarino CB, Duncan JS, Kasperaviciute D, Tate SK, Caboclo LO, Sander JW, Clayton L, Linney KN, Shianna KV, Gumbs CE, Smith J, Cronin KD, Maia JM, Doherty CP, Pandolfo M, Leppert D, Middleton LT, Gibson RA, Johnson MR, Matthews PM, Hosford D, Kalviainen R, Eriksson K, Kantanen AM, Dorn T, Hansen J, Kramer G, Steinhoff BJ, Wieser HG, Zumsteg D, Ortega M, Wood NW, Huxley-Jones J, Mikati M, Gallentine WB, Husain AM, Buckley PG, Stallings RL, Podgoreanu MV, Delanty N, Sisodiya SM, Goldstein DB. Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes. *Am J Hum Genet*. 2010;86:707–718.
60. Mefford HC, Muhle H, Ostertag P, von Spiczak S, Buysse K, Baker C, Franke A, Malafosse A, Genton P, Thomas P, Gurnett CA, Schreiber S, Bassuk AG, Guipponi M, Stephani U, Helbig I, Eichler EE. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet*. 2010;6:e1000962.
61. Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nat Rev Genet*. 2010;11:415–425.
62. Ng SB, Nickerson DA, Bamshad MJ, Shendure J. Massively parallel sequencing and rare disease. *Hum Mol Genet*. 2010;19:R119–124.
63. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, Beck AE, Tabor HK, Cooper GM, Mefford HC, Lee C, Turner EH, Smith JD, Rieder MJ, Yoshiura K, Matsumoto N, Ohta T, Niikawa N, Nickerson DA, Bamshad MJ, Shendure J. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat Genet*. 2010;42:790–793.
64. Bilguvar K, Ozturk AK, Louvi A, Kwan KY, Choi M, Tatli B, Yalnizoglu D, Tuysuz B, Caglayan AO, Gokben S, Kaymakcalan H, Barak T, Bakircioglu M, Yasuno K, Ho W, Sanders S, Zhu Y, Yilmaz S, Dincer A, Johnson MH, Bronen RA, Kocer N, Per H, Mane S, Pamir MN, Yalcinkaya C, Kumandas S, Topcu M, Ozmen M, Sestan N, Lifton RP, State MW, Gunel M. Whole-exome sequencing identifies recessive WDR62 mutations in severe brain malformations. *Nature*. 2010;467:207–210.
65. Ruzzo E, Heinzen EL, Poduri A, Wedel R, Ottman R, Goldstein DB. Whole-genome sequencing in multiplex epilepsy families: an approach to identify rare susceptibility variants. Abstract 3.290. American Epilepsy Society Annual Meeting, 2010; www.aesnet.org.
66. Bansal V, Libiger O, Torkamani A, Schork NJ. Statistical analysis strategies for association studies involving rare variants. *Nat Rev Genet*. 2010;11:773–785.
67. Winawer MR. Phenotype definition in epilepsy. *Epilepsy Behav*. 2006;8:462–476.
68. Anderson VE, Hauser WA, Rich SS. Genetic heterogeneity in the epilepsies. *Adv Neurol*. 1986;44: 59–75.
69. Winawer MR, Shinnar S. Genetic epidemiology of epilepsy or what do we tell families? *Epilepsia*. 2005;46(suppl 10):24–30.
70. Ottman R, Annegers JF, Hauser WA, Kurland LT. Higher risk of seizures in offspring of mothers than of fathers with epilepsy. *Am J Hum Genet*. 1988;43: 257–264.
71. Ottman R, Hauser WA, Susser M. Genetic and maternal influences on susceptibility to seizures. An analytic review. *Am J Epidemiol*. 1985;122:923–939.
72. Annegers JF, Hauser WA, Elveback LR, Anderson VE, Kurland LT. Seizure disorders in offspring of parents with a history of seizures—a maternal-paternal difference? *Epilepsia*. 1976;17:1–9.
73. Ottman R, Lee JH, Hauser WA, Risch N. Are generalized and localization-related epilepsies genetically distinct? *Arch Neurol*. 1998;55:339–344.
74. Ottman R, Annegers JF, Hauser WA, Kurland LT. Seizure risk in offspring of parents with generalized versus partial epilepsy. *Epilepsia*. 1989;30:157–161.
75. Winawer M, Ottman R, Rabinowitz D. Concordance of disease form in kindreds ascertained through affected individuals. *Stat Med*. 2002;21:1887–1897.
76. Winawer MR, Rabinowitz D, Barker-Cummings C, Scheuer ML, Pedley TA, Hauser WA, Ottman R. Evidence for distinct genetic influences on generalized

and localization-related epilepsy. *Epilepsia.* 2003;44: 1176–1182.
77. Winawer MR, Rabinowitz D, Pedley TA, Hauser WA, Ottman R. Genetic influences on myoclonic and absence seizures. *Neurology*. 2003;61:1576–1581.
78. Kinirons P, Rabinowitz D, Gravel M, Long J, Winawer M, Senechal G, Ottman R, Cossette P. Phenotypic concordance in 70 families with IGE-implications for genetic studies of epilepsy. *Epilepsy Res*. 2008; 82:21–28.
79. Winawer MR, Marini C, Grinton BE, Rabinowitz D, Berkovic SF, Scheffer IE, Ottman R. Familial clustering of seizure types within the idiopathic generalized epilepsies. *Neurology.* 2005;65:523–528.

Chapter 51

Strategies for Studying the Epilepsy Genome

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Current leading theories on the etiology of the group of diseases called epilepsy implicate both genes and factors in the environment. Genetic research has the potential to identify molecular and cellular mechanisms that can be targeted directly for therapeutic intervention. Although nearly two decades have passed since the discovery of the first epilepsy-causing gene mutation[1] and mutations in about a dozen other human genes are now well characterized,[2,3] the vast majority of genetic variation that contributes to the development of epilepsy is undiscovered. Thus, there is a critical need to continue to elucidate the epilepsy genome. This chapter will review the comprehensive genome-wide approaches that are being used to achieve this goal.

THE EPILEPSY GENOME

The epilepsy genome has expanded slowly until now. Initially, it consisted of a handful of genes that were identified using traditional linkage and positional cloning methods in a few rare or sometimes even single Mendelian families. The genes discovered in this way are sufficient to cause epilepsy by themselves when mutated. Twenty years later, it is well understood that most genetic influences on epileptogenesis are more complex and that the majority of epilepsy cases result from multiple genetic susceptibility factors interacting with each other, with genetic background alleles, and with environmental factors.[4,5] Discovering genetic variants that have only partial effects and that act in concert to determine common complex clinical phenotypes involving seizures is a daunting task that requires new approaches.

The size of the epilepsy genome is unknown, but some perspective is provided by prior human studies as well as by studies of model organisms. In humans, although relatively few genes are considered proven at this time to be causative for epilepsy when mutated, there are over 100 genes that have been documented to

have at least one nominally or statistically significant genetic association result,[6] thus identifying them as potential susceptibility factors. Studies in mice with targeted gene defects indicate that over 400 different genes (~2% of all genes) are known to cause spontaneous seizures or alter the seizure threshold when they are inactivated.[7] The possibility that a similar large subset of all genes may influence seizure susceptibility in humans supports the contention that most of the genetic vulnerability to development of epilepsy remains to be characterized.

MODELING COMMON FORMS OF EPILEPSY FOR GENOMIC STUDIES

Phenotypic Models

Several key issues in studying the genetics of epilepsies involve the choice of patients. Given the enormous clinical heterogeneity associated with the disease, a variety of experimental designs and patient cohorts can be considered for use. Most studies to date have involved patients with two or more unprovoked seizures, although patients with one unprovoked seizure and an abnormal electroencephalogram (EEG) or magnetic resonance imaging (MRI) scan may also be genetically informative. More controversial is whether patients with no clinical seizures, but with a family history of epilepsy and an epileptiform EEG, should be enrolled and considered along a spectrum of presumed genetic susceptibility to epilepsy.[8] A major distinguishing feature in classification of the epilepsy etiology is the presence or absence of an identifiable remote symptomatic cause, such as stroke, head trauma, brain tumor, or central nervous system (CNS) infection. Cases with no known identifiable cause are presumed to be determined, at least in part, by genetic vulnerability and have been most commonly recommended and used for genetic studies.[9] However, even cases with no identifiable cause based on medical records, study interviews, and neuroimaging could harbor occult structural lesions below the level of resolution of current MRI scans. Thus, genetic studies of focal (also called *localization-related* or *partial*) epilepsy of unknown cause could, in fact, be genetic studies of microscopic malformations of cortical development with epilepsy as a secondary symptom. Cases with obvious acquired risk factors for epilepsy may also be influenced by genetic factors associated with susceptibility to epilepsy,[10] but such cases likely involve at least partly different sets of genes compared to those that influence idiopathic (presumed genetic) epilepsies. Nongenetic influences in acquired epilepsy may be so strong that the potential effect of genetic factors is mollified. Thus, one major study design question is whether to include both symptomatic and idiopathic cases or to focus on the groups separately. Most previous studies have attempted to focus separately on idiopathic and acquired epilepsies. A caveat for studies designed to focus only on idiopathic cases is the potential for environmental influences, such as minor traumatic brain injury, to go unaccounted for, particularly if these events occurred at a time long before the onset of seizures and the diagnosis of epilepsy.

Another key aspect of the epilepsy phenotype that may have a major impact on genetic studies is whether seizures are focal or generalized. Clearly, there are families with members who exhibit both focal and generalized seizures[11]; some patients have both types,[12] while other patients cannot be easily classified. Changes in seizure semiology or epilepsy classification over the course of development[13,14] further complicate the phenotypic landscape in epilepsy research. The potential difference between bona fide generalized epilepsy and focal epilepsy with seizures that are secondarily generalized[15] also warrants consideration. Studies suggest that generalized epilepsy has a higher recurrence risk ratio than focal epilepsy,[16] indicating possibly more genetic influence with the former. At least one large genetic study of focal epilepsy did not find any statistically significant associations between cases and controls,[17] although both symptomatic and idiopathic cases were included, potentially diluting genetic signals. In contrast, a recent study of idiopathic generalized epilepsy reported a statistically significant association with genetic alterations on chromosome 15q13.3.[18] These results have reinforced suspicions, perhaps prematurely, that cohorts of generalized epilepsy are more likely to be genetically informative than cohorts of focal epilepsy. The issue

is by no means settled, however, and virtually all questions regarding phenotype in studies of epilepsy genetics remain unresolved.[19] As a result, there are several available study designs for epilepsy genetics investigations. At one end of the design spectrum, patients may be segregated into groups according to very strict demographic and/or clinical categories. Factors such as age at seizure onset, gender, and seizure profile (including parameters like seizure type, frequency, and circadian pattern), may each have unique genetic signatures. Thus, there is a rationale for dividing patients into very specific diagnostic categories to allow all such factors to be analyzed separately.[20] At the other end of the spectrum, studies can be conducted using a single status for affected individuals. This model assumes that there are some genetic factors that predispose to all or most types of common epilepsy. This hypothesis is supported by the phenotypic heterogeneity observed in families in which the same single-gene mutation results in different clinical phenotypes.[21]

Hypothetical Genetic Models

A substantial body of experimental evidence now supports a multifactorial, polygenic basis for common forms of epilepsy. A major question affecting the direction of current research is whether common, sporadic forms of idiopathic epilepsy are influenced by genetic variants that are common in the general population or by genetic variants that are unique to each individual, or a mixture of both.[22] Until recently, sporadic cases of common idiopathic epilepsies were investigated primarily under the *common variant* hypothesis, which posits that susceptibility to common forms of epilepsy results from the assortment of a specific subset of DNA polymorphisms, typically single nucleotide polymorphisms (SNPs). Single nucleotide polymorphisms are single base substitutions, insertions, or deletions that occur throughout the genome and many SNPs alter gene function. They are considered common if their minor allele frequency is >1% when measured in several hundred chromosomes. In addition to SNPs, there are larger insertions, deletions, or duplications known as *copy number variations* (CNVs) that can span a few nucleotides to millions of bases. Common CNVs have been identified that are associated with epilepsy,[18,23] and certain epilepsy-related CNVs are associated with a wider spectrum of diseases including schizophrenia and autism.[24,25] The composition of all possible common genetic variants that act as epilepsy susceptibility alleles contributes to the specific clinical features of the disease in any individual. Since so many putative epilepsy susceptibility alleles exist, the number of different possible subsets of susceptibility alleles is large and thus the phenotypic diversity of the disease is high. Strategies to elucidate genetic variants that predispose to epilepsy under this hypothesis are based largely on resources such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/index.html) and other similar databases, including those hosted by HapMap, 1000 Genomes, UCSC, and Illumina, that catalog common human genetic variations for use in association studies.

Equally compelling is the *rare variant* hypothesis of common forms of epilepsy. Although still based on the perspective that the disease is genetically complex, the mechanism of epilepsy under this hypothesis is based on the premise that novel SNPs, indels, or other polymorphisms such as CNVs, unique to each individual, act together and in concert with common genetic variants and environmental factors to cause disease. Evaluation of the rare variant hypothesis requires different analytical approaches than those used with the common variant hypothesis.[26,27]

Based on recent findings concerning other complex human diseases, it is likely that both common genetic variation found in the population and rare variants private to each individual contribute to the etiology of epilepsy. Thus, there may be thousands of alleles that constitute the broader epilepsy genome, and combinations of these will increase susceptibility or resistance to epilepsy in any given individual. Ultimately, any variant, common or rare, may be considered to be a putative susceptibility allele if there is statistical evidence to show that it is present significantly more often in cases compared to controls. Variants identified as putative susceptibility alleles also require independent confirmation and validation, the hallmark of a true association between a variant and a phenotype. This is usually achieved by the study of additional patient and control populations. This presents an obstacle to the

confirmation of rare variants, which, by definition, are infrequent in the general population. Validation of variants as bona fide susceptibility alleles comes from biological studies performed in vitro, using biochemical or cell-based assay systems.[28] Less frequently, in vivo validation schemes are utilized.[29] Rare variants may have effects of a larger magnitude compared to common variants, and this can facilitate the validation process.

In the following sections, we describe two new genome-wide approaches being used to establish the genetic landscape in the epilepsies: genome-wide association studies (GWAS), which focus on common variants as epilepsy susceptibility alleles, and whole-genome sequencing (WGS), which aims to identify rare variants associated with susceptibility to epilepsy. We also present a summary of recent data generated in our laboratories and discuss how, together, these whole-genome approaches have the potential to allow full elucidation of the epilepsy genome.

NEW RESEARCH APPROACHES TO THE EPILEPSY GENOME

Genetic Association

SNPS AND CANDIDATE GENES

Until recently, available technologies limited genetic association studies in epilepsy to the analysis of candidate genes. These studies have taken advantage of results of the Human Genome Project,[30] which led to the establishment of SNPs as the most common form of genetic variation.[31] Single nucleotide polymorphisms comprise a large set of biallelic genomic variants that are found on average approximately every 100–300 bp throughout the genome, although less frequently in protein-coding DNA sequences. Overall, there are estimated to be up to 10 million SNPs in the human genome. Given this extraordinary abundance, SNPs offer a powerful means of assessing genetic association, allowing essentially any gene to be surveyed for variants that may associate with a disease. The most popular experimental design for genetic association studies involves direct comparison of SNP genotypes between patients and controls, the so-called *candidate gene case-control study*.

PITFALLS AND LIMITATIONS

A variety of pitfalls must be avoided in the conduct of association studies involving the case-control candidate gene approach. In epilepsy, failure of a number of high-priority candidate genes to show consistent replication is the likely result of some of these pitfalls, such as misinterpretation of data, mismatching of case and control populations, and lack of statistical power.[32] Study design obstacles specific for epilepsy phenotypes have also been cited as reasons for lack of replication in candidate gene studies.[33] Nonetheless, careful control of experimental variables and adherence to standardized design guidelines can facilitate a candidate gene approach and lead to confirmed susceptibility loci.[6] An often-cited limitation of candidate gene studies is that they rely on prior information about epilepsy and its biological basis, and this bias reduces the chance of identifying novel mechanisms. Restricting genes to those that are positional candidates, based on human or animal linkage studies, has enhanced the candidate gene approach.[34–36] Thus, if candidate gene association studies can be optimized to identify key molecules that funnel into final common pathways related to seizure biology, new ideas for treating epilepsy may be developed without having to identify all of the genetic factors involved. A Web site devoted to cataloging all candidate gene association studies in epilepsy has been established as a valuable resource (http://www.epigad.org/page/show/homepage).

GENOTYPING SNPs AND CONSTRUCTING HAPLOTYPES

The previous emphasis on candidate gene association studies was driven in part by a desire to exploit the biological information that was known on epilepsy, but more so by available genotyping methodologies. In particular, the development of SNP DNA markers and related Taqman genotyping technology[37] facilitated the widespread appeal for studies of candidate genes. Methods have been developed to prioritize SNPs for selection in candidate gene studies based on various characteristics including location (e.g., exon vs. intron) and predicted effect on protein abundance or function.[38] Further studies of SNPs have since led to elucidation of the haplotype block structure of the human genome, and the concept of *tagging*

SNPs was introduced to enhance the efficiency of genotyping strategies. The HapMap project integrates linkage disequilibrium data on SNPs to generate haplotypes and also begins to take the population structure of the human species into account with respect to ethnicity and race.[39] Despite attempts to carefully match comparison groups, however, hidden racial/ethnic differences between assembled populations of patients and controls are an impediment to success in genetic association studies. Genomic control genotyping is a valid means to account for the influence of cryptic population substructure[40]; however, this method is resource-intensive and has not been employed commonly in epilepsy studies. Ancestry informative markers (AIMs) have been developed as an alternative means to control for population substructure.[41] Population substructure does not significantly influence the outcome of genetic association determined using the transmission disequilibrium test (TDT) in families,[42] and such designs may be considered preferable to case-control studies. On the other hand, the families required for TDT are generally harder to recruit compared to individual sporadic cases,[43] and this is reflected by the relative dearth of candidate gene studies in epilepsy that have employed the TDT.

GENOME-WIDE ASSOCIATION STUDIES

The logical extension of genetic association analysis from candidate genes to the full genome was predicted in a landmark paper published by Risch and Merikangas[44] when the concept of a full-genome study of genetic association was first described. Now the genome-wide association study (GWAS) has been applied to a multitude of traits and disease states. The GWAS involves analysis of a dense set of SNP markers, as many as 1 million or even more, spaced across all chromosomes in patients and controls, allowing unbiased genome scans to look for genetic variations associated with a particular disease. The method is robust and permits multiple study designs.[45] Key aspects of study design that influence the conduct of GWAS are similar to those affecting candidate gene association studies, including both technical genotyping issues and statistical data analysis issues.

From a technical standpoint, the development of microarray-based SNP genotyping[46] has made it economically feasible to analyze hundreds of thousands of markers in thousands of individuals; however, the very large number of genotypes generated in GWAS present unique problems for data quality control.[47] Basic genotype parameters should be checked to eliminate errors, including analysis of the Hardy-Weinberg Equilibrium (HWE) for all markers in both case and control populations. Genotypes from study groups that contain related individuals (e.g., parent-progeny trios) should be checked for consistency with Mendelian inheritance patterns. Missing genotypes are also a potential cause of data analysis artifacts if the loss is nonrandom and related to a systematic error.

SAMPLE SIZE AND STATISTICAL POWER

The common variant hypothesis predicts weak genetic effects (i.e., odds ratios of 1.20–1.25); thus, very large sample sizes are required to boost the genetic "signal" over the "noise" produced by environmental variables and other genetic factors. Power analyses conducted with standard tools such as QUANTO[48] indicate that comparisons must involve thousands of patients and equal numbers of control individuals to detect gene effects of this magnitude with a relatively high degree of confidence (e.g., 80% power). An alternate way to increase the signal-to-noise ratio is to reduce phenotypic heterogeneity (i.e., noise) by refining the disease affection model, thus enriching for a smaller subset of susceptibility alleles.

DATA ANALYSIS

The goal of genetic association data analysis is to determine whether the distribution of alleles at specific genetic loci is significantly different between patients and controls.[26] Analysis of data derived from unrelated individuals can be accomplished using conventional statistical methods such as the chi-square test with genotype × disease status contingency tables. Covariates may be incorporated using approaches involving analysis of variance or logistic regression. Dividing case and control groups into random halves and conducting data analysis in two stages allows for replication of significant results,[49] the hallmark of a true association. Increases in statistical power can be achieved primarily by expanding the sample size, an effort facilitated by the establishment

of consortia between research groups[50,51] or by meta-analysis of multiple datasets generated independently.[52]

SIGNIFICANCE THRESHOLDS AND MULTIPLE TESTING

Determining thresholds for declaring statistical significance in genetic studies of complex trait phenotypes has been a long-standing concern that led to early establishment of generally accepted guidelines.[53] More recent studies carried out by the HapMap consortium to determine the appropriate statistical significance threshold for GWAS have led to the suggestion that a *P*-value threshold of 10^{-8} is required to overcome potential type 1 error and for statistical significance to be achieved.[39] On the other hand, *P*-values should not be summarily disregarded if they do not meet or exceed the current standard for genome-wide significance and should be examined in the context of the study being conducted, the population under study, and the statistical analysis being performed. Permutation analysis is an alternative method to correct for multiple testing that is also useful for analyzing data from a full genome scan.[54]

GWAS IN FOCAL EPILEPSIES

Although several GWAS have been published for many common human illnesses (http://www.genome.gov/gwastudies/), only one GWAS has been completed and published in epilepsy.[17] Data for this study were collected from a multinational, clinically heterogeneous group of focal epilepsy patients of Caucasian descent (N = 3445), including many individuals with symptomatic causes of disease such as tumors, head trauma, and stroke.[17] Genome-wide association analysis compared to neurologically normal controls (N = 3668) failed to identify genetic variants that satisfied the most stringent criteria for reaching genome-wide statistical significance; however, a number of strong findings were reported, as shown in Table 51–1.

GWAS IN IDIOPATHIC GENERALIZED AND FOCAL EPILEPSIES

We have recently completed an epilepsy GWAS on patients of European ancestry with idiopathic generalized (N = 412) and (non-symptomatic) focal (N = 295) epilepsy. The results presented in Table 51–2 show SNPs with the lowest *P*-values (i.e., *top hits*) following comparison of all epilepsy patients (N = 707 patients) to a large group of neurologically normal controls (N = 6158) by contingency analysis. All individuals were genotyped on the Illumina 610Q platform. Only markers with a *P*-value of 10^{-8} or lower are shown. None of the loci or genes from the list of top hits corresponds with loci or genes identified in the study by Kasperaviciute et al.[17] Separate comparison of generalized and focal patients versus controls revealed both overlapping and independent top hits as well as novel hits not observed in the combined analysis (data not shown). The candidate genes identified from our analysis thus far are largely related to developmental neurobiology based on the expression and function of the encoded proteins. Similar to results from

Table 51–1 **Top Hits* in Focal Epilepsy GWAS**

Chromosome	SNP	Gene	Function	Type
6q14.1	Rs346291	*AL132875.2*	Fatty acid elongation	Pseudogene
6q14.1	Rs9341799	*AL132875.2*	Fatty acid elongation	Pseudogene
16p13.3	Rs2601828	*ADCY9*	Produces cAMP	Intronic
16p12.1	Rs1989647	*PRKCB*	Protein phosphorylation	Intronic
3p24.3	Rs1490157	*ZNF385D*	Zinc-finger protein	Intronic
3p24.3	Rs1320292	*ZNF385D*	Zinc-finger protein	Intronic
3p24.3	Rs1387822	*ZNF385D*	Zinc-finger protein	Intronic
2p23	Rs951997	*MOGAT1*	Diacylglycerol synthesis	Intronic
2q	Rs16834756	*GALNT13*	Mucin glycoslylation	Intronic
10q21.3	Rs1942006	*CTNNA3*	Cell-cell adhesion	Intergenic

*$1 \times 10^{-7} < P < 5 \times 10^{-5}$ (logistic regression or Cochran-Mantel-Haenszel test).
Source: Data are as reported in ref. 17.

Table 51–2 **Top Hits in Idiopathic Epilepsy GWAS***

Chr	SNP	Gene	Function	*P*-value	Odds Ratio
13	rs9572727	*DACH1*	Transcription factor	1.71E-14	2.475
5	rs10040564	*NEUROG1*	Transcription factor	1.97E-13	3.13
8	rs1480692	*SGCZ*	Sarcoglycan protein	5.68E-10	2.485
4	rs2389145	*TRAM1L1*	Secretory across the endoplasmic reticulum	9.20E-10	2.784
16	rs4781689	*MYH11/NDE1*	Contractile/neural migration	1.30E-09	0.4342
3	rs892365	*CADM2*	Cell–cell adhesion	5.60E-09	0.5413
8	rs12678653	*RIMS2*	Synapse exocytosis	8.74E-09	2.265
16	rs4420517	*XYLT1*	Glycosaminoglycan synthesis	1.68E-08	0.3172

*Chi-square analysis (Hakonarson, Buono, et al., unpublished).

the focal epilepsy GWAS, there is surprisingly little evidence for involvement of genes that encode ion channel proteins, a result that contrasts with the *channelopathy* concept of the epilepsies.[55]

Other GWAS in epilepsy are in progress (e.g., Epicure). However, published data from those studies have focused on CNVs, novel genetic deletions, and duplications that are more consistent with rare variant hypotheses of epilepsy pathogenesis. Our preliminary work identified new CNVs associated with idiopathic generalized epilepsy as well as replication of previous CNV results.[18,23] Thus, we detected deletions at 15q11.2, 15q13.3, and 16p13.11 in 1.7% of our idiopathic generalized epilepsy patients compared to 0.5% of controls and found them associated with this common form of epilepsy ($P = 1.5 \times 10^{-3}$). Such results on CNVs have stimulated interest in the potential utility of whole-exome and whole-genome sequencing in individual patients, as will be described below.

Next-Generation Sequencing

The results of the initial GWAS described above suggest that common genetic variation makes only a modest contribution to epilepsy susceptibility and are consistent with results concerning other human diseases of the nervous system. Thus, the fraction of unaccounted variation (the *heritability gap*) left by GWAS and other genetic association approaches is hypothesized to be associated with unique or private genetic variation discoverable only through in-depth sequence analysis of individual genomes. This is now possible through *next-generation sequencing* (NGS), in which hundreds of thousands of short DNA molecules are sequenced in parallel, permitting in-depth (i.e., "deep") DNA sequence analysis in individual patients at low cost.

NGS STRATEGIES

A variety of strategies are now being employed to take advantage of massively parallel DNA sequencing[56] to yield new discoveries in epilepsy. These include whole-genome sequencing (WGS)[57] and whole-exome sequencing (WES),[58] the latter involving sequence analysis of only gene coding regions. Another strategy being employed collects mRNAs from pathological tissue of an individual with a known disease, or from a subject after medical treatment, and converts these mRNAs into to cDNAs for sequencing of the *transcriptome*.[59] In this way, gene expression profiles (transcription) influenced by disease or medical treatment can be identified using the new sequencing technology. Finally, targeted sequencing of specific disease genes can be done on a much larger scale. Thus, if a specific gene mutation is linked to or associated with an epilepsy phenotype, it would be possible to sequence the entire coding and putative regulatory regions of that specific target gene in hundreds or thousands of patients to determine if the same or novel mutations are discovered that can be linked to or associated with disease. In addition, a set of 100 or more target genes can be interrogated all at once by NGS in additional patients to determine if rare mutations exist. Recent studies exemplify the use of NGS technologies to identify disease-causing DNA variations.[58–66]

NGS-BASED GENE DISCOVERY IN THE EPILEPSIES

At the time of this writing, there are no published papers in the epilepsy field using the new sequencing strategies; however, an abstract at the 2010 annual meeting of the American Epilepsy Society describes the use of WGS on the two most distantly related members of each of nine separate families affected with nonacquired epilepsy to look for potential epilepsy mutations.[66] On average, each relative pair shared ~100 rare variations that could potentially be related to their disease; however, very few were found in multiple families. These data confirm that the epilepsy genome is complex and potentially very large, as previously emphasized.

Our laboratories have recently performed WGS on four individuals from a family with a rare inherited type of epilepsy known as *autosomal dominant nocturnal frontal lobe epilepsy* (ADNFLE). Prior work has shown that mutations in the genes that encode subunits of the nicotinic acetylcholine receptor genes *CHRNA2*, *CHRNA4*, and *CHRNB2* are sufficient to cause ADNFLE (http://www.ncbi.nlm.nih.gov/omim). Additional families have been studied in which linkage to chromosomes 3 and 8 was demonstrated, but causative genes were not identified, suggesting that significant genetic heterogeneity exists even for this rare familial form of epilepsy.[67–69] We obtained DNA from an affected individual and three children of the proband. One child was unaffected. The family was too small to use traditional linkage and positional cloning strategies, and after screening the *CHRN* genes and finding no mutations, we were not able to proceed with analysis for several years. In late 2010, we used WGS at ~15x coverage and generated full genomic sequence data in less than 30 days. Bioinformatic analysis conducted to date reveals that the affected relatives share ~100 rare exonic variations that are not found in the unaffected relative. However, only several candidates exist with functional variation in exons of brain-expressed genes. . The prime candidate is a putative disease-causing SNP that leads to a nonconservative amino acid change in a gamma-aminobutyric acid (GABA) receptor-related protein that is predicted to be deleterious to the tertiary structure of the protein. The mutation was not found in the unaffected relative or in any public database or in hundreds of controls tested. The finding is currently being validated by direct sequencing, further analysis of additional samples from an expansion of the original pedigree, and functional studies of the mutation in a biological assay. This would be one of the first examples of WGS being used in isolation to identify a causative mutation in a small family with any autosomal dominant transmitted disease.

FUTURE PROSPECTS FOR NGS

Next-generation sequencing will become a significant tool for clinical utility once causative mutations linked to specific monogenic phenotypes are defined. However, since complex human traits could be caused by hundreds to thousands of alleles, each with the potential to interact with another, it may take decades before NGS data make a substantial impact on clinical decision making in epilepsy patients without a family history of epilepsy. Nonetheless, NGS is a major technological improvement that will enable rapid discovery of the many rare alleles related to disease phenotype and response to medications if enough genomes are sequenced. It may take decades, but over time it will be possible to integrate NGS information from many sources and determine the variations linked to the converging common biological pathways that cause epilepsy when altered. The epilepsy genome will slowly be elucidated as WGS/WES and other methods are applied to families and unrelated sporadic cases with various seizure disorders. At a minimum, a large list of candidate disease-related alleles will be identified and systematically studied until improved statistical modeling and candidate filtering methods are developed. At best, large-scale studies will show a plethora of rare variations that eventually cluster into a finite set of genes and gene networks related to specific epilepsy phenotypes. Identification of these variations within converging common biological pathways linked to epilepsy will provide accurate diagnostics and insight into molecular mechanisms that will offer new opportunities for therapeutic intervention.

CONCLUDING REMARKS

Complex human traits, including susceptibility to common diseases, are influenced by both

genetic and environmental factors. Common forms of human epilepsy are such multifactorial diseases. Epidemiological studies in twins, sporadic epilepsy patients, and families with a strong history of epilepsy provide strong evidence that genetics play an important role; however, only a small number of genetic risk factors have been identified to date. Whole genome approaches including GWAS and NGS offer the potential to identify many or all of the genetic risk factors for the epilepsies. Although it will likely be many years before such goals are achieved and the epilepsy genome is fully elucidated, whole genome strategies promise to reveal the full nature of epilepsy pathogenesis and exert a major impact on the development of new therapies.

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REFERENCES

1. Steinlein OK, Mulley JC, Propping P, Wallace RH, Phillips HA, Sutherland GR, Scheffer IE, Berkovic SF. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet*. 1995;11(2):201–203.
2. Rees MI. The genetics of epilepsy—the past, the present and future. *Seizure*. 2010;19(10):680–683.
3. Baulac S, Baulac M. Advances on the genetics of Mendelian idiopathic epilepsies. *Clin Lab Med*. 2010;30:911–929.
4. Dibbens LM, Heron SE, Mulley JC. A polygenic heterogeneity model for common epilepsies with complex genetics. *Genes Brain Behav*. 2007;6(7):593–597.
5. Ferraro TN, Buono RJ. Polygenic epilepsy. *Adv Neurol*. 2006;97:389–398.
6. Tan NC, Berkovic SF. The Epilepsy Genetic Association Database (epiGAD): analysis of 165 genetic association studies, 1996–2008. *Epilepsia*. 2010;51(4):686–689.
7. Frankel WN. Genetics of complex neurological disease: challenges and opportunities for modeling epilepsy in mice and rats. *Trends Genet*. 2009;25(8):361–367.
8. Martínez-Juárez IE, Alonso ME, Medina MT, Durón RM, Bailey JN, López-Ruiz M, Ramos-Ramírez R, León L, Pineda G, Castroviejo IP, Silva R, Mija L, Perez-Gosiengfiao K, Machado-Salas J, Delgado-Escueta AV. Juvenile myoclonic epilepsy subsyndromes: family studies and long-term follow-up. *Brain*. 2006;129(pt 5):1269–1280.
9. Delgado-Escueta AV, Greenberg DA, Weissbecker K, Serratosa JM, Liu A, Treiman LJ, Sparkes R, Park MS, Barbetti A. The choice of epilepsy syndromes for genetic analysis. *Epilepsy Res Suppl*. 1991;4: 147–159.
10. Wagner AK, Miller MA, Scanlon J, Ren D, Kochanek PM, Conley YP. Adenosine A1 receptor gene variants associated with post-traumatic seizures after severe TBI. *Epilepsy Res*. 2010;90(3):259–272
11. Magnin E, Vidailhet M, Depienne C, Saint-Martin C, Bouteiller D, LeGuern E, Apartis E, Rumbach L, Labauge P. Familial cortical myoclonic tremor with epilepsy (FCMTE): clinical characteristics and exclusion of linkages to 8q and 2p in a large French family. *Rev Neurol (Paris)*. 2009;165(10):812–820.
12. Jeha LE, Morris HH, Burgess RC. Coexistence of focal and idiopathic generalized epilepsy in the same patient population. *Seizure*. 2006;15(1):28–34.
13. Fogarasi A, Tuxhorn I, Janszky J, Janszky I, Rásonyi G, Kelemen A, Halász P. Age-dependent seizure semiology in temporal lobe epilepsy. *Epilepsia*. 2007;48(9): 1697–1702.
14. Morrell MJ. Hormones and epilepsy through the lifetime. *Epilepsia*. 1992;33(suppl 4):S49–S61.
15. Schindler K, Leung H, Lehnertz K, Elger CE. How generalised are secondarily "generalised" tonic-clonic seizures? *J Neurol Neurosurg Psychiatry*. 2007;78(9): 993–996.
16. Briellmann RS, Torn-Broers Y, Berkovic SF. Idiopathic generalized epilepsies: do sporadic and familial cases differ? *Epilepsia*. 2001;42(11):1399–1402.
17. Kasperaviciūte D, Catarino CB, Heinzen EL, Depondt C, Cavalleri GL, Caboclo LO, Tate SK, Jamnadas-Khoda J, Chinthapalli K, Clayton LM, Shianna KV, Radtke RA, Mikati MA, Gallentine WB, Husain AM, Alhusaini S, Leppert D, Middleton LT, Gibson RA, Johnson MR, Matthews PM, Hosford D, Heuser K, Amos L, Ortega M, Zumsteg D, Wieser HG, Steinhoff BJ, Krämer G, Hansen J, Dorn T, Kantanen AM, Gjerstad L, Peuralinna T, Hernandez DG, Eriksson KJ, Kälviäinen RK, Doherty CP, Wood NW, Pandolfo M, Duncan JS, Sander JW, Delanty N, Goldstein DB, Sisodiya SM. Common genetic variation and susceptibility to partial epilepsies: a genome-wide association study. *Brain*. 2010;133(pt 7):2136–2147.
18. Helbig I, Mefford HC, Sharp AJ, Guipponi M, Fichera M, Franke A, Muhle H, de Kovel C, Baker C, von Spiczak S, Kron KL, Steinich I, Kleefuss-Lie AA, Leu C, Gaus V, Schmitz B, Klein KM, Reif PS, Rosenow F, Weber Y, Lerche H, Zimprich F, Urak L, Fuchs K, Feucht M, Genton P, Thomas P, Visscher F, de Haan GJ, Møller RS, Hjalgrim H, Luciano D, Wittig M, Nothnagel M, Elger CE, Nürnberg P, Romano C, Malafosse A, Koeleman BP, Lindhout D, Stephani U, Schreiber S, Eichler EE, Sander T., 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. *Nat Genet*. 2009;41(2): 160–162.

19. Greenberg DA, Subaran R. Blinders, phenotype, and fashionable genetic analysis: a critical examination of the current state of epilepsy genetic studies. *Epilepsia*. 2011;52(1):1–9.
20. Ottman R. Analysis of genetically complex epilepsies. *Epilepsia*. 2005;46(suppl 10):7–14.
21. Mahoney K, Moore SJ, Buckley D, Alam M, Parfrey P, Penney S, Merner N, Hodgkinson K, Young TL. Variable neurologic phenotype in a GEFS+ family with a novel mutation in SCN1A. *Seizure*. 2009;18(7): 492–497.
22. Schork NJ, Murray SS, Frazer KA, Topol EJ. Common vs. rare allele hypotheses for complex diseases. *Curr Opin Genet Dev*. 2009;19(3):212–219.
23. Mefford HC, Muhle H, Ostertag P, von Spiczak S, Buysse K, Baker C, Franke A, Malafosse A, Genton P, Thomas P, Gurnett CA, Schreiber S, Bassuk AG, Guipponi M, Stephani U, Helbig I, Eichler EE. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet*. 2010;6(5):e1000962.
24. Ben-Shachar S, Lanpher B, German JR, Qasaymeh M, Potocki L, Nagamani SC, Franco LM, Malphrus A, Bottenfield GW, Spence JE, Amato S, Rousseau JA, Moghaddam B, Skinner C, Skinner SA, Bernes S, Armstrong N, Shinawi M, Stankiewicz P, Patel A, Cheung SW, Lupski JR, Beaudet AL, Sahoo T. Microdeletion 15q13.3: a locus with incomplete penetrance for autism, mental retardation, and psychiatric disorders. *J Med Genet.* 2009;46(6):382–388.
25. Sharp AJ, Mefford HC, Li K, Baker C, Skinner C, Stevenson RE, Schroer RJ, Novara F, De Gregori M, Ciccone R, Broomer A, Casuga I, Wang Y, Xiao C, Barbacioru C, Gimelli G, Bernardina BD, Torniero C, Giorda R, Regan R, Murday V, Mansour S, Fichera M, Castiglia L, Failla P, Ventura M, Jiang Z, Cooper GM, Knight SJ, Romano C, Zuffardi O, Chen C, Schwartz CE, Eichler EE A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. *Nat Genet*. 2008;40(3):322–328.
26. Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT. Basic statistical analysis in genetic case-control studies. *Nat Protoc*. 2011;6(2): 121–133
27. Liu DJ, Leal SM. Replication strategies for rare variant complex trait association studies via next-generation sequencing. *Am J Hum Genet*. 2010;87(6):790–801
28. Cossette P, Loukas A, Lafrenière RG, Rochefort D, Harvey-Girard E, Ragsdale DS, Dunn RJ, Rouleau GA. Functional characterization of the D188V mutation in neuronal voltage-gated sodium channel causing generalized epilepsy with febrile seizures plus (GEFS). *Epilepsy Res.* 2003;53(1–2):107–117.
29. Tang B, Dutt K, Papale L, Rusconi R, Shankar A, Hunter J, Tufik S, Yu FH, Catterall WA, Mantegazza M, Goldin AL, Escayg A. A BAC transgenic mouse model reveals neuron subtype-specific effects of a Generalized Epilepsy with Febrile Seizures Plus (GEFS+) mutation. *Neurobiol Dis*. 2009;35(1):91–102.
30. McPherson JD, Marra M, Hillier L, Waterston RH, Chinwalla A, Wallis J, Sekhon M, Wylie K, Mardis ER, Wilson RK, Fulton R, Kucaba TA, Wagner-McPherson C, Barbazuk WB, Gregory SG, Humphray SJ, French L, Evans RS, Bethel G, Whittaker A, Holden JL, McCann OT, Dunham A, Soderlund C, Scott CE, Bentley DR, Schuler G, Chen HC, Jang W, Green ED, Idol JR, Maduro VV, Montgomery KT, Lee E, Miller A, Emerling S, Kucherlapati, Gibbs R, Scherer S, Gorrell JH, Sodergren E, Clerc-Blankenburg K, Tabor P, Naylor S, Garcia D, de Jong PJ, Catanese JJ, Nowak N, Osoegawa K, Qin S, Rowen L, Madan A, Dors M, Hood L, Trask B, Friedman C, Massa H, Cheung VG, Kirsch IR, Reid T, Yonescu R, Weissenbach J, Bruls T, Heilig R, Branscomb E, Olsen A, Doggett N, Cheng JF, Hawkins T, Myers RM, Shang J, Ramirez L, Schmutz J, Velasquez O, Dixon K, Stone NE, Cox DR, Haussler D, Kent WJ, Furey T, Rogic S, Kennedy S, Jones S, Rosenthal A, Wen G, Schilhabel M, Gloeckner G, Nyakatura G, Siebert R, Schlegelberger B, Korenberg J, Chen XN, Fujiyama A, Hattori M, Toyoda A, Yada T, Park HS, Sakaki Y, Shimizu N, Asakawa S, Kawasaki K, Sasaki T, Shintani A, Shimizu A, Shibuya K, Kudoh J, Minoshima S, Ramser J, Seranski P, Hoff C, Poustka A, Reinhardt R, Lehrach H; International Human Genome Mapping Consortium. A physical map of the human genome. *Nature.* 2001;409(6822):934–941.
31. Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, Sherry S, Mullikin JC, Mortimore BJ, Willey DL, Hunt SE, Cole CG, Coggill PC, Rice CM, Ning Z, Rogers J, Bentley DR, Kwok PY, Mardis ER, Yeh RT, Schultz B, Cook L, Davenport R, Dante M, Fulton L, Hillier L, Waterston RH, McPherson JD, Gilman B, Schaffner S, Van Etten WJ, Reich D, Higgins J, Daly MJ, Blumenstiel B, Baldwin J, Stange-Thomann N, Zody MC, Linton L, Lander ES, Altshuler D;, International SNP Map Working Group. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature*. 2001;409(6822):928–933.
32. Jorgensen TJ, Ruczinski I, Kessing B, Smith MW, Shugart YY, Alberg AJ. Hypothesis-driven candidate gene association studies: practical design and analytical considerations. *Am J Epidemiol*. 2009;170(8): 986–993.
33. Pal DK, Strug LJ, Greenberg DA. Evaluating candidate genes in common epilepsies and the nature of evidence. *Epilepsia.* 2008;49(3):386–392.
34. Ferraro TN, Golden GT, Smith GG, Martin JF, Lohoff FW, Gieringer TA, Zamboni D, Schwebel CL, Press DM, Kratzer SO, Zhao H, Berrettini WH, Buono RJ. Fine mapping of a seizure susceptibility locus on mouse Chromosome 1: nomination of *Kcnj10* as a causative gene. *Mamm Genome*. 2004;15(4): 239–251.
35. Buono RJ, Lohoff FW, Sander T, Sperling MR, O'Connor MJ, Dlugos DJ, Ryan SG, Golden GT, Zhao H, Scattergood TM, Berrettini WH, Ferraro TN. Association between variation in the human KCNJ10 potassium ion channel gene and seizure susceptibility. *Epilepsy Res.* 2004;58(2–3):175–183.
36. Lenzen KP, Heils A, Lorenz S, Hempelmann A, Höfels S, Lohoff FW, Schmitz B, Sander T. Supportive evidence for an allelic association of the human KCNJ10 potassium channel gene with idiopathic generalized epilepsy. *Epilepsy Res*. 2005;63(2–3):113–118.
37. Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. *Genet Anal*. 1999;14(5–6):143–149.
38. Pettersson FH, Anderson CA, Clarke GM, Barrett JC, Cardon LR, Morris AP, Zondervan KT. Marker

selection for genetic case-control association studies. *Nat Protoc*. 2009;4(5):743–752.
39. International HapMap Consortium. A haplotype map of the human genome. *Nature*. 2005;437(7063): 1299–1320.
40. Devlin B, Roeder K. Genomic control for association studies. *Biometrics*. 1999;55(4):997–1004.
41. Shriver MD, Smith MW, Jin L, Marcini A, Akey JM, Deka R, Ferrell RE. Ethnic-affiliation estimation by use of population-specific DNA markers. *Am J Hum Genet*. 1997;60(4):957–964.
42. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet*. 1993;52(3):506–516.
43. Ottman R, Berenson K, Barker-Cummings C. Recruitment of families for genetic studies of epilepsy. *Epilepsia*. 2005;46(2):290–297.
44. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science*. 1996;273(5281): 1516–1517.
45. Kraft P, Cox DG. Study designs for genome-wide association studies. *Adv Genet*. 2008;60:465–504.
46. Oliphant A, Barker DL, Stuelpnagel JR, Chee MS. BeadArray technology: enabling an accurate, cost-effective approach to high-throughput genotyping. *Biotechniques*. 2002;suppl:56–58, 60–61.
47. Laurie CC, Doheny KF, Mirel DB, Pugh EW, Bierut LJ, Bhangale T, Boehm F, Caporaso NE, Cornelis MC, Edenberg HJ, Gabriel SB, Harris EL, Hu FB, Jacobs KB, Kraft P, Landi MT, Lumley T, Manolio TA, McHugh C, Painter I, Paschall J, Rice JP, Rice KM, Zheng X, Weir BS, GENEVA Investigators. Quality control and quality assurance in genotypic data for genome-wide association studies. *Genet Epidemiol*. 2010;34(6):591–602.
48. Gauderman WJ. Sample size requirements for association studies of gene–gene interaction. *Am J Epidemiol*. 2002;155(5):478–484.
49. Satagopan JM, Elston RC Optimal two-stage genotyping in population-based association studies. *Genet Epidemiol*. 2003;25(2):149–157.
50. UK Parkinson's Disease Consortium, Wellcome Trust Case Control Consortium 2, Spencer CC, Plagnol V, Strange A, Gardner M, Paisan-Ruiz C, Band G, Barker RA, Bellenguez C, Bhatia K, Blackburn H, Blackwell JM, Bramon E, Brown MA, Brown MA, Burn D, Casas JP, Chinnery PF, Clarke CE, Corvin A, Craddock N, Deloukas P, Edkins S, Evans J, Freeman C, Gray E, Hardy J, Hudson G, Hunt S, Jankowski J, Langford C, Lees AJ, Markus HS, Mathew CG, McCarthy MI, Morrison KE, Palmer CN, Pearson JP, Peltonen L, Pirinen M, Plomin R, Potter S, Rautanen A, Sawcer SJ, Su Z, Trembath RC, Viswanathan AC, Williams NW, Morris HR, Donnelly P, Wood NW. Dissection of the genetics of Parkinson's disease identifies an additional association 5′ of SNCA and multiple associated haplotypes at 17q21. *Hum Mol Genet*. 2011;20(2):345–353.
51. International Multiple Sclerosis Genetics Consortium (IMSGC). Comprehensive follow-up of the first genome-wide association study of multiple sclerosis identifies KIF21B and TMEM39A as susceptibility loci. *Hum Mol Genet*. 2010;19(5):953–962.
52. Kavvoura FK, Ioannidis JP. Methods for meta-analysis in genetic association studies: a review of their potential and pitfalls. *Hum Genet*. 2008;123(1):1–14.
53. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet*. 1995;11(3):241–247.
54. Browning BL. PRESTO: rapid calculation of order statistic distributions and multiple-testing adjusted P-values via permutation for one and two-stage genetic association studies. *BMC Bioinformatics*. 2008;9:309.
55. Heron SE, Scheffer IE, Berkovic SF, Dibbens LM, Mulley JC. Channelopathies in idiopathic epilepsy. *Neurotherapeutics*. 2007;4(2):295–304.
56. Brenner S, Johnson M, Bridgham J, Golda G, Lloyd DH, Johnson D, Luo S, McCurdy S, Foy M, Ewan M, Roth R, George D, Eletr S, Albrecht G, Vermaas E, Williams SR, Moon K, Burcham T, Pallas M, DuBridge RB, Kirchner J, Fearon K, Mao J, Corcoran K. Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. *Nat Biotechnol*. 2000;18(6):630–634. Erratum in: *Nat Biotechnol*. 2000;18(10):1021.
57. Ng PC, Kirkness EF. Whole genome sequencing. *Methods Mol Biol*. 2010;628:215–226.
58. Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, Nayir A, Bakkaloğlu A, Ozen S, Sanjad S, Nelson-Williams C, Farhi A, Mane S, Lifton RP. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *Proc Natl Acad Sci USA*. 2009;106(45):19096–19101.
59. Cirulli ET, Singh A, Shianna KV, Ge D, Smith JP, Maia JM, Heinzen EL, Goedert JJ, Goldstein DB;. Screening the human exome: a comparison of whole genome and whole transcriptome sequencing. *Genome Biol*. 2010;11(5):R57.
60. Arnold CN, Xia Y, Lin P, Ross C, Schwander M, Smart NG, Muller U, Beutler B. Rapid identification of a disease allele in mouse through whole genome sequencing and bulk segregation analysis. *Genetics*. 2011;187(3):633–641.
61. Bolze A, Byun M, McDonald D, Morgan NV, Abhyankar A, Premkumar L, Puel A, Bacon CM, Rieux-Laucat F, Pang K, Britland A, Abel L, Cant A, Maher ER, Riedl SJ, Hambleton S, Casanova JL. Whole-exome-sequencing-based discovery of human FADD deficiency. *Am J Hum Genet*. 2010;87(6): 873–881.
62. Wang JL, Yang X, Xia K, Hu ZM, Weng L, Jin X, Jiang H, Zhang P, Shen L, Guo JF, Li N, Li YR,Lei LF, Zhou J, Du J, Zhou YF, Pan Q, Wang J, Wang J, Li RQ, Tang BS. TGM6 identified as a novel causative gene of spinocerebellar ataxias using exome sequencing. *Brain*. 2010;133(pt 12):3510–3518.
63. Haack TB, Danhauser K, Haberberger B, Hoser J, Strecker V, Boehm D, Uziel G, Lamantea E, Invernizzi F, Poulton J, Rolinski B, Iuso A, Biskup S, Schmidt T, Mewes HW, Wittig I, Meitinger T, Zeviani M, Prokisch H. Exome sequencing identifies ACAD9 mutations as a cause of complex I deficiency. *Nat Genet*. 2010;42(12):1131–1134.
64. Meder B, Haas J, Keller A, Heid C, Just S, Borries A, Boisguerin V, Scharfenberger-Schmeer M, Stähler P, Beier M, Weichenhan D, Strom TM, Pfeufer A, Korn B, Katus HA, Rottbauer W. Targeted next-generation sequencing for the molecular genetic diagnostics of cardiomyopathies. *Circ Cardiovasc Genet*. 2011;4(2):110–122..
65. Bonnefond A, Durand E, Sand O, De Graeve F, Gallina S, Busiah K, Lobbens S, Simon A,

Bellanné-Chantelot C, Létourneau L, Scharfmann R, Delplanque J, Sladek R, Polak M, Vaxillaire M, Froguel P. Molecular diagnosis of neonatal diabetes mellitus using next-generation sequencing of the whole exome. *PLoS One*. 2010;26;5(10):e13630.

66. Ruzzo E, Heinzen E., Poduri A., Wedel R., Ottman R., Goldstein D. Whole-genome sequencing in multiplex epilepsy families: an approach to identify rare susceptibility variants *Epilepsia*. Abst. 3.290. Epub on www.aesorg.net.
67. Phillips HA, Scheffer IE, Crossland KM, Bhatia KP, Fish DR, Marsden CD, Howell SJ, Stephenson JB, Tolmie J, Plazzi G, Eeg-Olofsson O, Singh R, Lopes-Cendes I, Andermann E, Andermann F, Berkovic SF, Mulley JC. Autosomal dominant nocturnal frontal-lobe epilepsy: genetic heterogeneity and evidence for a second locus at 15q24. *Am J Hum Genet*. 1998;63(4):1108–1116.
68. De Marco EV, Gambardella A, Annesi F, Labate A, Carrideo S, Forabosco P, Civitelli D, Candiano IC, Tarantino P, Annesi G, Quattrone A. Further evidence of genetic heterogeneity in families with autosomal dominant nocturnal frontal lobe epilepsy. *Epilepsy Res*. 2007;74(1):70–73.
69. Combi R, Ferini-Strambi L, Montruccoli A, Bianchi V, Malcovati M, Zucconi M, Dalprà L, Tenchini ML. Two new putative susceptibility loci for ADNFLE. *Brain Res Bull*. 2005;67(4):257–263

Chapter 52

Sodium Channel Mutations and Epilepsy

William A. Catterall

INTRODUCTION

While many pathophysiological changes contribute to seizure susceptibility, recent work suggests that genetic factors are especially important. Polygenic inheritance patterns have been associated with febrile seizures and idiopathic epilepsy and may be important in determining susceptibility to acquired epilepsy following brain injury. Monogenic inheritance patterns are seen in a number of epilepsies associated with mutations in ligand-gated or voltage-gated ion channels. The genes most frequently associated with epilepsy encode brain sodium channels.

VOLTAGE-GATED SODIUM CHANNELS

Voltage-gated Na^+ channels in the brain are complexes of a 260 kD α subunit in association with auxiliary β subunits ($\beta1$–$\beta4$) of 33 to 40 kD (see ref. 1 and Chapter 4). The α subunit contains the voltage sensors and the

ion-conducting pore.[1] The β subunits modify the kinetics and voltage dependence of gating and serve as cell adhesion molecules interacting with the extracellular matrix, other cell adhesion molecules, and the cytoskeleton.[2,3] The mammalian genome contains nine functional voltage-gated sodium channel α subunits, which differ in patterns of tissue expression and biophysical properties. The $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$ channel subtypes, encoded by the *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A* genes, respectively, are the primary sodium channels in the central nervous system[1,4–6] (see also Chapter 4). $Na_V1.1$ and $Na_V1.3$ channels are primarily localized in cell bodies,[7,8] $Na_V1.2$ channels in unmyelinated or premyelinated axons and dendrites,[7,8] and $Na_V1.6$ channels in myelinated axons and in dendrites.[9–11] These channels participate in the generation of both somatodendritic and axonal action potentials, and also conduct subthreshold persistent and resurgent Na^+ currents following action potentials.[12–16] In rodents, $Na_V1.3$ channels are highly expressed in the brain during embryonic life, and their expression declines after birth as $Na_V1.1$, $Na_V1.2$, and $Na_V1.6$ channels take over the primary role in action potential generation.[17,18] $Na_V1.1$ expression is first detectable at postnatal day 7 and increases steadily through young adulthood.[17,18]

SODIUM CHANNEL MUTATIONS IN GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS

Screening of human patients with inherited epilepsy first led to the identification of mutations of $Na_V1.1$ channels in two large families with the autosomal dominant epilepsy disorder generalized epilepsy with febrile seizures plus, OMIM 604233 (GEFS+).[19] More than 20 different missense mutations were subsequently identified in GEFS+ patients, spread throughout the α subunit (Fig. 52–1). A mutation in the $Na_V\beta1$ subunit also causes GEFS+ epilepsy, probably by impairing expression and function of $Na_V1.1$ channels.[20] Mutations in GEFS+ alter multiple biophysical properties of the Na^+ channels expressed in nonneuronal cells[21] (see also below). However, GEFS+ is

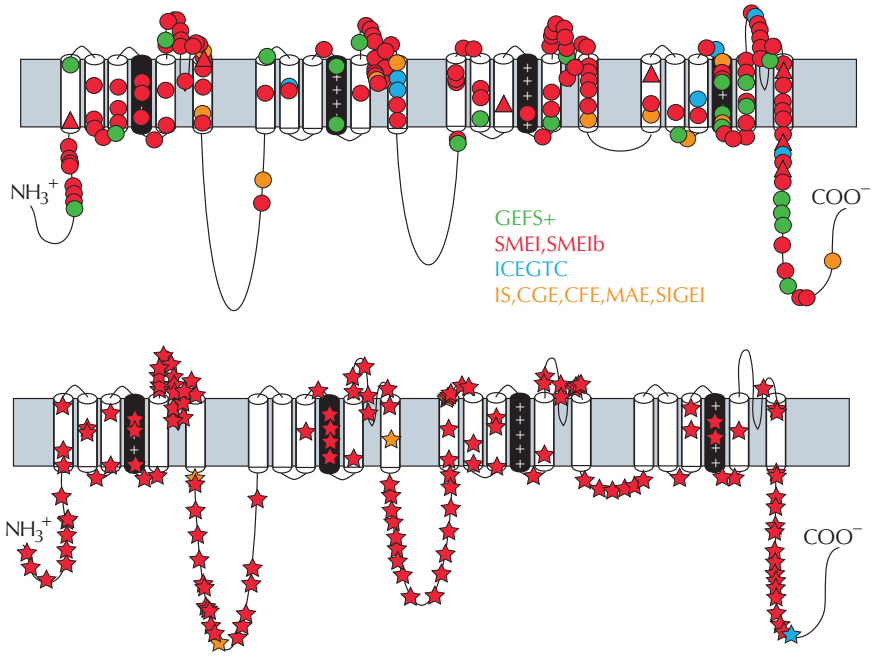

Figure 52–1. Mutations in NaV1.1 channels in patients with epilepsy. Top panel: missense mutations (circles) and in-frame deletions (triangles). Bottom panel: truncation mutations (stars). The clinical type of epilepsy is indicated by color: GEFS+, generalized epilepsy with febrile seizures plus; SMEI, severe myoclonic epilepsy of infancy.

usually a relatively mild epilepsy syndrome. Seizures are typically well controlled by antiepileptic drugs, and no cognitive impairment is observed. Nevertheless, it has been difficult to determine the molecular mechanisms and genotype–phenotype correlations for GEFS+ epilepsy.

Effects of GEFS+ Mutations Expressed in Nonneuronal Cells

Functional effects of GEFS+ mutations were first studied by expression in nonneuronal cells and whole-cell voltage-clamp analysis. The initial study of two mutations inserted in rat $Na_V1.1$ and expressed in *Xenopus* oocytes revealed that one was a gain-of-function mutation because of destabilized slow inactivation, whereas the second was a loss-of-function mutation because of enhanced slow inactivation.[22] In contrast, the first three mutations inserted in human $Na_V1.1$ and studied by expression in human somatic cells revealed a different functional effect: all three caused impaired inactivation and an increased persistent sodium current, leading to the hypothesis that gain of function of mutant sodium channels due to loss of inactivation is responsible for GEFS+ epilepsy.[23] However, further studies of several GEFS+ mutations expressed in mammalian cells[24,25] or *Xenopus* oocytes[26–28] have again revealed a mixture of loss-of-function and gain-of-function effects that were caused by several different changes in the biophysical properties of $Na_V1.1$ channels. These studies did not clearly define the functional basis for hyperexcitability in GEFS+ epilepsy.

Defective Protein Folding and Partial Rescue of GEFS+ Mutants

Studies of GEFS+ mutations in families with variable disease penetrance revealed that loss of function resulted from folding and/or trafficking defects that prevented channel expression in the absence of β subunits and reduced expression significantly in the presence of β subunits.[29,30] Remarkably, these two GEFS+ mutations can also be partially rescued by treatment with antiepileptic drugs, which apparently stabilize the mutant channels by contributing their binding energy to stabilization of the correctly folded protein.[29,30] These results indicate that loss-of-function effects can result from changes in biophysical properties and/or defects in folding and cell surface expression.

GEFS+ Mutations in Mouse Genetic Models

Considering the confusing picture emerging from studies of the functional effects of GEFS+ mutations in transfected nonneuronal cells, it is important to determine the functional effects of these mutations in neurons in vivo. To this end, the GEFS+ mutation R1648H was incorporated into the mouse genome using a Bac transgene strategy, providing an animal model of GEFS+ that permitted detailed analysis of the effect of mutations on neuronal sodium currents in vivo. The transfected $Na_V1.1$ channel contained both the GEFS+ mutation and an additional amino acid substitution that prevents block by the pore blocker tetrodotoxin (TTX), allowing selective block of endogenous sodium channels by TTX.[31] Mice expressing the transgene had increased sodium channel expression and a reduced threshold for seizure induction by kainic acid. The level of transgene-induced sodium current was much greater in inhibitory neurons than in excitatory neurons, as expected from previous studies of $Na_V1.1$ knockout mice showing selective effects in inhibitory neurons.[32, 33] The R1648H channels showed reduced function in both excitatory and inhibitory neurons, but the biophysical mechanisms were different: reduced peak sodium currents and enhanced slow inactivation in inhibitory neurons versus negatively shifted voltage dependence of fast inactivation in excitatory neurons. These functional effects were predicted to have the net result of reduced excitability of inhibitory neurons. Thus, this GEFS+ mutation causes selective impairment of excitability of GABAergic inhibitory neurons in vivo,[31] as previously demonstrated for complete loss-of-function mutations that cause severe myoclonic epilepsy of infancy in mice[33] (see also below). More recent studies of a mouse model in which the R1648H mutation has been inserted into the mouse genome under the native promoter led to similar

conclusions.[34] In light of these results, GEFS+ epilepsy may be caused by mutational effects that selectively impair firing of GABAergic inhibitory neurons.

SEVERE MYOCLONIC EPILEPSY OF INFANCY

Severe myoclonic epilepsy of infancy (SMEI; also known as *Dravet syndrome*) begins during the first year of life, with seizures often associated with elevated body temperature due to fever or bathing, and progresses to prolonged, clustered, or continuous seizures and to status epilepticus.[35,36] During the second year of life, patients develop comorbidities including psychomotor delay, ataxia, and cognitive impairment. Medically refractory seizures including frequent and prolonged episodes of status epilepticus contribute to an unfavorable long-term outcome.[36,37] Thus, SMEI is a much more severe form of epilepsy than GEFS+. Therefore, it was surprising that identification of familial *SCN1A* mutations in GEFS+ epilepsy was soon followed by a report of *SCN1A* mutations in children with SMEI, OMIM 607208.[38] These children carry de novo mutations in one allele of the *SCN1A* gene, leading to haploinsufficiency of $Na_V1.1$ channels.[38–43] More than 600 *SCN1A* mutations in the coding sequences of the *SCN1A* gene have been identified (Fig. 52–1), accounting for more than 70% of cases[21] (http://www.molgen.ua.ac.be/SCN1AMutations/home/Default.cfm). Since only coding regions of the gene have been sequenced, it is possible that the many of the remaining 30% of SMEI patients harbor mutations in regulatory regions of the gene outside of the coding sequences that impair or prevent channel expression. In addition, duplications and deletions of segments of the *SCN1A* gene can impair expression and/or function.[44] Mutation hot spots, including several sites of CpG deamination, account for approximately 25% of new mutations.[45,46] More than half of the SMEI mutations cause loss of function due to stop codons or deletions, demonstrating that haploinsufficiency of *SCN1A* is pathogenic. Missense mutations of $Na_V1.1$ channels in patients with SMEI are concentrated in the transmembrane segments of the protein, where they may prevent channel folding or severely impair channel function (Fig. 52–1). In addition, recent studies show that homozygous loss-of-function mutations in the $Na_V\beta1$ subunits cause SMEI, probably by impairing expression of $Na_V1.1$ channels on the cell surface.[47]

Loss of Excitability of GABAergic Interneurons and Hyperexcitability in SMEI

It was a surprise to find that haploinsufficiency of a Na_V channel causes epilepsy, because reduced sodium current should lead to hypoexcitability rather than hyperexcitability. To understand the mechanistic basis for hyperexcitability and comorbidities in SMEI, an animal model was generated by targeted deletion or mutation of the *Scn1a* gene in mouse.[32,33] Homozygous null $Na_V1.1(-/-)$ mice developed ataxia and died on postnatal day (P) 15.[32,33] Heterozygous $Na_V1.1(+/-)$ mice exhibited spontaneous seizures and sporadic deaths beginning after P21, with a striking dependence on genetic background.[33] Loss of $Na_V1.1$ did not change voltage-dependent activation or inactivation of sodium channels in hippocampal neurons.[33] However, the sodium current density was substantially reduced in inhibitory interneurons of $Na_V1.1(+/-)$ and $Na_V1.1(-/-)$ mice, but not in their excitatory pyramidal neurons (Table 52–1). This reduction in sodium current caused a loss of sustained high-frequency firing of action potentials in hippocampal and cortical interneurons[32,33] (Fig. 52–2A), thereby impairing their in vivo inhibitory function that depends on generation of high-frequency bursts of action potentials. These results suggest that reduced sodium currents in GABAergic inhibitory interneurons in $Na_V1.1(+/-)$ heterozygotes may cause the hyperexcitability that leads to epilepsy in patients with SMEI. Loss of excitability of GABAergic inhibitory interneurons would allow hyperexcitability of dentate granule and pyramidal neurons, and this gain-of-function effect may cause epilepsy. Failure of firing of additional classes of interneurons in the cerebral cortex and thalamus may also contribute to this complex seizure phenotype. Considered together with the later results on GEFS+ mutations expressed in mice (see above), these studies suggest that selective loss of function of

Table 52–1 Functional Impact of Deletion of the $Na_V1.1$ Channel

Functional Effect	Heterozygous Knockout (%)	Homozygous Knockout (%)
Na^+ current in hippocampal pyramidal cells (% WT)	100 ± 5.1	96 ± 6.0
Na^+ current in hippocampal interneurons (% WT)	47.0 ± 7.4	27.5 ± 5.4
Na^+ current in Purkinje neurons (% WT) peak; persistent; resurgent	57.6 ± 0.6; 44.9 ± 4.1; 49.6 ± 5.5	41.6 ± 0.5; 41.0 ± 3.7; 31.2 ± 3.5
Ataxia	Significant at P21	Severe at P11–14
Na^+ current in RNT neurons	77.5 ± 2	54.1 ± 2
Rebound action potential firing of RNT neurons	50	50
Ventral/dorsal Ca^{2+} transients in the suprachiasmatic nucleus	50	30
Circadian rhythm defect	++	Not tested
Thermally induced seizures	First observed at P20, increasing thereafter	Not tested
Spontaneous seizures	First observed at P21, increasing thereafter	P11–14
Premature death	Increasing premature death after P21	Death at P15

Source: Yu et al., 2006; Kalume et al., 2007; Kalume et al., 2010; Han et al., 2010. RNT, reticular nucleus of the thalamus.

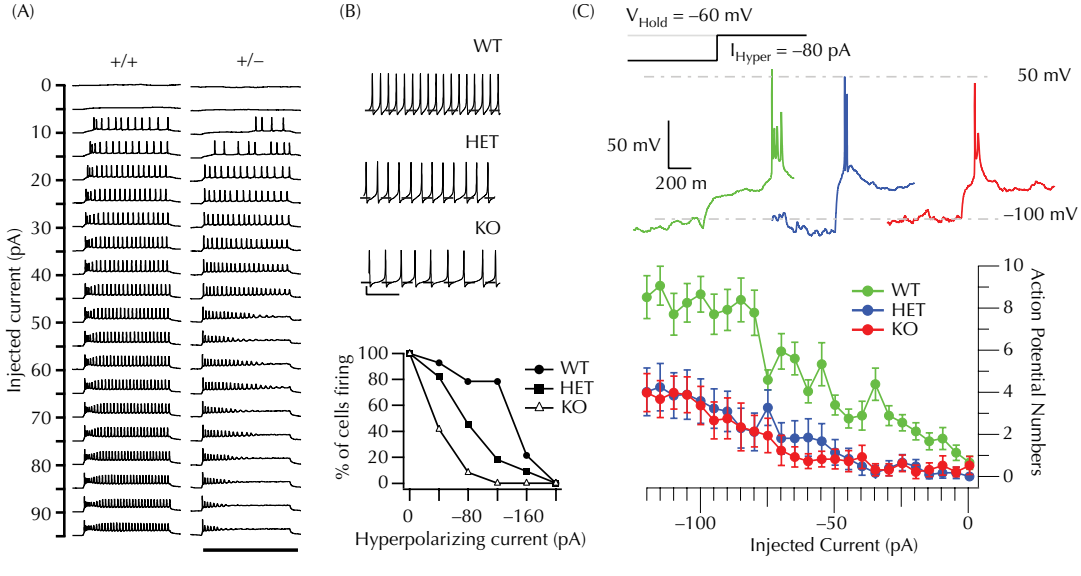

Figure 52–2. Action potentials in different brain regions in wild-type, heterozygous, and null mutant neurons in $Na_V1.1$ knockout mice. **A.** Action potential traces recorded from wild-type (+/+) and heterozygous (+/–) interneurons during 800 ms injections of depolarizing current in +10 pA increments from a holding potential of –80 mV.[53] **B.** Top: rates of spontaneous firing of cerebellar Purkinje neurons with a 50 ms depolarization from a holding potential of –90 mV to potentials ranging from –80 to +30 mV in 5 mV increments. Bottom: resistance of action potential firing to hyperpolarizing current injection. **C.** Hyperpolarization-release firing of RNT neurons. The number of action potentials fired after a hyperpolarizing pulse is illustrated above and plotted versus the hyperpolarizing current below.

Na^+ channels in GABAergic inhibitory neurons may be a common mechanism underlying both of these epilepsy syndromes.

Selective Deletion of $Na_V1.1$ Channels in Inhibitory Neurons

In order to definitively test the hypothesis that selective loss of Na^+ channels in GABAergic inhibitory neurons is the causative change in SMEI, Lox P sites were inserted on both sides of the last coding exon of the *Scn1A* gene in mice, and the resulting Floxed mice were mated with a mouse strain expressing the Cre recombinase in GABAergic inhibitory neurons in the cerebral cortex and hippocampus under the DLX5,6 promoter, which is selectively expressed in migrating inhibitory neuron precursors destined for these brain areas. These mice die prematurely and have spontaneous seizures that are as severe as those of $Na_V1.1$ KO mice,[48] confirming that loss of action potential firing in inhibitory neuron cause SMEI.

Loss of Excitability of GABAergic Interneurons and Comorbidities in SMEI

Ataxia, failure of motor coordination, and other comorbidities contribute substantially to the developmental delay and functional impairments of SMEI patients and are major determinants of their poor quality of life, burden of care, and premature deaths[49,50] (Dravet syndrome: http://www.ilae-epilepsy.org/ctf/dravet.html). How might loss of $Na_V1.1$ channels cause ataxia and failure of motor coordination? Purkinje cells are GABAergic inhibitory neurons that serve as the output pathway for information on movement, coordination, and balance from the cerebellar cortex. Degeneration of Purkinje neurons and abnormal expression of voltage-gated ion channels within them are associated with ataxia.[51–54] Behavioral assessment indicated severe motor deficits in homozygous $Na_V1.1$ knockout mice, including irregularity of stride length during locomotion, impaired motor reflexes in grasping, and mild tremor in limbs when immobile, consistent with cerebellar dysfunction.[33,55] A milder impairment of normal gait was observed in the heterozygotes after P21.[55] $Na_V1.1$ and $Na_V1.6$ channels are the primary sodium channel isoforms expressed in cerebellar Purkinje neurons.[55] The amplitudes of whole-cell peak, persistent, and resurgent sodium currents in Purkinje neurons were reduced by 58% to 69%, without a detectable change in the kinetics or voltage dependence of channel activation or inactivation (Table 52–1). Current-clamp recordings revealed that the firing rates of Purkinje neurons from mutant mice were substantially reduced, with no effect on the threshold for action potential generation[55] (Fig. 52–2B). Moreover, the firing of $Na_V1.1^{+/-}$ and $Na_V1.1^{-/-}$ mice is less resistant to extinction by injection of hyperpolarizing current compared to wild-type mice (Fig. 52–2B). These results show that $Na_V1.1$ channels play a crucial role in the excitability of cerebellar Purkinje neurons, with major contributions to peak, persistent, and resurgent forms of sodium current and to sustained action potential firing. Loss of these channels in Purkinje neurons may be sufficient to cause ataxia and related motor deficits.

Children with SMEI also have a significant impairment of sleep,[56] and $Na_V1.1^{+/-}$ mice have reduced non-REM sleep.[57] Sodium currents and action potential firing are impaired in GABAergic neurons of the reticular nucleus of the thalamus (RNT), which participate in a trisynaptic circuit with thalamic relay cells and cortical pyramidal cells that generates the sleep spindles in the cerebral cortex that drive sleep behavior.[57] In contrast, there is no effect on sodium current in thalamic relay cells. The RNT neurons fire bursts of action potentials upon release from hyperpolarization, which are required for the burst of cortical activity that generates sleep spindles. Recordings from dissociated RNT neurons reveal a major deficit in rebound action potential firing (Fig. 52–2C), suggesting that this is the basis for the reduction of non–rapid eye movement (non-REM) sleep in SMEI.

$Na_V1.1^{+/-}$ mice also have impaired circadian rhythms.[58] Circadian rhythms are generated in the suprachiasmatic nucleus (SCN) of the hypothalamus, which receives direct input from the retina via the optic nerve. $Na_V1.1$ channels are expressed in the GABAergic neurons of the ventral SCN, and expression is impaired in $Na_V1.1^{+/-}$ mice. Communication from ventral to dorsal SCN, which is required for normal

circadian rhythm, is also impaired as a consequence of reduced $Na_V1.1$ channel expression.[58] These results suggest that impaired firing of GABAergic neurons in the SCN is responsible for the circadian defect in SMEI. Therefore, selective decrease in action potential firing in GABAergic neurons in different parts of the brain may be responsible for both the comorbidity of ataxia and the comorbidity of sleep and circadian rhythm disturbance in SMEI.

Thermally Induced Seizures in a Mouse Model of SMEI

Children with SMEI frequently have seizures with elevated body temperature as their first symptom of the disease.[59] Experiments with a mouse model of SMEI demonstrated that haploinsufficiency of $Na_V1.1$ channels is sufficient to allow induction of seizures by elevated body temperature.[60] P17–18 mice with SMEI did not have thermally induced seizures, but nearly all P20–22 and P30–46 mice with SMEI had myoclonic seizures followed by generalized seizures with elevated core body temperature. Spontaneous seizures were only observed in mice older than P21, indicating that mice with SMEI become susceptible to temperature-induced seizures before spontaneous seizures. Interictal spike activity was seen at normal body temperature in most P30–46 mice with SMEI but not in P20–22 or P17–18 mice, suggesting that interictal epileptic activity correlates with seizure susceptibility. These results define a critical developmental transition for susceptibility to seizures in a mouse model of SMEI and reveal a close correspondence between human and mouse SMEI in the striking temperature and age dependence of SMEI onset and progression.

Balancing Excitation and Inhibition with Genetic Compensation and Drug Treatment

The net electrophysiological properties of a neuron are the product of its total ion channel activity, so inheritance of genetic variants of ion channels may contribute to polygenic inheritance or variable penetrance among family members. Since SMEI is apparently caused by loss of sodium current and failure of firing of GABAergic interneurons,[33,55] it may be compensated for by mutations that reduce the sodium current and action potential firing of excitatory neurons and thereby rebalance excitation and inhibition in the brain. Such genetic compensation can be studied by mating mouse lines having different well-defined genetic deficiencies. $Na_V1.6$ channels encoded by the *Scn8a* gene are highly expressed in excitatory neurons, and their functional properties are well suited to driving repetitive firing.[50,61,62] Double heterozygous mice with haploinsufficiency for both *Scn1a* and *Scn8a* did indeed have reduced susceptibility to drug-induced seizures and an increased lifespan compared to $Na_V1.1$ heterozygotes.[63] These results support the concept that loss-of-function mutations in $Na_V1.1$ channels in SMEI cause an imbalance of excitation over inhibition in the brain and that this imbalance can be partially compensated for by a corresponding reduction in the activity of $Na_V1.6$ channels.

In principle, the imbalance between excitation and inhibition can also be corrected by drug treatment. One practical result of the discovery that haploinsufficiency of $Na_V1.1$ channels causes SMEI is avoidance of treatment with non-subtype-selective sodium channel-blocking antiepileptic drugs, which exacerbate symptoms in patients with reduced expression of *SCN1A*.[64,65] Unfortunately, there are no drugs that selectively inhibit $Na_V1.6$ channels. However, an alternative approach to rebalancing excitation and inhibition is to enhance GABAergic neurotransmission by drug treatment. The reduced frequency of action potentials in GABAergic inhibitory neurons in SMEI would decrease the phasic release of GABA and impair inhibitory neurotransmission. Drugs such as tiagabine increase the concentration of GABA in the synaptic cleft by inhibiting its reuptake into nerve terminals and glia, and benzodiazepines such as clonazepam increase the response of the postsynaptic $GABA_A$ receptors to GABA. Using febrile seizures in a mouse model of SMEI[60] as a test system, we found that combinations of tiagabine and clonazepam are effective in completely preventing thermally induced myoclonic and generalized tonic-clonic seizures.[66] These encouraging results suggest that similar combination drug therapies may be useful for children with SMEI.

POTENTIAL ROLE OF MUTATIONS IN $Na_V1.1$ CHANNELS IN FEBRILE SEIZURES IN CHILDHOOD

Febrile seizures are common in childhood, but the basis for their prevalence is unknown. Recent evidence suggests that mild loss-of-function mutations or polymorphisms in $Na_V1.1$ channels may cause a significant proportion of febrile seizures. Mantegazza et al.[67] characterized a mild loss-of-function mutation in $Na_V1.1$ channels in a family with familial febrile seizures. This mutation caused reduction of peak sodium currents and a positive shift in the voltage dependence of activation when expressed in nonneuronal cells, providing the first evidence for association of mild loss of function of $Na_V1.1$ channels with familial febrile seizures.

There has been considerable controversy regarding claims that routine childhood vaccination may be associated with the onset of both febrile and afebrile seizures and mental decline. Berkovic et al.[68] studied 14 children with this diagnosis and identified *SCN1A* mutations in 11 of them. Their observations indicate that vaccination and its associated fever may trigger the first seizure episode of an underlying genetic disorder, GEFS+ or SMEI, which often presents first as febrile seizures. These studies further implicate dysfunction of $Na_V1.1$ channels in childhood febrile seizures.

Most recently, human genetic studies have also suggested an association of genetic alterations in $Na_V1.1$ channels with nonfamilial febrile seizures.[69] In normal development, the mRNAs encoding $Na_V1.1$, $Na_V1.2$, and $Na_V1.3$ channels undergo a regulated change in alternative splicing of exon 5,[70,71] which has a striking effect on the voltage dependence of channel activation.[72] Regulation of this alternative splicing process is disrupted by a single nucleotide polymorphism (SNP IVS5N+5 G>A).[73,74] The presence of this SNP has been correlated with an altered response to antiepileptic drugs[73,74] and with the risk of febrile seizures in a large cohort of epilepsy patients from Germany and Austria.[69] On the other hand, correlation of this SNP with febrile seizures was not observed in a similar study of Australian epilepsy patients.[75] Nevertheless, even considering these negative data, the combination of results from studies of familial febrile seizures,[67] vaccination-related seizures,[68] responsiveness to antiepileptic drugs,[73,74] and febrile seizures in a German/Austrian cohort of patients[69] all point to a key role of $Na_V1.1$ channels as molecular determinants of the risk of epilepsy in the general population and raise the possibility that febrile seizures in children are often caused by mild loss-of-function mutations or polymorphisms of $Na_V1.1$ channels in combination with environmental precipitating factors.

A UNIFIED LOSS-OF-FUNCTION HYPOTHESIS FOR $Na_V1.1$ GENETIC EPILEPSIES

$Na_V1.1$ channels are highly expressed in many GABAergic inhibitory neurons and are responsible for essentially all of the sodium current in the cell bodies of hippocampal interneurons.[33] Loss of function of these sodium channels greatly impairs the ability of these inhibitory neurons to fire action potentials at high frequency and therefore would greatly reduce their phasic release of GABA.[33] It is likely that this loss of action potential firing by inhibitory neurons leads to an imbalance between excitation and inhibition in the brain and consequently to febrile seizures and epilepsy. Although more work is needed to develop definite genotype–phenotype correlations for the $Na_V1.1$ epilepsies, we extend here the previous proposal[76] suggesting the unifying hypothesis that the spectrum of severity of the $Na_V1.1$-associated forms of epilepsy results from a spectrum of increasing severity of loss-of-function mutations of $Na_V1.1$ channels and increasing impairment of action potential firing in GABAergic inhibitory neurons (Fig. 52–3). Mild impairment of $Na_V1.1$ channel function causes febrile seizures; moderate to severe impairment of $Na_V1.1$ function by missense mutations and/or altered mRNA processing causes the range of phenotypes observed in GEFS+ epilepsy; and very severe to complete loss of function causes SMEI. The severity of the phenotype in these genetic diseases is also influenced strongly by genetic background effects, as illustrated by striking differences in phenotypes among GEFS+ patients with the same missense mutation,[77] different severity of disease of SMEI patients with complete loss-of-function mutations,[78,79] and dramatic differences in sensitivity among mouse strains

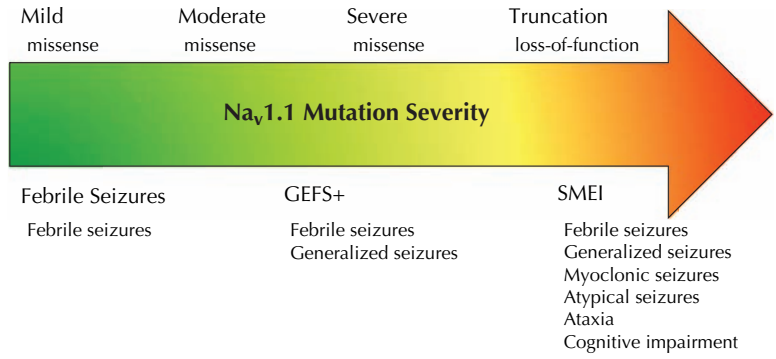

Figure 52–3. The unified loss-of-function hypothesis for Na$_V$1.1 genetic epilepsies. Increasing severity of loss-of-function mutations of Na$_V$1.1 channels, noted above the arrow, causes progressively more severe epilepsy syndromes ranging from familial febrile seizures to GEFS+ and finally SMEI, noted below the arrow. Major symptoms of each syndrome are also listed.

to the same loss-of-function mutations.[33] This unifying hypothesis may bring increasing clarity to the understanding of the genotype–phenotype correlations in this family of epilepsy syndromes.

MUTATIONS OF OTHER NA$_V$ CHANNELS IN EPILEPSY

Benign familial neonatal-infantile seizures (BFNIS) is a mild seizure syndrome caused by dominant mutations in Na$_V$1.2 channels.[80] Affected individuals have onset of seizures in early infancy.[81] Typically, ictal episodes begin as partial seizures, which often become generalized. Febrile seizures are rare.[81] Fortunately, these seizures respond favorably to treatment with antiepileptic drugs, and they generally remit by 1 year of age.[81]

Na$_V$1.2 channels are closely related to Na$_V$1.1 channels in amino acid sequence and in their primary expression in the central nervous system.[5] They are primarily expressed in unmyelinated axons in adult rodent brain,[7] and they are replaced by Na$_V$1.6 channels during formation of the myelin sheath of myelinated axons.[82] As with Na$_V$1.1 channels, their expression increases during the first 4 weeks of postnatal life in rodents.[17,18] The relationship between the functional alterations in Na$_V$1.2 channels and the hyperexcitability of BFNIS is not clear. Some missense mutations cause a gain of sodium channel function via negative shifts in the voltage dependence of activation or impairment of inactivation.[83] On the other hand, some missense mutations cause reduced Na^+ channel activity.[84] In addition, one missense mutation caused a selective gain of sodium channel function in the embryonic isoform of Na$_V$1.2 channels,[85] which has a more positive voltage dependence of activation than the adult isoform.[70,72] These seizures remitted during neonatal development, following a time course similar to that of the normal increase in voltage sensitivity of the wild-type Na$_V$1.2 channel.[85] These results suggest that increased excitability of the mutant embryonic isoform of Na$_V$1.2 channels can cause early-onset epilepsy. This epilepsy remits with development because the enhanced excitability of the mutant embryonic isoform of the Na$_V$1.2 channel is comparable to that of wild-type channels after alternative splicing to the mature form of Na$_V$1.2 is completed. Thus, in this case, remission of the seizure phenotype is apparently caused by the fact that the mutant Na$_V$1.2 channel is no more excitable than wild-type Na$_V$1.2 after the wild type is converted to the adult isoform by alternative splicing of its mRNA. In addition to BFNIS, de novo dominant mutations in Na$_V$1.2 channels can cause other, more severe seizure syndromes.[86–88] These results broaden the spectrum of epilepsies that can be caused by mutations in Na$_V$1.2 channels, which is now similar to the spectrum of seizure syndromes observed for Na$_V$1.1 mutations (Fig. 52–3).

Surprisingly, no mutations in Na$_V$1.3 or Na$_V$1.6 channels have been described that cause epilepsy. Na$_V$1.3 channels are primarily expressed in embryonic life, so severe

mutations within them may cause premature termination of pregnancy, and mild mutations may not have major effects because expression of these channels declines soon after birth. On the other hand, $Na_V1.6$ channels drive the excitability of excitatory neurons,[89] so gain of function should cause hyperexcitability and epilepsy. The lack of such mutations in the human population may indicate that even mild increases in the function of $Na_V1.6$ channels have such deleterious consequences that individuals with gain-of-function mutations die prematurely from causes other than epilepsy.

DISCLOSURE STATEMENT

The author has no conflicts of interest to disclose.

REFERENCES

1. Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron*. 2000;26:13–25.
2. Isom LL. The role of sodium channels in cell adhesion. *Front Biosci*. 2002;7:12–23.
3. Isom LL, Ragsdale DS, De Jongh KS, Westenbroek RE, Reber BFX, Scheuer T, Catterall WA. Structure and function of the beta-2 subunit of brain sodium channels, a transmembrane glycoprotein with a CAM-motif. *Cell*. 1995;83:433–442.
4. Goldin AL. Resurgence of sodium channel research. *Annu Rev Physiol*. 2001;63:871–894.
5. Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Berwald Netter Y, Noda M, Tamkun MM, Waxman SG, Wood JN, Catterall WA. Nomenclature of voltage-gated sodium channels. *Neuron*. 2000;28:365–368.
6. Trimmer JS, Rhodes KJ. Localization of voltage-gated ion channels in mammalian brain. *Annu Rev Physiol*. 2004;66:477–519.
7. Westenbroek RE, Merrick DK, Catterall WA. Differential subcellular localization of the RI and RII Na^+ channel subtypes in central neurons. *Neuron*. 1989;3:695–704.
8. Westenbroek RE, Noebels JL, Catterall WA. Elevated expression of type II Na^+ channels in hypomyelinated axons of shiverer mouse brain. *J Neurosci*. 1992;12:2259–2267.
9. Krzemien DM, Schaller KL, Levinson SR, Caldwell JH. Immunolocalization of sodium channel isoform NaCh6 in the nervous system. *J Comp Neurol*. 2000;420:70–83.
10. Caldwell JH, Schaller KL, Lasher RS, Peles E, Levinson SR. Sodium channel $Na_V1.6$ is localized at nodes of Ranvier, dendrites, and synapses. *Proc Natl Acad Sci USA*. 2000;97:5616–5620.
11. Jenkins SM, Bennett V. Ankyrin-G coordinates assembly of the spectrin-based membrane skeleton, voltage-gated sodium channels, and L1 CAMs at Purkinje neuron initial segments. *J Cell Biol*. 2001;155:739–746.
12. Stuart GJ, Sakmann B. Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature*. 1994;367:69–72.
13. Johnston D, Magee JC, Colbert CM, Christie BR. Active properties of neuronal dendrites. *Annu Rev Neurosci*. 1996;19:165–186.
14. Callaway JC, Ross WN. Spatial distribution of synaptically activated sodium concentration changes in cerebellar Purkinje neurons. *J Neurophysiol*. 1997;77:145–152.
15. Raman IM, Bean BP. Properties of sodium currents and action potential firing in isolated cerebellar Purkinje neurons. *Ann NY Acad Sci*. 1999;868:93–96.
16. Khaliq ZM, Raman IM. Relative contributions of axonal and somatic Na channels to action potential initiation in cerebellar Purkinje neurons. *J Neurosci*. 2006;26:1935–1944.
17. Beckh S, Noda M, Lübbert H, Numa S. Differential regulation of three sodium channel messenger RNAs in the rat central nervous system during development. *EMBO J*. 1989;8:3611–3616.
18. Gordon D, Merrick D, Auld V, Dunn R, Goldin AL, Davidson N, Catterall WA. Tissue-specific expression of the RI and RII sodium channel subtypes. *Proc Natl Acad Sci USA*. 1987;84:8682–8686.
19. Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. *Nat Genet*. 2000;24:343–345.
20. Wallace RH, Wang DW, Singh R, Scheffer IE, George JAL, Phillips HA, Saar K, Reis A, Johnson EW, Sutherland GR, Berkovic SF, Mulley JC. Febrile seizures and generalized epilepsy associated with a mutation in the sodium channel β1 subunit gene SCN1B. *Nat Genet*. 1998;19:366–370.
21. Meisler MH, Kearney JA. Sodium channel mutations in epilepsy and other neurological disorders. *J Clin Invest*. 2005;115:2010–2017.
22. Spampanato J, Escayg A, Meisler MH, Goldin AL. Functional effects of two voltage-gated sodium channel mutations that cause generalized epilepsy with febrile seizures plus type 2. *J Neurosci*. 2001;21:7481–7490.
23. Lossin C, Wang DW, Rhodes TH, Vanoye CG, George AL Jr. Molecular basis of an inherited epilepsy. *Neuron*. 2002;34:877–884.
24. Lossin C, Rhodes TH, Desai RR, Vanoye CG, Wang D, Carniciu S, Devinsky O, George AL Jr. Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel SCN1A. *J Neurosci*. 2003;23:11289–11295.
25. Kahlig KM, Misra SN, George AL Jr. Impaired inactivation gate stabilization predicts increased persistent current for an epilepsy-associated SCN1A mutation. *J Neurosci*. 2006;26:10958–10966.
26. Spampanato J, Escayg A, Meisler MH, Goldin AL. Generalized epilepsy with febrile seizures plus type 2 mutation W1204R alters voltage-dependent gating of $Na_V1.1$ sodium channels. *Neuroscience*. 2003;116:37–48.
27. Spampanato J, Kearney JA, de Haan G, McEwen DP, Escayg A, Aradi I, MacDonald BT, Levin SI,

Soltesz I, Benna P, Montalenti E, Isom LL, Goldin AL, Meisler MH. A novel epilepsy mutation in the sodium channel SCN1A identifies a cytoplasmic domain for beta subunit interaction. *J Neurosci*. 2004;24:10022–10034.
28. Barela AJ, Waddy SP, Lickfett JG, Hunter J, Anido A, Helmers SL, Goldin AL, Escayg A. An epilepsy mutation in the sodium channel SCN1A that decreases channel excitability. *J Neurosci.* 2006;26:2714–2723.
29. Rusconi R, Scalmani P, Cassulini RR, Giunti G, Gambardella A, Franceschetti S, Annesi G, Wanke E, Mantegazza M. Modulatory proteins can rescue a trafficking defective epileptogenic $Na_v1.1$ Na^+ channel mutant. *J Neurosci.* 2007;27:11037–11046.
30. Rusconi R, Combi R, Cestele S, Grioni D, Franceschetti S, Dalpra L, Mantegazza M. A rescuable folding defective $Na_v1.1$ (SCN1A) sodium channel mutant causes GEFS+: common mechanism in $Na_v1.1$ related epilepsies? *Hum Mutat*. 2009;30:E747–E760.
31. Tang B, Dutt K, Papale L, Rusconi R, Shankar A, Hunter J, Tufik S, Yu FH, Catterall WA, Mantegazza M, Goldin AL, Escayg A. A BAC transgenic mouse model reveals neuron subtype-specific effects of a Generalized Epilepsy with Febrile Seizures Plus (GEFS+) mutation. *Neurobiol Dis*. 2009;35:91–102.
32. Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, Takeuchi T, Itohara S, Yanagawa Y, Obata K, Furuichi T, Hensch TK, Yamakawa K. $Na_v1.1$ localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. *J Neurosci*. 2007;27:5903–5914.
33. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci.* 2006;9:1142–1149.
34. Martin MS, Dutt K, Papale LA, Dubé CM, Dutton SB, De Haan G, Shankar A, Tufik S, Meisler MH, Baram TZ, Goldin AL, Escayg A. (2010) Altered function of the SCN1A voltage-gated sodium channel leads to GABAergic interneuron abnormalities. *J Biol. Chem*. 2010;285:9823–9834.
35. Engel J Jr. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. *Epilepsia.* 2001;42:796–803.
36. Dravet C, Bureau M, Guerrini R, Giraud N, Roger J. Severe myoclonic epilepsy in infants. In: Roger J, Dravet C, Bureau M, Dreifus FE, Perret A, Wolf P, eds. *Epileptic Syndromes in Infancy, Childhood, and Adolescence*. London: John Libbey; 1992:75–102.
37. Oguni H, Hayashi K, Awaya Y, Fukuyama Y, Osawa M. Severe myoclonic epilepsy in infants—a review based on the Tokyo Women's Medical University series of 84 cases. *Brain Dev*. 2001;23:736–748.
38. Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet*. 2001;68:1327–1332.
39. Ohmori I, Ouchida M, Ohtsuka Y, Oka E, Shimizu K. Significant correlation of the SCN1A mutations and severe myoclonic epilepsy in infancy. *Biochem Biophys Res Commun*. 2002;295:17–23.
40. Fujiwara T, Sugawara T, Mazaki-Miyazaki E, Takahashi Y, Fukushima K, Watanabe M, Hara K, Morikawa T, Yagi K, Yamakawa K, Inoue Y. Mutations of sodium channel α subunit type 1 (SCN1A) in intractable childhood epilepsies with frequent generalized tonic-clonic seizures. *Brain*. 2003;126:531–546.
41. Claes L, Ceulemans B, Audenaert D, Smets K, Lofgren A, Del-Favero J, Ala-Mello S, Basel-Vanagaite L, Plecko B, Raskin S, Thiry P, Wolf NI, Van Broeckhoven C, De Jonghe P. De novo SCN1A mutations are a major cause of severe myoclonic epilepsy of infancy. *Hum Mutat*. 2003;21:615–621.
42. Sugawara T, Mazaki-Miyazaki E, Fukushima K, Shimomura J, Fujiwara T, Hamano S, Inoue Y, Yamakawa K. Frequent mutations of SCN1A in severe myoclonic epilepsy in infancy. *Neurology*. 2002;58:1122–1124.
43. Fukuma G, Oguni H, Shirasaka Y, Watanabe K, Miyajima T, Yasumoto S, Ohfu M, Inoue T, Watanachai A, Kira R, Matsuo M, Muranaka H, Sofue F, Zhang B, Kaneko S, Mitsudome A, Hirose S. Mutations of neuronal voltage-gated Na^+ channel alpha 1 subunit gene SCN1A in core severe myoclonic epilepsy in infancy (SMEI) and in borderline SMEI (SMEB). *Epilepsia*. 2004;45:140–148.
44. Marini C, Scheffer IE, Nabbout R, Mei D, Cox K, Dibbens LM, McMahon JM, Iona X, Carpintero RS, Elia M, Cilio MR, Specchio N, Giordano L, Striano P, Gennaro E, Cross JH, Kivity S, Neufeld MY, Afawi Z, Andermann E, Keene D, Dulac O, Zara F, Berkovic SF, Guerrini R, Mulley JC. SCN1A duplications and deletions detected in Dravet syndrome: implications for molecular diagnosis. *Epilepsia*. 2009;50(7):1670–8.
45. Kearney JA, Wiste AK, Stephani U, Trudeau MM, Siegel A, Ramachandran Nair R, Elterman RD, Muhle H, Reinsdorf J, Shields WD, Meisler MH, Escayg A. Recurrent de novo mutations of SCN1A in severe myoclonic epilepsy of infancy. *Pediatr Neurol*. 2006;34:116–120.
46. Depienne C, Trouillard O, Saint-Martin C, Gourfinkel-An I, Bouteiller D, Carpentier W, Keren B, Abert B, Gautier A, Baulac S, Arzimanoglou A, Cazeneuve C, Nabbout R, LeGuern E. Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333 patients. *J Med Genet*. 2009;46:183–191.
47. Patino GA, Claes LR, Lopez-Santiago LF, Slat EA, Dondeti RS, Chen C, O'Malley HA, Gray CB, Miyazaki H, Nukina N, Oyama F, De Jonghe P, Isom LL. A functional null mutation of SCN1B in a patient with Dravet syndrome. *J Neurosci*. 2009;29:10764–10778.
48. Cheah CS, Yu FH, Westenbroek RE, Kalume FK, Oakley JC, Rubenstein JL, Catterall WA. Conditional deletion of Nav1.1 channels in inhibitory interneurons is sufficient to cause the seizures and premature death in a mouse model of SMEI. *Society of Neuroscience* 2010, Online 255.16/R1.
49. Dravet C. Dravet's syndrome history. *Dev Med Child Neurol.* 2011;53:1–6.
50. Dravet C, Bureau M, Oguni H, Fukuyama Y, Cokar O. Severe myoclonic epilepsy in infancy: Dravet syndrome. *Adv Neurol.* 2005;95:71–102.
51. Grusser-Cornehls U, Baurle J. Mutant mice as a model for cerebellar ataxia. *Prog Neurobiol*. 2001;63:489–540.

52. Raman IM, Bean BP. Resurgent sodium current and action potential formation in dissociated cerebellar Purkinje neurons. *J Neurosci*. 1997;17:4517–4526.
53. Sausbier M, Hu H, Arntz C, Feil S, Kamm S, Adelsberger H, Sausbier U, Sailer CA, Feil R, Hofmann F, Korth M, Shipston MJ, Knaus HG, Wolfer DP, Pedroarena CM, Storm JF, Ruth P. Cerebellar ataxia and Purkinje cell dysfunction caused by Ca^{2+}-activated K^+ channel deficiency. *Proc Natl Acad Sci USA*. 2004;101:9474–9478.
54. Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD Jr, Hawkes R, Frankel WN, Copeland NG, Jenkins NA. Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell*. 1996;87:607–617.
55. Kalume F, Yu FH, Westenbroek RE, Scheuer T, Catterall WA. Reduced sodium current in Purkinje neurons from Nav1.1 mutant mice: implications for ataxia in severe myoclonic epilepsy in infancy. *J Neurosci*. 2007;27:11065–11074.
56. Nolan KJ, Camfield CS, Camfield PR. Coping with Dravet syndrome: parental experiences with a catastrophic epilepsy. *Dev Med Child Neurol*. 2006;48: 761–765.
57. Kalume FK, Oakley JC, Westenbroek RE, Scheuer T, Catterall WA. Reduced excitability of GABAergic interneurons in the reticular nucleus of the thalamus and sleep impairment in a mouse model of Severe Myoclonic Epilepsy of Infancy. *Society of Neuroscience* 2010. Online 255.15/Q18.
58. Han S, Yu FU, Schwartz MD, Bosma MM, De La Iglesia HO, Catterall WA. Reduced $Na_v1.1$ expression in the suprachiasmatic nucleus alters circadian rhythm by impairing GABAergic transmission in a mouse model of severe myoclonic epilepsy in infancy. *Society of Neuroscience* 2010. Online 698.9/JJJ11.
59. Oguni H, Hayashi K, Osawa M, Awaya Y, Fukuyama Y, Fukuma G, Hirose S, Mitsudome A, Kaneko S. Severe myoclonic epilepsy in infancy: clinical analysis and relation to SCN1A mutations in a Japanese cohort. *Adv Neurol*. 2005;95:103–117.
60. Oakley JC, Kalume F, Yu FH, Scheuer T, Catterall WA. Temperature- and age-dependent seizures in a mouse model of severe myoclonic epilepsy in infancy. *Proc Natl Acad Sci USA*. 2009;106:3994–3999.
61. Raman IM, Bean BP. Ionic currents underlying spontaneous action potentials in isolated cerebellar Purkinje neurons. *J Neurosci*. 1999;19:1664–1674.
62. Chen Y, Yu FH, Sharp EM, Beacham D, Scheuer T, Catterall WA. Functional properties and differential neuromodulation of $Na_v1.6$ channels. *Mol Cell Neurosci*. 2008;38:607–615.
63. Martin MS, Tang B, Papale LA, Yu FH, Catterall WA, Escayg A. The voltage-gated sodium channel Scn8a is a genetic modifier of severe myoclonic epilepsy of infancy. *Hum Mol Genet*. 2007; 16:2892–2899.
64. Loscher W. Preclinical assessment of proconvulsant drug activity and its relevance for predicting adverse events in humans. *Eur J Pharmacol*. 2009;610:1–11.
65. Guerrini R, Dravet C, Genton P, Belmonte A, Kaminska A, Dulac O. Lamotrigine and seizure aggravation in severe myoclonic epilepsy. *Epilepsia*. 1998;39:508–512.
66. Oakley JC, Kalume F, Yu FH, Westenbrock RE, Schever T, Catterall WA. Mechanism-based combination drug therapy in a mouse of severe myoclonic epilepsy of infancy. *American Epilepsy Soc Abst*. 2010; 3.026.
67. Mantegazza M, Gambardella A, Rusconi R, Schiavon E, Annesi F, Cassulini RR, Labate A, Carrideo S, Chifari R, Canevini MP, Canger R, Franceschetti S, Annesi G, Wanke E, Quattrone A. Identification of an Nav1.1 sodium channel (SCN1A) loss-of-function mutation associated with familial simple febrile seizures. *Proc Natl Acad Sci USA*. 2005;102:18177–18182.
68. Berkovic SF, Harkin L, McMahon JM, Pelekanos JT, Zuberi SM, Wirrell EC, Gill DS, Iona X, Mulley JC, Scheffer IE. De-novo mutations of the sodium channel gene SCN1A in alleged vaccine encephalopathy: a retrospective study. *Lancet Neurol*. 2006;5: 488–492.
69. Schlachter K, Gruber-Sedlmayr U, Stogmann E, Lausecker M, Hotzy C, Balzar J, Schuh E, Baumgartner C, Mueller JC, Illig T, Wichmann HE, Lichtner P, Meitinger T, Strom TM, Zimprich A, Zimprich F. A splice site variant in the sodium channel gene SCN1A confers risk of febrile seizures. *Neurology*. 2009;72:974–978.
70. Sarao R, Gupta SK, Auld VJ, Dunn RJ. Developmentally regulated alternative RNA splicing of rat brain sodium channel mRNAs. *Nucleic Acids Res*. 1991;19:5673–5679.
71. Gazina EV, Richards KL, Mokhtar MB, Thomas EA, Reid CA, Petrou S. Differential expression of exon 5 splice variants of sodium channel alpha subunit mRNAs in the developing mouse brain. *Neuroscience*. 2010;166;195–200.
72. Auld VJ, Goldin AL, Krafte DS, Marshall J, Dunn JM, Catterall WA, Lester HA, Davidson N, Dunn RJ. A rat brain sodium channel β subunit with novel gating properties. Neuron. 1988;1:449–461.
73. Tate SK, Depondt C, Sisodiya SM, Cavalleri GL, Schorge S, Soranzo N, Thom M, Sen A, Shorvon SD, Sander JW, Wood NW, Goldstein DB. Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. *Proc Natl Acad Sci USA*. 2005;102: 5507–5512.
74. Tate SK, Singh R, Hung CC, Tai JJ, Depondt C, Cavalleri GL, Sisodiya SM, Goldstein DB, Liou HH. A common polymorphism in the SCN1A gene associates with phenytoin serum levels at maintenance dose. *Pharmacogenet Genomics*. 2006;16:721–726.
75. Petrovski S, Scheffer IE, Sisodiya SM, O'Brien TJ, Berkovic SF. Lack of replication of association between SCN1A SNP and febrile seizures. *Neurology*. 2009;73:1928–1930.
76. Ragsdale DS. How do mutant $Na_v1.1$ sodium channels cause epilepsy? *Brain Res Rev*. 2008;58: 149–159.
77. Scheffer IE, Zhang YH, Jansen FE, Dibbens L. Dravet syndrome or genetic (generalized) epilepsy with febrile seizures plus? *Brain Dev*. 2009;31:394–400.
78. Mulley JC, Scheffer IE, Petrou S, Dibbens LM, Berkovic SF, Harkin LA. SCN1A mutations and epilepsy. *Hum Mutat*. 2005;25:535–542.
79. Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, Sadleir LG, Andermann E, Gill D, Farrell K, Connolly M, Stanley T, Harbord M, Andermann F, Wang J, Batish SD, Jones JG, Seltzer WK, Gardner A, Sutherland G, Berkovic SF, Mulley JC,

Scheffer IE. The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain.* 2007;130:843–852.
80. Heron SE, Crossland KM, Andermann E, Phillips HA, Hall AJ, Bleasel A, Shevell M, Mercho S, Seni MH, Guiot MC, Mulley JC, Berkovic SF, Scheffer IE. Sodium-channel defects in benign familial neonatal-infantile seizures. *Lancet.* 2002;360:851–852.
81. Berkovic SF, Heron SE, Giordano L, Marini C, Guerrini R, Kaplan RE, Gambardella A, Steinlein OK, Grinton BE, Dean JT, Bordo L, Hodgson BL, Yamamoto T, Mulley JC, Zara F, Scheffer IE. Benign familial neonatal-infantile seizures: characterization of a new sodium channelopathy. *Ann Neurol.* 2004;55: 550–557.
82. Rasband MN. The axon initial segment and the maintenance of neuronal polarity. *Nat Rev Neurosci.* 2010;11:552–562.
83. Scalmani P, Rusconi R, Armatura E, Zara F, Avanzini G, Franceschetti S, Mantegazza M. Effects in neocortical neurons of mutations of the $Na_v1.2$ channel causing benign familial neonatal-infantile seizures. *J Neurosci.* 2006;26:10100–10109.
84. Misra SN, Kahlig KM, George AL Jr. Impaired $Na_v1.2$ function and reduced cell surface expression in benign familial neonatal-infantile seizures. *Epilepsia.* 2008;49:1535–1545.
85. Xu R, Thomas EA, Jenkins M, Gazina EV, Chiu C, Heron SE, Mulley JC, Scheffer IE, Berkovic SF, Petrou S. A childhood epilepsy mutation reveals a role for developmentally regulated splicing of a sodium channel. *Mol Cell Neurosci.* 2007;35:292–301.
86. Liao Y, Anttonen AK, Liukkonen E, Gaily E, Maljevic S, Schubert S, Bellan-Koch A, Petrou S, Ahonen VE, Lerche H, Lehesjoki AE. SCN2A mutation associated with neonatal epilepsy, late-onset episodic ataxia, myoclonus, and pain. *Neurology.* 2010;75:1454–1458.
87. Kamiya K, Kaneda M, Sugawara T, Mazaki E, Okamura N, Montal M, Makita N, Tanaka M, Fukushima K, Fujiwara T, Inoue Y, Yamakawa K. A nonsense mutation of the sodium channel gene SCN2A in a patient with intractable epilepsy and mental decline. *J Neurosci.* 2004;24:2690–2698.
88. Ogiwara I, Ito K, Sawaishi Y, Osaka H, Mazaki E, Inoue I, Montal M, Hashikawa T, Shike T, Fujiwara T, Inoue Y, Kaneda M, Yamakawa K. De novo mutations of voltage-gated sodium channel alphaII gene SCN2A in intractable epilepsies. *Neurology.* 2009;73: 1046–1053.
89. Chen Y, Yu F, Sharp E, Beacham D, Scheuer T, Catterall W. Functional properties and differential modulation of Nav1.6 channels. *Mol Cell Neurosci.* 2008;38:607–615.

Chapter 53

Potassium Channelopathies of Epilepsy

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INTRODUCTION

Channelopathies are inherited genetic changes in ion channel genes that generate a disease. Given the pivotal role of voltage-dependent potassium channels in moderating neuronal excitability, it is not surprising that these channels are well represented among the channelopathies contributing to epilepsy. Voltage-dependent potassium channels are regarded as the "initial responders" that shape and moderate depolarization of excitable cells. However, this oversimplification belies a heterogeneous mix of diverse voltage-dependent potassium channels in the nervous system that are specialized for function in different subcellular compartments, in different neuronal types, and, indeed, even at different voltages. Consistent with this, the uncovering of epilepsy channelopathies during the past two decades has revealed a number of potassium channels that contribute to epilepsy in distinct ways when genetically altered. This chapter provides a brief overview of the various potassium channelopathies that have so far been identified to contribute to epilepsy. In so doing, it also highlights the distinct functions that different potassium channels serve in regulating excitability and how they contribute to epilepsy when such functions are altered.

WHAT ARE POTASSIUM CHANNELS?

The minimal structures that comprise potassium channels are best represented by the

inward rectifier potassium channels (*KCNJ* family, Fig. 53–1). These channels include two transmembrane segments that flank a pore-loop domain. The transmembrane segments form an aqueous channel, and the pore-loop domain filters ions to selectively allow potassium flux. These components are conserved in all potassium channels. Binding of magnesium and polyamines at the intracellular vestibule of the channel is a property unique to the inward rectifier class of potassium channels that controls ion flux. Binding and occlusion of the pore occur at relatively positive but not negative voltages, therefore giving these channels the property of inward rectification (larger inward than outward conductance).[1] Conduction through the channel is also regulated by conformational changes in the channel, either near the selectivity filter or at the second transmembrane segment that allows some inward rectifiers, such as the adenosine triphosphate (ATP)-sensitive potassium channels, to gate channel opening in response to intracellular signals such as ATP concentration.[2]

Evolution and gene duplication have diversified the potassium channel family to include the twin pore channels (Fig. 53–1), which are thought to contribute to the "leak" resting potassium current and therefore affect resting membrane potentials.[3] As well, an additional 4-transmembrane segment (S1–S4 segment) has been appended to the channel pore (S5–S6 segment) to create the 6-transmembrane channel family (Fig. 53–1). The 6-transmembrane family has diversified extensively, both to maintain potassium-selective channels and evolve members of nonselective cation channels not shown here. Among the 6-transmembrane potassium channels, some have evolved an intracellular domain that mediates the response to intracellular ion fluxes such as calcium-activated (*KCNN*) and sodium-activated (*KCNT*) potassium channels. The voltage-activated potassium channel family has charged transmembrane residues, located in the S4 segment, but additional residues may occur in the S1–S3 segment, which senses the membrane electric field and allosterically couples to the channel gate to promote channel opening. The voltage sensor domain not only affects the voltage sensitivity of channels (the relative change in open probability per change in voltage) but also determines the voltage ranges at which channels open. For example, voltage sensors may

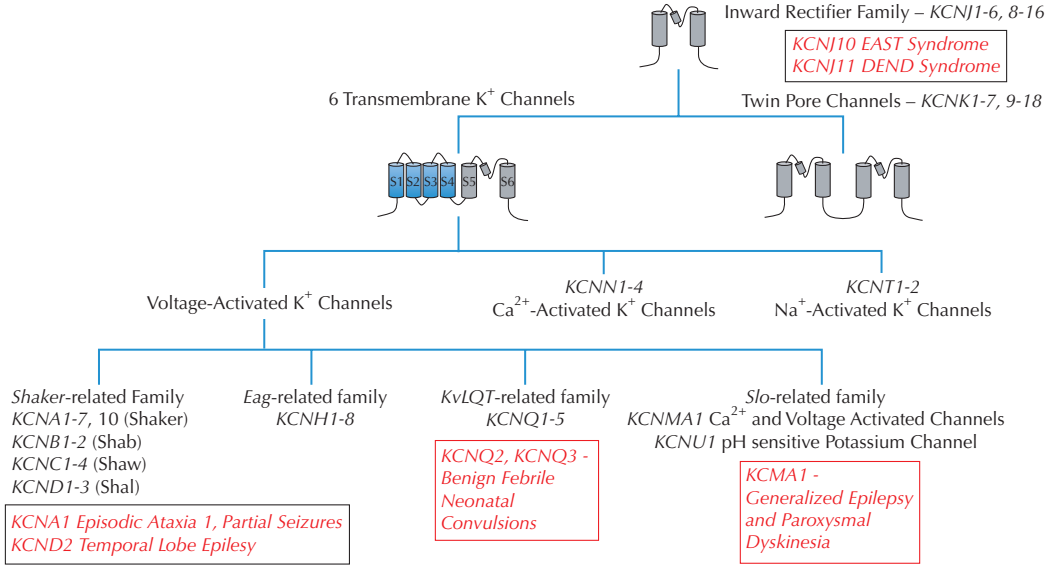

Figure 53–1. The potassium channel gene family and epilepsy channelopathies**.** Family members are organized according to transmembrane structure. Genes within each family that are known to occur with epilepsy channelopathy mutations are boxed below in red. *shaker*-related channel genes that encode nonconducting subunits (*KCNF, KCNG, KCNS*, and *KCNV* channel genes) are not included in the diagram.

be tuned to activate at voltages below threshold to affect the resting membrane potential, near threshold to affect the action potential firing frequency, or at high voltages to contribute only to spike repolarization.

A subset of voltage-dependent potassium channels can undergo inactivation. This includes some members of the *shaker* and *eag* families of potassium channels. Inactivation is a transition from channel opening to closing despite continued depolarization. Inactivation is often mediated by an amino-terminal sequence (ball-and-chain sequence) that plugs the pore following channel opening and is called *N-type inactivation*.[4] N-type inactivation is often relatively quick (in the tens to hundreds of milliseconds) and is mediated by amino terminal sequences intrinsic to the channel pore-forming subunit, or it can be conferred by accessory subunits that contain a ball-and-chain sequence. Inactivation can also be of a slow type (occurring over hundreds of milliseconds to seconds), called *P-* or *C-type inactivation*, that occurs by conformational changes at the pore.[4–6] Fast inactivation of potassium channels is seen as A-type potassium currents. These currents repolarize membrane voltages during one or the first few action potentials but inactivate over multiple spikes.[7] This may lead to a broadening of action potentials or higher action potential frequency during the late components of a spike train.[7]

Members of the voltage-dependent potassium channels include a very large family, perhaps encompassing 32 channel genes in humans (see the HUGO list at http://www.genenames.org/genefamily/kcn.php) and outside the scope of this introduction. Figure 53–1 highlights those genes that have human epilepsy mutations. From this chart, we can see that the inward rectifier potassium channel family contain two members, *KCNJ10* and *KCNJ11*, that contribute to inherited epilepsies as part of more complex disorders (ataxia, sensorineural deafness, and kidney tubulopathy [EAST] and developmental delay, epilepsy, and neonatal diabetes [DEND] syndromes, respectively). The remaining and majority of the epilepsy potassium channelopathies reside among the voltage-dependent potassium family members. These include two members of the *shaker*-related family (*KCNA1* and *KCND2*), two members of the KvLQT family (*KCNQ2* and *KCNQ3*), and one member of the *Slo*-related family, *KCNMA1*. Below, we will provide a review of these various channel genes that contribute to epilepsy when mutated and attempt to describe what is known regarding the physiological basis for increased excitability.

KCNA1

Historically, human *KCNA1* (Kv1.1) mutations were first identified as an autosomal dominant ataxia channelopathy[8] that has subsequently been classified as episodic ataxia type 1 (EA1).[9] Episodic ataxia type1 occurs as brief episodes of discoordination induced by startle, stress, or heavy exertion. Patients may also experience continuous muscle movement (myokymia) either during or between attacks of ataxia.[9] It was later found that a subset of individuals with *KCNA1* mutations, T226R and A242P, also experience partial seizures.[10–12] The T226R mutation causes EA1, myokymia, and partial-onset epilepsy.[12] The A242P mutation does not cause ataxia, but does cause partial epilepsy and myokymia.[10,11] An interesting observation was that the T226R mutation is penetrant for epilepsy only in some families or family members,[12,13] suggesting that the KCNA1 epilepsy phenotype is sensitive to secondary environmental or genetic influences. As well, *KCNA1* mutations have been identified that exhibit different comorbidities, such as partial epilepsy and myokymia but no ataxia, or mutations that exhibit myokymia alone.[10] Thus, the different nature of *KCNA1* mutations appears to also confer phenotypic variability.

Effect of *KCNA1* Epilepsy Mutations on Potassium Currents

KCNA1 encodes potassium channels related to the *Drosophila shaker* family of voltage-gated potassium channels. *KCNA1* currents differ from the *shaker* A-type currents due to a lack of intrinsic fast inactivation. However, in some cells, coassembly with the Kvbeta1 accessory subunits[14] or heteromeric assembly with *KCNA4* subunits[15,16] confers fast inactivation. *KCNA1* channels are often ascribed to a low-voltage-threshold, fast-activated delayed rectifier potassium current that inactivates over a slow time period (hundreds of milliseconds). The two *KCNA1* mutations causing epilepsy, T226R[12] and A242P, are located in the

S2 transmembrane domain[10,11] The common feature is that both mutations are likely to be hypomorphs since they dramatically reduce expression or trafficking of channels in expression systems.[10,12,17,18] The T226R mutation also reduces channel function by shifting the conductance voltage relationship to positive potentials and dramatically slowing the activation time.[17]

The correlation between *KCNA1* hypomorphic mutations and epilepsy is also strengthened by knockout studies of the *KCNA1* gene in mice.[19] *KCNA1* knockout mice have frequent spontaneous seizures, although ataxia is not apparent. Electrophysiological studies suggest normal intrinsic excitability but enhanced excitability related to axonal repolarization and propagation. These results are consistent with *KCNA1* channel preferential expression in axons and presynaptic terminals.[20,21] Studies of *KCNA1* mutations expressed in neurons also suggest that epilepsy mutations (T226R) perturb presynaptic function rather than intrinsic excitability.[22]

KCNA1 and Sudden Unexplained Death in Epilepsy

Recently, work on Kv1.1 channels has significantly advanced the understanding of the potential mechanisms of sudden unexplained death in epilepsy (SUDEP), which refers to death of unknown cause in individuals who have epilepsy. Death in these individuals is approximately 40-fold higher than in individuals without epilepsy.[23] The work of Glasscock and colleagues indicates that increased excitability at parasympathetic nerves may contribute to SUDEP.[24] This was revealed using *KCNA1* knockout mice that are predisposed to SUDEP and have cardiac arrhythmias, including atrioventricular conduction block that is increased during seizures. The authors used the parasympathetic antagonist atropine to correct the arrhythmias, suggesting that *KCNA1* channel defects may increase vagus nerve activity sufficiently to cause SUDEP.

KCNA1 Channelopathy as a Consequence of *LGI1* Mutations

Voltage-dependent potassium channels are heavily regulated to alter their properties or expression, depending on the needs of the neuron. Mutation of the *LGI1* protein presents one example of a situation in which genetic alteration of a potassium channel regulatory protein may contribute indirectly to epilepsy. The *LGI1* protein was first identified as a candidate gene that is absent or downregulated in malignant brain tumors.[25] Its acronym is based on the finding that it is a *leucine*-rich *gene* that is *inactivated* in *gliomas*. The *LGI1* gene encodes a single transmembrane protein with leucine-rich repeats in the extracellular amino terminus that is similar to a family (F-20) of cell adhesion proteins and receptors.[25] Linkage analysis and cloning have identified mutations in this gene as a cause of the inherited disorder autosomal dominant lateral temporal lobe epilepsy (ADLTE), also named *autosomal dominant partial epilepsy with auditory features* (ADPEAF).[26–29] ADLTE occurs as simple partial seizures, of temporal lobe origin, with acoustic and sensory hallucinations. Some individuals also have a less frequent, secondarily generalized seizure. The *LGI1* locus carries mutations in about 50% of all ADLTE families.[28,29]

A connection between *LGI1* and *KCNA1* channels was discovered using biochemical purification of *KCNA1* complexes that revealed coassembly of *LGI1* protein.[30] The *KCNA1* channel also copurified a number of other proteins, including *KCNA4* (Kv1.4) channels and *KCNB1* (Kvbeta1), both of which assemble with and confer fast N-type inactivation properties on *KCNA1* channels. A pivotal finding was that *LGI1* occludes *KCNB1*-mediated increases in inactivation rates. This presumably creates a more sustained A-type current and may reduce excitability in neurons. The finding that ADLTE *LGI1* mutants do not have antagonistic effects on *KCNB1* inactivation suggests that *LGI1* mutations may increase excitability in ADLTE patients through increase inactivation of *KCNA1*-containing, A-type currents.[30]

A more thorough understanding of *LGI1* mutation on the neurophysiology of ADLTE was provided using genetic mouse models of the disease. Knockout of the *LGI1* gene causes severe myoclonic seizures in early life (12–20 days), and mice die shortly thereafter.[31] Interestingly, heterozygous *LGI1* null mice do not show a seizure phenotype, whereas ADLTE LGI1 individuals are heterozygous for a mutant allele.[28] A transgenic study suggests that this can be explained by the mutant *LGI1*

protein in ADLTE patients acting in a dominant negative manner on wild-type protein.[32]

Consistent with *LGI1* protein action on *KCNA1* channels, *LGI1* knockout had no effect on intrinsic properties of neurons, but it increased spontaneous glutamate-mediated excitatory postsynaptic potentials.[31] In addition, transgenic expression of the ADLTE truncation mutant of *LGI1* in mice also increased excitatory synaptic transmission.[32] However, studies suggest that *LGI1* has multiple interacting protein targets and that the mechanism for increased excitability is complex. Besides producing mutant *LGI1* effects on presynaptic *KCNA1* channels.[32] *LGI1* interacts with the postsynaptic protein *ADAM22*[33] and has effects on synapse maturation[32] that may also contribute to seizures when mutated.

KCND2

KCND2 AND A-TYPE CURRENTS

The *KCND2* channel contributes to voltage-gated potassium currents that undergo fast inactivation and are broadly classified as A-type currents (I_A). Among the genes contributing to A-type current, *KCND2* and its family members (*KCND1–3*, also called Kv4.1-Kv4.3) are activated at subthreshold voltages and enriched in somato-dendritic compartments, and therefore have the more specialized designation of the I_{SA} current.[34] One of these family members (*KCND3*) also underlies the transient outward (I_{TO}) current of the cardiac action potential in humans.[35]

KCND2 Channelopathy Mutation

The *KCND2* potassium channel is an ion channel that has been heavily studied for its role in acquired epilepsy, whereas its role as a potassium channelopathy gene requires further study. Evidence of *KCND2* channels as a channelopathy mutation is limited to a report of a single individual identified in a genetic screen of individuals with temporal lobe epilepsy.[36] Sequencing of the *KCND2* locus identified a carboxyl terminal 5 nucleotide deletion that leads to a premature stop codon and truncation of the last 44 amino acids of the protein.[36] Electrophysiology studies in transfected HEK293 cells did not detect discernible effects on channel gating. However, the average current density of transfected cells is reduced by approximately half, suggesting that the mutation is a hypomorph due to reduced current density. Whether this mutation is responsible for, or coincident with, epilepsy in this single patient is uncertain, particularly since genetic ablation of *KCND2* is insufficient to cause spontaneous seizures in mice.

Acquired Changes in *KCND2* and Seizures

Our understanding of I_{SA} current and KCND channels in epilepsy mainly concerns their role in the control of dendritic excitability. These currents have often been studied in CA1 dendrites that are sufficiently large at distances from the soma at which they can be detected by sophisticated patch-clamp recording techniques. The I_{SA} current is expressed as a gradient, being more enriched at distal versus proximal dendrites.[37] The contribution of this current by *KCND2* is confirmed by immunohistochemistry[38] and also by gene knockout of *KCND2* that largely eliminates the dendritic I_{SA} current.[39] An important function of this I_{SA} gradient is to limit the amplitude of backpropagating action potentials to distal dendrites.[37,40] This provides a repolarizing current that limits excitability and activation of *N*-methyl-D-aspartate (NMDA) receptors that otherwise enhance the long-term potentiation of synapses. Importantly, the I_{SA} current is inhibited by both protein kinase A and protein kinase C phosphorylation that is upstream of ERK kinase phosphorylation of channels. The sensitivity of *KCND2* to these kinases makes dendrites particularly prone to enhanced excitability following changes in any number of signaling cascades that alter these kinases. With regard to epilepsy, ERK inhibition of Kv4.2, coupled with transcriptional downregulation, appears to mediate enhanced dendritic excitability and seizures following pilocarpine-induced status epilepticus.[41] In addition models of cortical and hippocampal malformations that are prone to seizures also show a correlative reduction in Kv4.2 expression and current in heterotopic cell regions.[41] Finally, knockout of Kv4.2 also demonstrates increased susceptibility to seizures following convulsant stimulation.[41]

KCNMA1

KCNMA1 AND THE BK CHANNEL

The *KCNMA1* gene encodes large-conductance potassium channels that are dually activated by calcium and voltage. In accordance with their name (*BK channel*, "big K conductance"), these channels tower over most voltage-gated channels in single-channel conductance (~250 pS, more than 20-fold larger than *shaker*-type potassium channels) and can potentially have dramatic effects on membrane voltage.[42] Interestingly, only the single *KCNMA1* gene encodes this class of potassium channel. Their large conductance combined with their broad distribution means that these channels are relatively easy to detect and have been characterized in many cell types. BK channels are observed in skeletal and smooth muscle cells, central nervous system (CNS) and peripheral nervous system (PNS) neurons, endothelial and kidney epithelial cells, and other cell types. Gene identification was made possible by cloning of the *Drosophila* Slowpoke gene locus and cDNA.[43] Therefore, these channels are also called *slo* channels or *mslo* or *hslo* for their mouse or human orthologues, respectively. Although they have structural homology to other voltage-dependent potassium channels, BK channels have an additional amino-terminal transmembrane domain (designated S0) and a large carboxyl terminal domain that has structural domains for two calcium-binding sites per subunit.[44] Finally, a family of four tissue-specific accessory β subunits modulates the biophysical properties and pharmacology of BK channels.[45]

KCNMA1 Gating Properties

Understanding the biophysical properties of BK channels allow us to infer some principles of channel function that are borne out by studies in native cells. The first is that BK channels are effectively activated by calcium concentrations (micromolar) not normally occurring at global concentrations (hundreds of nanomolars). The BK current evoked by action potential-shaped voltage waveforms provides an estimate of 14 μM calcium required to evoke half-maximal current.[46] Thus, BK channels often require colocalization with a calcium source for significant activation.[47] Second, there are examples of BK regulation by numerous mechanisms, including alternative splicing, accessory subunits, phosphorylation status, and redox state. Therefore, BK channel gating properties appear to be tightly regulated for their local calcium environment. Finally, BK channel opening is sensitive in a roughly additive fashion to both calcium and voltage.[48] Thus, BK channels are well tailored for repolarization of action potentials that are coincident with calcium transients, such as occur during action potentials in the soma or in presynaptic nerve terminals. The combined voltage and calcium sensitivity also means that BK channels deactivate following repolarization of action potentials, which restrict their contribution to the fast component of the afterhyperpolarization. Other purely calcium-sensitive potassium channels, such as SK channels, are not deactivated by repolarization and usually contribute to a more sustained (medium) afterhyperpolarization.[49]

BK Channel Function in Neurons

Owing to their broad tissue distribution, the physiological roles of BK channels are quite diverse. Indeed, limiting the focus of this review on epilepsy overlooks a large body of knowledge concerning BK channels outside the nervous system. These include control of tonic and phasic smooth muscle constriction, in renal control of potassium secretion, and control of hormone release of many secretory cells. However, even in the nervous system, BK channels have been studied within many different neuronal compartments and within many neuronal types.

BK channel effects are often uncovered with the highly specific scorpion toxin iberiotoxin, or with charybdotoxin, which also blocks intermediate-conductance potassium channels (IK channels).[42] Worth noting is that the affinity for scorpion toxin is reduced by some beta accessory subunits and shows little block with the accessory beta4 subunit.[50] However, organic blockers, such as paxilline, which have more recently come into use, block BK channels independently of beta subunit composition.[51] Blocking BK channels causes broadening of the action potential and, in some neurons, eliminates the fast component of the afterhyperpolarization.[49] In CA1 and lateral amygdala

pyramidal neurons, BK channel effects are limited to the first few spikes in the action potential train.[52,53] This is likely due to inactivation of BK channels in these cells during repetitive firing and is manifested as frequency-dependent action potential broadening. Interestingly, the consequence of BK channel block on firing patterns is neuron-specific. In CA1 and dentate gyrus (DG) neurons of the hippocampus, BK channels increase rather than decrease the firing rate.[54,55] Both result from action potential sharpening that secondarily affects other currents to increase excitability. For example, in CA1 neurons, BK channel sharpening of action potentials removes sodium channel inactivation and reduces delayed rectifier potassium channel inactivation.[54] In DG neurons, sharpening of action potentials reduces recruitment of SK-type potassium channels to increase the firing rate.[55]

BK Channels and Acquired Epilepsy

The first evidence that BK channels may enhance the excitability related to seizures was obtained in cultured cortical neurons.[56] The gamma-aminobutyric acid (GABA) antagonist and convulsant pentylenetetrazol (PTZ) causes bursting activity in cultured cortical neurons. Treatment of cells with the BK channel blocker iberiotoxin inhibited bursting activity in PTZ- treated and pro-epileptic L mouse cortical neurons. In a picrotoxin seizure model, it was also observed that increased firing rates in cortical neurons could be attenuated by BK channel block.[57] Interestingly, the effectiveness of BK channel block occurs during the second but not the first picrotoxin treatment. This suggests that BK channels are part of a maladaptive upregulation in cortical neurons following seizures. Further, it was shown that systemic injection of the BK channel blocker paxilline was effective in protecting against subsequent picrotoxin-induced seizures.[58]

Human BK Channel Epilepsy Channelopathy

The BK channel pore-forming subunit knockout mice have a plethora of defects, including slowed growth rates, cerebellar dysfunction and ataxia, defects in circadian rhythms, and a number of defects due to smooth muscle hypercontractility.[59–64] Interestingly, seizures have not been reported in *KCNMA1* knockout mice. Rather, it is a gain-of-function mutation that causes nonconvulsive seizures in humans.[65] The effect is partially penetrant, showing apparent absence type seizures in 9 of 16 affected family members. The polymorphism also causes 12 of the 16 family members to have a paroxysmal nonkinesigenic dyskinesia. The single aspartate-to-glycine (D434G) amino acid change resides in one of the calcium activation domains (RCK domain, regulator of conductance of potassium) in the pore-forming alpha subunit. Interestingly, expression of the mutant channels in *Xenopus* oocytes or cultured cells demonstrates an increased current due to faster activation and an increased open probability.[65] More detailed biophysical studies suggest that the mutation affects calcium-dependent gating[46,66,67] and may also directly reduce the energetic barrier to opening.[46]

The mechanisms by which the BK channel D434G gain-of-function mutation causes epilepsy in humans will require a transgenic animal model for greater understanding. Reduced excitability in inhibitory neurons may explain the seizures; however, BK channel expression predominates in excitatory principal neurons of most brain regions, including the cortex and hippocampus.[68,69] An exception is brainstem vestibular neurons, where BK channels moderate high-frequency action potential firing.[70,71] Alternatively, it may be that the human BK channel gain of function acts in a manner similar to that seen in CA1 pyramidal neurons, where BK channels sharpening of action potentials secondarily increases excitability.[54] A similar effect is also seen in the BK channel β4 knockout mice. The β4 accessory subunit inhibits BK channel opening through a slowing of activation,[46] and therefore the β4 knockout mice may also be considered a gain-of-function model. In these animals, epilepsy is also observed, although because of the strong localization of β4 in the hippocampus, the seizures appear to be secondarily generalized with a temporal lobe origin.[55] Thus, there are at least two genetic models[55,65] and one acquired seizure model[57,58] to support the paradoxical findings that BK potassium channels increase excitability and can lead to seizures.

KCNQ Channelopathy Mutations

The M-type potassium current (I_M) regulates the resting membrane potential and spike frequency adaptation and can contribute to slow afterhyperpolarization following action potentials.[72,73] A large number of mutations in two genes encoding the subunits that comprise the M-channel, *KCNQ2* (Kv7.2) and *KCNQ3* (Kv7.3), result in the seizure disorder benign familial neonatal convulsions (BFNC). In BFNC, seizures typically begin within the first 3 days of life and can be partial or generalized. Importantly, the seizures remit spontaneously and normal development follows in the majority of cases, although the incidence of later seizures is higher than in the general population in some families.[74–77] The initial findings implicating the Kv7.2 and Kv7.3 channelopathies in BFNC were the first to link mutations in potassium channels to a familial epilepsy. While 4 different mutations in *KCNQ3* have been identified to date, at least 63 different mutations in *KCNQ2* have been observed in families with BFNC.[77] In addition, de novo *KCNQ2* mutations have been identified in patients with benign neonatal seizures whose seizures also remit.[78] Many of these mutations result in a haploinsufficiency of these channels, while other mutations, such as those that occur in the pore region of the channels, result in a reduction of function.[76] In addition, a number of mutations in *KCNQ2* are located in transmembrane regions of channels and can alter gating properties.[76,79] Therefore, it is thought that the neonatal seizures that occur as a consequence of these mutations are due to a reduction of function of the M-type channels and a concomitant increase in excitability of neurons.

Mutations in *KCNQ2* that cause BFNC have also been associated with other disorders as well, such as rolandic epilepsy,[76,80,81] drug-resistant epilepsy, and/or mental retardation.[79,82,83] Therefore, mutations in these channels have attracted considerable attention over the last decade, especially as a novel anticonvulsant, retigabine (RGB), has as its primary mechanism of action a shift in the activation curve for I_M to a more hyperpolarized potential.[84] This finding, coupled with the genetic findings described above, have garnered enthusiasm for consideration of the M-channel as a potential and novel therapeutic target for the treatment of seizures.

KCNQ Mutations and Animal Models

To study the contribution of mutations in the M-channel to seizure disorders, a number of animal models have been developed. These include the *Kcnq2* knockout mouse, the dominant-negative *Kcnq2* G279S mutation, the *Szt1* spontaneous deletion mouse strain, the *Kcnq2* A306T knockin mouse, and the *Kcnq3* G311V knockin mouse.[85–89] Mice homozygous for both the *Kcnq2* knockout and *Szt1* mutations are lethal, dying soon after birth due to a lung defect. However, mice heterozygous for the *Kcnq2* knockout and the *Szt1* mutation, are viable. In addition, all of the knockin mice that have been generated to date are viable, even when homozygous for the mutations.[88] Electrophysiology experiments performed on CA1 neurons in the in vitro hippocampal brain slices obtained from these mouse models have uniformly found decreased $I_{K(M)}$ amplitude, decreased current density, and, when examined, a reduction in action potential accommodation compared to their wild-type C57BL/6 littermates.[77,86,90] In addition to the observed alterations found in the membrane currents, significant differences in $I_{K(M)}$ pharmacology in CA1 neurons recorded from *Szt1* mice have been observed.[90,91] These differences have intriguing parallels to the results of in vivo experiments in the *Szt1* mice, including a highly significant decrease in sensitivity to RGB.[90,91] These results have profound implications for pharmacotherapy, as they suggest that antiepileptic drugs that target the M-channel, such as retigabine and flupertine, may be less effective in patients with underlying *KCNQ2* mutations that result in a haploinsufficiency.

KCNQ1

A member of the Kv7 family of potassium channels, Kv7.1, is encoded by the *KCNQ1* gene and forms the α subunit of this potassium channel.[92] Kv7.1 is expressed in cardiac tissue, and mutations in this gene have been linked to the long QT syndrome (LQTS) and fatal cardiac arrhythmias. Over 300 mutations have been identified in this gene, and many of those mutations result in a prolongation of the cardiac action potential and sudden death.[93] It had

been thought that this gene was not expressed in the CNS, but recent work has convincingly demonstrated that in fact *KCNQ1* is expressed in brain.[94] In two mouse lines with LQTS mutations, *Kcnq1* A340E and T311I, mice heterozygous and homozygous for the mutations have cardiac abnormalities, as well as exhibiting both partial and generalized seizures.[94] In addition, at least one mouse in the study was observed to die following a prolonged period of seizure activity, bradycardia, cardiac depression, and ultimately cardiac arrest.[94] As SUDEP may be responsible for a large percentage of deaths in patients with epilepsy, these findings provide support for evaluating the LQTS genes in patients with epilepsy and concomitant cardiac irregularities.

KCNJ10

The inward-rectifying potassium channel (Kir4.1) is highly expressed in glial cells in the CNS, as well as in cochlear, cardiac, kidney, and other tissues, and is encoded by the *KCNJ10* gene. Kir channels expressed by glial cells buffer extracellular potassium in the CNS following neuronal action potentials and are primarily responsible for maintaining low extracellular levels of potassium in the CNS. Quantitative trait loci mapping experiments first identified a variation in the mouse *Kcnj10* gene that was correlated with an increase in seizure susceptibility in the DBA/2 mouse,[95] and a variation in the human *KCNJ10* has also been found to be associated with common forms of human epilepsy.[96–98] Furthermore, coding region mutations in the *KCNJ10* gene that are linked to a familial form of epilepsy with ataxia, sensorineural deafness, and kidney tubulopathy (EAST syndrome) have recently been identified.[99,100] When *KCNJ10* is expressed in CHO cells, these mutations are found to result in a loss of (or greatly reduced) function.[101] In addition, recent work has demonstrated that astrocytes from DBA/2 mice, which harbor the initially described variant, have a decreased Kir current and also exhibit a reduction in glutamate transport compared to the more seizure-resistant strain of mice, C57Bl/6.[102] Therefore, variants of *KCNJ10* may be associated with common forms of human epilepsy by reducing seizure thresholds. However, there are also rare mutations in this gene that can underlie familial types of epilepsy that are part of a more complex phenotype.

KCNJ11

The *KCNJ11* gene encodes the Kir 6.2 protein that comprises the pore region of the ATP-sensitive potassium channel (K_{ATP}).[103] Not only is Kir 6.2 highly expressed in brain, but it is also found in pancreatic β cells. When the intracellular levels of ATP are high, the ion channel in both neurons and β cells is closed, allowing for depolarization, action potential generation, and the release of neurotransmitters and insulin, respectively.[104] However, to conserve energy, when intracellular levels of ATP are low, the ion channel opens and causes a hyperpolarization of both neurons and β cells, thus inhibiting action potential generation, calcium influx, and the release of either neurotransmitters or insulin, respectively. Mutations in the *KCNJ11* gene that reduce the affinity for ATP and increase the probability of channel opening result in a syndrome characterized by developmental delay, epilepsy, and neonatal diabetes (DEND).[105,106] The diabetes results from a reduced ability to release insulin due to membrane hyperpolarization that occurs as the ion channel remains open. It is hypothesized that increased expression of K_{ATP} channels in inhibitory neurons may underlie the epilepsy that occurs in DEND. Such a gain-of-function mutation in a potassium channel is paradoxical and resembles that observed in the BK channels described above. In a recently published case study of a DEND patient, treatment with a sulfunylurea drug, glibenclamide, successfully treated the diabetes and permitted insulin therapy to be halted, most likely as a consequence of blocking the K_{ATP} channel.[106,107] This block aids in the depolarization of the β cells of the pancreas and allows insulin release to resume. Interestingly, following treatment with glibenclamide, the seizures were substantially diminished and the hypsarrhythmia observed on the patient's electroencephalogram was resolved.[106] While generalized sharp-wave activity was still observed during sleep, this patient demonstrated substantial psychomotor improvement. This suggests that early diagnosis and treatment of DEND could result in preventing seizures and concomitant psychomotor impairments.[105]

CONCLUSIONS

An exceedingly large number of familial and de novo channelopathies in several different types of potassium channels have already been found to underlie, or be associated with, many types of epilepsy. Given that the role of most potassium channels is to contribute to the maintenance of membrane hyperpolarization and repolarization, it is not surprising that loss-of-function mutations contribute to epilepsy. However, recently described potassium channelopathies resulting in gain of function can also, paradoxically, result in epilepsy. Furthermore, as many LQTS mutations arise in potassium channels, a link between epilepsy, SUDEP, and LQTS, as has now been observed for *KCNQ1*, may begin to inform prevention strategies for patients at risk for SUDEP. Finally, animal models harboring human mutations found in potassium channels have contributed greatly to our understanding of the mechanisms whereby specific channelopathies contribute to epilepsy, and it is anticipated that as this field continues to develop, advances in treatment strategies for patients will also be elucidated from such animal models.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Bichet D, Haass FA, Jan LY. Merging functional studies with structures of inward-rectifier K(+) channels. *Nat Rev Neurosci*. 2003;4:957–967.
2. Proks P, Ashcroft FM. Modeling K(ATP) channel gating and its regulation. *Prog Biophys Mol Biol*. 2009;99:7–19.
3. Kim D. Physiology and pharmacology of two-pore domain potassium channels. *Curr Pharm Des*. 2005;11: 2717–2736.
4. Kurata HT, Fedida D. A structural interpretation of voltage-gated potassium channel inactivation. *Prog Biophys Mol Biol*. 2006;92:185–208.
5. Cuello LG, Jogini V, Cortes DM, Pan AC, Gagnon DG, Dalmas O, Cordero-Morales JF, Chakrapani S, Roux B, Perozo E. Structural basis for the coupling between activation and inactivation gates in K(+) channels. *Nature*. 2010;466:272–275.
6. Cuello LG, Jogini V, Cortes DM, Perozo E. Structural mechanism of C-type inactivation in K(+) channels. *Nature*. 2010;466:203–208.
7. Bean BP. The action potential in mammalian central neurons. *Nat Rev Neurosci*. 2007;8:451–465.
8. Browne DL, Gancher ST, Nutt JG, Brunt ER, Smith EA, Kramer P, Litt M. Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. *Nat Genet*. 1994;8: 136–140.
9. Tomlinson SE, Hanna MG, Kullmann DM, Tan SV, Burke D. Clinical neurophysiology of the episodic ataxias: insights into ion channel dysfunction in vivo. *Clin Neurophysiol*. 2009;120:1768–1776.
10. Eunson LH, Rea R, Zuberi SM, Youroukos S, Panayiotopoulos CP, Liguori R, Avoni P, McWilliam RC, Stephenson JB, Hanna MG, Kullmann DM, Spauschus A. Clinical, genetic, and expression studies of mutations in the potassium channel gene KCNA1 reveal new phenotypic variability. *Ann Neurol*. 2000;48: 647–656.
11. Liguori R, Avoni P, Baruzzi A, Di Stasi V, Montagna P. Familial continuous motor unit activity and epilepsy. *Muscle Nerve*. 2001;24:630–633.
12. Zuberi SM, Eunson LH, Spauschus A, De Silva R, Tolmie J, Wood NW, McWilliam RC, Stephenson JB, Kullmann DM, Hanna MG. A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain*. 1999;122(pt 5): 817–825.
13. Kinali M, Jungbluth H, Eunson LH, Sewry CA, Manzur AY, Mercuri E, Hanna MG, Muntoni F. Expanding the phenotype of potassium channelopathy: severe neuromyotonia and skeletal deformities without prominent episodic ataxia. *Neuromuscul Disord*. 2004;14:689–693.
14. Pongs O, Schwarz JR. Ancillary subunits associated with voltage-dependent K^+ channels. *Physiol Rev*. 2009;90:755–796.
15. Imbrici P, D'Adamo MC, Kullmann DM, Pessia M. Episodic ataxia type 1 mutations in the KCNA1 gene impair the fast inactivation properties of the human potassium channels Kv1.4–1.1/Kvbeta1.1 and Kv1.4–1.1/Kvbeta1.2. *Eur J Neurosci*. 2006;24: 3073–3083.
16. Wang FC, Parcej DN, Dolly JO. alpha subunit compositions of Kv1.1-containing K^+ channel subtypes fractionated from rat brain using dendrotoxins. *Eur J Biochem*. 1999;263:230–237.
17. Rea R, Spauschus A, Eunson LH, Hanna MG, Kullmann DM. Variable K(+) channel subunit dysfunction in inherited mutations of KCNA1. *J Physiol*. 2002;538:5–23.
18. Spauschus A, Eunson L, Hanna MG, Kullmann DM. Functional characterization of a novel mutation in KCNA1 in episodic ataxia type 1 associated with epilepsy. *Ann NY Acad Sci*. 1999;868:442–446.
19. Smart SL, Lopantsev V, Zhang CL, Robbins CA, Wang H, Chiu SY, Schwartzkroin PA, Messing A, Tempel BL. Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. *Neuron*. 1998;20:809–819.
20. Rasband MN, Trimmer JS. Subunit composition and novel localization of K^+ channels in spinal cord. *J Comp Neurol*. 2001;429:166–176.
21. Wang H, Kunkel DD, Schwartzkroin PA, Tempel BL. Localization of Kv1.1 and Kv1.2, two K channel proteins, to synaptic terminals, somata, and dendrites in the mouse brain. *J Neurosci*. 1994;14:4588–4599.
22. Heeroma JH, Henneberger C, Rajakulendran S, Hanna MG, Schorge S, Kullmann DM. Episodic ataxia type 1 mutations differentially affect neuronal

excitability and transmitter release. *Dis Model Mech*. 2009;2:612–619.
23. Annegers JF, Coan SP. SUDEP: overview of definitions and review of incidence data. *Seizure.* 1999;8: 347–352.
24. Glasscock E, Yoo JW, Chen TT, Klassen TL, Noebels JL. Kv1.1 potassium channel deficiency reveals brain-driven cardiac dysfunction as a candidate mechanism for sudden unexplained death in epilepsy. *J Neurosci*. 2010;30:5167–5175.
25. Chernova OB, Somerville RP, Cowell JK. A novel gene, LGI1, from 10q24 is rearranged and downregulated in malignant brain tumors. *Oncogene.* 1998;17: 2873–2881.
26. Kalachikov S, Evgrafov O, Ross B, Winawer M, Barker-Cummings C, Martinelli Boneschi F, Choi C, Morozov P, Das K, Teplitskaya E, Yu A, Cayanis E, Penchaszadeh G, Kottmann AH, Pedley TA, Hauser WA, Ottman R, Gilliam TC. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nat Genet*. 2002;30:335–341.
27. Morante-Redolat JM, Gorostidi-Pagola A, Piquer-Sirerol S, Saenz A, Poza JJ, Galan J, Gesk S, Sarafidou T, Mautner VF, Binelli S, Staub E, Hinzmann B, French L, Prud'homme JF, Passarelli D, Scannapieco P, Tassinari CA, Avanzini G, Marti-Masso JF, Kluwe L, Deloukas P, Moschonas NK, Michelucci R, Siebert R, Nobile C, Perez-Tur J, Lopez de Munain A. Mutations in the LGI1/Epitempin gene on 10q24 cause autosomal dominant lateral temporal epilepsy. *Hum Mol Genet*. 2002;11:1119–1128.
28. Nobile C, Michelucci R, Andreazza S, Pasini E, Tosatto SC, Striano P. LGI1 mutations in autosomal dominant and sporadic lateral temporal epilepsy. *Hum Mutat*. 2009;30:530–536.
29. Ottman R, Winawer MR, Kalachikov S, Barker-Cummings C, Gilliam TC, Pedley TA, Hauser WA. LGI1 mutations in autosomal dominant partial epilepsy with auditory features. *Neurology*. 2004;62: 1120–1126.
30. Schulte U, Thumfart JO, Klocker N, Sailer CA, Bildl W, Biniossek M, Dehn D, Deller T, Eble S, Abbass K, Wangler T, Knaus HG, Fakler B. The epilepsy-linked Lgi1 protein assembles into presynaptic Kv1 channels and inhibits inactivation by Kvbeta1. *Neuron*. 2006;49:697–706.
31. Yu YE, Wen L, Silva J, Li Z, Head K, Sossey-Alaoui K, Pao A, Mei L, Cowell JK. Lgi1 null mutant mice exhibit myoclonic seizures and CA1 neuronal hyperexcitability. *Hum Mol Genet*. 2010;19: 1702–1711.
32. Zhou YD, Lee S, Jin Z, Wright M, Smith SE, Anderson MP. Arrested maturation of excitatory synapses in autosomal dominant lateral temporal lobe epilepsy. *Nat Med*. 2009;15:1208–1214.
33. Fukata Y, Adesnik H, Iwanaga T, Bredt DS, Nicoll RA, Fukata M. Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission. *Science.* 2006;313:1792–1795.
34. Jerng HH, Qian Y, Pfaffinger PJ. Modulation of Kv4.2 channel expression and gating by dipeptidyl peptidase 10 (DPP10). *Biophys J*. 2004; 87: 2380–2396.
35. Zhu XR, Wulf A, Schwarz M, Isbrandt D, Pongs O. Characterization of human Kv4.2 mediating a rapidly-inactivating transient voltage-sensitive K^+ current. *Receptors Channels*. 1999;6:387–400.
36. Singh B, Ogiwara I, Kaneda M, Tokonami N, Mazaki E, Baba K, Matsuda K, Inoue Y, Yamakawa K. A Kv4.2 truncation mutation in a patient with temporal lobe epilepsy. *Neurobiol Dis*. 2006;24:245–253.
37. Hoffman DA, Magee JC, Colbert CM, Johnston D. K^+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature*. 1997;387: 869–875.
38. Maletic-Savatic M, Lenn NJ, Trimmer JS. Differential spatiotemporal expression of K^+ channel polypeptides in rat hippocampal neurons developing in situ and in vitro. *J Neurosci.* 1995;15:3840–3851.
39. Chen X, Yuan LL, Zhao C, Birnbaum SG, Frick A, Jung WE, Schwarz TL, Sweatt JD, Johnston D. Deletion of Kv4.2 gene eliminates dendritic A-type K^+ current and enhances induction of long-term potentiation in hippocampal CA1 pyramidal neurons. *J Neurosci*. 2006;26:12143–12151.
40. Migliore M, Hoffman DA, Magee JC, Johnston D. Role of an A-type K^+ conductance in the back-propagation of action potentials in the dendrites of hippocampal pyramidal neurons. *J Comput Neurosci*. 1999;7:5–15.
41. Bernard C, Anderson A, Becker A, Poolos NP, Beck H, Johnston D. Acquired dendritic channelopathy in temporal lobe epilepsy. *Science*. 2004;305:532–535.
42. Calderone V. Large-conductance, ca(2+)-activated k(+) channels: function, pharmacology and drugs. *Curr Med Chem*. 2002;9:1385–1395.
43. Atkinson NS, Robertson GA, Ganetzky B. A component of calcium-activated potassium channels encoded by the *Drosophila* slo locus. *Science*. 1991;253:551–555.
44. Cui J, Yang H, Lee US. Molecular mechanisms of BK channel activation. *Cell Mol Life Sci.* 2009;66: 852–875.
45. Orio P, Rojas P, Ferreira G, Latorre R. New disguises for an old channel: MaxiK channel beta-subunits. *News Physiol Sci.* 2002;17:156–161.
46. Wang B, Rothberg BS, Brenner R. Mechanism of increased BK channel activation from a channel mutation that causes epilepsy. *J Gen Physiol.* 2009;133:283–294.
47. Fakler B, Adelman JP. Control of K(Ca) channels by calcium nano/microdomains. *Neuron.* 2008;59:873–881.
48. Horrigan FT, Aldrich RW. Coupling between voltage sensor activation, Ca^{2+} binding and channel opening in large conductance (BK) potassium channels. *J Gen Physiol*. 2002;120:267–305.
49. Sah P, Faber ES. Channels underlying neuronal calcium-activated potassium currents. *Prog Neurobiol*. 2002;66:345–353.
50. Meera P, Wallner M, Toro L. A neuronal beta subunit (KCNMB4) makes the large conductance, voltage- and Ca^{2+}-activated K^+ channel resistant to charybdotoxin and iberiotoxin. *Proc Natl Acad Sci USA*. 2000;97:5562–5567.
51. Hu H, Shao LR, Chavoshy S, Gu N, Trieb M, Behrens R, Laake P, Pongs O, Knaus HG, Ottersen OP, Storm JF. Presynaptic Ca^{2+}-activated K^+ channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release. *J Neurosci*. 2001;21:9585–9597.
52. Faber ES, Sah P. Ca^{2+}-activated K^+ (BK) channel inactivation contributes to spike broadening during repetitive firing in the rat lateral amygdala. *J Physiol*. 2003;552:483–497.
53. Shao LR, Halvorsrud R, Borg-Graham L, Storm JF. The role of BK-type Ca^{2+}-dependent K^+ channels in

spike broadening during repetitive firing in rat hippocampal pyramidal cells. *J Physiol.* 1999;521(pt 1): 135–146.
54. Gu N, Vervaeke K, Storm JF. BK potassium channels facilitate high-frequency firing and cause early spike frequency adaptation in rat CA1 hippocampal pyramidal cells. *J Physiol.* 2007;580:859–882.
55. Brenner R, Chen QH, Vilaythong A, Toney GM, Noebels JL, Aldrich RW. BK channel beta4 subunit reduces dentate gyrus excitability and protects against temporal lobe seizures. *Nat Neurosci.* 2005;8: 1752–1759.
56. Jin W, Sugaya A, Tsuda T, Ohguchi H, Sugaya E. Relationship between large conductance calcium-activated potassium channel and bursting activity. *Brain Res.* 2000;860:21–28.
57. Shruti S, Clem RL, Barth AL. A seizure-induced gain-of-function in BK channels is associated with elevated firing activity in neocortical pyramidal neurons. *Neurobiol Dis.* 2008;30:323–330.
58. Sheehan JJ, Benedetti BL, Barth AL. Anticonvulsant effects of the BK-channel antagonist paxilline. *Epilepsia.* 2009;50:711–720.
59. Werner ME, Zvara P, Meredith AL, Aldrich RW, Nelson MT. Erectile dysfunction in mice lacking the large-conductance calcium-activated potassium (BK) channel. *J Physiol.* 2005;567:545–556.
60. Thorneloe KS, Meredith AL, Knorn AM, Aldrich RW, Nelson MT. Urodynamic properties and neurotransmitter dependence of urinary bladder contractility in the BK channel deletion model of overactive bladder. *Am J Physiol Renal Physiol.* 2005;289:F604–F610.
61. Sausbier M, Hu H, Arntz C, Feil S, Kamm S, Adelsberger H, Sausbier U, Sailer CA, Feil R, Hofmann F, Korth M, Shipston MJ, Knaus HG, Wolfer DP, Pedroarena CM, Storm JF, Ruth P. Cerebellar ataxia and Purkinje cell dysfunction caused by Ca^{2+}-activated K^+ channel deficiency. *Proc Natl Acad Sci USA.* 2004;101:9474–9478.
62. Sausbier M, Arntz C, Bucurenciu I, Zhao H, Zhou XB, Sausbier U, Feil S, Kamm S, Essin K, Sailer CA, Abdullah U, Krippeit-Drews P, Feil R, Hofmann F, Knaus HG, Kenyon C, Shipston MJ, Storm JF, Neuhuber W, Korth M, Schubert R, Gollasch M, Ruth P. Elevated blood pressure linked to primary hyperaldosteronism and impaired vasodilation in BK channel-deficient mice. *Circulation.* 2005;112: 60–68.
63. Ruttiger L, Sausbier M, Zimmermann U, Winter H, Braig C, Engel J, Knirsch M, Arntz C, Langer P, Hirt B, Muller M, Kopschall I, Pfister M, Munkner S, Rohbock K, Pfaff I, Rusch A, Ruth P, Knipper M. Deletion of the Ca^{2+}-activated potassium (BK) alpha-subunit but not the BKbeta1-subunit leads to progressive hearing loss. *Proc Natl Acad Sci USA.* 2004;101:12922–12927.
64. Meredith AL, Wiler SW, Miller BH, Takahashi JS, Fodor AA, Ruby NF, Aldrich RW. BK calcium-activated potassium channels regulate circadian behavioral rhythms and pacemaker output. *Nat Neurosci.* 2006;9:1041–1049.
65. Du W, Bautista JF, Yang H, Diez-Sampedro A, You SA, Wang L, Kotagal P, Luders HO, Shi J, Cui J, Richerson GB, Wang QK. Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. *Nat Genet.* 2005;37:733–738.
66. Yang J, Krishnamoorthy G, Saxena A, Zhang G, Shi J, Yang H, Delaloye K, Sept D, Cui J. An epilepsy/dyskinesia-associated mutation enhances BK channel activation by potentiating Ca^{2+} sensing. *Neuron.* 2010;66: 871–883.
67. Diez-Sampedro A, Silverman WR, Bautista JF, Richerson GB. Mechanism of increased open probability by a mutation of the BK channel. *J Neurophysiol.* 2006;96:1507–1516.
68. Knaus HG, Schwarzer C, Koch RO, Eberhart A, Kaczorowski GJ, Glossmann H, Wunder F, Pongs O, Garcia ML, Sperk G. Distribution of high-conductance Ca(2+)-activated K^+ channels in rat brain: targeting to axons and nerve terminals. *J Neurosci.* 1996;16: 955–963.
69. Misonou H, Menegola M, Buchwalder L, Park EW, Meredith A, Rhodes KJ, Aldrich RW, Trimmer JS. Immunolocalization of the Ca^{2+}-activated K^+ channel Slo1 in axons and nerve terminals of mammalian brain and cultured neurons. *J Comp Neurol.* 2006;496: 289–302.
70. Smith MR, Nelson AB, Du Lac S. Regulation of firing response gain by calcium-dependent mechanisms in vestibular nucleus neurons. *J Neurophysiol.* 2002;87: 2031–2042.
71. Nelson AB, Krispel CM, Sekirnjak C, du Lac S. Long-lasting increases in intrinsic excitability triggered by inhibition. *Neuron.* 2003;40:609–620.
72. Tzingounis AV & Nicoll RA. Contribution of KCNQ2 and KCNQ3 to the medium and slow afterhyperpolarization currents. *Proc Natl Acad Sci USA.* 2008;105:19974–19979.
73. Marrion NV. Control of M-current. *Annu Rev Physiol.* 1997;59:483–504.
74. Charlier C, Singh NA, Ryan SG, Lewis TB, Reus BE, Leach RJ, Leppert M. A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. *Nat Genet.* 1998;18:53–55.
75. Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, Ronen GM, Bjerre I, Quattlebaum T, Murphy JV, McHarg ML, Gagnon D, Rosales TO, Peiffer A, Anderson VE, Leppert M. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat Genet.* 1998;18:25–29.
76. Singh NA, Westenskow P, Charlier C, Pappas C, Leslie J, Dillon J, Anderson VE, Sanguinetti MC, Leppert MF, Consortium BP. KCNQ2 and KCNQ3 potassium channel genes in benign familial neonatal convulsions: expansion of the functional and mutation spectrum. *Brain.* 2003;126:2726–2737.
77. Singh N, Otto JF, Leppert M, White HS, Wilcox KS. Mouse models of benign familial neonatal convulsions (BFNC): mutations in KCNQ (Kv7) genes. In: Baraban SC, ed. *Animal Models of Epilepsy: Methods and Innovations.* Totowa, NJ: Humana Press; 2008:107–120.
78. Claes LR, Ceulemans B, Audenaert D, Deprez L, Jansen A, Hasaerts D, Weckx S, Claeys KG, Del-Favero J, Van Broeckhoven C, De Jonghe P. De novo KCNQ2 mutations in patients with benign neonatal seizures. *Neurology.* 2004;63:2155–2158.
79. Dedek K, Fusco L, Teloy N, Steinlein OK. Neonatal convulsions and epileptic encephalopathy in an Italian family with a missense mutation in the fifth transmembrane region of KCNQ2. *Epilepsy Res.* 2003;54:21–27.

80. Maihara T, Tsuji M, Higuchi Y, Hattori H. Benign familial neonatal convulsions followed by benign epilepsy with centrotemporal spikes in two siblings. *Epilepsia*. 1999;40:110–113.
81. Coppola G, Castaldo P, Miraglia del Giudice E, Bellini G, Galasso F, Soldovieri MV, Anzalone L, Sferro C, Annunziato L, Pascotto A, Taglialatela M. A novel KCNQ2 K^+ channel mutation in benign neonatal convulsions and centrotemporal spikes. *Neurology*. 2003;61:131–134.
82. Borgatti R, Zucca C, Cavallini A, Ferrario M, Panzeri C, Castaldo P, Soldovieri MV, Baschirotto C, Bresolin N, Dalla Bernardina B, Taglialatela M, Bassi MT. A novel mutation in KCNQ2 associated with BFNC, drug resistant epilepsy, and mental retardation. *Neurology*. 2004;63:57–65.
83. Schmitt B, Wohlrab G, Sander T, Steinlein OK, Hajnal BL. Neonatal seizures with tonic clonic sequences and poor developmental outcome. *Epilepsy Res*. 2005;65:161–168.
84. Wickenden AD, Yu W, Zou A, Jegla T, Wagoner PK. Retigabine, a novel anti-convulsant, enhances activation of KCNQ2/Q3 potassium channels. *Mol Pharmacol*. 2000;58:591–600.
85. Watanabe H, Nagata E, Kosakai A, Nakamura M, Yokoyama M, Tanaka K, Sasai H. Disruption of the epilepsy KCNQ2 gene results in neural hyperexcitability. *J Neurochem*. 2000;75:28–33.
86. Peters HC, Hu H, Pongs O, Storm JF, Isbrandt D. Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. *Nat Neurosci*. 2005;8:51–60.
87. Yang Y, Beyer BJ, Otto JF, O'Brien TP, Letts VA, White HS, Frankel WN. Spontaneous deletion of epilepsy gene orthologs in a mutant mouse with a low electroconvulsive threshold. *Hum Mol Genet*. 2003;12:975–984.
88. Singh NA, Otto JF, Dahle EJ, Pappas C, Leslie JD, Vilaythong A, Noebels JL, White HS, Wilcox KS, Leppert MF. Mouse models of human KCNQ2 and KCNQ3 mutations for benign familial neonatal convulsions show seizures and neuronal plasticity without synaptic reorganization. *J Physiol*. 2008;586:3405–3423.
89. Otto JF, Singh NA, Dahle EJ, Leppert MF, Pappas CM, Pruess TH, Wilcox KS, White HS. Electroconvulsive seizure thresholds and kindling acquisition rates are altered in mouse models of human Kcnq2 and Kcnq3 mutations for benign familial neonatal convulsions. *Epilepsia*. 2009;50(7):1752–9
90. Otto JF, Yang Y, Frankel WN, White HS, Wilcox KS. A spontaneous mutation involving Kcnq2 (Kv7.2) reduces M-current density and spike frequency adaptation in mouse CA1 neurons. *J Neurosci*. 2006;26:2053–2059.
91. Otto JF, Yang Y, Frankel WN, Wilcox KS, White HS. Mice carrying the szt1 mutation exhibit increased seizure susceptibility and altered sensitivity to compounds acting at the m-channel. *Epilepsia*. 2004;45:1009–1016.
92. Gutman GA, Chandy KG, Grissmer S, Lazdunski M, McKinnon D, Pardo LA, Robertson GA, Rudy B, Sanguinetti MC, Stuhmer W, Wang X. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev*. 2005;57:473–508.
93. Peroz D, Rodriguez N, Choveau F, Baro I, Merot J, Loussouarn G. Kv7.1 (KCNQ1) properties and channelopathies. *J Physiol*. 2008;586:1785–1789.
94. Goldman AM, Glasscock E, Yoo J, Chen TT, Klassen TL, Noebels JL. Arrhythmia in heart and brain: KCNQ1 mutations link epilepsy and sudden unexplained death. *Sci Transl Med*. 2009;1:2ra6.
95. Ferraro TN, Golden GT, Smith GG, Martin JF, Lohoff FW, Gieringer TA, Zamboni D, Schwebel CL, Press DM, Kratzer SO, Zhao H, Berrettini WH, Buono RJ. Fine mapping of a seizure susceptibility locus on mouse chromosome 1: nomination of Kcnj10 as a causative gene. *Mamm Genome*. 2004;15:239–251.
96. Buono RJ, Lohoff FW, Sander T, Sperling MR, O'Connor MJ, Dlugos DJ, Ryan SG, Golden GT, Zhao H, Scattergood TM, Berrettini WH, Ferraro TN. Association between variation in the human KCNJ10 potassium ion channel gene and seizure susceptibility. *Epilepsy Res*. 2004;58:175–183.
97. Heuser K, Nagelhus EA, Taubøll E, Indahl U, Berg PR, Lien S, Nakken S, Gjerstad L, Ottersen OP. Variants of the genes encoding AQP4 and Kir4.1 are associated with subgroups of patients with temporal lobe epilepsy. *Epilepsy Res*. 2010;88:55–64.
98. Lenzen KP, Heils A, Lorenz S, Hempelmann A, Hofels S, Lohoff FW, Schmitz B, Sander T. Supportive evidence for an allelic association of the human KCNJ10 potassium channel gene with idiopathic generalized epilepsy. *Epilepsy Res*. 2005;63:113–118.
99. Bockenhauer D, Feather S, Stanescu HC, Bandulik S, Zdebik AA, Reichold M, Tobin J, Lieberer E, Sterner C, Landoure G, Arora R, Sirimanna T, Thompson D, Cross JH, van't Hoff W, Al Masri O, Tullus K, Yeung S, Anikster Y, Klootwijk E, Hubank M, Dillon MJ, Heitzmann D, Arcos-Burgos M, Knepper MA, Dobbie A, Gahl WA, Warth R, Sheridan E, Kleta R. Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCNJ10 mutations. *N Engl J Med*. 2009;360:1960–1970.
100. Scholl UI, Choi M, Liu T, Ramaekers VT, Hausler MG, Grimmer J, Tobe SW, Farhi A, Nelson-Williams C, Lifton RP. Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME syndrome) caused by mutations in KCNJ10. *Proc Natl Acad Sci USA*. 2009;106:5842–5847.
101. Reichold M, Zdebik AA, Lieberer E, Rapedius M, Schmidt K, Bandulik S, Sterner C, Tegtmeier I, Penton D, Baukrowitz T, Hulton SA, Witzgall R, Ben-Zeev B, Howie AJ, Kleta R, Bockenhauer D, Warth R. KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. *Proc Natl Acad Sci USA*. 2010;107:14490–14495.
102. Inyushin M, Kucheryavykh LY, Kucheryavykh YV, Nichols CG, Buono RJ, Ferraro TN, Skatchkov SN, Eaton MJ. Potassium channel activity and glutamate uptake are impaired in astrocytes of seizure-susceptible DBA/2 mice. *Epilepsia*. 2010;51(9):1707–13.
103. Kubo Y, Adelman JP, Clapham DE, Jan LY, Karschin A, Kurachi Y, Lazdunski M, Nichols CG, Seino S, Vandenberg CA. International Union of Pharmacology. LIV. Nomenclature and molecular

relationships of inwardly rectifying potassium channels. *Pharmacol Rev*. 2005;57:509–526.
104. Yellen G. Ketone bodies, glycolysis, and KATP channels in the mechanism of the ketogenic diet. *Epilepsia*. 2008;49(suppl 8):80–82.
105. Cooper EC, Pan Z. Putting an end to DEND: a severe neonatal-onset epilepsy is treatable if recognized early. *Neurology*. 2007;69:1310–1311.
106. Shimomura K, Horster F, de Wet H, Flanagan SE, Ellard S, Hattersley AT, Wolf NI, Ashcroft F, Ebinger F. A novel mutation causing DEND syndrome: a treatable channelopathy of pancreas and brain. *Neurology*. 2007;69: 1342–1349.
107. Mlynarski W, Tarasov AI, Gach A, Girard CA, Pietrzak I, Zubcevic L, Kusmierek J, Klupa T, Malecki MT, Ashcroft FM. Sulfonylurea improves CNS function in a case of intermediate DEND syndrome caused by a mutation in KCNJ11. *Nat Clin Pract Neurol*. 2007;3:640–645.

Chapter 54

The Voltage-Gated Calcium Channel and Absence Epilepsy

Jeffrey L. Noebels

> The term epilepsy . . . should be degraded to stand for our ignorance of conditions that cause not only convulsions, but temporary failures of motor and mental functions . . . a failure of any part of the "circle" where the mind lives.
>
> John Hughlings Jackson, "A Digression on Epilepsy," *British Medical Journal,* 1866

The discovery that absence epilepsy, a "temporary failure of motor and mental function," can be linked to disruption of a single gene is now entering its 30th year.[1] Before then, few clues indicated that generalized epilepsies could be monogenic or, even more surprisingly, that an inherited alteration of one base pair in a calcium ion channel responsible for neurotransmitter release at virtually all central synapses might lead to the development of the spike-wave absence seizure phenotype, first described in a child by Berger a half century earlier.[2] Nevertheless, the detection of spontaneous cortical spike-wave discharges in the ataxic mutant mouse *tottering* led to the identification of a missense point mutation in *Cacna1a*,[3] the gene encoding the P/Q subunit calcium channel, and a parallel clinical syndrome in humans.[4] Since then, the molecular details of how dysfunction of this voltage-gated calcium channel pore-forming subunit, joined later by other auxiliary subunit members of the voltage-gated calcium ion channel family,[5,6]

leads to the distinctive spike-wave electroencephalographic (EEG) phenotype in mouse and human are steadily emerging. Together they provide an exemplary pathogenic road map revealing how early disruption of synaptic signaling patterns in the developing brain due to genetic impairment of a solitary ion channel entrains a downstream cascade of ion channel remodeling that favors neuronal burst firing and abnormal thalamocortical synchrony.

TOTTERING, ITS EPILEPTOGENIC ALLELES, AND INTERACTING SUBUNITS

The prototypical mutation, *tottering*, was the first of several spontaneous mutant gene loci underlying spike-wave seizures detected by EEG screening beginning in the late 1970s.[7] Now recognized as a single nucleotide mutation in *Cacna1a*, the gene encoding the pore-forming alpha subunit of the P/Q-type high-voltage-activated calcium channel, it shares the behavioral absence phenotype with other mutant alleles in the mouse,[8–12] the human,[13] and recently in the rat[14] (Fig. 54–1). Like many other ion channel disorders, the murine phenotype behaves as a true recessive since heterozygotes of most models appear unaffected; however, semidominant or true dominant phenotypes have recently been identified in *tottering 4J* and *wobbler* alleles.[10,11] These mutations recapitulate major neurological elements of the *tottering* syndrome, including nonconvulsive spike-wave absence epilepsy, ataxia, and episodic nonepileptic dyskinesias of variable developmental onset and severity[1]; we now appreciate that the disorder depends upon the loss of one or more functional domains of the channel protein, since full deletion of *Cacna1a* leads to a similar, more severe phenotype.[15] Interestingly, the syndrome contains a latent but beneficial neuroprotective element; the pathological activation of cortical spreading depression is strikingly diminished due to a major reduction in depolarization-induced release of glutamate and gamma-aminobutyric acid (GABA).[16] In contrast, gain-of-function

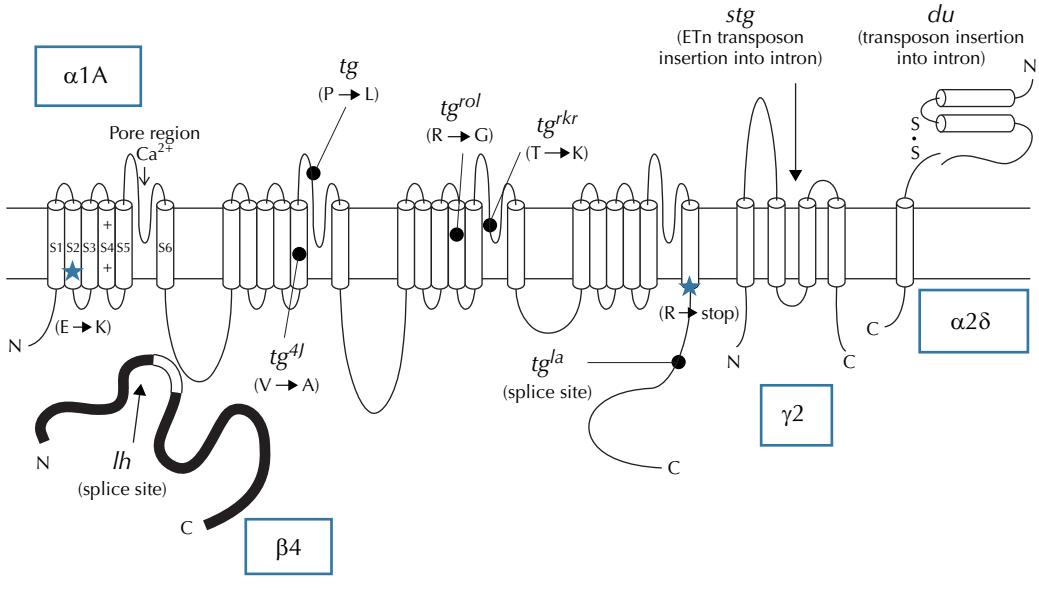

Figure 54–1. Genes for voltage-gated calcium channel subunits bearing absence epilepsy mutations. Approximate positions of spontaneous loss-of-function mutations in *tottering* (*tg*) and its alleles (*rolling*, *rocker*, *leaner*, and *tg 4J*) are shown by the black circles in the pore-forming alpha subunit (*Cacna1a*). The positions of two loss-of-function human mutations producing absence epilepsy, ataxia with/without episodic dyskinesia are shown with stars. The *lethargic* mutation interrupts interaction of the cytoplasmic regulatory beta subunit (*CacnB4*) with the alpha subunit, and *ducky* results in a defective membrane-spanning a2delta subunit (*Cacna2d2*). The *stargazer* mutation shown in the transmembrane gamma subunit (*Cacng2*) was originally considered to be a member of the voltage-gated calcium channel, as found in heteromeric muscle channels.

mutations in *Cacna1a* facilitate neurotransmitter release at central terminals[17,18] and are associated in the forebrain with convulsive seizures and episodic hemiplegic migraine mediated by a greatly reduced threshold for cortical spreading depression in the human and the mouse.[19–21] Together, these diametrically opposed presentations of calcium channelopathy offer a clear example of the spectrum of network excitability effects that arise from mutations within a single ion channel gene.

HOW LOSS-OF-FUNCTION *CACNA1A* MUTATIONS IMPAIR NEURONAL NETWORKS

Somatodendritic Compartment

Cacna1a mutations from mice displaying spike-wave epilepsy reduce P/Q current density in somatodendritic membranes. For the *tg* and *tg(rol)* mutations this is due to the lower voltage sensitivity of single-channel current activation[22–25] and in part to reduced membrane expression.[26] Human absence epilepsy mutations also reduce the P/Q current.[4,13] Interestingly, lower membrane current density may also contribute to dysfunction in gain-of-function hemiplegic migraine mutations.[27,28] In this case, the balance of the dual opposing effects of increased conductance and prolonged kinetics of single P/Q channels in the face of reduced current density may give rise to a complex array of intermediate and potentially network-specific excitability changes in these genotypes. Diminished P/Q current within somatodendritic compartments, where it is coupled to Ca^+-activated potassium channels, alters spontaneous firing patterns and postsynaptic plasticity at dendritic spines.[29,30]

Axon Initial Segment

Recent evidence indicates that P/Q channels are also active at the axon initial segment, where they play a role in membrane repolarization during spike firing by enhancing Ca^+-activated BK potassium channels.[31] Loss of P/Q current in this compartment is therefore of mechanistic interest as a pro-absence mechanism, since *KCNMA1*, one of the genes encoding brain BK channels, is highly expressed in neocortex, nucleus reticularis thalami (nRT), and relay nuclei of thalamus, and individuals with *KCNMA1* mutations show an absence epilepsy phenotype.[32]

Presynaptic Terminal and Exocytosis

Mutations in calcium channel subunits alter interactions with proteins coupled to exocytosis at the presynaptic terminal, revealing an inherant synaptic defect underlying absence epilepsy. P/Q channels are a major source of calcium ions that trigger the evoked release of neurotransmitter; this function is shared to a highly variable extent with N-type and R-type channels at central terminals, which possess distinct biophysical properties and individual contributions to the release machinery.[33] For example, P/Q channels show strong activity-dependent facilitation at the presynaptic terminal, which N channels do not.[34] Since evoked release at different interneurons depends selectively upon the P/Q type, the N type, or both,[35] the functional complexity of an inherited P/Q-type lesion makes it difficult to predict the emergent effects on excitability in any given synaptic network. In addition, in the immature brain, there is a developmental subunit switch in the channel dependence of synaptic release, where at some but not all synapses, the excitation-secretion machinery converts its reliance upon N-type release coupling to the P/Q type in the third postnatal week.[36,37] Interestingly, this switch is coincident with the postnatal appearance (P14–19) of spike-wave seizures in *tg* and other SW mutant mice.[38,39] While mechanistic details of the subunit switch are still emerging,[40] it may precipitate the onset of seizures in P/Q-type-deficient mutants, since a shift to P/Q reallocates the release machinery dependence on a source of calcium ions from functional N-type channels to defective or unavailable mutant P/Q channels, as demonstrated at *tottering* central synapses.[41] The specificity of P/Q splice variants in different cell types adds to additional release complexity within the thalamocortical network.[42,43]

When excitation-secretion coupling is assessed at central presynaptic terminals in the adult *tottering* mouse hippocampus, excitatory transmitter release is intact yet depends

almost entirely on rescue by N-type currents, since blockade by a specific P/Q toxin eliminates the majority of evoked release and spares a minor residual component presumably due to R-type current.[41] Similar results have also been seen at peripheral terminals,[44] where P/Q deficiency also increases jitter and reduces preterminal synaptic synchrony during rapid stimulation at the neuromuscular junction.[45] Intrahipppocampal inhibition is impaired, as assessed by the abnormally prolonged burst duration in *tottering* pyramidal cells.[46] At intracortical terminals of thalamic relay neurons in *tottering* mice, evoked excitatory responses in layer IV pyramidal cells are again spared (implying N-type release dependence), while evoked feedforward inhibition is impaired in this layer but not in layer V.[37] In contrast, at excitatory terminals in *tottering* thalamus, there is a defect in glutamate release.[47] Thus, P/Q deficiency has no uniform effect on synaptic signaling during brain development, and it may also alter somatodendritic firing patterns even in cells where it spares P/Q-independent exocytosis. Interestingly, impairment of the two other calcium channel alpha subunits involved in transmitter release, N type and R type, do not impact thalamocortical synchrony in the same way. Removal of N-type channels by *Cacna1b* deletion does not lead to an absence epilepsy phenotype, but it alters background cortical EEG rhythmicity at gamma frequencies.[48,49] On the other hand, deletion of *Cacna1e* eliminates R-type channels that contribute only a nominal amount to release at most measured synapses, yet it does alter the spike-wave discharge phenotype.[50]

CALCIUM CHANNEL REGULATORY SUBUNIT "RESHUFFLING" AND EMERGENT SPIKE-WAVE NETWORKS

Lethargic (*Cacnb4 lh*) and *ducky* (*Cacna2d2 du*) mutants display remarkable phenotypic similarities to the *tg* mutant. The roles of the auxiliary β[51] and α2δ2[52] subunits have been studied in detail in heterologous and neuronal systems. Although there are distinct functional and anatomical expression differences between individual family members, both types increase the current amplitude, accelerate inactivation kinetics and facilitate gating, and shift the voltage dependence of inactivation in the hyperpolarizing direction. When expressed singly with an alpha subunit in heterologous systems, loss of any one of these family members would be expected to significantly alter calcium channel signaling; however, when promiscuously binding family members are coexpressed and available in the same cell in vivo, deletion of one regulatory subunit gene leads to complex changes in the neuronal network as a whole (Fig. 54–2A). Rather than affecting all cells in which they are expressed, the extent of their loss in vivo depends on their replacement by alternative subunit family members, with functional rescue at certain alpha subunits in some cells and failure at others. Beta subunits also show variable efficacy in mediating release at presynaptic terminals.[53] This "reshuffling" phenomenon was evaluated in the *lethargic* brain,[54] where coimmunoprecipitation studies confirmed that β4 is the preferred binding subunit for both P/Q and N-type alpha subunits, and coimmunoprecipitation studies revealed novel heteromeric associations with β1 and β3, allowing for the rescue of calcium currents and functional release at various synapses in these mice (Fig. 54–2C). Indeed, electrophysiological analysis revealed that in *lethargic* Purkinje cells, somatic high-voltage–activated (HVA) currents were unaffected (Fig. 54–2D), yet release at excitatory synapses onto thalamic neurons in *tottering* and *lethargic* is sharply reduced.[47] No alteration in release at inhibitory synapses was found in *lethargic* thalamus, indicating that GABAergic neurotransmission in nRT terminals is relatively intact. This suggests that unlike *tottering*, the *lethargic* absence phenotype arises out of a global thalamocortical network lesion that is defined by the presence or absence of rescuing regulatory subunit family members. When brain expression patterns of the related beta subunit family members are compared (Fig. 54–2B), it is evident that thalamic nuclei lack significant expression of β1 and β3 subunits, pointing to a likely explanation for excitability and release defects in these cells. Thus, incomplete overlap of coexpressed heteromeric subunit family members defines a critical map of synaptic vulnerability in ion channel regulatory subunit mutant brain.

As with alpha subunits, additional complexity arises when cell-specific splicing patterns of regulatory subunits are considered. For

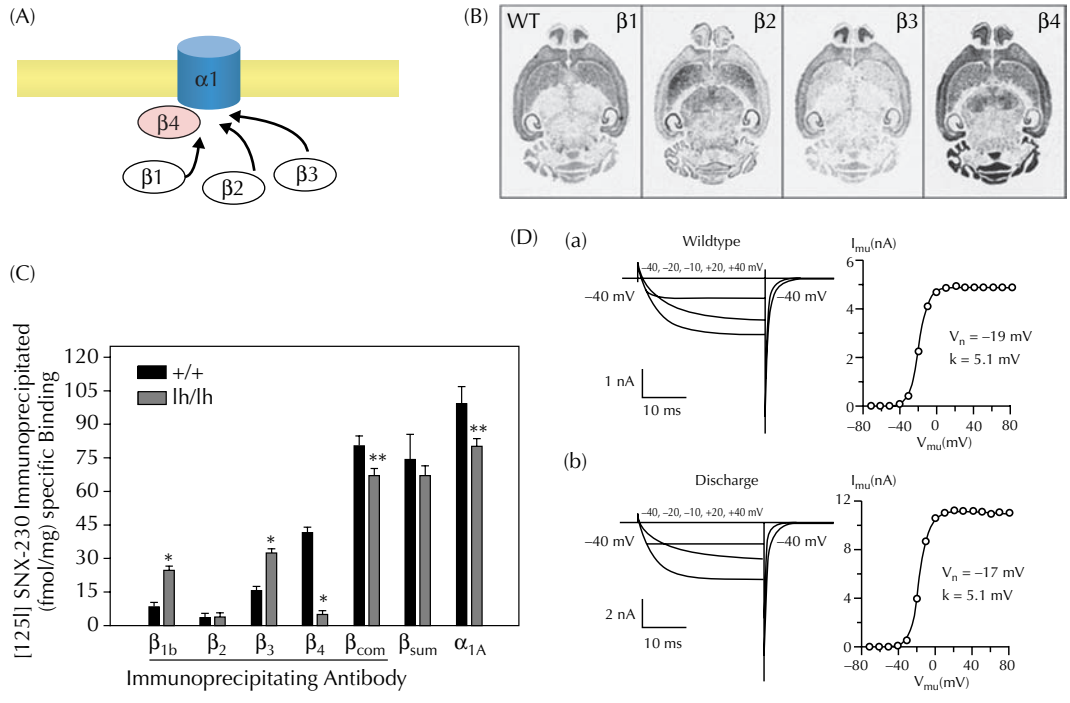

Figure 54–2. The brain lesion arising from genetic loss of a calcium ion channel regulatory subunit is more complex than that of a pore-forming alpha subunit. In **A**, due to the promiscuity of alpha subunits, absence of a single regulatory subunit (β4) in the lethargic mouse allows compensatory reshuffling of alternative family members, resulting in novel channel properties. In **B**, the distinct expression patterns of β1–4 family members in situ means that reshuffled P/Q-type alpha subunits may differ in subunit composition in different brain regions according to subunit availability. In **C**, coimmunoprecipitation patterns in lethargic brain show that α1a subunits, deprived of β4 interactions in lethargic mice, bind β1 and β3 partners instead, allowing rescue of the P/Q-type current in cerebellar Purkinje cells. **D**. Patch-clamp recordings confirm the functional rescue of normal P/Q-type currents in lethargic Purkinje cells. Different patterns of partial channel rescue throughout brain networks due to the presence or absence of available regulatory subunits may account for the spike-wave and ataxic phenotype in these mice. Adapted from ref. 54.

example, the cytoplasmic β4 subunit is alternatively spliced in different cell types, with the Cavβ4a spliceform present in Purkinje cells while Cavβ4b appears in basket cells.[55] Alternative α2δ subunit rescue is also likely to explain the pattern of release defects at selected synapses in *ducky* mice, at least at the neuromuscular junction, where it has been studied.[56]

DYSAFFERENTATION AS A CONVERGENT PATHWAY TO ABSENCE EPILEPSY IN DEVELOPING BRAIN

Among the possible sites of action of mutated P/Q channels and their regulatory subunits in epileptogenesis, several lines of evidence suggest that aberrant transmitter release is a critical defect underlying the expression of a spike-wave phenotype. The data suggest that reduced calcium entry coupled to release machinery, or the absence of key interaction domains in the α1a channel with *N*-ethylmaleimide-sensitive fusion protein receptor (SNARE) proteins essential for exocytosis, or the SNARE proteins themselves, or postsynaptic receptor loss, or deficient GABA reuptake may each suffice to alter afferent input in the thalamocortical network and produce an absence phenotype. Examples of each of these molecular steps have been defined. For instance, mutations in the first intracellular loop of *CACNA1A* block exocytosis by preventing P/Q modulation by SNARE proteins.[57] Loss of β4 not only alters calcium channel kinetics and trafficking, but eliminates a scaffolding function of this protein with synap-

totagmin1 at synapses.[58] Loss of SNAP25 in the mutant mouse *Coloboma* leads to an autosomal dominant spike-wave phenotype.[39] Loss of stargazin, the protein product of *Cacng2*, reduces alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor-mediated synaptic transmission in the mutant mouse *stargazer*, which may contribute to destabilization of the thalamocortical network.[59] Increased tonic inhibition due to loss of GABA reuptake also favors the spike-wave phenotype. For example, defective GABA reuptake by the GAT1 glial transporter exacerbates tonic inhibition, and VGAT1-deficient mice display spike-wave epilepsy; *stargazer* and *lethargic* mice share reduced GABA reuptake, while *tottering* mice do not.[60] Clearly, many molecular lesions converge on the spike-wave phenotype; however, each may exert direct and indirect downstream effects. While the loss of synaptic strength may directly produce dynamic changes in thalamocortical oscillations, a second possibility is that it incurs secondary molecular remodeling in these cells, resulting in maladaptive downstream mechanisms leading to the spike-wave phenotype.

RESCULPTING THALAMIC EXCITABILITY: INVOLVEMENT OF T-TYPE CURRENTS

Low voltage-activated, T-type currents have long been implicated in the generation of aberrant spike-wave discharges in thalamocortical circuitry based on their ability to confer rebound bursting in thalamic neurons,[61,62] the association of gain-of-function polymorphisms in human *CACNA1H*[63–65] and *CACNA1G*[66] genes in sporadic absence epilepsy, and their sensitivity to blockade by ethosuximide.[67] In animal models, targeted deletions of α1G channels suppress both $GABA_B$-receptor agonist-induced spike-wave discharges in wild-type mice and spontaneous discharges in calcium channel mutant mice,[68,69] indicating a contributing role for this gene in seizure expression. In addition, five distinct genetic mouse models of absence, *tottering*, *lethargic*, *stargazer*, *Coloboma*, and *Cacna1a* –/– show striking enhancement of thalamic T-type currents.[38,39,69] T-type current-mediated bursting is also unmasked in the *HCN2*-null mouse, where loss of Ih current generates spontaneous absence seizures,[70] and in the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) rat, which exhibits elevated thalamic T-type currents and increased *Cacna1g* and *Cacna1h* mRNA expression.[71–73]

Evaluating the T-Type Contribution

In each of these models, elevated T-type current is one of many possible contributing pathogenic defects that could facilitate aberrant cortical synchronizations. Indeed, when this current was genetically reduced in *Cacna1a* –/– mice through a series of crosses with *Cacna1g* –/– mice to generate compound mutants with varying amounts of T-type channel protein, it was found that spike-wave discharges could persist even in the presence of normal rather than elevated thalamic current.[69] However, when T-type current was directly increased by overexpression of *Cacna1g* using a bac-transgenic construct under the control of its native promoter, T-type channel and functional current elevation alone was sufficient to induce a pure absence phenotype in an otherwise wild-type mouse brain (Figure 54–3).[74]

Other P/Q Channel-Induced Downstream Plasticity

Not unexpectedly, other forms of molecular and cellular plasticity have been described in the *tottering* forebrain and may participate in the epilepsy phenotype. One of the earliest, seen in both *tg* and *roller* alleles, is the presence of noradrenergic hyperinnervation.[75,76] Given the strong dependence of the compensatory N-type channels (but not P/Q-type channels) on noradrenergic modulation, and the fact that neonatal lesions in this pathway produced an essentially complete rescue of the *tottering* phenotype,[77] noradrenergic signaling appears to participate in spike-wave expression, although the exact reasons why elimination of this pathway at birth prevents spike-wave patterns remains to be elucidated. Gamma-aminobutyric acid receptor density and stoichiometry are also significantly altered in the *tottering* forebrain.[78,79] Baseline cyclic adenosine monophosphate (cAMP) levels are increased in the *tottering* forebrain, where they may play a role in potentiating T-type currents.[80,81]

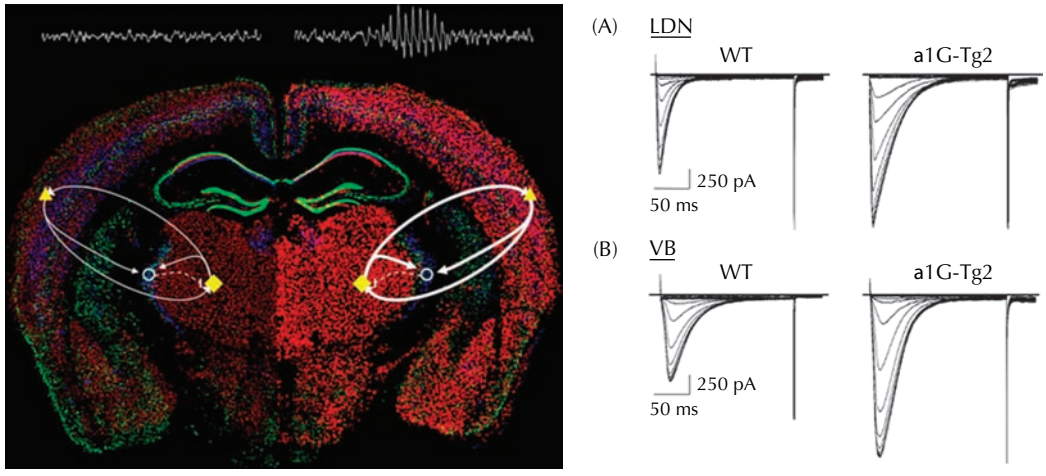

Figure 54–3. Enhancement of A1G in thalamocortical networks produces spike-wave absence epilepsy. Split composite image of in situ hybridization patterns of three genes—*Cacna1g* (red), *Cacna1h* (green), and *Cacna1i* (blue)—in adult mouse brain. The left side of the brain depicts native levels of expression and representative baseline cortical EEG activity in the wild-type mouse. The right side of the brain shows elevated transcript levels in the bac-transgenic *Cacna1g*-overexpressing mouse strain, with a typical cortical spike-wave EEG discharge above. The bac transgene drives *Cacna1g* under the control of its endogenous promoter, selectively elevating the T-type current to conform with its native pattern in brain circuitry. At the right, current traces from patch-clamp recordings of neurons in the lateral dorsal nucleus (LDN, (**A**) and the ventrobasal nucleus (VB),(**B**) in wild-type and transgenic mice show representative elevations in T-type currents in mutant neurons overexpressing *Cacna1g*. From ref. 74.

TRANSLATIONAL ADVANCES TOWARD THE TREATMENT OF INHERITED CALCIUM CHANNEL EPILEPSIES

When Does *Cacna1a*-Mediated Pathogenesis Begin?

In contrast with the neonatal and infantile epilepsies due to sodium and potassium channelopathy occurring in the first year of life, a notable characteristic of absence epilepsy is the association of various syndromes with specific developmental ages; human spike-wave seizures appear rarely in infancy but typically are recognized much later in childhood and adolescence. Unlike absence models in inbred rat strains where seizures begin in adulthood, monogenic mouse models also display an early postnatal onset (~P13–17 days) corresponding to that of human childhood.[38,39] While this interval coincides with the early enhancement of T-type currents identified in these models, it is also a period of intense refinement of synaptic innervation and molecular plasticity in the immature mouse brain, including the switch from N- to P/Q-type release dominance at selected thalamocortical synapses. Since inherited P/Q calcium channel mutations disrupt channel function at the inception of nervous system development and are responsible for extensive changes in synaptic microarchitecture in the P/Q mutant brain,[82] it is of interest to determine whether the evolving network lesion depends upon early embryonic or delayed postnatal effects of the functional release defect on synaptogenesis.

To address this question, a conditional mutant mouse was constructed that allowed ablation of P/Q channels in the critical postnatal period.[83] *Purky* is a viable mouse mutant, created by crossing a delayed-onset cre-driver PCP2 (L7) Purkinje cell promoter with floxed *Cacna1a* mice to ablate the *Cacna1a* gene throughout the brain beginning in the second postnatal week. Detailed analysis of *purky* Purkinje neurons revealed that P/Q currents are degraded by 3–4 weeks of age and are absent when the mice are examined at 6 months, consistent with a loss of P/Q channel antibody staining, abnormal spike firing patterns, and impaired transmitter release. Despite the delayed loss of P/Q channels, *purky* mice display the complete neurological phenotype of *tottering* mice, including severe ataxia, episodic dyskinesias,

and spike-wave absence seizures.[83] The *purky* mutant demonstrates that despite the role of P/Q channels in synaptic competition in developing brain, delayed postnatal onset of inherited P/Q channelopathy rather than embryonic disruption of thalamocortical circuitry is sufficient for the expression of the epileptic phenotype. This finding removes a potential therapeutic obstacle for postnatal rescue by defining a generous developmental window following birth that will allow the opportunity to pharmacologically restore function in inherited P/Q calcium channelopathy prior to the onset of seizures.

EPISTATIC INTERACTIONS

While the nonselective partial T-type antagonist ethosuximide is currently the most effective antiabsence drug, many patients remain pharmacoresistent.[84] The possibility of higher-affinity antagonists that are more selective for specific T-type channels is attractive[85,86]; however, given the heterogeneity of absence epilepsy, even these may not prove to be universally effective. A promising route to the development of additional targets for therapy of monogenic absence epilepsies is to identify modifier genes that suppress the aberrant excitability phenotype. This can be accomplished by unbiased forward genetic approaches as used in high-throughput *Drosophila* mutagenesis screening,[87] or by slower "rational" hypothesis testing that combines known murine mutations based on plausible combinatorial biological outcomes. For example, to determine whether the *tottering* P/Q transmitter release defect could be rescued and spike-wave epilepsy prevented by prolonging nerve terminal depolarization and increasing evoked release, Glasscock et al.[88] crossed *tottering* mutant mice with a Kv1.1-deficient knockout mouse strain. Kv1.1 is expressed at axonal juxtaparanodes and presynaptic nerve terminals, and its removal delays membrane repolarization, augments presynaptic nerve terminal excitability, and potentiates neurotransmitter release.[89,90] Electroencephalographic recordings from doubly homozygous *Cacana1a–/–, Kv1.1 –/–* mutant mice) revealed that spike-wave seizures were indeed abolished in the double mutant offspring (Fig. 54–4A). As a test to determine whether the phenotypic rescue of *tottering* seizures could be reproduced in the adult brain once seizures had already appeared, 4-aminopyridine (4AP), a nonspecific potassium channel blocker, was administered to adult *tottering* mutants. Like ethosuximide,

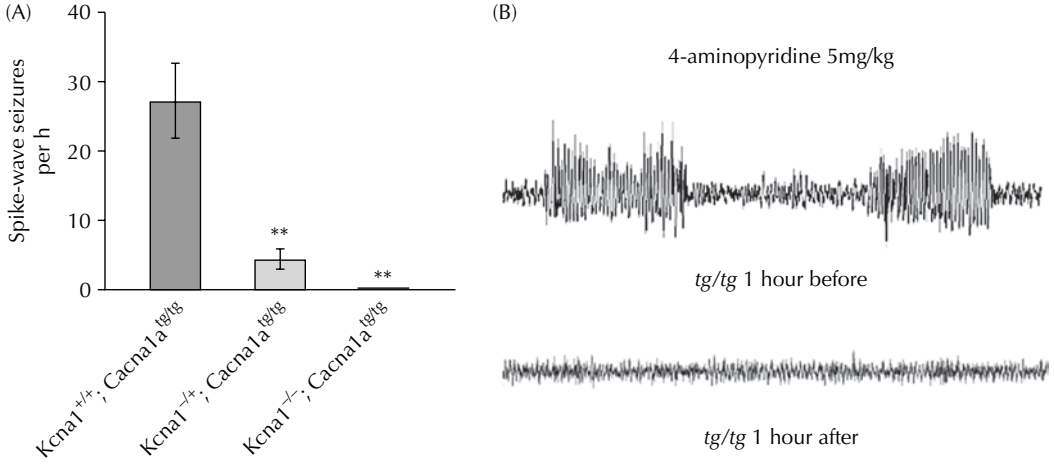

Figure 54–4. Potassium channel modification of the P/Q calcium channel spike-wave phenotype. **A.** Epistatic masking of spike-wave seizures in double mutants bearing *Cacna1a* (P/Q^{tg}) and Kv1.1 –/– mutations. There is a striking gene dosage effect showing an allelic dose-dependent decrease in seizure frequency with the removal of a single copy of the *Kcna1a* gene (middle) and complete suppression of seizure activity upon full ablation of the *Kcna1a* gene. In **B**, phenocopy experiments demonstrated suppression of cortical spike-wave absence seizures in adult *tottering* mutants following intraperitoneal injections of low doses of 4-AP, a nonspecific potassium channel blocker. From ref. 88.

4AP entirely abolished all spike-wave discharge activity in the *tottering* mutant within minutes of intraperitoneal injection (Fig. 54–4B). It is of interest that 4AP and noradrenergic blockade have also been found effective in reducing the severity of induced dyskinesias.[77,91,92] These phenocopy experiments demonstrate the ability of alternative ion channel targets to suppress phenotypes by rebalancing excitability in epileptogenic brain networks.

SUMMARY

Voltage-gated calcium channels were the first of now many genetic starting points for tracing the molecular mechanisms of spike-wave seizures, and in so doing, we have gained equally valuable information regarding the neurobiology of the P/Q-type calcium channel and its family members. Perhaps the most important lesson is that the solution of the neurological phenotype requires two steps: first, analyzing how the mutation affects the biology of the channel in a single neuron; second, determining how and when the misbehavior converts normal rebound bursting patterns in the thalamocortical network into an unstable pathological substrate for epilepsy in the developing brain. The "temporary failure of motor and mental functions" defined by Jackson arises not from enhanced excitatory release, as might be expected in paroxysmal hypersynchronous activity, but from the opposite: reduced synaptic strength within the thalamocortical network due to impaired calcium entry and diminished interactions with transmitter release machinery. This reduction in synaptic strength joins other molecular triggers, such as loss of vesicular exocytosis proteins or postsynaptic AMPA receptors, to induce downstream elevations in low voltage-activated T-type calcium channels, which may suffice to generate a spike-wave seizure phenotype. There is ample clinical and genetic evidence to suggest that there will be no uniform thalamocortical molecular pathology underlying this disorder and that alternative routes to the clinical spectrum of absence epilepsy remain to be discovered. Comparisons of their monogenic mechanisms will prove highly instructive in subsequent attempts to unravel the most elusive causes of sporadic generalized epilepsies, those that arise out of polygenic combinations. This category comprises the majority of individuals with absence seizures, and to them we remain obliged to confess our continuing ignorance of the causes of their condition.

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DISCLOSURE STATEMENT

The author has no conflicts of interest to disclose.

REFERENCES

1. Noebels JL, Sidman RL. Inherited epilepsy: spike-wave and focal motor seizures in the mutant mouse tottering. *Science*. 1979;204:1334–1336.
2. Berger H. Uber das Elektrenkephalogramm des Menschen. IV. *Arch Psychiatrie Nervenkrankheit*. 1933;101:452–469.
3. Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD, Hawkes R, Frankel WN, Copeland NG, Jenkins NA. Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell*. 1996;87: 607–617.
4. Jouvenceau A, Eunson LH, Spauschus A, Ramesh V, Zuberi SM, Kullmann DM, Hanna MG. Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. *Lancet*. 2001;358:801–807.
5. Burgess DL, Jones JM, Meisler MH, Noebels JL. Mutation of the Ca^{2+} channel beta subunit gene Cchb4 is associated with ataxia and seizures in the lethargic (lh) mouse. *Cell*. 1997;88:385–392.
6. Barclay J, Balaguero N, Mione M, Ackerman SL, Letts VA, Brodbeck J, Canti C, Meir A, Page KM, Kusumi K, Perez-Reyes E, Lander ES, Frankel WN, Gardiner RM, Dolphin AC, Rees M. Ducky mouse phenotype of epilepsy and ataxia is associated with mutations in the Cacna2d2 gene and decreased calcium channel current in cerebellar Purkinje cells. *J Neurosci*. 2001;21:6095–6104.
7. Noebels JL. Mutational analysis of inherited epilepsies. *Adv Neurol*. 1986;44:97–113.
8. Zwingman TA, Neumann PE, Noebels JL, Herrup K. *Rocker* is a new variant of the voltage-dependent calcium channel gene Cacna1a. *J Neurosci*. 2001;21: 1169–1178.
9. Lorenzon NM, Lutz CM, Frankel WN, Beam KG. Altered calcium channel currents in Purkinje cells of

the neurological mutant mouse leaner. *J Neurosci.* 1998;18:4482–4489.
10. Miki T, Zwingman TA, Wakamori M, Lutz CM, Cook SA, Hosford DA, Herrup K, Fletcher CF, Mori Y, Frankel WN, Letts VA. Two novel alleles of tottering with distinct Ca(v)2.1 calcium channel neuropathologies *Neuroscience*. 2008;155:31–44
11. Xie G, Clapcote SJ, Nieman BJ, Tallerico T, Huang Y, Vukobradovic I, Cordes SP, Osborne LR, Rossant J, Sled JG, Henderson JT, Roder JC. Forward genetic screen of mouse reveals dominant missense mutation in the P/Q-type voltage-dependent calcium channel, CACNA1A. *Genes Brain Behav*. 2007;6:717–727.
12. Brill J, Klocke R, Paul D, Boison D, Gouder N, Klugbauer N, Hofmann F, Becker CM, Becker K. entla, a novel epileptic and ataxic Cacna2d2 mutant of the mouse. *J Biol Chem*. 2004;279:7322–7330.
13. Imbrici P, Jaffe SL, Eunson LH, Davies NP, Herd C, Robertson R, Kullmann DM, Hanna MG. Dysfunction of the brain calcium channel Cav2.1 in absence epilepsy and episodic ataxia. *Brain*. 2004;127:2682–2692.
14. Tokuda S, Kuramoto T, Tanaka K, Kaneko S, Takeuchi IK, Sasa M, Serikawa T. The ataxic groggy rat has a missense mutation in the P/Q-type voltage-gated Ca^{2+} channel alpha1A subunit gene and exhibits absence seizures. *Brain Res*. 2007;1133:168–177.
15. Jun K, Piedras-Renteria ES, Smith SM, Wheeler DB, Lee SB, Lee TG, Chin H, Adams ME, Scheller RH, Tsien RW, Shin HS. Ablation of P/Q-type Ca(2+) channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the alpha(1A)-subunit. *Proc Natl Acad Sci USA*. 1999;96:15245–15250.
16. Ayata C, Shimizu-Sasamata M, Lo EH, Noebels JL, Moskowitz MA. Impaired neurotransmitter release and elevated threshold for cortical spreading depression in mice with mutations in the alpha1A subunit of P/Q type calcium channels. *Neuroscience*. 2000;95: 639–645.
17. Pietrobon D. Insights into migraine mechanisms and CaV2.1 calcium channel function from mouse models of familial hemiplegic migraine. *J Physiol*. 2010;588: 1871–1878.
18. Adams PJ, Rungta RL, Garcia E, van den Maagdenberg AM, Macvicar BA, Snutch TP. Contribution of calcium-dependent facilitation to synaptic plasticity revealed by migraine mutations in the P/Q-type calcium channel. *Proc Natl Acad Sci USA*. 2010;107:18694–18699.
19. Beauvais K, Cave-Riant F, De Barace C, Tardieu M, Tournier-Lasserve E, Furby A. New CACNA1A gene mutation in a case of familial hemiplegic migraine with status epilepticus. *Eur Neurol.* 2004;52:58–61.
20. Kors EE, Melberg A, Vanmolkot KR, Kumlien E, Haan J, Raininko R, Flink R, Ginjaar HB, Frants RR, Ferrari MD, van den Maagdenberg AM. Childhood epilepsy, familial hemiplegic migraine, cerebellar ataxia, and a new CACNA1A mutation. *Neurology*. 2004;63:1136–1137.
21. Chan YC, Burgunder JM, Wilder-Smith E, Chew SE, Lam-Mok-Sing KM, Sharma V, Ong BK. Electroencephalographic changes and seizures in familial hemiplegic migraine patients with the CACNA1A gene S218L mutation. *J Clin Neurosci*. 2008;15:891–894.
22. Lorenzon NM, Lutz CM, Frankel WN, Beam KG. Altered calcium channel currents in Purkinje cells of the neurological mutant mouse leaner. *J Neurosci*. 1998;18:4482–4489.
23. Wakamori M, Yamazaki K, Matsunodaira H, Teramoto T, Tanaka I, Niidome T, Sawada K, Nishizawa Y, Sekiguchi N, Mori E, Mori Y, Imoto K. Single tottering mutations responsible for the neuropathic phenotype of the P-type calcium channel. *J Biol Chem*. 1998;273:34857–34867.
24. Dove LS, Abbott LC, Griffith WH. Whole-cell and single-channel analysis of P-type calcium currents in cerebellar Purkinje cells of leaner mutant mice. *J Neurosci*. 1998;18:7687–7699.
25. Mori Y, Wakamori M, Oda S, Fletcher CF, Sekiguchi N, Mori E, Copeland NG, Jenkins NA, Matsushita K, Matsuyama Z, Imoto K. Reduced voltage sensitivity of activation of P/Q-type Ca^{2+} channels is associated with the ataxic mouse mutation rolling Nagoya (tg(rol)). *J Neurosci*. 2000;20:5654–5662.
26. Leenders, AGM, van den Maagdenberg AMJM, Lopes da Silva FH, Sheng ZH, Molenaar PC, Ghijsen WEJM. Neurotransmitter release from tottering mice nerve terminals with reduced expression of mutated P- and Q-type Ca^{2+}-channels. *Eur J Neurosci*. 2002;15: 13–18.
27. Tottene A, Fellin T, Pagnutti S, Luvisetto S, Striessnig J, Fletcher C, Pietrobon D. Familial hemiplegic migraine mutations increase Ca(2+) influx through single human CaV2.1 channels and decrease maximal CaV2.1 current density in neurons. *Proc Natl Acad Sci USA.* 2002;99:13284–13289.
28. Jeng CJ, Sun MC, Chen YW, Tang CY. Dominant-negative effects of episodic ataxia type 2 mutations involve disruption of membrane trafficking of human P/Q-type Ca^{2+} channels. *J Cell Physiol*. 2008;214: 422–433.
29. Womack MD, Chevez C, Khodakhah K. Calcium-activated potassium channels are selectively coupled to P/Q-type calcium channels in cerebellar Purkinje neurons. *J Neurosci.* 2004;24:8818–8822.
30. Hartmann J, Konnerth A. Determinants of postsynaptic Ca^{2+} signaling in Purkinje neurons. *Cell Calcium*. 2005;37:459–466.
31. Yu Y, Maureira C, Liu X, McCormick D. P/Q and N channels control baseline and spike-triggered calcium levels in neocortical axons and synaptic boutons. *J Neurosci*. 2010;30:11858–11869.
32. Du W, Bautista JF, Yang H, Diez-Sampedro A, You SA, Wang L, Kotagal P, Lüders HO, Shi J, Cui J, Richerson GB, Wang QK. Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. *Nat Genet*. 2005;37:733–738.
33. Li L, Bischofberger J, Jonas P. Differential gating and recruitment of P/Q-, N-, and R-type Ca^{2+} channels in hippocampal mossy fiber boutons. *J Neurosci*. 2007;27:13420–13429.
34. Ishikawa T, Kaneko M, Shin HS, Takahashi T. Presynaptic N-type and P/Q-type Ca^{2+} channels mediating synaptic transmission at the calyx of Held of mice. *J Physiol*. 2005;568:199–209.
35. Reid CA, Bekkers JM, Clements JD. Presynaptic Ca^{2+} channels: a functional patchwork. *Trends Neurosci*. 2003;26:683–687.
36. Iwasaki S, Momiyama A, Uchitel OD, Takahashi T. Developmental changes in calcium channel types mediating central synaptic transmission. *J Neurosci*. 2000;20:59–65.
37. Sasaki S, Huda K, Inoue T, Miyata M, Imoto K. Impaired feedforward inhibition of the thalamocortical projection in epileptic Ca^{2+} channel mutant mice, tottering. *J Neurosci*. 2006;26:3056–3065.

38. Zhang Y, Mori M, Burgess DL, Noebels JL. Mutations in high-voltage-activated calcium channel genes stimulate low-voltage-activated currents in mouse thalamic relay neurons. *J Neurosci*. 2002;22:6362–7631.
39. Zhang Y, Vilaythong AP, Yoshor D, Noebels JL. Elevated thalamic low-voltage-activated currents precede the onset of absence epilepsy in the SNAP25-deficient mouse mutant coloboma. *J Neurosci*. 2004;24: 5239–5248
40. Cao YQ, Tsien RW. Different relationship of N- and P/Q-type Ca^{2+} channels to channel-interacting slots in controlling neurotransmission at cultured hippocampal synapses. *J Neurosci.* 2010;30:4536–4546.
41. Qian J, Noebels JL. Presynaptic Ca(2+) influx at a mouse central synapse with Ca(2+) channel subunit mutations. *J Neurosci*. 2000;20:163–170.
42. Jurkat-Rott K, Lehmann-Horn F. The impact of splice isoforms on voltage-gated calcium channel alpha1 subunits. *J Physiol*. 2004;554:609–619.
43. Liao P, Zhang HY, Soong TW. Alternative splicing of voltage-gated calcium channels: from molecular biology to disease. *Pflugers Arch*. 2009;458:481–487.
44. Plomp JJ, Vergouwe MN, Van den Maagdenberg AM, Ferrari MD, Frants RR, Molenaar PC. Abnormal transmitter release at neuromuscular junctions of mice carrying the tottering alpha(1A) Ca(2+) channel mutation. *Brain.* 2000;123:463–471.
45. Depetris RS, Nudler SI, Uchitel OD, Urbano FJ. Altered synaptic synchrony in motor nerve terminals lacking P/Q-calcium channels. *Synapse*. 2008;62: 466–471.
46. Noebels JL, Rutecki PA. Altered hippocampal network excitability in the hypernoradrenergic mutant mouse tottering. *Brain Res*. 1990;524:225–230.
47. Caddick SJ, Wang C, Fletcher CF, Jenkins NA, Copeland NG, Hosford DA. Excitatory but not inhibitory synaptic transmission is reduced in lethargic (Cacnb4(lh)) and tottering (Cacna1atg) mouse thalami. *J Neurophysiol*. 1999;81:2066–2074.
48. Beuckmann CT, Sinton CM, Miyamoto N, Ino M, Yanagisawa M. N-type calcium channel alpha1B subunit (Cav2.2) knock-out mice display hyperactivity and vigilance state differences. *J Neurosci*. 2003;23: 6793–6797
49. Llinás RR, Choi S, Urbano FJ, Shin HS. Gamma-band deficiency and abnormal thalamocortical activity in P/Q-type channel mutant mice. *PNAS*. 2007;104: 17819–17824.
50. Weiergräber M, Henry M, Ho MS, Struck H, Hescheler J, Schneider T. Altered thalamocortical rhythmicity in Ca(v)2.3-deficient mice. *Mol Cell Neurosci.* 2008;39:605–618
51. Buraei Z, Yang J. The beta subunit of voltage-gated Ca^{2+} channels. *Physiol Rev.* 2010;90:1461–1506.
52. Bauer CS, Tran-Van-Minh A, Kadurin I, Dolphin AC. A new look at calcium channel $\alpha 2\delta$ subunits. *Curr Opin Neurobiol*. 2010;20:563–571.
53. Xie M, Li X, Han J, Vogt DL, Wittemann S, Mark MD, Herlitze S. Facilitation versus depression in cultured hippocampal neurons determined by targeting of Ca^{2+} channel Cav B4 versus CavB2 subunits to synaptic terminals. *J Cell Biol.* 2007;178:489–502.
54. Burgess DL, Biddlecome GH, McDonough SI, Diaz ME, Zilinski CA, Bean BP, Campbell KP, Noebels JL. Beta subunit reshuffling modifies N- and P/Q-type Ca^{2+} channel subunit compositions in lethargic mouse brain. *Mol Cell Neurosci*. 1999;13:293–311.
55. Vendel AC, Terry MD, Striegel AR, Iverson NM, Leuranguer V, Rithner CD, Lyons BA, Pickard GE, Tobet SA, Horne WA. Alternative splicing of the voltage-gated Ca^{2+} channel beta4 subunit creates a uniquely folded N-terminal protein binding domain with cell-specific expression in the cerebellar cortex. *J Neurosci*. 2006;26:2635–2644.
56. Kaja S, Todorov B, van de Ven RC, Ferrari MD, Frants RR, van den Maagdenberg AM, Plomp JJ. Redundancy of Cav2.1 channel accessory subunits in transmitter release at the mouse neuromuscular junction. *Brain Res*. 2007;1143:92–101.
57. Serra SA, Cuenca-León E, Llobet A, Rubio-Moscardo F, Plata C, Carreño O, Fernàndez-Castillo N, Corominas R, Valverde MA, Macaya A, Cormand B, Fernández-Fernández JM. A mutation in the first intracellular loop of CACNA1A prevents P/Q channel modulation by SNARE proteins and lowers exocytosis. *PNAS.* 2010;107:1672–1677.
58. Weiss N. The calcium channnel B4a subunit: a scaffolding protein between voltage-gated calcium channel and presynaptic vesicle release machinery. *J Neurosci*. 2006;26:6117–6118.
59. Menuz K, Nicoll RA. Loss of inhibitory neuron AMPA receptors contributes to ataxia and epilepsy in stargazer mice. *J Neurosci*. 2008;28:10599–10603.
60. Cope DW, Di Giovanni G, Fyson SJ, Orbán G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V. Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. *Nat Med*. 2009;15:1392–1398.
61. Huguenard JR, McCormick DA. Thalamic synchrony and dynamic regulation of global forebrain oscillations. *Trends Neurosci*. 2007;30:350–356.
62. Steriade M. Neuronal substrates of spike-wave seizures and hypsarrhythmia in corticothalamic systems. *Adv Neurol*. 2006;97:149–154.
63. Chen Y, Lu J, Pan H, Zhang Y, Wu H, Xu K, Liu X, Jiang Y, Bao X, Yao Z, Ding K, Lo WH, Qiang B, Chan P, Shen Y, Wu X. Association between genetic variation of CACNA1H and childhood absence epilepsy. *Ann Neurol*. 2003;54:239–243.
64. Khosravani H, Altier C, Simms B, Hamming KS, Snutch TP, Mezeyova J, McRory JE, Zamponi GW. Gating effects of mutations in the Cav3.2 T-type calcium channel associated with childhood absence epilepsy. *J Biol Chem*. 2004;279:9681–9684.
65. Vitko I, Bidaud I, Arias JM, Mezghrani A, Lory P, Perez-Reyes E. The I-II loop controls plasma membrane expression and gating of Ca(v)3.2 T-type Ca^{2+} channels: a paradigm for childhood absence epilepsy mutations. *J Neurosci*. 2007;27:322–330.
66. Singh B, Monteil A, Bidaud I, Sugimoto Y, Suzuki T, Hamano S, Oguni H, Osawa M, Alonso ME, Delgado-Escueta AV, Inoue Y, Yasui-Furukori N, Kaneko S, Lory P, Yamakawa K. Mutational analysis of CACNA1G in idiopathic generalized epilepsy. Mutation in brief #962. *Online Hum Mutat*. 2007;28:524–525.
67. Coulter DA, Huguenard JR, Prince DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann Neurol*. 1989;25:582–593.
68. Kim D, Song I, Keum S, Lee T, Jeong MJ, Kim SS, McEnery MW, Shin HS. Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking alpha(1G) T-type Ca(2+) channels. *Neuron*. 2001;31:35–45.

69. Song I, Kim D, Choi S, Sun M, Kim Y, Shin HS. Role of the alpha1G T-type calcium channel in spontaneous absence seizures in mutant mice. *J Neurosci*. 2004;24:5249–5257.
70. Ludwig A, Budde T, Stieber J, Moosmang S, Wahl C, Holthoff K, Langebartels A, Wotjak C, Munsch T, Zong X, Feil S, Feil R, Lancel M, Chien KR, Konnerth A, Pape HC, Biel M, Hofmann F. Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. *EMBO J*. 2003;22:216–224.
71. Tsakiridou E, Bertollini L, de Curtis M, Avanzini G, Pape HC. Selective increase in T-type calcium conductance of reticular thalamic neurons in a rat model of absence epilepsy. *J Neurosci*. 1995;15:3110–3117.
72. Talley EM, Solórzano G, Depaulis A, Perez-Reyes E, Bayliss DA. Low-voltage-activated calcium channel subunit expression in a genetic model of absence epilepsy in the rat. *Brain Res Mol Brain Res.* 2000;75: 159–165.
73. Powell KL, Cain SM, Ng C, Sirdesai S, David LS, Kyi M, Garcia E, Tyson JR, Reid CA, Bahlo M, Foote SJ, Snutch TP, O'Brien TJ. A Cav3.2 T-type calcium channel point mutation has splice-variant-specific effects on function and segregates with seizure expression in a polygenic rat model of absence epilepsy. *J Neurosci*. 2009;29:371–380.
74. Ernst WL, Zhang Y, Yoo JW, Ernst SJ, Noebels JL. Genetic enhancement of thalamocortical network activity by elevating alpha 1g-mediated low-voltage-activated calcium current induces pure absence epilepsy. *J Neurosci*. 2009;29:1615–1625.
75. Levitt P, Noebels JL. Mutant mouse tottering: selective increase of locus ceruleus axons in a defined single-locus mutation. *Proc Natl Acad Sci USA*. 1981;78:4630–4634.
76. Muramoto O, Kanazawa I, Ando K. Neurotransmitter abnormality in Rolling mouse Nagoya, an ataxic mutant mouse. *Brain Res*. 1981;215:295–304.
77. Noebels JL. A single gene error of noradrenergic axon growth synchronizes central neurones. *Nature.* 1984;310:409–411.
78. Tehrani MH, Baumgartner BJ, Liu SC, Barnes EM Jr. Aberrant expression of $GABA_A$ receptor subunits in the tottering mouse: an animal model for absence seizures. *Epilepsy Res.* 1997;28:213–223
79. Payne HL, Donoghue PS, Connelly WM, Hinterreiter S, Tiwari P, Ives JH, Hann V, Sieghart W, Lees G, Thompson CL. Aberrant GABA(A) receptor expression in the dentate gyrus of the epileptic mutant mouse stargazer. *J Neurosci*. 2006;26:8600–8608.
80. Tehrani MH, Barnes EM Jr. Basal and drug-induced cAMP levels in cortical slices from the *tottering* mouse. *Epilepsy Res*. 1990;7:205–209.
81. Leresche N, Hering J, Lambert RC. Paradoxical potentiation of neuronal T-type Ca^{2+} current by ATP at resting membrane potential. *J Neurosci.* 2004;24: 5592–5602.
82. Miyazaki T, Hashimoto K, Shin HS, Kano M, Watanabe M. P/Q-type Ca^{2+} channel alpha1A regulates synaptic competition on developing cerebellar Purkinje cells. *J Neurosci*. 2004;24:1734–1743.
83. Mark MD, Maejima T, Kuckelsberg D, Yoo JY, Hyde RA, Shah V, Gutierrez D, Kruse K Noebels JL, Herlitze S. Delayed postnatal loss of P/Q type calcium channels recapitulates the absence epilepsy, dyskinesia, and ataxia phenotypes of genomic Cacna1A mutations. *J Neurosci.* 2011;31:4311–4326.
84. Glauser TA, Cnaan A, Shinnar S, Hirtz DG, Dlugos D, Masur D, Clark PO, Capparelli EV, Adamson PC, Childhood Absence Epilepsy Study Group. Ethosuximide, valproic acid, and lamotrigine in childhood absence epilepsy. *N Engl J Med*. 2010;362: 790–799.
85. Snutch TP, Cain SM. Contributions of T-type calcium channel isoforms to neuronal firing. *Channels* (*Austin*). 2010;4:44–51.
86. Dreyfus FM, Tscherter A, Errington AC, Renger JJ, Shin HS, Uebele VN, Crunelli V, Lambert RC, Leresche N. Selective T-type calcium channel block in thalamic neurons reveals channel redundancy and physiological impact of I(T)window. *J Neurosci*. 2010;30:99–109.
87. Song J, Tanouye MA. From bench to drug: human seizure modeling using *Drosophila. Prog Neurobiol*. 2008;84:182–191.
88. Glasscock E, Qian J, Yoo JW, Noebels JL. Masking epilepsy by combining two epilepsy genes. *Nat Neurosci*. 2007;10:1554–1558.
89. Smart SL, Lopantsev V, Zhang CL, Robbins CA, Wang H, Chiu SY, Schwartzkroin PA, Messing A, Tempel BL. Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. *Neuron*. 1998;20:809–819.
90. Zhou L, Messing A, Chiu SY. Determinants of excitability at transition zones in Kv1.1-deficient myelinated nerves. *J Neurosci*. 1999;19:5768–5781
91. Weisz CJ, Raike RS, Soria-Jasso LE, Hess EJ. Potassium channel blockers inhibit the triggers of attacks in the calcium channel mouse mutant tottering. *J Neurosci*. 2005;25:4141–4145.
92. Fureman BE, Hess EJ. Noradrenergic blockade prevents attacks in a model of episodic dysfunction caused by a channelopathy. *Neurobiol Dis.* 2005;20:227–232.

Chapter 55

Mutated $GABA_A$ Receptor Subunits in Idiopathic Generalized Epilepsy

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INTRODUCTION AND HISTORICAL PERSPECTIVES

Gamma-aminobutyric acid (GABA) was first identified as a biochemical substance in 1910 by Ackermann, who showed its production by decarboxylation of glutamic acid in bacteria.[1] In 1950, the presence of GABA in considerable amounts in the mammalian brain was discovered by different investigators,[2–4] and GABA was later established as the main inhibitory neurotransmitter in the brain.[5,6] Jasper and colleagues provided the first evidence that GABA is released in the brain under physiological conditions. Notably, they found variation in the release of GABA at the surface of the brain in relation to the state of activation of the cerebral cortex.[7]

GABAergic inhibition is mediated by two classes of receptor, ionotropic and metabotropic.

The GABA receptors type A ($GABA_A$) and C ($GABA_C$) are ligand-gated chloride channels that mediate fast inhibition. In turn, GABA receptors type B ($GABA_B$) mediate a variety of inhibitory effects through second messengers, affecting notably potassium and calcium conductance.[8,9] For several decades, abnormal GABAergic transmission associated with decreased inhibition of the central nervous system has been proposed as a major hypothesis for the development of epileptic seizures. Several forms of indirect evidence initially supported the role of this *GABA hypothesis of epileptogenesis.*[5,9–11] Early studies showed that pharmacological agents decreasing GABA levels or GABA-mediated inhibition through its receptors are pro-convulsant. In turn, increased GABA content or activation of its receptors is generally associated with an anti-convulsant effect. Also, whereas anti-GABA drugs can generate epileptic discharges in various animal models, pro-GABA agents generally decrease epileptic activity in these models. In addition, $GABA_A$ receptors are the molecular target of anti-convulsant drugs used in clinical practice, either by acting directly on its receptor (e.g., barbiturates, benzodiazepines) or by increasing GABA levels (e.g., vigabatrin). Finally, altered $GABA_A$ and $GABA_B$ receptor function has been observed in the brains of epileptic humans and in animal models of the disease.[10,12,13]

More recently, genetic studies have provided additional evidence supporting the role of GABA in epilepsy. First, mice deficient for two subunits of the $GABA_A$ receptors (*GABRB3*, *GABRG2*),[14,15] as well as two $GABA_B$ receptors (*GABBR1*, *GABBR2*), have been associated with epileptic seizures.[16–18] In humans, Minassian et al. found that typical and atypical absence attacks are present in Angelman syndrome patients whose chromosome 15q11–13 deletion includes *GABRB3.*[19] It is thus possible that haploinsufficiency of this gene may explain the severe epilepsy in this syndromic form of epilepsy associated with mental retardation and autistic features. However, the study of familial forms of idiopathic generalized epilepsies provided the first direct evidence that impaired $GABA_A$ receptor function is associated with epilepsy in humans.[20–24] We will review the various mutations in $GABA_A$ receptors associated with a variety of epilepsy phenotypes in humans, as well as their functional consequences in cellular and animal models.

DEFINITION AND CLASSIFICATION

Epilepsy is a pathological state characterized by recurrent unprovoked epileptic seizures.[25–27] Epileptic individuals may exhibit a wide range of clinical presentations, not only in terms of seizure manifestations, but also in terms of age of onset, etiology, prognosis, and so on. Therefore, epilepsy is clearly not a single condition, but rather a diverse family of disorders, called *the epilepsies*, having in common an abnormally increased predisposition to seizures.[26]

Despite great heterogeneity among the various epilepsies, clusters of clinical signs and symptoms have been observed among epileptic individuals, allowing physicians to identify relatively unique clinical entities called *epileptic syndromes*. In 1985, the International League Against Epilepsy (ILEA) started to make an inventory of the epileptic syndromes widely accepted in the epilepsy community, a process that is still underway.[27–30] The International Classification of the Epilepsies and Epileptic Syndromes rests on two criteria: (1) *seizure type*, which may be generalized or partial, and (2) *etiology*, which may be idiopathic or symptomatic.[28] Additional criteria include age of onset, evolution of the syndrome, associated interictal signs, symptoms and electroencephalographic (EEG) patterns, and genetic basis.[27,28,30]

Symptomatic epilepsies have multiple heterogeneous causes, including brain injury, central nervous system infection, and metabolic disorders.[27,28] In turn, the majority of individuals with epilepsy (65%) have recurrent unprovoked seizures without detectable structural lesions in the brain and without showing any neurological abnormality between seizures. For these individuals, no underlying cause can be identified, and they are said to have an *idiopathic* epileptic syndrome.[27,28,30] From the beginning of the ILAE classification, the term *idiopathic epilepsies* has been used for those in which there is no underlying cause other than a possible hereditary predisposition.[27,28,30]

GENETIC EVIDENCE FOR IDIOPATHIC EPILEPSIES

There is increasing evidence that genetic factors play an important role in idiopathic

epilepsies. These strong genetic factors have been highlighted by genetic epidemiology, including familial aggregation and twin studies, as well as by the study of unusually large families with epilepsy. Among the epilepsy syndromes, idiopathic generalized epilepsies (IGEs) have the most significant hereditary component and represent approximately 40% of all the epilepsies.

Familial Aggregation Studies

Lennox first observed that generalized spike-and-wave (GSW) discharges, the classical EEG pattern associated with IGE, aggregates in families.[25,31] Following this original finding, familial aggregation studies consistently showed a threefold increased risk of epilepsy (~3%) in siblings of probands with idiopathic or cryptogenic epilepsy compared to the general population.[32–34] In contrast, symptomatic epilepsies do not increase this risk. A higher risk (6% to 8%) is observed for individuals with IGE associated with typical GSW. The occurrence of GSW in another member of the family (parent or sibling) further increases the risk in the sibling at 12% to 15%.

Twin Studies

Several studies of epilepsy in twins have consistently shown a higher concordance rate for the disease in monozygotic (MZ) pairs compared with dizygotic (DZ) pairs.[35–39] Unfortunately, most of these studies were performed before the ILAE classification of the epileptic seizures and syndromes was developed, thereby limiting the appreciation of the relative genetic contribution to the various epileptic syndromes. In contemporary twin studies, the case-wise concordances for IGEs (MZ = 0.76; DZ = 0.33) were greater than those for partial epilepsies (MZ = 0.36; DZ = 0.05), with intermediate values for febrile seizures (MZ = 0.58; DZ = 0.14).[40,41] Interestingly, in 94% of concordant MZ pairs, affected twins exhibit the same epilepsy syndrome, suggesting the existence of syndrome-specific genetic determinants rather than a broad genetic predisposition to seizures.[40]

Complex Inheritance Patterns in Idiopathic Epilepsy

The concordance rate in identical twins is thus very high for IGE, suggesting an almost complete genetic etiology for these latter syndromes.[40,42] However, clinical observations indicate that, in the vast majority of these families, a single-gene mode of inheritance is implausible and complex inheritance involving two or more genes is most likely.[43–45] These observations are further supported by genetic epidemiology studies.[32,34,40] Indeed, the relative risk of being affected for a relative of an individual with IGE (λ_R) is almost 100 for MZ twins (λ_M), 7.5 for sibs (λ_S), and 1.5 for nieces/nephews (λ_N). For IGE and cryptogenic partial epilepsy, the relative risk of being affected for a relative (λ_R) is 55 for MZ twins (λ_M), 3.5 for sibs (λ_S), and 1.5 for nieces/nephews (λ_N). Such a rapid decrease in the λ_R with each degree of relationship suggests a multiplicative (epistatic) interaction among many contributing loci for IGE and partial epilepsy.[42,46]

FAMILIAL FORMS OF IDIOPATHIC EPILEPSIES

Although the common forms of idiopathic epilepsies are complex genetic traits, several groups of investigators have identified large epilepsy families in which the pattern of inheritance is compatible with a Mendelian trait.[47,48] The identification of such large families allowed gene mapping and eventually the identification of mutations in several predisposing genes for the disease. Successful examples for idiopathic epilepsy are presented in Table 55–1 and discussed briefly here.

Molecular Mechanisms Underlying Familial Epilepsies

At least 25 genes predisposing to familial forms of epilepsy in humans have been identified, as well as more than 100 epilepsy genes in mice. The molecular mechanisms underlying these inherited epilepsies can be divided into four broad functional categories:

1. *Primary defects of membrane excitability*, caused by mutations in genes

Table 55–1 **Genes Predisposing to Idiopathic Epilepsy in Humans**

Genes	Protein Products	Idiopathic Epilepsy Syndrome	References
Voltage-dependent ion channels			
Sodium channels			
SCN1A	Nav1.1	GEFS+, partial epilepsy with FS	49, 117–119
SCN1B	β1 subunit	GEFS+	50, 120, 121
SCN2A	Nav1.2	BFNIS (benign familial neonatal infantile seizures)	122–125
Potassium channels			
KCNQ2	Kv7.2	BFNS	51, 69, 126–129
KCNQ3	Kv7.3	BFNS	52, 129, 130
KCNA1	Kv1.1	Focal epilepsy and episodic ataxia	131–133
KCNMA1	KCa1.1	GE and paroxysmal dyskinesia	134
Calcium channels			
CACNA1A	Cav2.1	AE and episodic ataxia	135, 136
CACNA1H	Cav3.2	CAE	137
		IGE	138, 139
CACNB4	β4 subunit	JME	53
		IGE	53
Chloride channels			
CLCN2	CLC-2	IGE	54
Ligand-gated ion channels			
Nicotinic acetylcholine receptors			
CHRNA2	α2 subunit	ADNFLE	59
CHRNA4	α4 subunit	ADNFLE	57, 140–142
CHRNB2	β2 subunit	ADNFLE	58, 143
$GABA_A$ receptors			
GABRA1	α1 subunit	JME	22
		CAE	77
		AE, FS, IGE	91
GABRG2	γ2 subunit	GEFS+	20, 72, 73, 75, 76, 91
		CAE with FS	21, 74
GABRB3	β3 subunit	CAE with FS	24, 78
GABRD	δ subunit	GEFS+	23, 79
Nonion channel genes			
LGI1	Leucine-rich repeat protein	ADPEAF	144–146
ATP1A2	Sodium-potassium ATPase	Familial hemiplegic migraine and epilepsy	147, 148
EFHC1	EF hand motif protein	JME	80
SLC2A1	GLUT1	Early-onset absence epilepsy	149
		Epilepsy and exercise-induced dyskinesia	150, 151

encoding voltage-gated sodium,[49,50] potassium,[51,52] calcium,[53] and chloride channels.[54–56]

2. *Defects in synaptic signaling*, caused by mutations in ligand-gated chloride (*GABRA1*, *GABRG2*, *GABRB3*, *GABRD*)[20–24] and ligand-gated sodium channel receptors (*CHRNA2*, *CHRNA4*, *CHRNB2*).[57–59]
3. *Impaired synaptic signaling and plasticity*, caused by defects in various proteins implicated in the *neurotransmitter release machinery* (e.g., mobilization of neurotransmitters, synaptic vesicle trafficking, exocytosis), such as *SYN1/2*, *Sv2A*, *AP3δ*, and *ZNT3*.[60–64]
4. *Abnormal brain network development and structure*, also called *cortical dysplasias*, caused by signaling defects in migration, proliferation, differentiation, and segmentation.[65–68] These latter forms of epilepsy are not considered idiopathic per se, although some conceptual overlaps may exist (see below).

Rare Syndromes Caused by Mutations in Complementary Subunits

Benign neonatal familial convulsions (BNFC) is the first epilepsy syndrome for which a gene could be localized, and mutations in *KCNQ2* and *KCNQ3*, encoding for two different subunits of the same potassium channel, have been associated with the disease.[51,52,69] Scheffer and Berkovic later identified a hitherto unrecognized IGE syndrome following single-gene inheritance, named *generalized epilepsy with febrile seizures plus* (GEFS+).[70] Recognition of this syndrome allowed the identification of at least two genes for GEFS+: the β1 and α1 subunits of the neuronal voltage-gated sodium channel (*SCN1B, SCN1A*).[49,50] Finally, *autosomal dominant nocturnal frontal lobe epilepsy* (ADNFLE) is caused by mutations in *CHRNA4*, *CHRNA2*, and *CHRNB2*, encoding for three different subunits of the ligand-gated neuronal nicotinic acetylcholine receptor (α4, α2, β2).[57–59] Although these unique Mendelian forms of IGE are rare in the general population, genetic studies of these families provided the first direct evidence for molecular mechanisms involved in idiopathic epilepsy syndromes. Interestingly, these specific epilepsy syndromes have been associated with their corresponding molecular mechanisms, with mutations identified in complementary subunits of the same protein complex.

Autosomal Dominant Forms of Classical Idiopathic Generalized Epilepsy

Various genetic mutations in families segregating classical IGE syndromes have been recently described, including those for juvenile myoclonic epilepsy (JME), childhood absence epilepsy (CAE), and epilepsy with grand mal seizures on awakening (EGMA).[71] In these rare epilepsy families, the phenotype is indistinguishable from that of classical IGE except for the occurrence of similar epilepsy syndromes in many affected individuals of the same pedigree. Mutations in the γ2 subunits of the $GABA_A$ receptor have been found in families segregating CAE with febrile seizures and GEFS families,[20,21,72–76] whereas mutations in the α1 subunits of the same receptor have been found in families with various IGE phenotypes, including JME and CAE.[22,77] More recently, mutations in the β3 subunits have been found in families with CAE.[24,78] In addition, mutations in the δ subunit of the $GABA_A$ receptor (*GABRD*) were found in small nuclear families with GEFS, CAE, and JME.[23,79] Finally, mutations in the voltage-gated chloride channel type 2 (*CLCN2*) were associated with CAE, JAE, JME, and EGMA,[54–56] and mutations in *EFHC1* were found in families with classical JME.[80] Overall, a total of six genes are implicated in these classical IGE syndromes with autosomal dominant inheritance, including four different subunits of the $GABA_A$ receptors, suggesting that impaired $GABA_A$ receptor function is an important mechanism that could lead to IGE in humans.

STRUCTURE AND FUNCTION OF $GABA_A$ RECEPTORS

Gamma-aminobutyric acid is an inhibitory neurotransmitter that activates two major classes of receptor in the central nervous system: the ionotropic $GABA_A$ and $GABA_C$ receptors, as well as the metabotropic $GABA_B$ receptors.[8,81] The $GABA_A$ and $GABA_C$ receptors are ligand-gated ion channels that are permeable to chloride and bicarbonate anions**.** The $GABA_B$ receptors are G-protein-coupled receptors that are localized on both pre- and postsynaptic membranes. Postsynaptic $GABA_B$ receptors activate inward rectifying potassium channels, which cause hyperpolarization of the neurons and generate inhibitory postsynaptic potentials (IPSPs). In turn, activation of presynaptic $GABA_B$ receptors decreases neurotransmitter release by inhibiting voltage-gated Ca^{2+} channels. This latter effect can be either inhibitory or excitatory, depending on which neurotransmitter is involved.

Distribution and Structural Diversity of the $GABA_A$ Receptors

The $GABA_A$ receptor is a heteropentamer assembled from height classes of subunit

(α1–6, β1–3, γ1–3, δ, π, ε, θ, and ρ1–3).[82] The subunits are grouped to form a central pore that constitutes the ion channel. The optimal subunit stoichiometry and composition required for functional expression of $GABA_A$ receptors is a 2:2:1 ratio of α, β, and γ subunits, respectively (Fig. 55–1). The variability in the composition of $GABA_A$ results in a functional heterogeneity of these receptors. Incorporation of a ρ subunit defines the bicuculline-insensitive $GABA_C$ receptors, whereas the $GABA_A$ receptors are blocked by the antagonist bicuculline.[83] Although there is a potential for a high variability of combinations, α1β2γ2 is the most abundant and represents approximately 60% of all $GABA_A$ receptors in brain.[82] Other major combinations are $\alpha_1\beta_{2/3}\gamma_2$, $\alpha_2\beta_3\gamma_2$, and $\alpha_3\beta_3\gamma_2$.[84] The varying subunit composition of the receptor is associated with variable GABA binding affinity, channel kinetics, and the response to pharmacological agents.[85] The different anti-convulsant drugs, such as the benzodiazepines and anxiolytics, preferentially interact with $GABA_A$ receptors containing α_x subtypes.[84] The binding sites for benzodiazepines are located at the interface between α_1 and γ_2.[86,87] Several subunits, such as δ, π, ε, θ, and ρ, are less well characterized and exhibit lower levels of expression, which could be tissue-specific. As an example, the π subunit is detected only in certain peripheral tissues, whereas ρ1–3 are expressed mainly in the retina.[88]

Genomic Evolution of $GABA_A$ Receptor Genes in *Homo sapiens*

The genomic location of all 19 genes coding for the various subunits of the $GABA_A$ receptor are now fully assigned, and 14 of them are arranged in gene clusters. Two four-gene clusters and two three-gene clusters have been identified, respectively on human chromosomes 4, 5, 15, and chromosome X (Fig. 55–2).[89] Each cluster contains genes coding for the α, β, or γ/ε class. This organization has been proposed to result from a series of gene duplication events from a single ancestral αβγ gene cluster that are believed to have occurred during early chordate evolution.[90] The *GABRQ* and *GABRE* genes encoded the θ and ε subunits, respectively, and are considered to be "β-like" and "γ-like," respectively.[90] The δ (*GABRD*) and ρ3 (*GABRR3*) subunit genes are orphans on chromosomes 1 and 3, respectively. The π-subunit gene (*GABRP*) is located on chromosome 5, but distal to and separate from the γ2α1α6β2 gene cluster. The genes coding for the ρ1 and

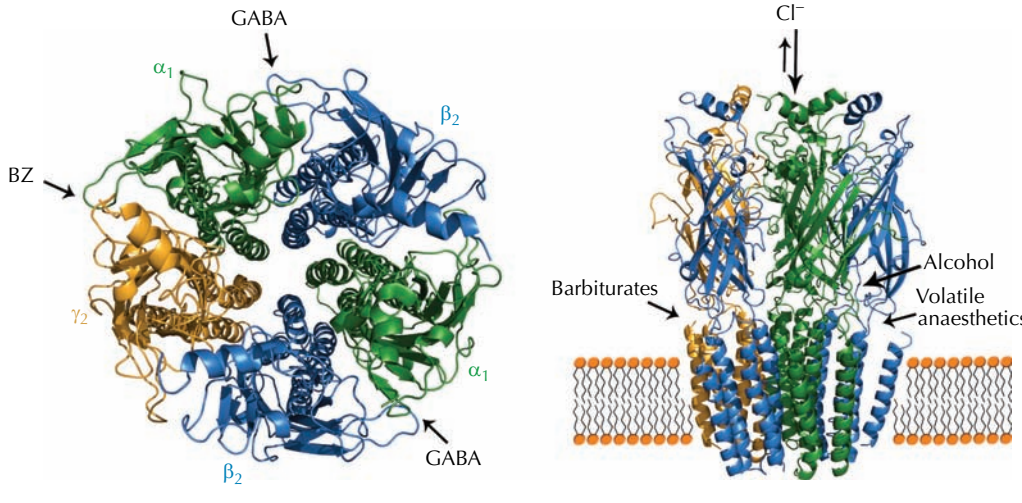

Figure 55–1. $GABA_A$ receptor models. Left: The top view of the structural model of the $GABA_A$ receptor[86] highlights the organization of the subunits within a pentameric complex as well as the proposed neurotransmitter GABA and benzodiazepine (BZ) binding sites. The locations of the GABA and BZ binding sites on the receptor occur at the interfaces of the αβ and αγ subunits, respectively. Right: The side view of the $GABA_A$ receptor model (insert) in a cell membrane is shown to illustrate some of the drug binding sites and chloride ions flux through the channel. Modified from ref. 91.

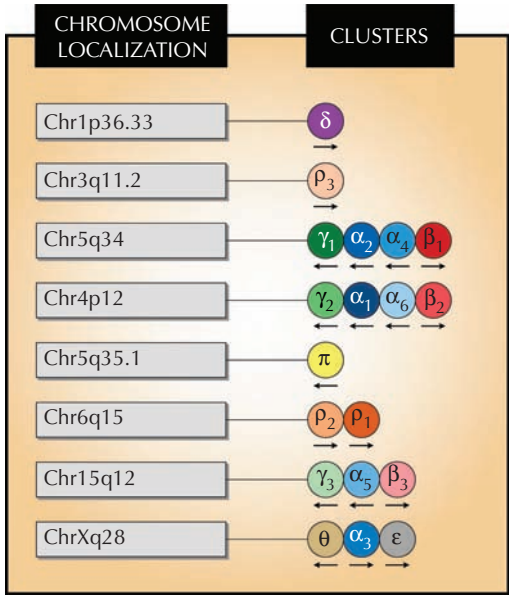

Figure 55–2. Clusters and chromosomal localization of $GABA_A$ receptor genes. The relative gene order and the transcriptional orientation (indicated by arrows) of the α, β, and γ subunit genes are conserved in each cluster. The chromosomal localization of each gene and cluster were obtained from the UCSC Genome Browser (Hg19 Assembly).

ρ2 subunits (*GABRR1-GABRR2*) have been mapped together on chromosome 6.

Functional Properties of the $GABA_A$ Receptors

Gamma-aminobutyric acid is the main inhibitory transmitter in the mammalian brain, and most of the cortical inhibitory effects of GABA are mediated through the $GABA_A$ receptor. In adult brain, the intracellular concentration of chloride inside the majority of neurons $[Cl^-]_i$ is lower than that in the extracellular space $[Cl^-]_e$ due to the activity of the K^+/Cl^- cotransporter. Therefore, when GABA activates $GABA_A$ receptors, the central pore opens and allows chloride influx, which results in hyperpolarization and a decreased probability of generating an action potential. The $GABA_A$ receptor may be modulated by several compounds, such as benzodiazepines, neurosteroïds, barbiturates, and anaesthetics. As an example, both benzodiazepines and barbiturates potentiate the action of GABA on $GABA_A$ receptors by increasing, respectively, the frequency and the length of opening of the central pore.[83] The $GABA_A$ receptor population located mainly at the synapse (α_1, α_2, α_3 or α_5 with β_x and γ_x subunits) mediates phasic inhibition in brain. Phasic inhibition is the synchronous opening of the ionotropic cluster of postsynaptic receptors participating in rhythmic activities in neuronal networks. This activation results in fast GABAergic inhibition and is sensitive to benzodiazepine modulation.[81] In contrast, the $GABA_A$ receptor population located in the extra- and perisynaptic regions (α_4 or α_6 subunits with β_x and δ subunits) mediates tonic inhibition.[81] Tonic inhibition is the activation of extrasynaptic $GABA_A$ receptors by low concentrations of ambient GABA. Tonic inhibition takes part in paracrine activity and plays a crucial role in regulating neuronal excitability. These receptors are composed mainly of δ subunit and are insensitive to benzodiazepine modulation.[81]

IMPAIRED FUNCTION OF MUTATED $GABA_A$ RECEPTORS

So far, epilepsy-causing mutations have been identified in four $GABA_A$ receptor genes: *GABRG2*, *GABRA1*, *GABRD*, and *GABRB3*, encoding respectively the γ2, α1, δ, and β3 subunits. The vast majority of these mutations have been identified in large families with a variable combination of generalized and febrile seizures. The pattern of inheritance is generally autosomal dominant, although complex inheritance and spontaneous mutation have been described as well.[23,77,78] Clinical manifestations in these families, as well as the functional impact of the mutations in $GABA_A$ receptor subunits, are summarized in Table 55–2.

Clinical Manifestations Associated with Mutations in $GABA_A$ Receptor Subunits

Mutations were first described in *GABRG2* in families with GEFS+, febrile seizures, and CAE.[20,21] Additional mutations in the same genes have been associated with similar

Table 55–2 **Clinical Manifestations and Functional Impact of $GABA_A$ Receptor Mutations**

Genes	Locus	Mutations	Phenotypes	Decreased GABA Currents	Decreased Surface Expression	Altered Benzodiazepine Sensitivity	Altered Zn_2+ sensitivity	Altered gating kinetics	References
GABRA1	5q34	A322D	JME	Yes	Yes	No	NA	Yes	22
		S326fs328X	CAE	Yes	Yes	NA	NA	NA	77
		D219N	FS/AE	No	Yes	NA	NA	Yes	91
		K353delins18X	Late IGE	Yes	Yes	NA	NA	NA	91
GABRB3	15q12	P11S	CAE/autism	Yes	No	NA	NA	NA	24, 78
		S15F	CAE	Yes	No	NA	NA	NA	24
		G32R	CAE	Yes	No	NA	NA	NA	24
GABRG2	5q34	K328M	GEFS+	Yes[20]/No[95]	No	Yes [20]/No[95]	NA	Yes	20
		R82Q	CAE/FS	Yes[21, 95, 98–100]/No[96, 97]	Yes	Yes[21, 96, 97]/No[95, 99]	No	Yes[96, 97]/No[95]	21
		Q390X	GEFS+	Yes	Yes	NA	Yes	NA	73
		IVS6+2T→G	CAE/FS	NA	NA	NA	NA	NA	74
		W429X	GEFS+	NA	NA	No	NA	NA	76
		R177G	FS	No	NA	Yes	No	Yes	72
		N79S	GTCS	NA	NA	NA	NA	NA	75
		P83S	FS/AE	No	NA	No	No	No	91
GABRD	1p36.33	E177A	GEFS+	Yes	NA	NA	NA	NA	23
		R220H*	GEFS+/IGE/FS	Yes	NA	NA	NA	NA	23, 79
		R220C	GEFS+	No	NA	NA	NA	NA	23

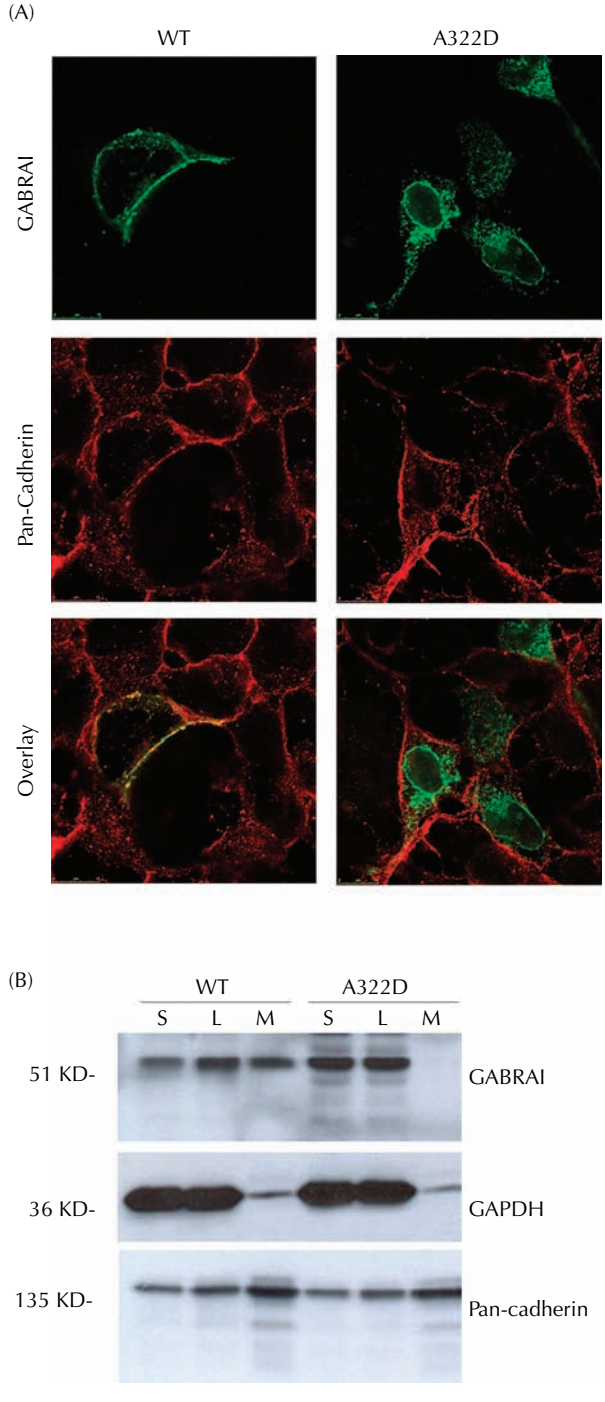

Figure 55–3. Effect of the A322D mutation on α1 subunit expression in transfected HEK 293 cells.A. Surface expression of the mutant α1 subunit. In contrast to the wild-type α1 subunit (left column), immunostaining of A322D *GABRA1* mutants did not colocalize with the marker for the cell membrane (pan-cadherin, red) when coexpressed with the wild-type β2 and γ2 subunits. B. Western blot of total (L: lysate), biotinylated (M: membrane fraction), and nonbiotinylated (S: supernatant) α1 proteins shows the absence of expression of the A322D mutation at the cell surface. Reduced glyceralehydephosphate dehydrogenase (GAPDH) was used to normalize the supernatant and lysate proteins, and pan-cadherin was used to normalize the membrane proteins. Modified from ref. 91.

phenotypes[72–76] but also have been found in one individual with severe myoclonic epilepsy of infancy (Dravet syndrome).[73] The phenotypes associated with *GABRB3* mutations appear so far to be more homogeneous, with almost exclusively absence seizures.[24,78] The first family reported with mutation in *GABRA1* had JME,[22] and a single individual with spontaneous mutation exhibited CAE.[77] We recently described two additional families with mutations in *GABRA1*: one family presents with a combination of febrile seizures and absences, whereas the other family exhibits late-onset generalized tonic-clonic seizures and photosensitivity.[91] The clinical phenotypes associated with mutations in *GABRA1* thus appear to be more heterogeneous. The small families with genetic variations in *GABRD* had either GEFS or JME syndromes.[23,79]

Mutations in $GABA_A$ Receptor Subunits Generally Produce Loss of Function

The functional impact of the mutations in various subunits of the $GABA_A$ receptors associated with epilepsy in humans has been studied by expressing recombinant receptors either in HEK 293 cells or in oocytes. These in vitro studies revealed that the majority of these mutations result in a reduction of GABA-activated chloride currents.[87,92] For at least three mutations in *GABRA1* (A322D, S326fs328X, K353delins18X),[77,78,93] it has been shown that the reduction in the amplitude of GABA-evoked current was due to reduced surface expression of receptor protein, caused by retention of mutant receptors in the endoplasmic reticulum (Fig. 55–3). Two *GABRA1* mutations (A322D and D219N) exhibit altered gating kinetics in addition to reduced surface expression.[22,91] Mutations in *GABRB3* are also associated with reduced GABA-evoked current density from whole cells, possibly because of increased glycosylation of the β3 protein.[24]

The nonsense mutation in *GABRG2* (Q390X), like those for *GABRA1* mutations, shows reduced GABA-evoked current and surface expression of the receptor protein.[73,94] However, the functional effects of the *GABRG2* missense mutations appear to be more complex. Reduced amplitude of GABA currents has been reported for the K328M and R82Q mutations, but this observation was less consistent among various investigations.[20,21,95–100] In addition, missense mutations in *GABRG2* (K328M, R177G, and possibly R82Q) can alter gating properties of the $GABA_A$ receptor.[72, 95–97] Two mutations in *GABRG2* (R82Q and P83S) are located close to the binding site of benzodiazepines. Interestingly, one of these mutations (R82Q) seems to alter the potentiation of GABA-evoked currents by the application of benzodiazepine.[21,96,97] However, these latter results are more controversial and have not been reproduced by other investigators.[95,99] It is intriguing that the other *GABRG2* mutation close to the benzodiazepine binding site (P83S) does not show obvious alteration in the function of the $GABA_A$ receptor despite strong segregation with febrile and absence seizures.[91] This observation suggests that there are additional, more subtle mechanisms by which mutation in $GABA_A$ receptors may lead to the disease.

The Complex Case of *GABRD* Mutations

Genetic variations in *GABRD* exhibit a more complex relationship to both epilepsy phenotypes and dysfunction of $GABA_A$ receptors. Two missense mutations in this gene are associated with GEFS+. One of these *GABRD* mutations (E177A), like mutations in *GABRG2* and *GABRA1*, results in decreased amplitude of GABA-evoked currents, but the other (R220C) does not. It is possible that this R220C is a rare neutral variant. Alternatively, as suggested above for another *GABRG2*, the R220C variant in *GABRD* could be associated with more subtle effects on the $GABA_a$ receptor, which remains to be determined. Another variant in *GABRD* (R220H) was first detected in a JME family, but it was also present in IGE and febrile seizures, as well as in control cases.[23,79] Interestingly, this R220H polymorphism is nonetheless associated with reduced peak amplitude of GABA-evoked current, which is expected to increase neuronal excitability. The role of this functional polymorphism in epilepsy with complex inheritance remains unclear. Such functional polymorphisms may represent candidate genes with small effects

on the phenotype, as is anticipated for common epilepsies with complex inheritance, such as JME and CAE. Consistent with this hypothesis, the P11S variation originally described as a mutation in *GABRB3*[24] was found to be a rare functional polymorphism in the French Canadian population.[78]

Animal Models with Mutations in $GABA_A$ Receptor Subunits

Knockout of genes encoding for various $GABA_a$ receptor subunits, including α1, α5, γ3, and δ, is not associated with the epileptic phenotype in mice.[101–104] However, targeted deletion of *GABRG2* in mice is lethal after birth, and mutants exhibit abnormal episodes of hyperactivity that might be compatible with neonatal seizures.[14] In addition, deletion of β3 in mice is associated with severe seizure disorder.[15,105] More recently, the R43Q mutation in *GABRG2*, which is associated with GEFS+ in humans, has been introduced in a mouse model. Interestingly, mice heterozygous for this knocked-in mutation exhibit manifestations of absence seizures, together with 6 to 7 Hz spike-and-wave discharges that are blocked by ethosuximide.[106] These authors conclude that reduced cortical inhibition is responsible for the epilepsy phenotype in this model.

MUTATIONS IN VOLTAGE-GATED CHLORIDE CHANNELS

Seven mutations have been identified in the *CLCN2* gene, five of which have been subject to functional studies.[54,56,107] Unfortunately, it appears that the mutations described by Haug et al. are not as penetrant as they were described to be, falsely, in the original report.[54,55] Nevertheless, these *CLCN2* mutations do segregate, although weakly, with epilepsy phenotypes. More importantly, at least five *CLCN2* mutations show alteration in the function of these voltage-gated chloride channels, including four mutations with loss of function.[54,56] These observations thus support the hypothesis that *CLCN2* is a predisposing gene for IGE.

CLCN2 encodes the voltage-gated chloride channel ClC-2, which is strongly expressed in the brain and notably localized in GABAergic interneurons.[108] ClC-2 channels play a role in establishing and maintaining a low intracellular chloride concentration, which is essential for neuronal inhibition mediated through $GABA_a$ receptors. Therefore, it is expected that loss of function of the ClC-2 channel may result in impaired chloride efflux, leading to an abnormal intracellular accumulation of chloride. These mutant channels are thus expected to lower the transmembrane gradient that is essential for GABAergic inhibition, resulting in increased excitability in neurons, which may lead to seizures. As with many $GABA_A$ receptor genes, *CLCN2* knockout mice do not exhibit the epileptic phenotype.[109,110]

Overall, mutations in *CLCN2* have been shown to alter the chloride gradient across the cellular membrane, which may eventually impair GABAergic neurotransmission in the brain. These findings are consistent with loss-of-function mutations found in at least four different subunits of $GABA_A$ for related epileptic syndromes. Taken together, these results suggest that impairment of the inhibition mechanisms mediated through the $GABA_A$ receptors may be central to the pathophysiology of classical IGE with autosomal dominant inheritance.

GABA PERFORMS CRITICAL FUNCTIONS IN THE DEVELOPING BRAIN

In the adult brain, the intracellular concentration of chloride inside the neurons $[Cl^-]_i$ is lower than that in the extracellular space $[Cl^-]_e$. Therefore, when GABA activates $GABA_A$ receptors, this heteropentamer opens its central pore and allows chloride influx (inside the cell), which results in hyperpolarization. However, in the immature brain, GABA produces depolarization because of a high concentration of intracellular chloride $[Cl^-]_i$ in developing neurons.[111] Under these conditions, $GABA_A$ receptor activation produces a chloride efflux (outside the cell), with the result that neurons are depolarized (Fig. 55–4). The developmental switch from GABA-mediated excitation to inhibition is mediated by the K^+-Cl^--coupled cotransporter, KCC2,[112,113] which is responsible for pumping out Cl^-. KCC2 is thus one of the major determinants of $[Cl^-]_i$ levels in

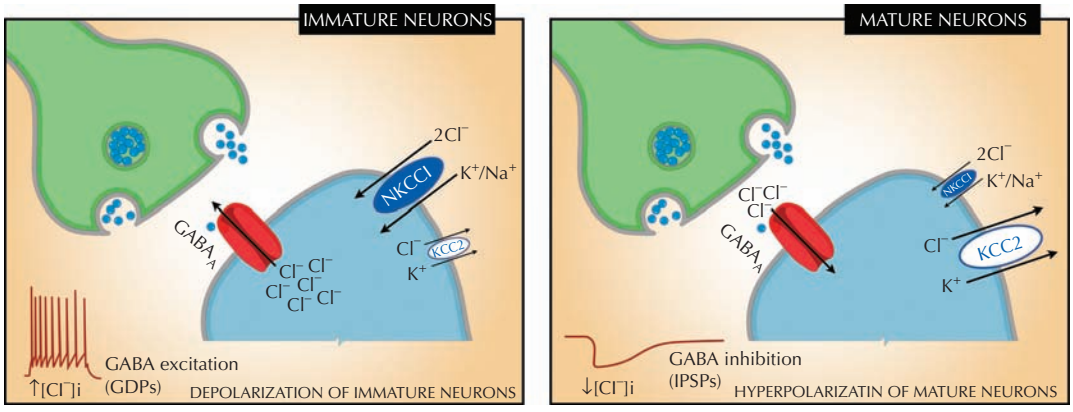

Figure 55–4. Developmental switch in chloride homeostasis during development. In immature neurons, hyperpolarizing and excitatory actions of the $GABA_A$ receptors are triggered by the higher intracellular [Cl^-] ions. The progressive switch from depolarizing to hyperpolarizing GABAergic signaling through the same receptors is generated by the developmental increase in the chloride cotransporter KCC2 and the decrease in NKCC1.

the adult brain. KCC2 is expressed at approximately the first days of life, during which reversal of the chloride transmembrane gradient occurs. Interestingly, this developmental switch mediated through KCC2 is induced by the activation of GABA synapses themselves.[114] The excitatory actions of GABA play a central role in the generation of primitive electrical activity recorded in developing circuits called *giant depolarizing potentials* (GDPs). Giant depolarizing potentials are long-lasting and recurrent depolarizing potentials that provide most of the activity in the developing brain.[111] These GDPs play an important role in modulating the developing network, including neuronal migration and growth, synapse formation, and plasticity of GABA synapses.[115] In addition, GABA has been shown to stimulate neuronal migration, cell division, and neuritic growth through other mechanisms.[111] It is generally believed that loss-of-function mutations found in familial IGE cause abnormal hyperexcitability in the mature brain by decreasing inhibitory transmission. However, one alternative and not mutually exclusive hypothesis is that impaired $GABA_A$ transmission caused by these mutations may lead to a significant decrease in excitatory GABA transmission in the developing brain. Should this be the case, one can expect that the critical functions performed by excitatory GABA transmission will also be impaired. The following and perhaps cumulative effects of the $GABA_A$ mutations could thus be anticipated: (1) decrease in the excitatory drive of GABAergic synapses in the developing brain; (2) decrease of the GDPs and their critical impact on the developing circuits; (3) delay in the developmental switch of the transmembrane gradient induced by KCC2, causing excitatory drives beyond the usual stages of development; and (4) abnormal neuronal migration, neurite elongation, and synapse formation. These potential effects, alone or in combination, could lead to aberrant neuronal networks that may be the substratum for triggering epileptic seizures in familial IGE. Interestingly, it has recently been found that impairment of *EFHC1*, another JME gene, causes marked disruption of cortical development *in vivo*.[116] We can thus speculate that abnormal development of the cortical network may represent a convergent mechanism in the pathogenesis of IGE.

FUTURE PERSPECTIVES

An increasing number of genes predisposing to epilepsy have been identified over the past 10 years. Among these genes, mutations in four subunits of the $GABA_A$ receptor appear to be important causes of familial epilepsy. So far, the majority of these mutations have been associated with a dramatic decrease of GABA-evoked currents in recombinant receptors. Because GABA is the main inhibitory transmitter in the adult brain, it is generally believed that this loss

of function would cause abnormal excitability of cortical neurons, thereby leading to clinical seizures. However, in contrast to the mature central nervous system, GABA is excitatory in the developing brain. This apparent paradox is caused by an inversion in the chloride gradient across the cell membrane that occurs during the first days of life. There is now increasing evidence supporting the hypothesis that excitatory GABA transmission plays a key role in various aspects of brain development, such as neuronal migration, shaping of dendritic trees, and synaptogenesis. It is thus very likely that epilepsy-causing mutations in $GABA_A$ receptors would be associated with abnormal development of neuronal networks, which may be one of the critical mechanisms leading to the disease. However, so far, this hypothesis has not been examined. Should it be validated, it would provide an unparalleled advance in our comprehension of the IGEs.

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DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Ackermann D. Über ein neues, auf bakteriellem Wege gewinnbares, Aporrhegma. *Hoppe-Seyler's Z Physiol Chemie.* 1910;69:273–281.
2. Roberts E, Frankel S. Gamma-aminobutyric acid in brain: its formation from glutamic acid. *J Biol Chem*. 1950;187(1):55–63.
3. Awapara J et al. Free gamma-aminobutyric acid in brain. *J Biol Chem*. 1950;187(1):35–39.
4. Udenfriend S. Identification of gamma-aminobutyric acid in brain by the isotope derivative method. *J Biol Chem*. 1950;187(1):65–69.
5. Elliott KA. Gamma-aminobutyric acid and other inhibitory substances. *Br Med Bull.* 1965;21:70–75.
6. Curtis DR et al. GABA, bicuculline and central inhibition. *Nature*. 1970;226(5252): 1222–1224.
7. Jasper HH, Khan RT, Elliott KA. Amino acids released from the cerebral cortex in relation to its state of activation. *Science*. 1965;147:1448–1449.
8. Ben-Ari Y et al. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev*. 2007;87(4):1215–1284.
9. Galanopoulou AS. GABA(A) receptors in normal development and seizures: friends or foes? *Curr Neuropharmacol*. 2008;6(1):1–20.
10. Olsen RW et al. GABA receptor function and epilepsy. *Adv Neurol*. 1999;79:499–510.
11. De Deyn PP, Marescau B, MacDonald RL. Epilepsy and the GABA-hypothesis: a brief review and some examples. *Acta Neurol Belg*. 1990;90(2):65–81.
12. Avoli M et al. Cellular and molecular mechanisms of epilepsy in the human brain. *Prog Neurobiol*. 2005;77(3):166–200.
13. Snead OC 3rd. Basic mechanisms of generalized absence seizures. *Ann Neurol*. 1995;37(2):146–157.
14. Gunther U et al. Benzodiazepine-insensitive mice generated by targeted disruption of the gamma 2 subunit gene of gamma-aminobutyric acid type A receptors. *Proc Natl Acad Sci USA*. 1995;92(17):7749–7753.
15. Homanics GE et al. Mice devoid of gamma-aminobutyrate type A receptor beta3 subunit have epilepsy, cleft palate, and hypersensitive behavior. *Proc Natl Acad Sci USA*. 1997;94(8):4143–4148.
16. Gassmann M et al. Redistribution of $GABA_B(1)$ protein and atypical $GABA_B$ responses in $GABA_B(2)$-deficient mice. *J Neurosci*. 2004;24(27):6086–6097.
17. Schuler V et al. Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). *Neuron*. 2001;31(1):47–58.
18. Prosser HM et al. Epileptogenesis and enhanced prepulse inhibition in GABA(B1)-deficient mice. *Mol Cell Neurosci*. 2001;17(6):1059–1070.
19. Minassian BA et al. Angelman syndrome: correlations between epilepsy phenotypes and genotypes. *Ann Neurol*. 1998;43(4):485–493.
20. Baulac S et al. First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. *Nat Genet*. 2001;28(1): 46–48.
21. Wallace RH et al. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet*. 2001;28(1):49–52.
22. Cossette P et al. Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nat Genet*. 2002;31(2):184–189.
23. Dibbens LM et al. GABRD encoding a protein for extra- or peri-synaptic $GABA_A$ receptors is a susceptibility locus for generalized epilepsies. *Hum Mol Genet*. 2004;13(13):1315–1319.
24. Tanaka M et al. Hyperglycosylation and reduced GABA currents of mutated GABRB3 polypeptide in remitting childhood absence epilepsy. *Am J Hum Genet*. 2008;82(6):1249–1261.
25. Pedley TA. EEG traits In: Engel J Jr, Pedley TA, eds. *Epilepsy: A Comprehensive Textbook*. New York: Lippincott-Raven; 1997:185–196.
26. Fisher RS et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia*. 2005;46(4):470–472.
27. Engel J Jr. Report of the ILAE classification core group. *Epilepsia*. 2006;47(9):1558–1568.

28. ILEA. Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. *Epilepsia.* 1989;30(4):389–399.
29. Engel J Jr. Classifications of the International League Against Epilepsy: time for reappraisal. *Epilepsia.* 1998;39(9):1014–1017.
30. Engel J Jr. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. *Epilepsia*. 2001;42(6):796–803.
31. Lennox WG. The heredity of epilepsy as told by relatives and twins. *JAMA.* 1951;146(6):529–536.
32. Hauser WA. Incidence and prevalence. In: Engel J Jr, Pedley TA, eds. *Epilepsy: A Comprehensive Textbook*. New York: Lippincott-Raven; 1997:47–57.
33. Annegers JF et al. The risks of seizure disorders among relatives of patients with childhood onset epilepsy. *Neurology*. 1982;32(2):174–179.
34. Jain S et al. Occurrence of epilepsies in family members of Indian probands with different epileptic syndromes. *Epilepsia*. 1997;38(2):237–244.
35. Corey LA et al. The occurrence of epilepsy and febrile seizures in Virginian and Norwegian twins. *Neurology*. 1991;41(9):1433–1436.
36. Inouye E. Observations on forty twin index cases with chronic epilepsy and their co-twins. *J Nerv Ment Dis*. 1960;130:401–416.
37. Marshall AG, Hutchinson EO, Honisett J. Heredity in common diseases. A retrospective survey of twins in a hospital population. *Br Med J.* 1962;1(5270):1–6.
38. Schiottz-Christensen E. Genetic factors in febrile convulsions. An investigation of 64 same-sexed twin pairs. *Acta Neurol Scand*. 1972;48(5):538–546.
39. Sillanpaa M et al. Genetic factors in epileptic seizures: evidence from a large twin population. *Acta Neurol Scand*. 1991;84(6):523–526.
40. Berkovic SF et al. Epilepsies in twins: genetics of the major epilepsy syndromes. *Ann Neurol.* 1998;43(4): 435–445.
41. Vadlamudi L et al. Epilepsy in twins: insights from unique historical data of William Lennox. *Neurology*. 2004;62(7):1127–1133.
42. Zara F et al. Mapping of genes predisposing to idiopathic generalized epilepsy. *Hum Mol Genet*. 1995;4(7):1201–1207.
43. Marini C et al. Genetic architecture of idiopathic generalized epilepsy: clinical genetic analysis of 55 multiplex families. *Epilepsia*. 2004;45(5):467–478.
44. ILAE. Concordance of clinical forms of epilepsy in families with several affected members. Italian League Against Epilepsy Genetic Collaborative Group. *Epilepsia*. 1993;34(5):819–826.
45. Rich SS et al. Complex segregation analysis of febrile convulsions. *Am J Hum Genet*. 1987;41(2):249–257.
46. Risch N. Linkage strategies for genetically complex traits. I. Multilocus models. *Am J Hum Genet.* 1990;46(2):222–228.
47. Helbig I et al. Navigating the channels and beyond: unravelling the genetics of the epilepsies. *Lancet Neurol*. 2008;7(3):231–245.
48. Baulac S, Baulac M. Advances on the genetics of Mendelian idiopathic epilepsies. *Clin Lab Med.* 2010;30(4):911–929.
49. Escayg A et al. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. *Nat Genet*. 2000;24(4):343–345.
50. Wallace RH et al. Febrile seizures and generalized epilepsy associated with a mutation in the Na^+-channel beta1 subunit gene SCN1B. *Nat Genet*. 1998;19(4): 366–370.
51. Biervert C et al. A potassium channel mutation in neonatal human epilepsy. *Science*. 1998;279(5349): 403–406.
52. Charlier C et al. A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. *Nat Genet*. 1998;18(1):53–55.
53. Escayg A et al. Coding and noncoding variation of the human calcium-channel beta4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. *Am J Hum Genet*. 2000;66(5):1531–1539.
54. Haug K et al. Mutations in CLCN2 encoding a voltage-gated chloride channel are associated with idiopathic generalized epilepsies. *Nat Genet*. 2003;33(4): 527–532.
55. Kleefuss-Lie A et al. CLCN2 variants in idiopathic generalized epilepsy. *Nat Genet*. 2009;41(9):954–955.
56. Saint-Martin C et al. Two novel CLCN2 mutations accelerating chloride channel deactivation are associated with idiopathic generalized epilepsy. *Hum Mutat*. 2009;30(3):397–405.
57. Steinlein OK et al. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet.* 1995;11(2):201–203.
58. De Fusco M et al. The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy. *Nat Genet*. 2000;26(3):275–276.
59. Aridon P et al. Increased sensitivity of the neuronal nicotinic receptor alpha 2 subunit causes familial epilepsy with nocturnal wandering and ictal fear. *Am J Hum Genet*. 2006;79(2):342–350.
60. Cole TB et al. Seizures and neuronal damage in mice lacking vesicular zinc. *Epilepsy Res*. 2000;39(2): 153–169.
61. Crowder KM et al. Abnormal neurotransmission in mice lacking synaptic vesicle protein 2A (SV2A). *Proc Natl Acad Sci USA*. 1999;96(26):15268–15273.
62. Fassio A et al. SYN1 loss-of-function mutations in autism and partial epilepsy cause impaired synaptic function. *Hum Mol Genet*. 2011;20(12):2297–2307.
63. Kantheti P et al. Mutation in AP-3 delta in the mocha mouse links endosomal transport to storage deficiency in platelets, melanosomes, and synaptic vesicles. *Neuron*. 1998;21(1):111–122.
64. Terada S et al. Impairment of inhibitory synaptic transmission in mice lacking synapsin I. *J Cell Biol.* 1999;145(5):1039–1048.
65. Feng Y, Walsh CA. Protein–protein interactions, cytoskeletal regulation and neuronal migration. *Nat Rev Neurosci*. 2001;2(6):408–416.
66. Fox JW et al. Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron*. 1998;21(6):1315–1325.
67. Hong SE. et al. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat Genet*. 2000;26(1):93–96.
68. Guerrini R, Barba C. Malformations of cortical development and aberrant cortical networks: epileptogenesis and functional organization. *J Clin Neurophysiol*. 2010;27(6):372–379.
69. Singh NA et al. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat Genet*. 1998;18(1):25–29.

70. Scheffer IE, Berkovic SF. Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes. *Brain*. 1997;120(pt 3): 479–490.
71. Reid CA, Berkovic SF, Petrou S. Mechanisms of human inherited epilepsies. *Prog Neurobiol*. 2009;87(1): 41–57.
72. Audenaert D et al. A novel GABRG2 mutation associated with febrile seizures. *Neurology*. 2006;67(4): 687–690.
73. Harkin LA et al. Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am J Hum Genet*. 2002; 70(2):530–536.
74. Kananura C et al. A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. *Arch Neurol*. 2002;59(7):1137–1141.
75. Shi X et al. Mutational analysis of GABRG2 in a Japanese cohort with childhood epilepsies. *J Hum Genet*. 2010;55(6):375–378.
76. Sun H et al. SCN1A, SCN1B, and GABRG2 gene mutation analysis in Chinese families with generalized epilepsy with febrile seizures plus. *J Hum Genet*. 2008;53(8):769–774.
77. Maljevic S et al. A mutation in the GABA(A) receptor alpha(1)-subunit is associated with absence epilepsy. *Ann Neurol*. 2006;59(6):983–987.
78. Lachance-Touchette P et al. Screening of GABRB3 in French-Canadian families with idiopathic generalized epilepsy. *Epilepsia*. 2010;51(9):1894–1897.
79. Dibbens LM et al. The role of neuronal GABA(A) receptor subunit mutations in idiopathic generalized epilepsies. *Neurosci Lett*. 2009;453(3):162–165.
80. Suzuki T et al. Mutations in EFHC1 cause juvenile myoclonic epilepsy. *Nat Genet*. 2004;36(8):842–849.
81. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci*. 2005;6(3):215–229.
82. Sieghart W, Sperk G. Subunit composition, distribution and function of GABA(A) receptor subtypes. *Curr Top Med Chem*. 2002;2(8):795–816.
83. Barnard EA et al. International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acid A receptors: classification on the basis of subunit structure and receptor function. *Pharmacol Rev*. 1998;50(2): 291–313.
84. Mohler H. GABA(A) receptor diversity and pharmacology. *Cell Tissue Res*. 2006;326(2):505–516.
85. Whiting PJ. The $GABA_A$ receptor gene family: new opportunities for drug development. *Curr Opin Drug Discov Dev*. 2003;6(5):648–657.
86. Ernst M et al. Comparative models of $GABA_A$ receptor extracellular and transmembrane domains: important insights in pharmacology and function. *Mol Pharmacol*. 2005;68(5):1291–1300.
87. Galanopoulou AS. Mutations affecting GABAergic signaling in seizures and epilepsy. *Pflugers Arch*. 2010;460(2):505–523.
88. Lukasiewicz PD. $GABA_C$ receptors in the vertebrate retina. *Mol Neurobiol*. 1996;12(3):181–194.
89. Russek SJ. Evolution of GABA(A) receptor diversity in the human genome. *Gene*. 1999;227(2):213–222.
90. Darlison MG, Pahal I, Thode C. Consequences of the evolution of the GABA(A) receptor gene family. *Cell Mol Neurobiol*. 2005;25(3–4):607–624.
91. Lachance-Touchette P et al. Novel α1 and γ2 $GABA_A$ receptor subunit mutations in families with idiopathic generalized epilepsy. *Eur J Neurosci*. 2011;34(2): 237–249.
92. Macdonald RL, Kang JQ, Gallagher MJ. Mutations in $GABA_A$ receptor subunits associated with genetic epilepsies. *J Physiol*. 2010;588(pt 11):1861–1869.
93. Krampfl K et al. Molecular analysis of the A322D mutation in the GABA receptor alpha-subunit causing juvenile myoclonic epilepsy. *Eur J Neurosci*. 2005;22(1):10–20.
94. Kang JQ et al. Slow degradation and aggregation in vitro of mutant $GABA_A$ receptor gamma2(Q351X) subunits associated with epilepsy. *J Neurosci*. 2010; 30(41):13895–13905.
95. Bianchi MT et al. Two different mechanisms of disinhibition produced by $GABA_A$ receptor mutations linked to epilepsy in humans. *J Neurosci*. 2002;22(13): 5321–5327.
96. Bowser DN et al. Altered kinetics and benzodiazepine sensitivity of a $GABA_A$ receptor subunit mutation [gamma 2(R43Q)] found in human epilepsy. *Proc Natl Acad Sci USA*. 2002;99(23):15170–15175.
97. Goldschen-Ohm MP et al. An epilepsy-related region in the GABA(A) receptor mediates long-distance effects on GABA and benzodiazepine binding sites. *Mol Pharmacol*. 2010;77(1):35–45.
98. Hales TG et al. The epilepsy mutation, gamma2(R43Q), disrupts a highly conserved inter-subunit contact site, perturbing the biogenesis of $GABA_A$ receptors. *Mol Cell Neurosci*. 2005;29(1):120–127.
99. Kang JQ, Macdonald RL. The $GABA_A$ receptor gamma2 subunit R43Q mutation linked to childhood absence epilepsy and febrile seizures causes retention of alpha1beta2gamma2S receptors in the endoplasmic reticulum. *J Neurosci*. 2004;24(40):8672–8677.
100. Kang JQ, Shen W, Macdonald RL. Why does fever trigger febrile seizures? $GABA_A$ receptor gamma2 subunit mutations associated with idiopathic generalized epilepsies have temperature-dependent trafficking deficiencies. *J Neurosci*. 2006;26(9):2590–2597.
101. Collinson N et al. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. *J Neurosci*. 2002;22(13):5572–5580.
102. Culiat CT et al. Phenotypic consequences of deletion of the gamma 3, alpha 5, or beta 3 subunit of the type A gamma-aminobutyric acid receptor in mice. *Proc Natl Acad Sci USA*. 1994;91(7):2815–2818.
103. Kralic JE et al. Molecular and pharmacological characterization of GABA(A) receptor alpha1 subunit knockout mice. *J Pharmacol Exp Ther*. 2002;302(3): 1037–1045.
104. Peng Z et al. GABA(A) receptor changes in delta subunit-deficient mice: altered expression of alpha4 and gamma2 subunits in the forebrain. *J Comp Neurol*. 2002;446(2):179–197.
105. DeLorey TM et al. Mice lacking the beta3 subunit of the $GABA_A$ receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. *J Neurosci*. 1998;18(20): 8505–8514.
106. Tan HO et al. Reduced cortical inhibition in a mouse model of familial childhood absence epilepsy. *Proc Natl Acad Sci USA*. 2007;104(44):17536–17541.

107. D'Agostino D et al. Mutations and polymorphisms of the CLCN2 gene in idiopathic epilepsy. *Neurology*. 2004;63(8):1500–1502.
108. Sik A, Smith RL, Freund TF. Distribution of chloride channel-2-immunoreactive neuronal and astrocytic processes in the hippocampus. *Neuroscience*. 2000;101(1):51–65.
109. Bosl MR et al. Male germ cells and photoreceptors, both dependent on close cell–cell interactions, degenerate upon ClC-2 Cl(-) channel disruption. *EMBO J*. 2001;20(6):1289–1299.
110. Nehrke K et al. Loss of hyperpolarization-activated Cl(–) current in salivary acinar cells from Clcn2 knockout mice. *J Biol Chem*. 2002; 277(26):23604–23611.
111. Ben-Ari Y. Excitatory actions of gaba during development: the nature of the nurture. *Nat Rev Neurosci*. 2002;3(9):728–739.
112. Rivera C, Voipio J, Kaila K. Two developmental switches in GABAergic signalling: the K^+-Cl^- cotransporter KCC2 and carbonic anhydrase CAVII. *J Physiol*. 2005;562(pt 1):27–36.
113. Rivera C et al. The K^+-Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*. 1999;397(6716):251–255.
114. Ganguly K et al. GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell*. 2001;105(4):521–532.
115. Owens DF, Kriegstein AR. Is there more to GABA than synaptic inhibition? *Nat Rev Neurosci*. 2002;3(9):715–727.
116. de Nijs L et al., EFHC1 interacts with microtubules to regulate cell division and cortical development. *Nat Neurosci*. 2009;12(10):1266–1274.
117. Lin WD, Chou IC, Tsai FJ. Novel human pathological mutations. Gene symbol: SCN1A. Disease: generalized epilepsy with febrile seizures plus. *Hum Genet*. 2010;127(4):482.
118. Sugawara T et al. Nav1.1 mutations cause febrile seizures associated with afebrile partial seizures. *Neurology*. 2001;57(4):703–705.
119. Wallace RH et al. Neuronal sodium-channel alpha1-subunit mutations in generalized epilepsy with febrile seizures plus. *Am J Hum Genet*. 2001;68(4): 859–865.
120. Audenaert D et al. A deletion in SCN1B is associated with febrile seizures and early-onset absence epilepsy. *Neurology*. 2003; 61(6):854–856.
121. Scheffer IE et al. Temporal lobe epilepsy and GEFS+ phenotypes associated with SCN1B mutations. *Brain*. 2007;130(pt 1):100–109.
122. Berkovic SF et al. Benign familial neonatal-infantile seizures: characterization of a new sodium channelopathy. *Ann Neurol*. 2004;55(4):550–557.
123. Herlenius E et al. SCN2A mutations and benign familial neonatal-infantile seizures: the phenotypic spectrum. *Epilepsia*. 2007;48(6):1138–1142.
124. Heron SE et al. Sodium-channel defects in benign familial neonatal-infantile seizures. *Lancet*. 2002; 360(9336):851–852.
125. Striano P et al. A novel SCN2A mutation in family with benign familial infantile seizures. *Epilepsia*. 2006;47(1):218–220.
126. Coppola G et al. A novel KCNQ2 K^+ channel mutation in benign neonatal convulsions and centrotemporal spikes. *Neurology*. 2003;61(1):131–134.
127. Lerche H et al. A reduced K^+ current due to a novel mutation in KCNQ2 causes neonatal convulsions. *Ann Neurol*. 1999;46(3):305–312.
128. Miraglia del Giudice E et al. Benign familial neonatal convulsions (BFNC) resulting from mutation of the KCNQ2 voltage sensor. *Eur J Hum Genet*. 2000;8(12):994–997.
129. Singh NA et al. KCNQ2 and KCNQ3 potassium channel genes in benign familial neonatal convulsions: expansion of the functional and mutation spectrum. *Brain*. 2003;126(pt 12):2726–2737.
130. Hirose S et al. A novel mutation of KCNQ3 (c. 925T $\rightarrow$ C) in a Japanese family with benign familial neonatal convulsions. *Ann Neurol*. 2000;47(6): 822–826.
131. Eunson LH et al. Clinical, genetic, and expression studies of mutations in the potassium channel gene KCNA1 reveal new phenotypic variability. *Ann Neurol*. 2000;48(4):647–656.
132. Spauschus A et al. Functional characterization of a novel mutation in KCNA1 in episodic ataxia type 1 associated with epilepsy. *Ann NY Acad Sci*. 1999;868: 442–446.
133. Zuberi SM et al. A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain*. 1999;122(pt 5):817–825.
134. Du W et al. Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. *Nat Genet*. 2005;37(7):733–738.
135. Imbrici P et al. Dysfunction of the brain calcium channel CaV2.1 in absence epilepsy and episodic ataxia. *Brain*. 2004;127(pt 12):2682–2692.
136. Jouvenceau A et al. Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. *Lancet*. 2001;358(9284):801–807.
137. Chen Y et al. Association between genetic variation of CACNA1H and childhood absence epilepsy. *Ann Neurol*. 2003;54(2):239–243.
138. Heron SE et al. Extended spectrum of idiopathic generalized epilepsies associated with CACNA1H functional variants. *Ann Neurol*. 2007;62(6):560–568.
139. Heron SE et al. Genetic variation of CACNA1H in idiopathic generalized epilepsy. *Ann Neurol*. 2004;55(4):595–596.
140. Hirose S et al. A novel mutation of CHRNA4 responsible for autosomal dominant nocturnal frontal lobe epilepsy. *Neurology*. 1999;53(8):1749–1753.
141. Phillips HA et al. A de novo mutation in sporadic nocturnal frontal lobe epilepsy. *Ann Neurol*. 2000;48(2): 264–267.
142. Steinlein OK et al. An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy. *Hum Mol Genet*. 1997;6(6):943–947.
143. Phillips HA et al. CHRNB2 is the second acetylcholine receptor subunit associated with autosomal dominant nocturnal frontal lobe epilepsy. *Am J Hum Genet*. 2001;68(1):225–231.
144. Gu W et al. The LGI1 gene involved in lateral temporal lobe epilepsy belongs to a new subfamily of leucine-rich repeat proteins. *FEBS Lett*. 2002;519(1–3): 71–76.
145. Kalachikov S et al. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nat Genet*. 2002;30(3):335–341.

146. Morante-Redolat JM et al. Mutations in the LGI1/Epitempin gene on 10q24 cause autosomal dominant lateral temporal epilepsy. *Hum Mol Genet*. 2002; 11(9):1119–1128.
147. Deprez L et al. Epilepsy as part of the phenotype associated with ATP1A2 mutations. *Epilepsia*. 2008;49(3):500–508.
148. Vanmolkot KR et al. Novel mutations in the Na^+, K^+-ATPase pump gene ATP1A2 associated with familial hemiplegic migraine and benign familial infantile convulsions. *Ann Neurol*. 2003;54(3):s360–366.
149. Suls A et al. Early-onset absence epilepsy caused by mutations in the glucose transporter GLUT1. *Ann Neurol*. 2009;66(3):415–419.
150. Suls A et al. Paroxysmal exercise-induced dyskinesia and epilepsy is due to mutations in SLC2A1, encoding the glucose transporter GLUT1. *Brain*. 2008; 131(pt 7): 1831–1844.
151. Weber YG et al. GLUT1 mutations are a cause of paroxysmal exertion-induced dyskinesias and induce hemolytic anemia by a cation leak. *J Clin Invest*. 2008;118(6):2157–2168.

Chapter 56

The $GABA_A\gamma2$(R43Q) Mouse Model of Human Genetic Epilepsy

Steven Petrou
Christopher A. Reid

UTILITY OF MOUSE MODELS IN UNDERSTANDING PATHOGENESIS IN EPILEPSY

Epilepsy is a complex disease of neuronal networks; therefore, an understanding of the consequences of genetic dysfunction at clinically relevant temporal and spatial scales requires in vivo models. Although in vitro models have and will continue to shed light on aspects of molecular pathogenesis, gaining knowledge of the effects of mutations on the emergent properties of brain networks is critical if we are to fully understand disease genesis. Genetically modified animal models provide an avenue to investigate disease mechanisms of human mutations at this level of organization. Genetically engineered knockin models, in which the human epilepsy mutation is integrated into the mouse genome and replaces the endogenous allele, are potentially one of the most useful in vivo models for understanding epileptogenesis in genetic epilepsy. These mouse models most closely replicate the human genetic disorder and are becoming the gold standard for investigating the mechanisms underlying specific human epilepsy syndromes.

THE $GABA_A$ RECEPTOR

Gamma-aminobutyric acid (GABA) is the predominant inhibitory transmitter within the central nervous system and acts through three receptor classes: the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors. The $GABA_A$ receptor is a transmitter-gated ion channel of the Cys-loop family. $GABA_A$ receptors are pentameric proteins with a central Cl^- permeant pore that is

formed from various combinations of proteins encoded by the α, β, γ, δ, ε, and θ subunit gene families. A vast array of functional $GABA_A$ receptors can be formed from combinations of these subunits in a brain-region and development-specific manner. *GABRG2* encodes the $GABA_A$ γ2 subunit in which most epilepsy-associated mutation have been found for the $GABA_A$ family.

$GABA_A$γ2 RECEPTORS AND GENETIC EPILEPSY

Two independent groups simultaneously identified mutations in *GABRG2* found in families with genetic epilepsy with febrile seizures plus (GEFS+) and childhood absence epilepsy (CAE).[1,2] Two further mutations in *GABRG2* have since been associated with GEFS+, Dravet syndrome, CAE, and febrile seizures.[3,4] A *GABRG2* mutation associated specifically with febrile seizures has also been described.[5] This chapter focuses on the $GABA_A$γ2(R43Q) mutation, for which a knockin mouse model has been engineered.[6]

$GABA_A$γ2(R43Q) FUNCTIONAL ANALYSIS IN HETEROLOGOUS EXPRESSION SYSTEMS

Several in vitro studies have been performed to determine the functional consequence of the various mutations. Analysis of the R43Q mutation was associated with changes in benzodiazepine sensitivity, receptor kinetics, assembly, trafficking, and cell surface expression.[7–10] These in vitro findings are consistent with a reduction in $GABA_A$ receptor-mediated current due to the mutation under the various conditions. The residues surrounding R43 in the γ2 subunit appear to be important for intersubunit interactions and are impaired by the Q43 mutant, providing a potential molecular basis for the kinetic and trafficking changes noted.[11] There has been some disagreement in the in vitro literature regarding whether there is altered diazepam sensitivity associated with the R43Q mutation or not, with some groups reporting altered diazepam binding or sensitivity [8,12,13] and others not.[7,9–11] The source of this discrepancy is not clear at this time and may relate to differences in subunit partners, cell lines, transfection methods, or culturing conditions that all potentially alter expression levels of the mutant R43Q subunit.

An elegant in vitro study used virally delivered R43Q subunits to demonstrate that cell surface expression of the mutant was reduced[14] (which was later confirmed in the R43Q mouse).[6] More importantly, however, Eugene et al. found that expression of the R43Q mutant subunit significantly reduced GABA-mediated tonic inhibition, presumably as a consequence of a dominant negative interaction with the GABAα5 subunit.[14] While it is intuitively satisfying to postulate that a reduction in tonic current, a major source of inhibition in the brain, could result in the absence epilepsy seen in R43Q patients, more recent data from the Crunelli laboratory clearly demonstrated a major *increase* in GABA-mediated tonic current in a rat absence model.[15] It is difficult to reconcile these findings unless one raises the idea that any disruption of the normal tonic inhibition levels within the thalamocortical circuit can result in pathological oscillations that could lead to absence seizures. Further experimental and computational studies are required to fully comprehend the fundamentals of the oscillatory behavior in this important network.

THE $GABA_A$γ2(R43Q) MOUSE MODEL

We have developed an animal model of GEFS+ based on a human *GABRG2* mutation. Patients with this mutation typically display febrile seizures and, less frequently, CAE and GEFS+ seizure types. The mouse recapitulates this core phenotype, displaying clear spike-and-wave discharges on electroencephalography (EEG) that are associated with behavioral arrest (Fig. 56–1). The mouse also displays a phenotype consistent with the febrile seizure seen in patients. Increasing the core temperature of many strains of mice caused thermal seizures that were evident on EEG and behaviorally. Using this simple assay, mice that were heterozygous for the R43Q mutation seized at lower temperatures than their wild-type counterparts (Fig. 56–2), potentially modeling the febrile seizures seen in patients with the same mutation. These mice

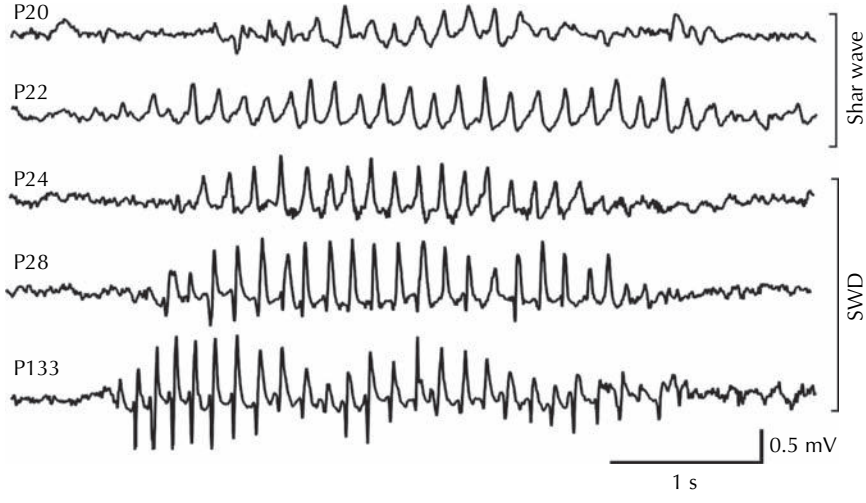

Figure 56–1. Evolution of SWDs in the heterozygous R43Q mice occurs over a short time period. The behavioral arrest phenotype (absence) was noted only after postnatal day 22 with the appearance of SWDs. From ref. 6.

are likely to provide significant insight into the mechanism underlying clinically relevant forms of epilepsy. They may also provide "disease state" screening tools for new antiepileptic drugs (AEDs) that distinguish them from chemical or physical seizure models.

Cortical Inhibition as a Common Deficit in R43Q Patients and Mice?

The thalamocortical circuit is comprised of three brain regions: the somatosensory cortex, the ventrobasal thalamus, and the thalamic reticular nucleus. This interconnected circuit has been implicated in the normal physiology of sleep and also, pathologically, in absence epilepsy. Analysis of synaptic inhibition in the heterozygous R43Q mouse points to a small reduction in cortical GABA$_A$ receptor-mediated transmission as a possible cellular mechanism underlying epilepsy (Fig. 56–3). No change in inhibition has been seen in the thalamic neurons, suggesting that cortical disinhibition is an important aspect of this particular form of genetic epilepsy and, potentially, of common forms of absence epilepsy with different genetic underpinnings. Transcranial magnetic stimulation analysis of cortical excitability in patients heterozygous for the R43Q mutation likewise suggested reduced cortical inhibition.[16] In patients with the mutation, paired pulse stimulation reduced short-interval cortical inhibition and increased intracortical facilitation, resulting in a more excitable motor cortex. In the earlier mouse studies, only inhibitory synapses onto layer 2/3 pyramidal neurons were investigated, and it is not known if other cortical inhibitory synapses were altered. In order to analyze the more global impact of this deficit on cortical function in R43Q mice, voltage-sensitive dye (VSD) studies were performed.[17] The cortical surface of the whisker barrel field in the mouse was exposed and loaded with a fluorescent VSD. A whisker that mapped to the exposed barrel region was found, and the stereotypical

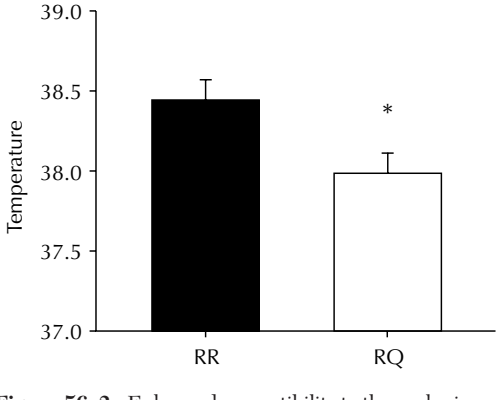

Figure 56–2. Enhanced susceptibility to thermal seizures in R43Q mice. Core temperature at which EEG seizures were first noted in control (RR, $n = 8$) and R43Q heterozygous mice (RQ, $n = 8$). Mice were heated with a stream of warm air during monitoring. $^*p < .05$.

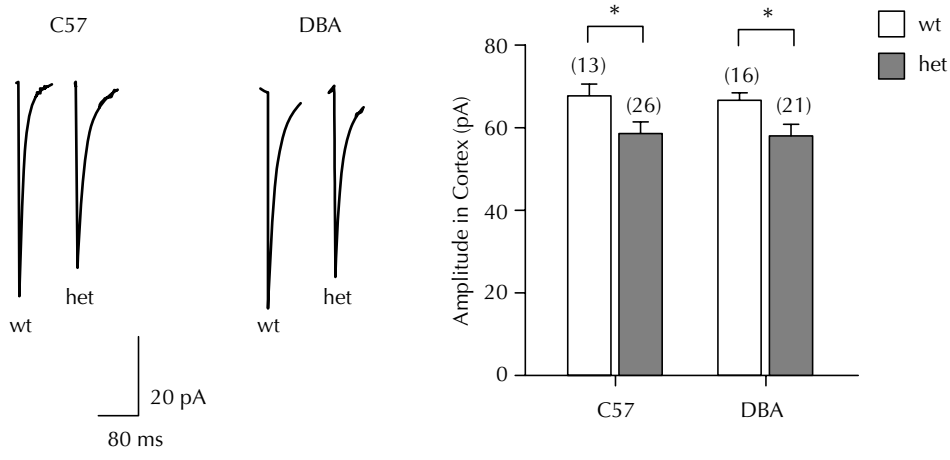

Figure 56–3. Miniature inhibitory postsynaptic current analysis in layer 2/3 pyramidal neurons reveals specific reduction in the cortical inhibition of heterozygous R43Q mice (het) in two different background strains. No such changes were observed in thalamic neurons.[6]

activation patterns caused by whisker deflection were recorded (Fig. 56–4). As can be seen in comparing the heterozygous R43Q and the control, whisker deflection caused a similar level of cortical activation (Fig. 56–4). Upon exposure to a subconvulsive dose of pentylenetetrazole (scPTZ; 40 mg/kg), the amplitude of cortical activation was reassessed. In this case, whisker deflection produced a significantly greater cortical response in the heterozygous mice compared to the controls. This result suggests that the deficit in cortical inhibition noted above (Fig. 56–3) can impact cortical function but only under conditions in which inhibitory reserves are compromised. Consistent with this argument is the episodic nature of seizures seen in both humans and mice, which suggests that spike-and-wave discharges (SWDs) and

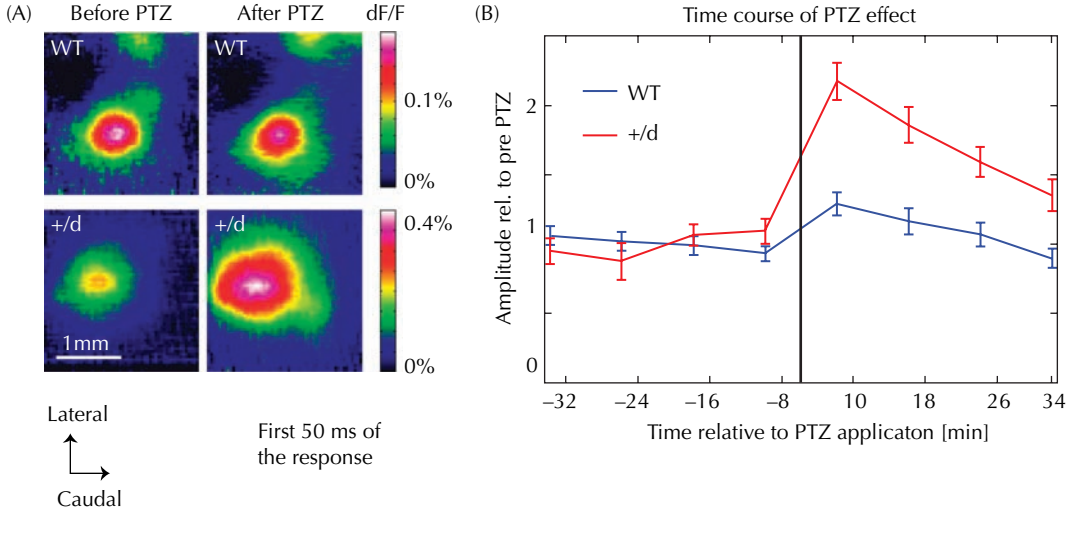

Figure 56–4. Voltage-sensitive dye imaging reveals enhanced cortical excitability in the R43Q mouse model. **A.** Imaging of a single whisker barrel demonstrates that the amplitude and extent of barrel activation following whisker stimulation are not enhanced by scPTZ in control mice (WT) and are significantly enhanced in heterozygous R43Q mice (+/d). **B.** Time course of signal amplitude showing that cortical excitability prior to administration of scPTZ was similar in controls and heterozygotes and that enhancement was prolonged for up to 30 min after exposure. Figure courtesy of H. Spors.

the attendant behavioral arrest may occur at times when the inhibitory drive is also compromised. This may be related to the finding that absence seizures emerge more readily following sleep deprivation in people[18] and during quite wakefulness in mice.[6]

Febrile Seizures in R43Q Patients and Mice

As mentioned above, febrile seizures are a common phenotype seen in R43Q patients and a common feature in many genetic forms of epilepsy. a potential cellular mechanism for febrile seizures in these patients emerged from a series of elegant in vitro experiments that demonstrated internalization of GABA$_A$ receptors containing mutant γ2(R43Q) subunits in response to heating of cell cultures. The loss of inhibitory receptors was proposed to reduce local inhibition and trigger the febrile seizures.[19] This mechanism was further investigated in the heterozygous R43Q mouse model by heating brain slices for amounts of time similar to those used in the in vitro experiments and then recording the amount of miniature inhibitory (GABA$_A$ receptor-mediated) postsynaptic current. Unlike the results found in the in vitro experiments, data from the mouse model showed that heat exposure failed to cause the expected reduction in phasic GABA$_A$ receptor-mediated inhibition[20] (Fig. 56–5). The failure of these in vivo experiments to corroborate the earlier in vitro data suggests that mutation-specific internalization during fever is not likely to be an important mechanism underlying febrile seizures in patients with the γ2(R43Q) mutation[20] (Fig. 56–5). This highlights the potential difficulty of translating in vitro findings to in vivo disease mechanisms and strengthens the need for syndrome specific models.

Developmental Role of the R43Q Mutation

Mutations that produce their effect by acutely altering gene function are also likely to alter the developmental trajectory of the circuits in which they reside. GABA$_A$ receptors in particular have been implicated as important players in normal neurodevelopment, and it would be expected that their dysfunction would likewise alter the course of neurodevelopment that could contribute to heightened seizure susceptibility. This concept has recently been tested by Chiu et al., who described a tetracycline-based conditional model based on the R43Q model that enabled activation of a hypomorphic Q43 allele at different times in the development of the mouse.[21] If the expression of the disease allele was suppressed during early development (in this case, from conception to postnatal day 21), scPTZ testing (Fig. 56–6) showed that these mice had significantly reduced seizure susceptibility. It is important to note that seizure susceptibility did not return to control levels, suggesting that a combination of acute and developmental effects contribute to determining seizure susceptibility in genetic epilepsy. Although the applicability of this mechanism to other forms of genetic epilepsy has not been investigated, it is likely to contribute more widely to pathogenesis in genetic epilepsy and provides an example of an emergent pathology that could not have demonstrated in simpler model systems. There are two therapeutic consequences of this finding: knowledge of the genetic lesion itself may not be sufficient to select therapy, and understanding of the impact of a mutation at multiple spatial and temporal scales will be necessary to enable the development of personalized medicine in epilepsy and other neurological disorders.

R43Q as a Model for Hypoglycemia-Induced Seizures

As discussed, GEFS+ has a genetic etiology. However, it is well known that environmental effects such as decreased vigilance and voluntary hyperventilation can induce absence seizures, which is one of the epilepsy phenotypes seen in R43Q patients with GEFS+. Not all environmental triggers of absence seizures are understood. The controlled experimental environment provided by using syndrome-specific animal models can be extremely valuable in investigating these issues, which may otherwise be difficult to achieve in highly heterogeneous patient populations. We have used the heterozygous R43Q mouse model to investigate the impact of low blood glucose on seizure

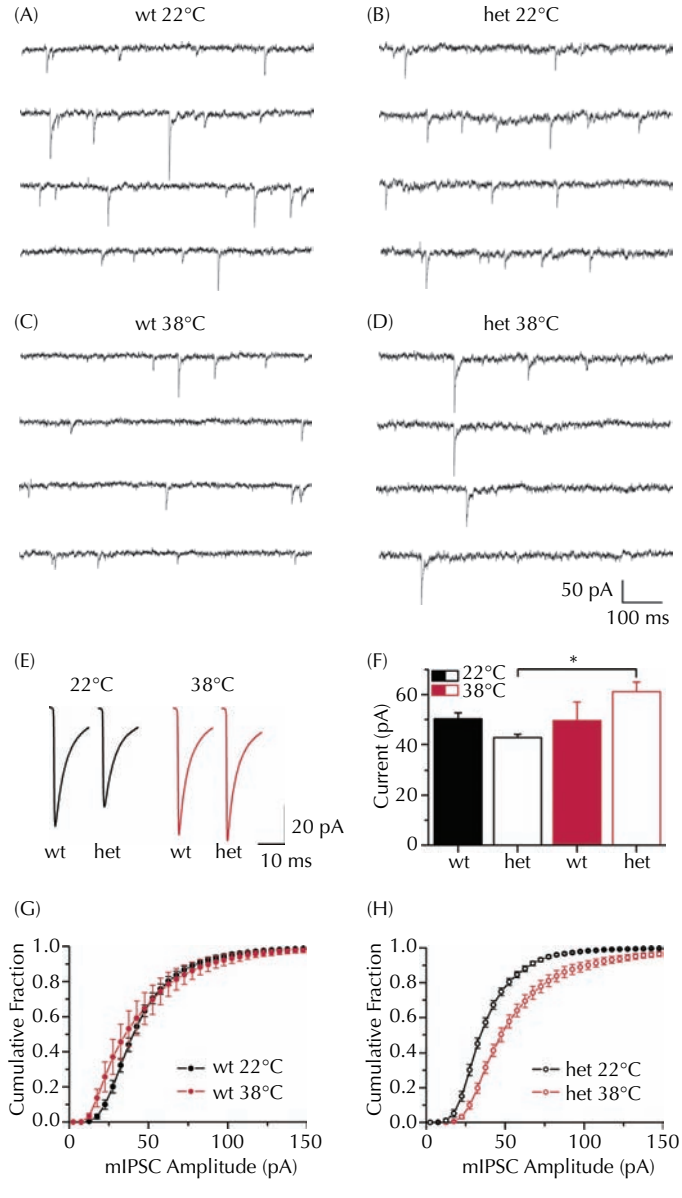

Figure 56–5. Temperature elevation causes a long-term increase in inhibitory currents in layer 2/3 cortical pyramidal neurons from the heterozygous R43Q mouse. **A–D.** Comparison of miniature inhibitory post synaptic currents (mIPSCs) from control (wt) and heterozygous R43Q mice (het) after incubation of slices at either 22°C or 38°C. **E–H.** Summary of mIPSC properties demonstrating the specific effect of preheating on neurons from the het mice only.[20]

precipitation.[22] We show that moderate lowering of blood glucose levels can precipitate seizures (Fig. 56–7). Low blood glucose may, therefore, represent an environmental risk factor in patients with epilepsy and, in particular, in those with preexisting genetic risk factors. These data provide a compelling reason to further investigate this phenomenon in absence epilepsy and have already led to clinical studies along these lines. This provides an exciting paradigm with which syndrome-specific models that recapitulate the human phenotype can be used to make predictions about human pathological outcomes.

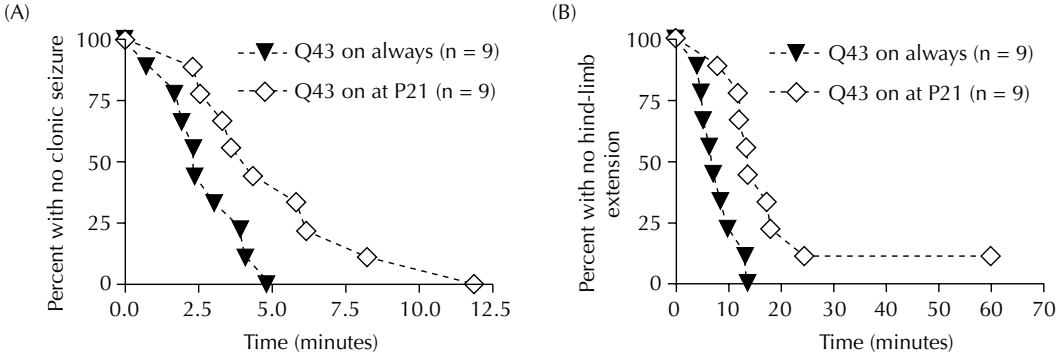

Figure 56–6. Suppression of the Q43 disease allele until day 21 reduces seizure susceptibility. Survival times for scPTZ-induced (**A)** tonic-clonic and (**B**) hind limb extension seizures.[21]

FUTURE CHALLENGES

The causal chain that extends from genetic lesion to behavioral seizure spans multiple temporal and spatial scales, crosses a range of axes from clinical to economic (for a review of the computational challenges facing the epilepsy field, see Lytton[23]), and operates on the complex substrate of the human brain. While the clinical need is clear; to develop a cure for all types of epilepsy, the challenge is to navigate this complex landscape efficiently. Genetically

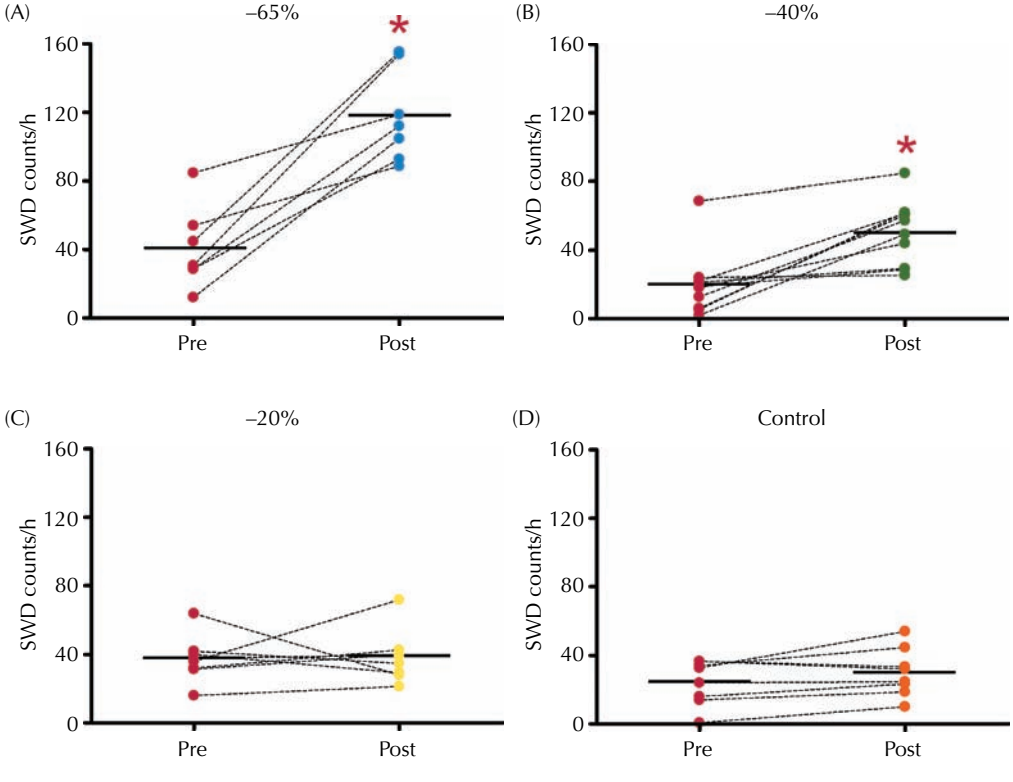

Figure 56–7. Hypoglycemic challenge increases SWDs in the heterozygous R43Q mouse model. Increasing levels of hypoglycemia are associated with an increased amount of SWDs (**A–C**). Vehicle controls did not show an increase (**D**).[22]

engineered mice, and more recently the transgenic rat model,[24] provide approaches to model many of the scales required to gain a full and predictive understanding of how our personal genome determines not only our seizure susceptibility but also the modulation of this susceptibility by external factors such as those imposed by disease (fever and chemical changes such as cytokines), diet, and lifestyle (hypoglycemia and other metabolic changes), as well as the important interaction with drugs that modify networks to abrogate seizures. The first challenge is perhaps to identify the critical path that will yield the greatest knowledge for a given scientific effort. Because epilepsy is a systems-level disorder, the methodological challenges are enormous, as experimental approaches must span genetic, molecular, synaptic, network, and behavioral levels, to name a few. Incorporating the latest methods, such as optogenetics and optophysiology, is also critical to revealing novel mechanisms and intervention strategies and for reporting on organizational scales relevant to disease genesis. A final challenge in the effort to achieve ideal therapeutic outcomes involves the translation of experimental findings into commercially sustainable drug discoveries and development.

DISCLOSURE STATEMENT

The authors have no conflict of interests to disclose.

REFERENCES

1. Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, Berkovic SF. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet*. 2001;28:49–52.
2. Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A, Prud'homme JF, Baulac M, Brice A, Bruzzone R, LeGuern E. First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. *Nat Genet*. 2001;28: 46–48.
3. Harkin LA, Bowser DN, Dibbens LM, Singh R, Phillips F, Wallace RH, Richards MC, Williams DA, Mulley JC, Berkovic SF, Scheffer IE, Petrou S. Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am J Hum Genet*. 2002;70:530–536.
4. Kananura C, Haug K, Sander T, Runge U, Gu W, Hallmann K, Rebstock J, Heils A, Steinlein OK. A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. *Arch Neurol*. 2002;59:1137–1141.
5. Audenaert D, Schwartz E, Claeys KG, Claes L, Deprez L, Suls A, Van Dyck T, Lagae L, Van Broeckhoven C, Macdonald RL, De Jonghe P. A novel GABRG2 mutation associated with febrile seizures. *Neurology*. 2006;67:687–690.
6. Tan HO, Reid CA, Single FN, Davies PJ, Chiu C, Murphy S, Clarke AL, Dibbens L, Krestel H, Mulley JC, Jones MV, Seeburg PH, Sakmann B, Berkovic SF, Sprengel R, Petrou S. Reduced cortical inhibition in a mouse model of familial childhood absence epilepsy. *Proc Natl Acad Sci USA*. 2007;104:17536–17541.
7. Bianchi MT, Song L, Zhang H, Macdonald RL. Two different mechanisms of disinhibition produced by $GABA_A$ receptor mutations linked to epilepsy in humans. *J Neurosci*. 2002;22:5321–5327.
8. Bowser DN, Wagner DA, Czajkowski C, Cromer BA, Parker MW, Wallace RH, Harkin LA, Mulley JC, Marini C, Berkovic SF, Williams DA, Jones MV, Petrou S. Altered kinetics and benzodiazepine sensitivity of a $GABA_A$ receptor subunit mutation [gamma 2(R43Q)] found in human epilepsy. *Proc Natl Acad Sci USA*. 2002;99:15170–15175.
9. Kang JQ, Macdonald RL. The $GABA_A$ receptor gamma2 subunit R43Q mutation linked to childhood absence epilepsy and febrile seizures causes retention of alpha1beta2gamma2S receptors in the endoplasmic reticulum. *J Neurosci*. 2004;24:8672–8677.
10. Sancar F, Czajkowski C. A $GABA_A$ receptor mutation linked to human epilepsy (gamma2R43Q) impairs cell surface expression of alphabetagamma receptors. *J Biol Chem*. 2004;279:47034–47039.
11. Hales TG, Tang H, Bollan KA, Johnson SJ, King DP, McDonald NA, Cheng A, Connolly CN. The epilepsy mutation, gamma2(R43Q) disrupts a highly conserved inter-subunit contact site, perturbing the biogenesis of $GABA_A$ receptors. *Mol Cell Neurosci*. 2005;29: 120–127.
12. Wallace RH, Wang DW, Singh R, Scheffer IE, George AL Jr, Phillips HA, Saar K, Reis A, Johnson EW, Sutherland GR, Berkovic SF, Mulley JC. Febrile seizures and generalized epilepsy associated with a mutation in the Na^+-channel beta1 subunit gene SCN1B. *Nat Genet*. 1998;19:366–370.
13. Goldschen-Ohm MP, Wagner DA, Petrou S, Jones MV. An epilepsy-related region in the GABA(A) receptor mediates long-distance effects on GABA and benzodiazepine binding sites. *Mol Pharmacol*. 2010;77: 35–45.
14. Eugene E, Depienne C, Baulac S, Baulac M, Fritschy JM, Le Guern E, Miles R, Poncer JC. GABA(A) receptor gamma 2 subunit mutations linked to human epileptic syndromes differentially affect phasic and tonic inhibition. *J Neurosci*. 2007;27:14108–14116.
15. Cope DW, Di Giovanni G, Fyson SJ, Orban G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V. Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. *Nat Med*. 2009;15:1392–1398.
16. Fedi M, Berkovic SF, Macdonell RA, Curatolo JM, Marini C, Reutens DC. Intracortical hyperexcitability in humans with a $GABA_A$ receptor mutation. *Cereb Cortex*. 2008;18:664–669.

17. Witsch JJ, Golkowski DJ, Petrou S, Spors H. In vivo recordings in a mouse model of a human genetic epilepsy. Abstract from the German Neuroscience meeting, Gottingen, 2007. Available at www.neuro.uni-goettingen.de/archiv/2007/pdf/Program.pdf
18. Borkowski WJ Jr, Ellington RJ, Sverdrup EK. Effect of sleep deprivation on the EEG of learning-impaired children with absence seizures. *Clin Electroencephalogr*. 1992;23:62–64.
19. Kang JQ, Shen W, Macdonald RL. Why does fever trigger febrile seizures? $GABA_A$ receptor gamma2 subunit mutations associated with idiopathic generalized epilepsies have temperature-dependent trafficking deficiencies. *J Neurosci*. 2006;26:2590–2597.
20. Hill E, Hosie S, Mulligan RS, Richards KL, Davies PJ, Dube CM, Baram TZ, Reid CA, Jones MV, Petrou S. Temperature elevation increases $GABA_A$-mediated cortical inhibition in a mouse model of genetic epilepsy. *Epilepsia*. 2011;52:179–184.
21. Chiu C, Reid CA, Tan HO, Davies PJ, Single FN, Koukoulas I, Berkovic SF, Tan SS, Sprengel R, Jones MV, Petrou S. Developmental impact of a familial $GABA_A$ receptor epilepsy mutation. *Ann Neurol*. 2008;64:284–293.
22. Reid CA, Kim TH, Berkovic SF, Petrou S. Low blood glucose precipitates spike-and-wave activity in genetically predisposed animals. *Epilepsia*. 2011;52:115–120.
23. Lytton WW. Computer modelling of epilepsy. *Nat Rev Neurosci*. 2008;9:626–637.
24. Zhu G, Okada M, Yoshida S, Ueno S, Mori F, Takahara T, Saito R, Miura Y, Kishi A, Tomiyama M, Sato A, Kojima T, Fukuma G, Wakabayashi K, Hase K, Ohno H, Kijima H, Takano Y, Mitsudome A, Kaneko S, Hirose S. Rats harboring S284L Chrna4 mutation show attenuation of synaptic and extrasynaptic GABAergic transmission and exhibit the nocturnal frontal lobe epilepsy phenotype. *J. Neurosci*. 2008;28:12465–12476.

Chapter 57

$GABA_A$ Receptor Subunit Mutations and Genetic Epilepsies

Robert L. Macdonald
Jing-Qiong Kang
Martin J. Gallagher

INTRODUCTION

Idiopathic epilepsy syndromes (IES) are common and constitute about 50% of the epilepsies diagnosed worldwide.[1] They vary in severity from the relatively benign febrile seizures (FS) and childhood absence epilepsy (CAE) to the severe epilepsy syndrome Dravet syndrome. A common IES is FS plus (FS+), which develops early in childhood with multiple FS that continue to occur beyond 6 years of age or are associated with afebrile seizures.[2] Generalized epilepsy with FS plus (GEFS+) is a familial epilepsy syndrome in which multiple family members have either FS, FS+, myoclonic-astatic epilepsy (MAE), and DS.[2]

Most IES have been thought to have a genetic basis,[3,4] and although complex polygenic inheritance is likely associated with most genetic epilepsy syndromes, rare monogenic mutations of transmembrane ion channels associated with IES in several large pedigrees and in sporadic cases with de novo mutations have been identified.[4–6] Mutations of ion channels that

either increase excitatory or reduce inhibitory neurotransmission produce neuronal hyperexcitability, thereby predisposing individuals harboring the mutant gene to experience seizures. In this chapter, we will focus on mutations in inhibitory GABA$_A$ receptor subunit genes that have been associated with IES.

GABA$_A$ RECEPTOR SUBUNIT GENES

Gamma-aminobutyric acid A (GABA$_A$) receptors are members of the cys-loop family of ligand-gated ion channels that also includes glycine, nicotinic cholinergic, and serotonin 5-HT3 receptors and are the primary mediators of fast inhibitory synaptic transmission in the central nervous system. The GABA$_A$ receptors are formed by pentameric assembly of different subunit subtypes (α1–α6, β1–β3, γ1–γ3, δ, ε, π, θ, and ρ1–ρ3) to form chloride ion channels,[7] and most GABA$_A$ receptors are thought to contain two α subunits, two β subunits, and one γ or δ subunit.[8] The GABA$_A$ receptors mediate both phasic synaptic and tonic perisynaptic or extrasynaptic inhibition, and several antiepileptic drugs, including benzodiazepines and barbiturates, act by enhancing GABA$_A$ receptor currents.[9] Therefore, it is not surprising that several different IES have been associated with mutations and variants in several GABA$_A$ receptor subunit *GABR* genes, including *GABRA1*, *GABRB3*, *GABRD*, and *GABRG2*[10,11] (see Fig. 57–1 and Table 57–1). The position of mutant amino acids in *GABRA1*, *GABRB3*, and *GABRD* associated with IES have been designated in the immature peptide that includes the signal sequence, but mutations in *GABRG2* have been designated in the

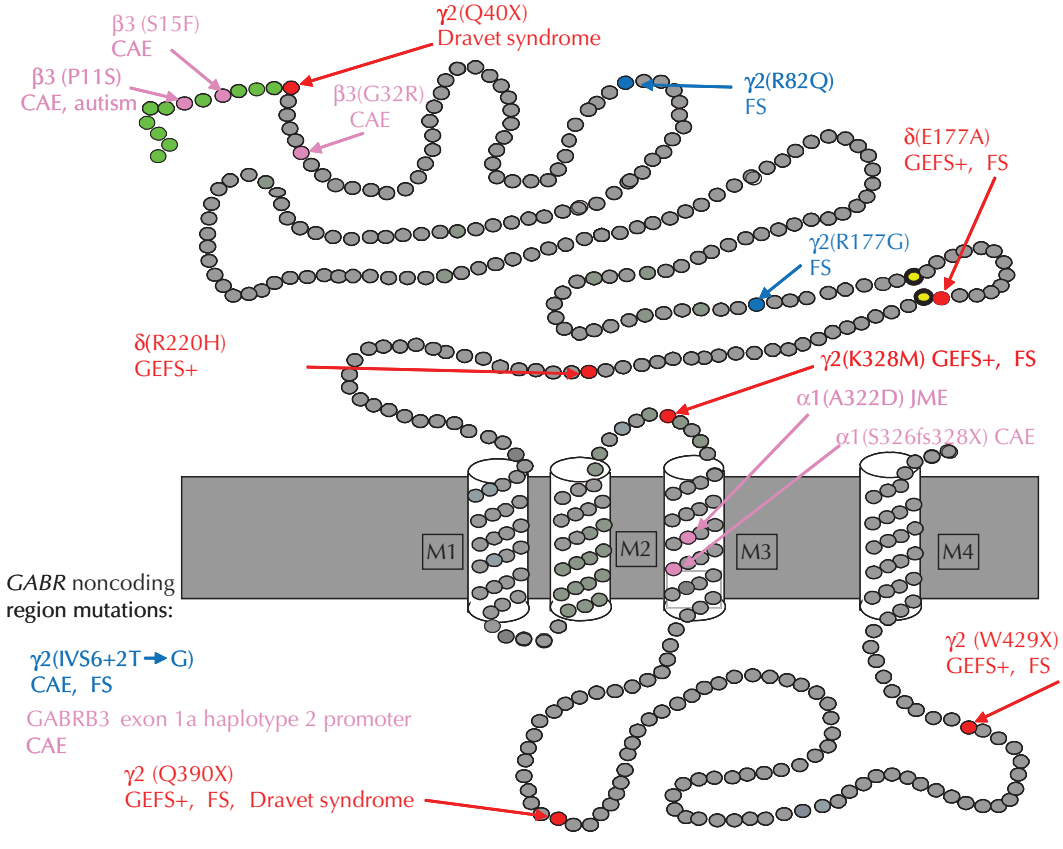

Figure 57–1. Gamma-aminobutyric acid A receptor subunit gene mutations associated with genetic epilepsy syndromes. Modified from ref. 11.

Table 57–1 **Epilepsy Mutations in *GABRs***

Mutation/Variant Original Reference	IES	Structural Feature	$GABA_A$ Receptor Dysfunction
CAE or JME (α1/β3 subunits): four missense, one promoter, and one frameshift mutation			
β3(P11S) Tanaka et al.[12]	CAE	Signal-peptide missense mutation	Abnormal N-linked glycosylation and reduced whole cell current
β3(S15F) Tanaka et al.[12]	CAE	Signal-peptide missense mutation	Abnormal N-linked glycosylation and reduced whole cell current
β3(G32R) Tanaka et al.[12]	CAE	N-terminus missense mutation	Abnormal N-linked glycosylation and reduced whole cell current
GABRB3 haplotype 2 Urak et al.[13]	CAE	Exon 1a haplotype 2 promoter	Impaired binding of N-Oct-3 transcription activator
α1(975delC, S326fs328X) Maljevic et al.[6]	CAE	Frameshift mutation and PTC in M3	NMD of mRNA followed by ERAD of subunit protein
α1(A322D) Cossette et al.[16]	JME	M3 missense mutation	Misfolded, reduced total and surface expression, reduced whole cell current
FS +/– CAE (γ2 subunit): two functional missense mutations and one nonfunctional nonsense mutation			
γ2(R82Q) Wallace et al.[5]	FS	N-terminus missense mutation	Impaired oligomerization, ER retention, reduced surface expression, reduced current
γ2(R177G) Audenaert et al.[18]	FS	N-terminus missense mutation	Altered whole cell current kinetics
GABRG2(IVS6+2T→G) Kananura et al.[19]	FS/CAE	Intron 6 splice-donor site mutation	Predicted to cause PTC at the exon 5–7 junction.
GEFS and/or DS (γ2/δ subunits): four functional missense and three nonfunctional nonsense mutations			
γ2(K328M) Baulac et al.[21]	GEFS+	M2/M3 loop missense mutation	Reduced single-channel mean open times, accelerated whole cell current deactivation
γ2(Q390X) Harkin et al.[22]	GEFS+/Dravet syndrome	M3/M4 loop nonsense mutation	ER retention, dominant negative reduction of wild-type receptors, reduced whole cell current
γ2(Q429X) Sun et al.[28]	GEFS+	M3/M4 loop nonsense mutation	Unstudied; likely unaffected by NMD and therefore produces truncated peptide
δ(E177A) Dibbens et al.[17]	GEFS+	N-terminus missense mutation	Reduced whole cell current, reduced single- channel mean open time
δ(R220C) Dibbens et al.[17]	GEFS+	N-terminus missense mutation	Reduced whole cell current, reduced single- channel mean open time
γ2(Q40X) Hirose[20]	Dravet syndrome	N-terminus nonsense mutation	Unstudied; likely triggers NMD

mature peptide. For consistency, in this chapter we will also designate the position of *GABRG2* mutations in the immature peptide.

IES ASSOCIATED WITH $GABA_A$ RECEPTOR SUBUNIT MUTATIONS

A wide range of IES have been shown to be associated with $GABA_A$ receptor subunit mutations[11] (Table 57–1). Childhood absence epilepsy alone has been associated with *GABRB*3 missense mutations located in the β3 subunit signal peptide (P11S, S15F) and the N-terminal region of the mature subunit (G32R),[12] as well as with a specific haplotype of the *GABRB3* promoter,[13] In addition, CAE is associated with a frameshift mutation in *GABRA1* (975delC, S326fs328X)[6] that produces a premature translation-termination codon (PTC). The β3 subunit (P11S) mutation has also been associated with autism pedigrees with some patients who also had epilepsy,[14,15] Juvenile myoclonic epilepsy (JME) has been associated with a missense mutation in *GABRA1* (A322D)[16] and a variant in *GABRD* (R220H).[17] Febrile seizures alone have been associated with missense mutations in *GABRG2* that are located in the N-terminal region of the mature γ2 subunit (R82Q, R177G)[5,18] and at the intron 6 splice donor site (IVS6 2T → G).[19] Generalized epilepsy with febrile seizures plus has been associated with missense and nonsense mutations in *GABRG2* (Q40X, K328M, Q390X, W429X)[20–23] and with variants in *GABRD* (E177A, R220H).[17] While a clear genotype/phenotype correlation has not been well established, to date mutations in *GABRB3* and *GABRA1* have been associated with CAE or JME alone, while *GABRG2* mutations and other *GABRD* variants have been associated with FS, FS with CAE, and GEFS+.

PATHOPHYSIOLOGY OF $GABA_A$ RECEPTOR SUBUNIT MUTATIONS ASSOCIATED WITH CAE AND JME

GABRB3(P11S, S15F, G32R)

GABRB3 has an alternative exon 1 (exon 1a) that encodes a variant signal peptide that translates a β3 subunit of identical length but with an altered mature peptide sequence. The two transcripts have different relative expression levels and distributions in fetal and adult brain, with exon 1a expression being enriched in fetal brain.[24] The *GABRB3* mutations P11S and S15F are located in exon 1a in the β3 subunit signal peptide. The *GABRB3* mutation G32R is in exon 2 and is located in the mature β3 subunit peptide near the N terminus. In HEK293T cells coexpressing each of the three β3 mutant subunits with α1 and γ2 subunits, whole cell peak currents and cell surface expression levels of the mutant β3 subunits were reduced.[12,14] It was suggested that these β3 subunit mutations may reduce $GABA_A$ receptor cell surface expression and whole cell current amplitudes by altering N-linked glycosylation of the β3 subunit.[12,14] However, the basis for the altered glycosylation of β3 subunits is unclear, and whether or not the altered glycosylation reduces receptor surface levels is also unclear. Furthermore, it is uncertain how the two signal peptide mutations alter the function of the mature β3 subunits since they are presumably cleaved prior to subunit folding and receptor assembly. Reduced expression of β3 subunit-containing $GABA_A$ receptors due to these mutations would be consistent with an epilepsy phenotype and would be expected to cause epilepsy early in development, since exon 1a is selectively expressed in fetal brain, and to remit in young adulthood since exon 1 is selectively expressed later in development.

In *GABRB3*, one of four haplotypes (haplotype 2) in the region from the exon 1a promoter to the beginning of intron 3 had a significant association with CAE.[13] Using an in vitro reporter gene assay with exon 1a promoter constructs, the haplotype 2 promoter was found to cause less transcriptional activity than the haplotype 1 promoter. Thus, it was suggested that the thymine-to-cytosine substitution in the haplotype 2 promoter impaired binding of the neuron-specific transcriptional activator N-Oct-3, leading to decreased transcription of *GABRB3* and thus presumably to a decrease in β3 subunit levels.

GABRA1(975delC, S326fs328X)

The *GABRA1* deletion mutation, 975delC, S326fs328X, causes a frameshift in *GABRA1*

that produces a premature translation-termination codon (PTC) and is associated with CAE[6] (see Fig. 57–1 and Table 57–1). Premature translation-termination codons in the last exon of a multi-exon gene or less than 50–55 nucleotides upstream of the last exon–exon junction result in production of a truncated protein. In contrast, PTCs not in the last exon of a multi-exon gene or more than 50–55 nucleotides upstream of the last exon–exon junction produce mRNA degradation through activation of nonsense-mediated decay (NMD), a cellular mRNA quality-control system that activates degradation of mutant mRNA to substantially reduce production of truncated proteins.[25] The *GABRA1* deletion mutation, 975delC, S326fs328X, is in exon 8 and is 84 base pairs upstream of intron 8; thus, this PTC activated NMD and reduced mutant α1 subunit mRNA.[26] However, the NMD was incomplete, but the truncated α1 subunit protein that was translated was degraded by endoplasmic reticulum (ER)-associated degradation (ERAD). These results suggested that the *GABRA1* mutation, S326fs328X, resulted in functional haploinsufficiency by reducing both mutant mRNA and subunit protein.

GABRA1(A322D)

The *GABRA1* missense mutation, A322D, replaces a small, neutral residue with a larger negatively charged aspartate residue in the M3 transmembrane helix and is associated with an autosomal dominant (AD) form of JME[16] (see Fig. 57–1 and Table 57–1). This non-conserved mutation was shown to impair α1 subunit folding by destabilizing insertion of the M3 domain into the lipid bilayer.[27] When mutant α1(A322D), β2, and γ2 subunits were coexpressed in HEK293T cells, both total and surface α1 subunit levels were reduced and an intermediate effect was found with heterozygous subunit expression. Loss of the misfolded mutant subunit was due to ERAD[28] and lysosomal degradation.[29] Peak GABA-evoked currents were significantly reduced with both heterozygous and homozygous α1(A322D) subunit expression, consistent with the impaired folding and assembly of the mutant α1(A322D) subunits.[6,30,31] Recently, we demonstrated that the presence of the nondegraded, misfolded α1(A322D) subunit produces small dominant negative effects that alter the composition and further reduce the expression of wild-type $GABA_A$ receptors.[32]

PATHOPHYSIOLOGY OF $GABA_A$ RECEPTOR SUBUNIT MUTATIONS ASSOCIATED WITH FS WITH OR WITHOUT CAE

In contrast to CAE and JME without FS associated with mutations in *GABRB3* and *GABRA1*, FS with or without CAE have only been associated with mutations in *GABRG2*. Two *GABRG2* missense mutations, R82Q[5] and R177G,[18] and one splice donor site mutation in *GABRG2*, IVS6+2T→G,[19] have been associated with FS with or without CAE.

GABRG2(R82Q)

The *GABRG2* missense mutation, R82Q, is located in the distal N terminus and is associated with FS[5] (see Fig. 57–1 and Table 57–1). An AD form of CAE was also present in the family pedigree, and it was demonstrated that an interaction of the γ2 subunit gene with another gene or genes is required for the CAE phenotype in this family.[33] Alignment of γ2 subunit and acetylcholine binding protein sequences revealed that R82 is positioned at the γ2/β2 subunit–subunit interface, and it was demonstrated that the mutation impaired γ2 and β2 subunit oligomerization.[34] This impaired oligomerization is likely the basis for this mutation's reduction of surface α1β2γ2 receptors,[35–38] ER retention of unassembled γ2(R82Q) subunits,[35,37] and reduction of $GABA_A$ receptor currents.[35,39] The R82Q mutation also caused intracellular retention and reduced surface expression of $GABA_A$ receptors in cortical pyramidal neurons,[40] reduced miniature inhibitory postsynaptic currents (IPSCs) in layer II/III cortical neurons, and electrographic and behavioral seizures in R82Q knockin mice. Endogenous expression of α5 subunits in cultured hippocampal neurons was reduced when coexpressed with γ2(R82Q) subunits, indicating that γ2(R82Q) subunits conferred a dominant negative effect.[38] In addition, it is possible that a deficit in γ2 subunits

caused a compensatory increase in other subunits, such as δ or β subunits. Since αβδ and αβ receptors are extrasynaptic or perisynaptic, this compensatory increase may result in a relative increase in tonic currents. Recently, it has been reported that extrasynaptic GABAergic "tonic" inhibition was increased in thalamocortical neurons from both genetic and pharmacological models of absence epilepsy,[41] consistent with this conclusion.

GABRG2(R177G)

The *GABRG2* missense mutation, R177G, is located in the N terminus and has been associated with FS[18] (see Fig. 57–1 and Table 57–1). The γ2 subunit R177 residue is conserved among γ2 subunits across species. Basic residues are conserved among other γ subunits, and in other cys-loop receptors, polar and charged amino acid residues occur at this position. Mutant α1β3γ2L(R177G) receptors had altered current kinetics and reduced benzodiazepine sensitivity,[18] but the underlying molecular mechanisms for FS associated with this mutation are unclear.

GABRG2(IVS6 + 2T→G)

The *GABRG2* splice-donor site mutation, IVS6 + 2T→G, is located in intron 6 and was identified in a family with FS and CAE[19] (see Fig. 57–1 and Table 57–1). The effect of this mutation on GABA$_A$ receptor function is unknown but was predicted to impair splicing of the intron 6. It was suggested that the mutation most likely would lead to a nonfunctional protein through exon skipping, which would result in a PTC at the fifth and seventh exon junction sites. If this is correct, the exon skipping-induced PTC would trigger NMD. However, when the mutation was made in the *GABRG2* intron 6 cloned in a bacterial artificial chromosome, a cryptic splice donor site in intron 6 was activated, resulting in retention of a portion of intron 6 and a frameshift that resulted in a PTC in exon 7 (Tian and Macdonald, unpublished). This exon 7 PTC had an exon–exon junction downstream and thus activated nonsense-mediated mRNA decay and loss of most of the mutant mRNA.

PATHOPHYSIOLOGY OF GABA$_A$ RECEPTOR SUBUNIT MUTATIONS ASSOCIATED WITH GEFS+ AND DRAVET SYNDROME

Generalized epilepsy with febrile seizures plus and Dravet syndrome have only been associated with *GABRG2* and *GABRD* mutations. One *GABRG2* missense mutation, (K328M),[21] three *GABRG2* nonsense mutations (Q40X, Q390X, and Q429X),[20,22,23] one *GABRD* mutation (R220C),[17] and a *GABRD* variant (E177A)[17] have been associated with GEFS+ with or without Dravet syndrome.

GABRG2(K328M)

The *GABRG2* missense mutation, K328M, is located in the short extracellular loop between transmembrane domains M2 and M3 and is associated with an AD GEFS+[21] (see Fig. 57–1 and Table 57–1). Brief GABA-evoked currents recorded from α1β3γ2L(K328M) receptors had unchanged current amplitudes but had accelerated deactivation.[39,42] In transfected hippocampal neurons, the K328M mutation also accelerated deactivation of IPSCs, thus reducing their duration.[38] Single-channel currents from α1β3γ2(K328M) receptors had reduced mean open times, consistent with accelerated macroscopic current deactivation.[39] Therefore, the γ2L(K328M) subunit mutation would reduce IPSC duration by accelerating its deactivation due to impaired stability of the channel open state.

GABRG2(Q390X)

The *GABRG2* nonsense mutation, Q390X, is located in the intracellular loop between transmembrane domains M3 and M4 and was identified in a family with GEFS+ and Dravet syndrome[22] (see Fig. 57–1 and Table 57–1). The PTC is located in the last (ninth) exon and therefore would not be expected to activate NMD. When cDNAs containing the mutant subunit were transfected into HEK293T cells, translation resulted in production of a truncated protein that lacked its C-terminal 78 amino acids and was retained in the ER.[43] Because the

γ2 subunit mutation (Q351X) prevents the cell surface trafficking of both α1β2γ2(Q351X) and α1β2 receptors, no GABA-evoked currents were recorded from cells transfected with α1, β2, and γ2(Q351X) subunits.[22,43] The γ2(Q390X) subunit also caused a dominant negative effect on wild-type receptors. Currents recorded following heterozygous expression of α1β2γ2/α1β2γ2(Q351X) receptors were reduced relative to hemizygous control currents, and γ2S and γ2S(Q390X) subunits and partnering α1 and β2 subunit levels were all reduced more than with hemizygous expression with only one wild-type allele, suggesting that the mutation produced a loss of function of the mutant allele and a dominant negative effect of the mutant γ2S(Q390X) subunit on wild-type receptor channels.

GABRG2(Q40X and Q429X)

The two *GABRG2* nonsense mutations, Q40X and Q429X, have been associated with Dravet syndrome and GEFS+, respectively.[20,23] The Q40X mutation likely triggers NMD, although this has not yet been demonstrated. In contrast, the Q429X mutation, which generates a PTC in the last *GABRG2* exon, would not be predicted to activate NMD and therefore would be expected to produce a truncated protein with loss of the C-terminal 39 amino acids.

GABRD(E177A, R220H, and R220C)

The *GABRD* susceptibility variants, E177A and R220H, and the *GABRD* mutation, R220C, are located in the δ subunit N terminus and are associated with an AD generalized epilepsy similar to GEFS+[17] (see Fig. 57–1 and Table 57–1). The *GABRD* (E177A) variant is adjacent to one of the two cysteines that form a disulfide bond, the signature feature of cys-loop receptors, and the *GABRD* (R220H) variant and (R220C) mutation are located between the cys-loop and the beginning of the first transmembrane domain (M1). The macroscopic current amplitudes of heterozygous and homozygous α1β2δ receptors containing the δ(E177A) subunit were significantly reduced due primarily to reduced single-channel mean channel open time.[44] There was also a small but significant reduction of mutant subunit surface levels with homozygous expression of receptors containing either variant.

DISCUSSION

Phenotype–Genotype Correlations

The pathophysiology of $GABA_A$ receptor subunit gene mutations associated with CAE and JME appears to involve a mechanism that produces developmentally regulated epilepsy and FS. Downregulation of *GABRB3* (exon 1a) function by decreasing β3 subunit surface expression (*GABRB3*[P11S, S15F]) or by decreasing *GABRB3* transcription (promoter mutation) associated with CAE should both result in epilepsy syndromes that have early onset and remit with age due to the expression of *GABRB3* exon 1a only early in development. In contrast, the *GABRA1* frameshift mutation (975delC, S326fs328X) should also occur early in development, corresponding to the onset of *GABRA1* expression, but should not remit with age if there is no functional compensation from other functionally equivalent subunits. In general, these patterns of epilepsy expression and genotype appear to be consistent. That said, it is unclear why mutations in *GABRG2* and *GABRD*, but not *GABRA1* and *GABRB3*, are associated with FS. It is tempting to speculate that this difference may have something to do with the ability of the nervous system to compensate for the loss of a $GABA_A$ receptor subunit. There are multiple α (α2–α6) and β (β1, β3) subunit subtypes that can substitute for α1 or β3 subunits and lessen the molecular defect caused by the impaired subunit function or expression, but γ2 or δ subunits do not have subunits that can readily be upregulated to compensate for their loss. Alternatively or in addition, it may be that decreased expression of γ2 or δ subunits may have some inherent temperature sensitivity that further reduces their expression or function.[45]

Expression of $GABA_A$ Receptor Subunit Mutations in Heterologous Cells and Neurons

The pathophysiology of mutant $GABA_A$ receptor subunits has been evaluated primarily by

expressing them in heterologous cells, but it is likely that $GABA_A$ receptor expression and function in heterologous cells and in neurons differ. While many fundamental features of subunit translation, folding, and oligomerization and receptor assembly and trafficking are likely similar, if not identical, in heterologous cells and neurons, heterologous cells do not express neuron-specific $GABA_A$ receptor-associating proteins that are involved in $GABA_A$ receptor biosynthesis, trafficking, and cell surface stability.[46] The processes involved in targeting $GABA_A$ receptors to dendrites in polar neurons and to subsynaptic and extrasynaptic sites are clearly not recapitulated in heterologous cells. Some, but not all, of these concerns can be overcome by expressing wild-type and mutant $GABA_A$ receptor subunits in cultured neurons. In addition to expressing neuron-specific proteins, cultured neurons form functional GABAergic synapses that obviate the need for exogenous GABA applications. Many of the initial observations in heterologous cells have been confirmed by study of mutant γ2(R82Q), γ2(K328M), γ2(Q390X), and α1(A322D) subunits transfected into cultured neurons.[32,37,38,43] For example, rapid agonist perfusion techniques applied to transfected HEK293T cells revealed that α1β2γ2(K328M) receptors deactivated significantly faster than wild-type receptors,[39] and mIPSCs recorded from hippocampal neurons transfected with γ2(K328M) subunits had shorter deactivation times than mIPSCs recorded from untransfected neurons or from neurons transfected with wild-type γ2 subunits.[38] In addition, studies in transfected neurons revealed new findings that could not have been predicted from heterologous expression alone. For example, while ER retention of mutant γ2(R82Q) subunits transfected into HEK293T cells[35,36] was confirmed in transfected hippocampal neurons,[37,38] expression of γ2(R82Q) subunits in cultured neurons also reduced nonsynaptic "tonic" GABA currents and reduced surface expression of α5 subunits.[37] These observations could not have been obtained in HEK293T cells that do not express neuron-specific proteins such as radixin, which associates with the α5 subunit.[47]

Although expression of mutant $GABA_A$ receptor subunits in cultured neurons has been useful, it is still likely that the details of the pathophysiology are incomplete using this approach. With transfection, it is not possible to regulate the levels of subunit transcription and translation; therefore, the relative amounts of wild-type and mutant subunits and assembly partners will not be physiologically correct. In addition, the epilepsy mutations may have different actions in different brain regions or in different neuronal cell types and thus could modify neuronal network function differently in different nervous system locations. Ultimately, many of these questions must be answered by studying genetically modified animals as well as human patients who possess these mutations.

Thus far, only the *GABRG2*(R82Q) mutation knockin mouse has been made and studied.[40,48] Consistent with the reports in transfected heterologous cells and neurons, γ2(R82Q) subunit neuronal surface expression was reduced.[40] Unexpectedly, mIPSC amplitudes were reduced in cortical neurons, but not in thalamic relay or reticular nuclei neurons.[40] Interestingly, the *GABRG2*(R82Q) subunit mutation produced an epilepsy phenotype only when expressed at a critical time in development,[48] indicating a long-term impact of the presence of mutant *GABRG2*(R82Q) protein by unknown mechanisms.

Pathophysiological Mechanisms of $GABA_A$ Receptor Subunit Mutations

As noted in this chapter, the types and locations of mutations in $GABA_A$ receptor subunit genes vary substantially. Missense and nonsense mutations have been reported in coding and noncoding regions. In general, there is an association of the epilepsy syndrome type with the gene, as noted above (i.e., CAE and JME associated with *GABRB3* and *GABRA1* mutations and FS with or without CAE, GEFS+ and DS associated with *GABRG2* and *GABRD* mutations) and a loose correlation with the severity of the mutation and the epilepsy phenotype (Fig. 57–1). Missense mutations in *GABRB3*, *GABRG2*, and *GABRD* are associated with CAE, FS, JME, and GEFS+, while nonsense mutations in *GABRG2* are associated with FS, GEFS+, and Dravet syndrome. In addition, mutations in noncoding regions in *GABRG2* or *GABRB3* and mutations in the signal peptide of *GABRB3* are associated with less severe epilepsies, FS, and CAE, but mutations in the mature peptide are also associated with GEFS+ and

Dravet syndrome in addition to FS and CAE. Whether or not the presence of these correlations is due only to chance, because of the small number of reported mutations, or to fundamental differences in the loss of function of specific subunits as a result of specific types of mutations remains to be seen.

It is also clear that the mutations, while all different, have some common pathophysiological features. Distal N-terminal missense mutations (*GABRB3*[P11S, S15F, G32R] and *GABRG2*[R82Q, R177G]) are associated with the less severe epilepsies CAE and FS, and proximal N-terminal missense mutations (*GABD*[E177A, R220H]) are associated with the more severe epilepsy GEFS+. The cytoplasmic M3/M4 loop nonsense mutations *GABRG2*(Q390X, W429X) are associated with the more severe epilepsies GEFS+ and Dravet syndrome.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Hauser WA. The prevalence and incidence of convulsive disorders in children. *Epilepsia*. 1994;35(suppl 2):S1–S6.
2. Scheffer IE, Berkovic SF. Generalized epilepsy with febrile seizures plus: a genetic disorder with heterogeneous clinical phenotypes. *Brain*. 1997;120:479–490.
3. Berkovic SF, Harkin L, McMahon JM, Pelekanos JT, Zuberi SM, Wirrell EC, Gill DS, Iona X, Mulley JC, Scheffer IE. De-novo mutations of the sodium channel gene SCN1A in alleged vaccine encephalopathy: a retrospective study. *Lancet Neurol.* 2006;5:488–492.
4. Hirose S, Mitsudome A, Okada M, Kaneko S. Genetics of idiopathic epilepsies. *Epilepsia*. 2005;46(suppl 1): 38–43.
5. Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG Williams DA, Sutherland GR, Mulley JC, Scheffer IE,, Berkovic SF. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet*. 2001;28: 49–52.
6. Maljevic S, Krampfl K, Cobilanschi J, Tilgen N, Beyer S, Weber YG, Schlesinger F, Ursu D, Melzer W, Cossette P, Bufler J, Lerche H, Heils A. A mutation in the GABA(A) receptor alpha(1)-subunit is associated with absence epilepsy. *Ann Neurol*. 2006;59:983–987.
7. Macdonald RL, Olsen RW. $GABA_A$ receptor channels. *Annu Rev Neurosci*. 1994;17:569–602. 8. Baumann SW, Baur R, Sigel E, Forced subunit assembly in alpha 1, beta 2, gamma 2 $GABA_A$ receptors. Insight into the absolute arrangement. *J Biol Chem*. 2002;277: 46020–46025.
9. Macdonald RL, Rogawski MA. Cellular effects of antiepileptic drugs. In: Engel J, Pedley TA, eds. *Epilepsy: A Comprehensive Textbook*. 2nd ed. Philadelphia: Lippincott, Williams, & Wilkins; 2007:1433–1445.
10. Macdonald RL, Kang JQ, Gallagher MJ, Feng H-J. $GABA_A$ receptor mutations epilepsy associated with generalized epilepsies. *Adv Pharmacol*. 2006;54: 147–169.
11. Macdonald RL, Kang J-Q, Gallagher MJ. Mutations in $GABA_A$ receptor subunits associated with genetic epilepsies. *J Physiol*. 2010;588:1861–1869.
12. Tanaka M, Olsen RW, Medina MT, Schwartz E, Alonso ME, Duron RM, Castro-Ortega R, Martinez-Juarez IE, Pascual-Castroviejo E, Machado-Salas J, Silva R, Bailey JN, Bai D, Ochoa A, Jara-Prado A, Pineda G, Macdonald RL, Delgado-Escueta AV. Hyperglycosylation and reduced GABA currents of mutated GABRB3 polypeptide in remitting childhood absence epilepsy. *Am J Hum Genet*. 2008;82: 1249–1261.
13. Urak L, Feucht M, Fathi N, Hornik K, Fuchs K. A GABRB3 promoter haplotype associated with childhood absence epilepsy impairs transcriptional activity. *Hum Mol Genet*. 2006;15:2533–2541.
14. Delahanty RJ, Kang JQ, Brune CW, Kistner EO, Courchesne E, Cox NJ, Cook EH Jr, Macdonald RL, Sutcliffe JS. Maternal transmission of a rare GABRB3 signal peptide variant is associated with autism. *Mol Psychiatry*. 2011;:16:86–96.;
15. Lachance-Touchette P, Martin C, Poulin C, Gravel M, Carmant L, Cossette P. Screening of GABRB3 in French-Canadian families with idiopathic generalized epilepsy. *Epilepsia*. 2010;51(9):1894–1897.
16. Cossette P, Liu L, Brisebois K, Dong H, Lortie A, Vanasse M, Saint-Hilaire JM, Carmant L, Verner A, Lu WY, Wang YT, Rouleau GA. Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nat Genet*. 2002;31:184–189.
17. Dibbens LM, Feng HJ, Richards MC, Harkin LA, Hodgson BL, Scott D, Jenkins M, Petrou S, Sutherland GR, Scheffer IE, Berkovic SF, Macdonald RL, Mulley JC. GABRD encoding a protein for extra- or peri-synaptic $GABA_A$ receptors is a susceptibility locus for generalized epilepsies. *Hum Mol Genet*. 2004;13:1315–1319.
18. Audenaert D, Schwartz E, Claeys KG, Claes L, Deprez L, Suls A, Van Dyck T, Lagae L, Van Broeckhoven C, Macdonald RL, De Jonghe P. A novel GABRG2 mutation associated with febrile seizures. *Neurology*. 2006;67:687–690.
19. Kananura C, Haug K, Sander T, Runge U, Gu W, Hallmann K, Rebstock J, Heils A, Steinlein OK. A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. *Arch Neurol*. 2002;59:1137–1141.
20. Hirose S. A new paradigm of channelopathy in epilepsy syndromes: intracellular trafficking abnormality of channel molecules. *Epilepsy Res*. 2006;70(suppl 1): S206–S217.
21. Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A, Prud'homme JF, Baulac M, Brice A, Bruzzone R, LeGuern E. First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. *Nat Genet*. 2001;28:46–48.

22. Harkin LA, Bowser DN, Dibbens LM, Singh R, Phillips F, Wallace RH, Richards MC, Williams DA, Mulley JC, Berkovic SF, Scheffer IE, Petrou S. Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am J Hum Genet*. 2002;70:530–536.
23. Sun H, Zhang Y, Liang J, Liu X, Ma X, Wu H, Xu K, Qin J, Qi Y, Wu X. SCN1A, SCN1B, and GABRG2 gene mutation analysis in Chinese families with generalized epilepsy with febrile seizures plus. *J Hum Genet*. 2008;53:769–774.
24. Kirkness EF, Fraser CM. A strong promoter element is located between alternative exons of a gene encoding the human gamma-aminobutyric acid-type A receptor beta 3 subunit (GABRB3). *J Biol Chem*. 1993;268:4420–4428.
25. Maquat LE. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. *Nat Rev Mol Cell Biol*. 2004;5:89–99.
26. Kang JQ, Shen W, Macdonald RL. Two molecular pathways (NMD and ERAD) contribute to a genetic epilepsy associated with the $GABA_A$ receptor GABRA1 PTC mutation, 975delC, S326fs328X. *J Neurosci*. 2009;29:2833–2844.
27. Gallagher MJ, Ding L, Maheshwari A, Macdonald RL. The GABA-A receptor epilepsy mutation A322D inhibits transmembrane helix formation and causes proteasomal degradation. *Proc Natl Acad Sci USA*. 2007;104:12999–13004.
28. Gallagher MJ, Shen W, Song L, Macdonald RL. Endoplasmic reticulum retention and associated degradation of a $GABA_A$ receptor epilepsy mutation that inserts an aspartate in the M3 transmembrane segment of the alpha 1 subunit. *J Biol Chem*. 2005;280: 37995–38004.
29. Bradley CA, Taghibiglou C, Collingridge GL, Wang YT. Mechanisms involved in the reduction of $GABA_A$ receptor alpha1-subunit expression caused by the epilepsy mutation A322D in the trafficking-competent receptor. *J Biol Chem*. 2008;283:22043–22050.
30. Fisher JL. A mutation in the $GABA_A$ receptor alpha 1 subunit linked to human epilepsy affects channel gating properties. *Neuropharmacology*. 2004;46(5): 629–637.
31. Gallagher MJ, Song L, Arain F, Macdonald RL. The juvenile myoclonic epilepsy GABA(A) receptor alpha1 subunit mutation A322D produces a symmetrical, subunit position-dependent reduction. *J Neurosci*. 2004;24:5570–5578.
32. Ding L, Feng HJ, Macdonald RL, Botzolakis EJ, Hu N, Gallagher MJ. The GABA-A receptor alpha 1 subunit mutation A322D associated with autosomal dominant juvenile myoclonic epilepsy reduces the expression and alters the composition of wild type GABA-A receptors. *J Biol Chem.* 2010;285:26390–26405.
33. Marini C, Harkin LA, Wallace RH, Mulley JC, Scheffer IE,, Berkovic SF. Childhood absence epilepsy and febrile seizures: a family with a GABA(A) receptor mutation. *Brain*. 2003;126:230–240.
34. Hales TG, Tang H, Bollan KA, Johnson SJ, King DP, McDonald NA, Cheng A, Connolly CN.. The epilepsy mutation, gamma2(R43Q) disrupts a highly conserved inter-subunit contact site, perturbing the biogenesis of $GABA_A$ receptors. *Mol Cell Neurosci*. 2005;29:120–127.
35. Kang JQ, Macdonald RL. The $GABA_A$ receptor gamma2 subunit R43Q mutation linked to childhood absence epilepsy and febrile seizures causes retention of alpha1beta2gamma2S receptors in the endoplasmic reticulum. *J Neurosci*. 2004;24:8672–8677.
36. Sancar, Czajkowski. A $GABA_A$ receptor mutation linked to human epilepsy (γ2R43Q) impairs cell surface expression of $\alpha\beta\gamma$ receptors. *J Biol Chem*. 2004;279:47034–47039.
37. Frugier G, Coussen F, Giraud M-F, Odessa M-F, Emerit MB, Boue-Grabot E, Garret M. A γ2(R43Q) mutation, linked to epilepsy in humans, alters $GABA_A$ receptor assembly and modifies subunit composition on the cell surface. *J Biol Chem*. 2007;282:3819–3828.
38. Eugène E, Depienne C, Baulac S, Baulac M, Fritschy JM, Le Guern E, Miles R, Poncer JC. GABA(A) receptor gamma 2 subunit mutations linked to human epileptic syndromes differentially affect phasic and tonic inhibition. *J Neurosci*. 2007;27:14108–14116.
39. Bianchi MT, Song L, Zhang H, Macdonald RL. Two different mechanisms of disinhibition produced by $GABA_A$ receptor mutations linked to epilepsy in humans. *J Neurosci*. 2002;22:5321–5327.
40. Tan HO, Reid CA, Single FN, Davis PJ, Chiu C, Murphy S, Clarke AL, Dibbens L, Krestel H, Mulley JC, Jones MV, Seeburg PH, Sakmann B, Berkovic SF, Sprengel R, Petrou S. Reduced cortical inhibition in a mouse model of familial childhood absence epilepsy. *Proc Natl Acad Sci USA*. 2007;104:17536–17541.
41. Cope DW, Di Giovanni G, Fyson SJ, Orban G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V. Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. *Nat Med*. 2009;15:1392–1398.
42. Hales TG, Deeb TZ, Tang H, Bollan KA, King DP, Johnson SJ, Connolly CN. An asymmetric contribution to γ-aminobutyric type A receptor function of a conserved lysine within T2–3 of α1, β2, and γ2 subunits. *J Biol Chem*. 2006;25:1734–1743.
43. Kang JQ, Shen W, Macdonald RL. The GABRG2 mutation, Q351X, associated with GEFS+ has both loss of function and dominant-negative suppression. *J Neurosci*. 2009;29:2845–2856.
44. Feng HJ, Kang JQ, Song L, Dibbens L, Mulley J, Macdonald RL. Delta subunit susceptibility variants E177A and R220H associated with complex epilepsy alter channel gating and surface expression of alpha4-beta2delta $GABA_A$ receptors. *J Neurosci*. 2006;26: 1499–1506.
45. Kang J-Q, Shen W, Macdonald RL. Why does fever trigger seizures? $GABA_A$ receptor γ2 subunit mutations associated with idiopathic generalized epilepsies have temperature-dependent trafficking deficiencies. *J Neurosci*. 2006;26:2590–2597.
46. Jacob TC, Moss SJ, Jurd R. GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci*. 2008;9:331–343.
47. Loebrich S, Bahring R, Katsuno T, Tsukita S, Kneussel M. Activated radixin is essential for $GABA_A$ receptor alpha5 subunit anchoring at the actin cytoskeleton. *EMBO J*. 2006;25:987–999.
48. Chiu C, Reid C A, Tan HO, Davies PJ, Single FN, Koukoulas I, Berkovic SF, Tan SS, Sprengel R, Jones MV, Petrou S. Developmental impact of a familial $GABA_A$ receptor epilepsy mutation. *Ann Neurol*. 2008;64:284–293.

Chapter 58

Nicotinic Acetylcholine Receptor Mutations

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INTRODUCTION

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) was described relatively recently as a rare but distinct familial idiopathic epilepsy in 1994.[1] Contrary to this late nosological "discovery," ADNFLE became the first idiopathic epilepsy that disclosed its underlying molecular pathogeneses, namely, nicotinic acetylcholine receptor (nAChR) mutations. As nAChR is a ligand-gated ion channel, at the same time, this molecular discovery made ADNFLE the first epilepsy to be recognized as one of channelopathies, or diseases resulting from channel dysfunction.[2] The notion that epilepsy can be a channelopathy, which was deduced from the molecular discovery on ADNFLE, has certainly contributed to knowledge of the genetics of epilepsy.[3] Supporting the notion, in fact, are some 20 other epilepsies that were recognized subsequently as *channelepsies*, that is, compounders of channelopathy and epilepsy, indicating epilepsies resulting from channel dysfunctions.[4] Such channelepsies include, among others, benign familial neonatal seizures (BFNS) resulting from mutations of potassium channels, genetic epilepsy with febrile seizures plus (GEFS+), and Dravet syndrome due to sodium channel mutations.[3] Studies of ADNFLE in association with nAChR mutations have also established a methodology for investigating the pathomechanisms of other

epilepsies. Thus, dysfunction of ion channels due to genetic abnormalities can be evaluated readily in vitro, as ion channels can be reconstituted in oocytes or cultivated cells using relevant cDNA expression systems. These ion channel dysfunctions enable us to understand the molecular and neuroscientific pathomechanisms underlying epilepsies. Furthermore, they allow us to genetically engineer animal models of epilepsy that harbor mutations homologous to those identified in human epilepsies.[5–7] Such genetically engineered animal models of epilepsy represent molecular pathomechanisms distinct from those of conventional animal models. These genetically engineered animal models, which more accurately represent the pathomechanisms in the human brain, will open new avenues for treating epilepsies and developing novel measures for epilepsy based upon a better understanding of their molecular basis. In this chapter, we demonstrate the clinicophysiological characteristics of ADNFLE and summarize the current understanding of the molecular pathomechanisms of ADNFLE resulting from nAChR mutations.

CLINICAL SPECTRUM OF ADNFLE

ADNFLE is a literally partial epilepsy inherited as an autosomal dominant trait with penetrance as high as 90%. There is also sporadic nocturnal frontal lobe epilepsy (NFLE), which is nonfamilial. Its clinical manifestations are indistinguishable from those of ADNFLE.[8,9] In fact, nAChR mutations may be found as a cause of sporadic NFLE.[10] As their names suggest, predominant ADNFLE/NFLE seizures are symptomatically comparable to those of frontal lobe epilepsy, whereas seizures occur exclusively, if at all, during non–rapid eye movement (NREM) sleep. Thus, ADNFLE/NFLE attacks occur during light sleep or even during the daytime and are characterized by clusters of brief seizures. These are often stereotyped and brief (5 s to 5 min), sometimes preceded by an aura including sensory or psychic symptoms. Such seizures may start with grunts, gasping, or vocalization followed by hyperkinetic movements including dystonia and sleep-working. These brief seizures may sometimes evolve into secondary generalized tonic-clonic seizures. Retained awareness during seizures is common. To some degree, sleep disturbance may be associated with the seizures; hence, affected individuals often complain of daytime fatigue after frequent seizure attacks during the night. Although the seizure pattern of ADNFLE/NFLE per se is stereotypic, within a family the manifestations may vary considerably, ranging from simple arousal from sleep to dramatic, often bizarre, hyperkinetic events with tonic or dystonic features.

Because of their mostly nocturnal occurrence, the attacks are often misdiagnosed as nightmares or parasomnias. Based on the observation of a large number of patients, Provini and colleagues have identified three distinct types of ADNFLE/NFLE seizure: *paroxysmal arousals*, *paroxysmal dystonia*, and *episodic wandering*. Paroxysmal arousal is characterized by brief and sudden recurrent motor paroxysmal behavior. Paroxysmal dystonia is a motor attack with dystonic-dyskinetic features. Episodic wandering is stereotypical and agitated somnambulism.[8] These cardinal manifestations may be a key to distinguishing NFLE from other parasomnias.

ADNFLE/NFLE onset ranges from infancy to adulthood. About 80% of patients develop ADNFLE/NFLE in the first two decades of life; mean age of onset is 14 years (14 ± 10 years). The age of onset, however, is known to vary among affected individuals, even those in the same pedigree. ADNFLE/NFLE is lifelong but not progressive. As an individual reaches middle age, attacks may become milder and less frequent. Still, as with seizure manifestations, there are intra- and interfamilial variations in the natural course of ADNFLE/NFLE.[9]

Apart from minor differences, the ADNFLE/NFLE clinical phenotype is mostly uniform. No proven relationship between phenotypes and genotypes of nAChR mutations (i.e., phenotype–genotype correlation) has been elucidated. To date, however, it has been reported that two clinical phenotypes may change according to the genotypes of the nAChR mutations. One such change is the intellectual impairment or cognitive deficit; the other is sensitivity to antiepileptic drugs (AEDs).[8,11,12] While in most cases neurological examinations are normal and there is no intellectual impairment, it is possible that the S284L mutation of *CHRNA4* is associated with a cognitive deficit.[9,10,13] (Details will be discussed in the following sections.)

Genotypes of nAChR mutations may be associated with sensitivity to AEDs in ADNFLE/NFLE. Carbamazepine (CBZ), often in relatively low doses, provides remission to about 70% of individuals with ADNFLE/NFLE. Hence, CBZ remains the first-choice AED for the treatment of ADNFLE/NFLE; however, resistance to AEDs, present in about 30% of affected individuals, requires a trial of other appropriate AEDs. Individuals with ADNFLE/NFLE associated with the *CHRNA4*-S284L mutation may respond only partially to CBZ but be more responsive to zonisamide (ZNS) or benzodiazepine.[11,12,14]

EEG AND BRAIN IMAGING IN ADNFLE PATIENTS

In recent years, studies with video-polysomnographic monitoring have identified a distinct form of clear-cut attacks originating from epileptic foci located in the frontal lobe and emerging almost exclusively during sleep (mainly NREM stage 2/3). During wakefulness, the electroencephalogram (EEG) of almost all patients with ADNFLE/NFLE is within normal limits and the interictal EEG during sleep is also normal.[8,15] A few ictal EEG recordings display clear-cut epileptic activity (spikes and spikes and waves), and in a few subjects, diffuse (background flattening) or focal EEG activity may be recorded (rhythmic theta or delta activity prominent over the anterior quadrants).[8,15,16] The interictal EEGs show no abnormality in more than 50% of patients with ADNFLE.[17] Therefore, without simultaneous video and polygraphic recordings of seizures, the lack of EEG epileptic abnormalities does not exclude ADNFLE/NFLE. Ictal EEGs show that seizure activity is of frontal origin and occurs predominantly in stage 2 NREM sleep[11] (Fig. 58–1). The failure to reveal abnormalities in many patients may be due to the inaccessibility of the locus to scalp EEG recordings.[11,17] Subdural grid recordings, sphenoidal or zygomatic recordings, positron emission tomography (PET), and single photon emission computed tomography (SPECT) also confirmed the frontal origin of seizures in patients with ADNFLE.[11,18–20] As for the relation between arousal and seizures, seizure onset usually preceded arousal in patients with ADNFLE. Seizure onset often coincided with the recording of K-complexes in the EEG and were sometimes triggered by sound stimuli.[21] In Japanese ADNFLE patients, the seizures were not always associated with arousal from sleep, but long seizures sometimes produced arousal.[11] A careful medical and family history of nocturnal symptoms, video-EEG monitoring of seizures, and gene analysis are enormously helpful in making an accurate diagnosis of ADNFLE.

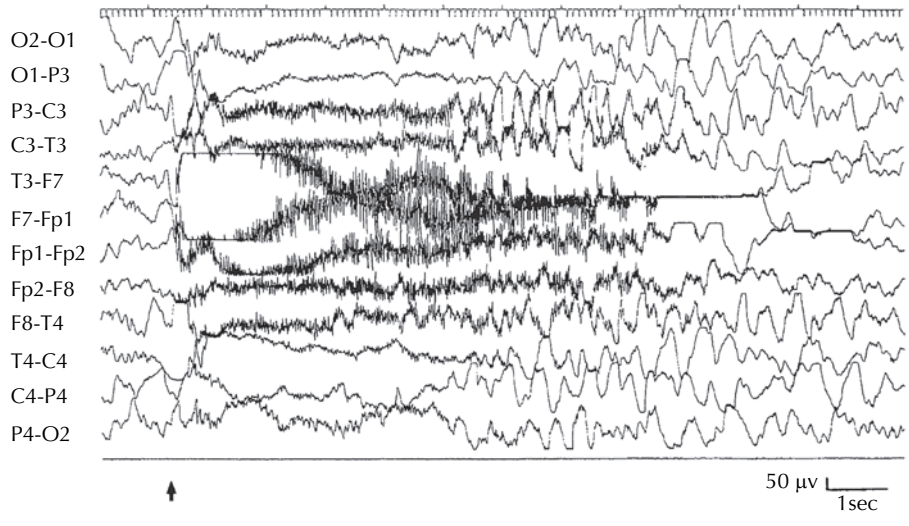

Figure 58–1. Ictal EEG of a Japanese ADNFLE patient with the mutation *CHRNA4*-S284L. Low-voltage spikes were initiated from the frontal region, gradually increased in amplitude, and were followed by high-voltage slow waves. Modified from ref. 11.

A Case

This boy's birth occurred via uncomplicated vaginal delivery after a normal pregnancy. At age 7 months, he had an afebrile general tonic convulsion during the daytime. The duration was 10 min. The interictal EEG showed no abnormality. He was diagnosed as having infantile convulsions and therefore was not treated with AEDs. At age 11 months, he began to have nocturnal seizures. His mother considered these to be nightmares. At age 1 year 3 months, he was admitted to a hospital for further evaluation. He had five to six seizures during stage 2 of NREM sleep at night and during midday naps. His seizures were sometimes provoked by movement and sound stimulation. The duration of each seizure was 10–15 s. During overnight video-EEG monitoring, five seizures were detected, and one of them was provoked by movement. All the seizures were identical in their clinical and electrographic manifestations. Magnetic resonance imaging (MRI) showed no abnormality, but SPECT detected low perfusion in both frontal lobes. Interictal EEG showed rare sporadic single spikes over the left frontopolar position. Ictal EEG showed low-voltage spikes initiating from the frontal region that gradually increased in amplitude and were followed by high-voltage slow waves (Fig. 58–1). The boy was treated with CBZ and cloxazolam. However, seizure control was not achieved.[11]

ADNFLE MUTATIONS IN nACHR SUBUNITS

ADNFLE, although classified as a partial epilepsy, turned out to be caused by mutations in genes that are ubiquitously expressed in brain. The first ADNFLE mutation had been found in 1995 in a large Australian family.[2] All affected family members available for mutation analysis carried a S280F amino acid exchange within the second transmembrane domain of *CHRNA4*. The same amino acid exchange was later found in families from Spain, Norway, and Scotland,[22–25] and additional ADNFLE mutations were identified within *CHRNA4* but also in the homologous gene *CHRNB2*.[10,23,26–32] All mutations identified so far are heterozygous. All but one of these mutations are amino acid exchanges that affect highly conserved residues. An exception is *CHRNA4*–865–873insGCT (also named 773ins3[27]), a three base pair insertion that adds a fourth leucine residue to a sequence stretch that already contains three consecutive leucines. The ADNFLE mutations cluster either in the second transmembrane (TM; *CHRNA4*) or in both the second and third TMs (*CHRNB2*). Mutations in the second TM, the major pore-forming part of each nAChR subunit, mostly affect amino acids that are part of the amino acid residue axis that rotates when agonists such as acetylcholine attach to the binding site, opening the ion channel. These mutations are therefore likely to interfere with the channel's kinetics. The pathomechanisms behind the mutations in the third TM are less well understood. It seems plausible that these mutated amino acids are in direct or indirect contact with residues in the second TM, because mutations in the third TM are also able to change the channel's opening and closing kinetics.[31] Recently, a single mutation described in a Chinese patient, C*HRNA4*-R336H (also named R308H[29]), was found downstream of TM2–3 in the second intracellular loop, a structure of unknown significance for receptor function.[29] This mutation has not yet been functionally characterized, and not enough clinical details are given in the original paper to allow a comparison with known ADNFLE phenotypes. A putative third ADNFLE gene is *CHRNA2*, a nAChR subunit gene that has been found mutated in a single Italian family with sleep-related epilepsy.[33]*CHRNA2* is one of the major nAChR subunit genes and is almost as ubiquitously expressed in brain as *CHRNA4* and *CHRNB2*. It nevertheless seems to be a much rarer cause of epilepsy than the latter two genes, as no additional mutations have been reported.[34] Overall, mutations of *CHRNA4*, *CHRNB2*, or *CHRNA2* are found in less than 20% of individuals with the ADNFLE/NFLE phenotypes,[35] suggesting genetic heterogeneity of ADNFLE/NFLE.

NEURONAL nACHRs

Neuronal AChRs are ligand-gated cationic channels composed of various combinations of five subunits arranged quasi-symmetrically around a central channel. They are encoded by nine α (α2–α10) and three β (β2–β4) subunit genes that show distinct patterns of expression

in both neuronal and nonneuronal tissues. Each subunit is composed of three different parts, which include the extracellular, cytoplasmatic, and transmembrane portions. The NH2 terminus is the largest extracellular domain, contains several glycosylation sites, and, in α subunits, contributes the main components of the high-affinity ligand binding sites that are located at the interface between neighboring subunits. This domain is important for receptor assembly and expresses the main immunogenic region. The four TM regions probably have a mixed α-helical/nonhelical secondary structure, with their helical segments forming both an inner ring (TM2) that shapes the pore and an outer shell (TM3–TM4) that shields the inner ring from membrane lipids. The different subunits assemble to build two classes of nAChRs: the homomeric or heteromeric α-bungarotoxin-sensitive receptors, which are composed solely of α subunits (α7–α10), and the heteromeric α-bungarotoxin-insensitive receptors, which consist of various combinations of α and β subunits (α2–α5, β2–β4). The mechanisms of nAChR activation, ion pore opening and closing, and desensitization are determined by highly conserved amino acid positions within the subunits. The function of the different neuronal nAChR subtypes depends on their subunit composition, their distribution within the brain, and their cellular location. Postsynaptic nAChRs often contribute to fast excitatory transmission, while preterminal and presynaptic nAChRs enhance neurotransmitter release (which, depending on the released transmitter, increases either inhibitory or excitatory synaptic impulses), and nonsynaptic nAChRs are likely to modulate neuronal excitability.[36] The nAChRs are involved in brain development and plasticity and participate in many different brain functions, including modulation of sensory inputs, locomotor activity, analgesia, attention, learning, memory, and reward mechanisms. It is therefore not surprising that genetic variants in nAChR subunit genes were found to be associated with different common disorders and behaviors, including Alzheimer's disease, schizophrenia, and smoking-related endophenotypes.[37–43] However, the only monogenic disorder so far known to be caused by high-penetrance mutations in neuronal nAChR is ADNFLE.

IN VITRO EXPRESSION OF ADNFLE MUTATIONS

The exact nAChR subtype compositions in different brain regions are not fully understood. This is due to the fact that various ligands are not subtype specific and highly specific antibodies are not yet available for all subunits. Probes for in situ hybridization on nAChR mRNAs are also not specific enough, which inevitably led to false-positive results.[44,45] The expression patterns for specific subunits may differ among species. Some nAChR subtypes, such as α2β3 and α4α6β2β3 receptor subtypes, are expressed in restricted regions of the brain and may have specific roles in brain function, while others, such as α4β2, are expressed ubiquitously and highly bind most nicotinic agonists.[46–49] To study the functional changes of mutated AChRs, whole-cell patch-clamp is often applied. Controversial data have been reported from in vitro expression studies, that is, loss of function and gain of function of receptors with *CHRNA4* mutations. Some reports indicated the acceleration of desensitization of receptors that leads to lowered Ca^{2+} permeability,[2,22,23,27,50] while increased affinity of receptors to Ach that leads to gain of function has also been reported.[10] Retardation of desensitization (V287L) or increased affinity to AChRs (V287M) has been reported for *CHRNB2* mutations.[51,52] In vitro expression studies showed that all mutations increase the receptors' sensitivity to acetylcholine, suggesting that a gain of function may be the basic mechanism behind ADNFLE.[28,34,52–54] However, the extent of the gain-of-function effect varies among mutations, and the mutated nAChRs display individual pathofunctional profiles for agonists such as nicotine or antiepileptic drugs like CBZ and ZNS. Most ADNFLE patients respond to CBZ, while patients with specific mutations, such as *CHRNA4*-S284L, are more likely to respond to ZNS.[11] A reduction of Ca^{2+} dependence may also be a common mechanism.[55] The functional involvement of the GABAergic system in the pathophysiology of ADNFLE deduced from animal models will be discussed separately.[5,7]

A recent study found that individuals with microdeletions of chromosomes affecting both *KCNQ2* and *CHRNA4* presented with only the BFNS phenotype and lacked ADNFLE/NFE semiology.[56] This finding, along with the

fact that all nAChR mutations identified in ADNFLE were heterozygous, suggests that the autosomal dominant inheritance mode of ADNFLE is attributable to the dominant negative or dominant positive effect, in accordance with the gain of function of mutant receptors. It is plausible that mutant subunits exert their effects as components of multimeric nAChRs.

ORIGIN OF SEIZURES

ADNFLE mutations in the nAChR subunits appear to modify the number and distribution of α4β2 nicotinic receptors in the living human brain. The distribution of α4β2 nicotinic receptors has been studied in nine ADNFLE patients carrying nicotinic receptor mutations (causative mutations): four patients with α4-S280F (numbered according to the reference sequence NP_000739.1; the mutation is also named S248F[2]); two with α4-S284L (also named S252L[26]); one with α4- T293I (also named T265I[28]); and two with β2-V287L) detected by a PET scan using [^{18}F]-F-A-85380, a radioligand with a high affinity for α4β2 nAChRs. This PET study demonstrated a regional nAChR density decrease in the prefrontal cortex, an observation consistent with partial epilepsy involving the frontal lobe. The increase in nAChR density in the mesencephalon suggests that these brain structures are involved in the pathophysiology of ADNFLE through the role of brainstem ascending cholinergic systems in arousal.[57] Electrophysiological studies of the receptors carrying the different mutations identified a common alteration in their properties corresponding to a gain of function. However, the precise mechanisms leading to the frontal lobe epilepsy remain elusive.

GENOTYPE–PHENOTYPE CORRELATIONS IN ADNFLE

The results of a pilot study in which 11 ADNFLE patients with different mutations were assessed by neuropsychological evaluation suggested that mild cognitive problems might be a common minor feature of the disorder.[57] However, even ADNFLE mutations that affect neighboring amino acid residues seem to differ significantly with respect to their major clinical features. Mutations such as *CHRNA4*-S280F and *CHRNB2*-V287M or V287L are usually associated with epilepsy as the sole major symptom, and additional neurological features have been reported in about 2% of patients with these mutations. This is especially obvious for *CHRNA4*-S280F, where, in 65 out of 67 known patients belonging to a total of five families with this mutation, the only reported major symptom is epilepsy.[2,22,23,25] In patients with mutations such as *CHRNA4*-S284L, *CHRNA4*-865–873insGCT (also named 776ins3[27]), or *CHRNB2*-I312M, additional major neurological features are reported in an average of 70% of patients. Comparison of unrelated families with the same ADNFLE mutation suggests that these additional major neurological features tend to be specific with each mutation. The mutation *CHRNA4*-S284L has been described in families from Japan, Korea, and Lebanon, and at least 11 of the 16 patients from these families are of borderline intelligence or mildly retarded.[10,13,26] Selective cognitive deficits that mostly affect verbal memory seem to be typical for the mutation *CHRNB2*-I312M and have been described in all four known patients from two families of different ethnic background.[13,32] Patients with *CHRNA4*–865–873insGCT show high comorbidity for psychiatric disorders such as the negative symptoms of schizophrenia.[24] The ethnic differences between families with the same mutation and the cosegregation of epilepsy and additional neurological features in multiplex pedigrees strongly suggest that the observed genotype–phenotype correlations are not coincidental, although so far, not enough families with these mutations are known to confirm this hypothesis. At first glance, it seems difficult to explain how mutations that affect amino acid residues located within the same functional domain, often only a few residues apart, could differ this drastically in their associated clinical features. It has been speculated that allosteric coupling between amino acids links functional elements within the nAChR that are far apart in the primary amino acid sequence.[58] In accordance with this theory, adjacent amino acids might differ with respect to the functional elements they interact with in other parts of the subunit. That would explain why functional

studies showed that ADNFLE mutations tend to differ from each other with respect to their biopharmacological profiles.[31] The nAChRs are involved in many important brain functions, and even such subtle functional differences are therefore likely to have an impact on the clinical phenotype.

ANIMAL MODELS OF ADNFLE: WHAT DID WE LEARN?

Several genetic animal models bearing mutant genes that have been identified in families with idiopathic epilepsy have been generated. However, to explore the pathogenesis of ADNFLE, it is important that the genetic epilepsy animal models fulfill the validation criteria for such models (face validity, construct validity, and predictive validity).[59,60] Face validity is the ability to fundamentally mimic the behavioral clinical characteristics of the disorder. Construct validity conforms to a theoretical rationale for the disorder. Predictive validity is the ability to predict previously unknown aspects of the behavior, genetics, and neurobiology of the disorder from the model.

Specific Criteria for Genetic Animal Models of ADNFLE

In general, the optimal animal model should mimic the human disorder in terms of etiology, biochemistry, symptomatology, and treatment.[61] The ADNFLE model should exhibit spontaneous epileptic seizures resembling paroxysmal arousals, nocturnal paroxysmal dystonia, and episodic nocturnal wandering, during NREM sleep. The interictal EEG may or may not be normal. The foci of ictal or interictal discharges should be localized in a frontal area or over the anterior quadrants. The age of onset of AFNFLE would ideally be around puberty. Carbamazepine might reduce both the frequency and complexity of seizures in model animals, as it does in more than 60% of patients with ADNFLE/NFLE, including those with *CHRNA4*-S280F and *CHRNA4*–865–873insGCT mutations.[14,15,62] However, CBZ efficacy is not obligate in animal models, just as some patients (individuals with *CHRNA4*-S284L,[10,11,63] *CHRNA4*-T293I,[28] *CHRNAB2*V287M,[52] and *CHRNAB2*-I312M[32]) are resistant to CBZ but susceptible to other AEDs, such as acetazolamide, benzodiazepine (BZP), topiramate, and zonisamide (ZNS), or are AED-resistant,[10,11,13–15,52,64] Among ADNFLE patients with the *CHRNA4*-S284L, mutation autism and mental retardation have been reported,[11,65] and ADNFLE patients with the *CHRNA4*–865–873insGCT mutation showed schizophrenia-like psychosis.[24] Therefore, behavioral studies should be part of the study design for animal models of ADNFLE. The knockin technique is probably appropriate to create genetic animal models of ADNFLE; however, the expression pattern and expression levels of the transgene in the genetic animal model must be confirmed because in some knockin techniques, promoters are used that do not occur naturally in rodents.

Validity of Genetic Animal Models of ADNFLE

The validity of four genetic animal models of ADNFLE is summarized in Table 58–1.[66] The phenotypes of the two strains of knockin mice with the *CHRNA4*-S280F mutation are different, since one strain exhibits spontaneous epileptic seizures (pS280F-KM), while the other does not (nS280F-KM).[5,6] The spontaneous epileptic seizures of pS280F-KM are observed during wakefulness but not during sleep.[5] They are characterized by paroxysmal onset and sudden termination. The ictal discharge of pS280F-KM shows complex patterns of spike-and-wave activity with a high-amplitude, low-frequency power spectrum; the background activities in the EEG of the pS284F-KM animal model show abnormal patterns characterized by a marked increase in δ-wave activity (0.5–4 Hz).[5] In contrast to pS284F-KM, no abnormalities are detected in the background EEG activities of nS284F-KM rat.[6]

The knockin mouse with *CHRNA4*–865–873insGCT (insL-KM) exhibits epileptic wandering-like spontaneous epileptic discharges with paroxysmal onset and sudden termination during wakefulness.[5] The EEG features of the insL-KM mice are similar to those of pS284F-KM. The ictal discharges of insL-KM show complex patterns of spike-and-wave activity with a high-amplitude, low-frequency power

Table 58–1 **Validity of Four ADNFLE Models**

Gene Mutation	insL	S280F	S280F	S284L
Genus (variety)	Mouse (C57BL/6J)	Mouse (C57BL/6J)	Mouse (C57BL/6J)	Rat (SD)
Technology	Knockin	Knockin	Knockin	Transgenic
Face validity				
Spontaneous seizure	Epileptic wandering during W	Epileptic wandering during W	No seizure	ADNFLE seizure during SWS
Background EEG	Slow-wave increase	Slow-wave increase	Normal	Normal
Construct validity				
Expression				
mRNA		Equal expression (whole brain)		Equal expression (Cor, Hip, Th)
protein			Equal expression (Cor, Hip, Th)	Equal expression (Cor, Hip, Th)
Predictive validity				
AED response			Suppressed by CBZ	Suppressed by ZNS
Nicotine-induced seizure	Potentiation generalized seizures	Potentiation generalized seizures	No difference	Potentiation partial seizures

Abbreviations: Cor, cortex; Hip, hippocampus; Th, thalamus; W, wakefulness.
Source: Data from ref. 66.

spectrum. The EEG of insL-KM during spontaneous seizures shows a more asymmetric and diffuse pattern than that of pS284F-KM. The EEG background activities of insL-KM also show a marked increase in δ-wave activity.[5]

The transgenic rat carrying the *CHRNA4*-S284L mutation (S284L-TG) exhibits the three distinct ADNFLE seizure characteristics during NREM sleep: paroxysmal arousals (brief episodes characterized by sudden frightened expression), paroxysmal dystonia (brief episodes of dystonic posturing), and epileptic wandering (episodes of longer duration ranging from 1 to 3 min, with head shaking accompanied by stereotyped paroxysmal ambulation and bizarre movements).[67] The onset of ictal discharges is synchronized with seizure behaviors. No abnormalities of background activities are observed in the EEG of the S284L-TG rat. The focus of both ictal and interictal discharges is the frontal sensorimotor cortex region. Usually the onset of interictal discharges starts after 6 weeks of age; however, the onset of spontaneous ADNFLE seizures is preceded by that of interictal discharges in the same transgenic rat. At 8 weeks of age, 90% of S284L-TG rats exhibit spontaneous seizures during NREM sleep. The characteristics of epileptic seizures of S284L transgenic rats are quite similar to those of ADNFLE patients with the same mutation.[67] The published phenotypic features of the above-described four genetic animal models of ADNFLE show that the face validity of the S248L-TG rat is adequate, whereas neither the pS280F-KM, nS280F-KM, nor insL-KM mice are suitable genetic animal models to study the clinical phenotype of ADNFLE. Both knockin mice, pS280F-KM and insL-KM, are considerably more sensitive to nicotine-induced seizures than wild-type mice. Indeed, in nicotine-induced seizure tests, these two types of knockin mice show a lower threshold dose of nicotine for generalized seizures with shorter latencies to seizure onset and exhibit longer seizure durations compared with their wild-type littermates.[5] Knockin mice with *CHRNA4*-S280F (nS280F-KM) that do not exhibit spontaneous epileptic seizures did not display a particularly different response in nicotine-induced seizure tests compared with wild-type mice. However, they developed ADNFLE-like epileptic wandering following administration of 1 mg/kg nicotine, and these seizures were prevented by supratherapeutic doses of CBZ.[6] No difference was observed between S284L-TG and non-TG littermates in the latency of nicotine-induced seizures. Nicotine-induced seizures in S284L-TG

transgenic rats were mainly partial seizures, whereas those in non-TG littermates were generalized seizures. In S284L-TG, subchronic administration (2 weeks) of diazepam and ZNS at therapeutically relevant doses reduced the frequency of interictal discharges by 43% and 48%, respectively, whereas at therapeutically relevant doses, CBZ had no effect on the frequency of interictal discharge.[67]

The results of the nicotine-induced seizure test showed different responses among the four genetic animal models of ADNFLE. However, the nS280F-KM and S284L-TG mice developed ADNFLE-like seizures, epileptic wandering (nS280F-KM and S284L-TG), paroxysmal arousal (S284L-TG), and paroxysmal dystonia (S284L-TG) in response to nicotine administration. Thus, the predictive validity of S284L-TG as a model for pharmacological effects in ADNFLE has been demonstrated, whereas neither that of nS280F-KM, pS252F-KM, nor insL-KM has been established.

The transgene promoter of S284L-TG is not a naturally occurring rodent promoter but a mammalian PDGF-β promoter that activates preferentially in certain brain tissues.[6,7] In situ hybridization using a nonselective probe (sensitive to both wild-type and S284L mutant *Chrna4* mRNA) showed no differences in the cerebral expression of *Chrna4* mRNA between non-TG and S284L-TG. The total amount of *Chrna4* mRNA (wild-type plus S284L *Chrna4*) in the frontal cortex of S284L-TG was almost equal to that in non-TG. The expression of wild-type versus S284L *Chrna4* was 45% versus 55%. In the focus region, there was no significant difference in the number of nAChR α4-immunopositive neurons between non-TG and S254L-TG. No distorted expression of wild-type or S284L *Chrna4* was observed in the cell populations of neurons, astrocytes, and oligodendrocytes. In spite of these findings, the wild-type *Chrna4* mRNA is predominantly expressed in the thalamus and cortex, whereas the S284L mutant *Chrna4* mRNA is mainly expressed in the cortex and thalamus. Laser-capture microdissection with single-cell reverse-transcription quantitative polymerase chain reaction (PCR) has demonstrated the lack of ectopic expression of the transgene in neurons and glial cells.

A question remains: why does the pS280F-KM but not the nS280F-KM exhibit spontaneous epileptic seizures? There is little or no information on the expression of mutant *Chrna4* gene or nAChR α4-subunit protein in the pS280F-KM, nS280F-KM, and insL-KM animals.[5,6] Although the promoter in these knockin techniques is a natural promoter, the mRNA and protein expression levels of S280F mutant *Chrna4* still need to be determined. At present, there are no suitable ADNFLE genetic animal models with construct validity. To study the pathogenesis and pathophysiology of ADNFLE, knockin mice with established construct validity or transgenic animal models with a natural promoter should be generated.[66]

Pathophysiology of ADNFLE

In spite of the limitations of ADNFLE model animals, it is interesting to note that S284L-TG rats showed attenuation of synaptic and extrasynaptic GABAergic transmission[1] and abnormal glutamate release during slow-wave sleep.[2] In mice bearing two engineered *chrna4* (S252F or +L263) mutations, the involvement of inhibitory synchronization of cortical networks via activation of mutant alpha4-containing nAChRs located on the presynaptic terminals and somatodendritic compartments of cortical GABAergic interneurons has been suggested.[5] Further analysis of the molecular biology of these abnormal neurotransmissions will help to uncover some of the mechanisms underlying ADNFLE, improving our understanding of its pathogenesis, epileptogenesis, and ictogenesis and ultimately leading to the development of new treatments.

KEY QUESTION: WHY DO nACHR MUTATIONS CAUSE EPILEPSY?

Most seizures are characterized by short bursts of abnormal neuronal activity that, by their origin and spreading pattern in brain, determine the clinical phenotype of the epilepsy. Seizures often arise from a state of hypersynchronization that causes groups of neurons or whole neuronal networks to fire simultaneously. In ADNFLE these episodes usually occur during NREM sleep phases[62] that can be subdivided into two main phases, one that consists of transient arousals and one that is characterized by

tonic activities in the EEG. The association between the seizures and NREM phases suggests that sleep-controlling brain structures are involved in the pathogenesis of ADNFLE. One of the main regulators of sleep is the central cholinergic system that controls both the circadian clock and the sleep-wake cycle. Important components of it are cholinergic neurons within the central reticular core of the brain, where the basic sleep-wake cycle is determined. From there, two major cholinergic cell groups are responsible for the cerebral activation that accompanies wakefulness and paradoxical sleep. One group of cells is located within the pontomesencephalic tegmentum, which projects rostrally into the nonspecific thalamocortical relay system. The other group of cells is located within the basal forebrain, which receives input from the brainstem reticular formation and projects as the ventral, extrathalamic relay upon the cerebral cortex.[67,68] Functional changes in nAChRs such as those induced by ADNFLE mutations might affect these cholinergic projections and cause aberrations in sleep stages that, for unknown reasons, render certain neuronal networks more vulnerable to sudden bursts of hyperactivity during NREM sleep. Such bursts of hyperactivity could have their origin in the gain-of-function effect caused by ADNFLE mutations in nAChRs located on GABAergic interneurons. At least two mechanisms are discussed that are both able to explain how changes in GABAergic interneuron activity might play a central role in seizure generation. The older hypothesis postulates that seizures are the result of reduced inhibition that causes an increase in excitatory neuronal activity. More recently, it has been shown that the opposite mechanism is at least as plausible, and is the one most likely involved in seizures caused by nAChR subunits. The basic principle underlying this mechanism is an enhancement of neuronal synchrony by inhibition. It has been shown that pyramidal cells connected to the same GABAergic interneuron synchronize their firing when released from the interneuron's inhibitory effect.[69] Interneurons innervating layer 2/3 pyramidal cells are known to carry $\alpha 4\beta 2$ nAChRs. Triggered by the gain-of-function effect ADNFLE mutations exhibit on these receptors, they could activate larger numbers of GABAergic interneurons that, in turn, could easily synchronize enough pyramidal cells to change local cortical activity from synchronization to hypersynchronization. Spreading of the hypersynchronization into the motor control area would then be likely to cause the types of seizures typically associated with ADNFLE. Such a mechanism, in which a gain of function of certain nAChR subunits causes hypersynchronization by increasing GABAergic inhibition, is supported by some of the above-described animal models. Gamma-aminobutyric acid antagonists such as picrotoxin have been shown to normalize the EEG and suppress spontaneous seizures in genetically altered pS280F-KM mice.[5] Nevertheless, many details of epileptogenesis in ADNFLE remain unknown and are the subject of further studies.

CONCLUSIONS

The nAChR mutations identified in ADNFLE/NFLE have increased our understanding of the pathogeneses not only of these unique and relatively rare epilepsies but also of other epilepsies. Our understanding should open new avenues to developing novel therapies against epilepsy based upon molecular pathomechanisms. Consecutive studies of ADNFLE/NFLE in association with nAChR mutations have for the first time provided compelling evidence that genetic predispositions closely associate with the pathogenesis of epilepsy on the molecular level. Thanks to the breakthrough in ADNFLE/NFLE research, novel methodologies have been established in epileptology. They range from evaluations of channel mutations in vitro to genetically engineered animal models bearing the mutations identified in human epilepsy. Continuing studies utilizing such methodologies should delineate all the pathomechanisms of single mutations or genetic predispositions that result in epilepsy. These could not have been learned through clinico-electrophysiological studies alone. Once the molecular pathomechanisms of epilepsy are revealed, it should be possible to cure epilepsy completely by blocking part of the pathomechanisms. Since the discovery of nAChR mutations in ADNFLE/NFLE, we have now reached the point where novel therapies based upon the molecular pathomechanisms of epilepsy can be designed. Such therapies will bring relief to individuals suffering from epilepsy and the adverse effects of conventional AEDs.

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The authors declare that no competing financial or nonfinancial interests exist.

REFERENCES

1. Scheffer IE, Bhatia KP, Lopes-Cendes I, et al. Autosomal dominant frontal epilepsy misdiagnosed as sleep disorder. *Lancet*. 1994;343:515–517.
2. Steinlein OK, Mulley JC, Propping P, et al. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet*. 1995;11:201–203.
3. Hirose S, Okada M, Kaneko S, et al. Are some idiopathic epilepsies disorders of ion channels? A working hypothesis. *Epilepsy Res*. 2000;41:191–204.
4. Lossin C. A catalog of SCN1A variants. *Brain Dev*. 2009;31:114–130.
5. Klaassen A, Glykys J, Maguire J, et al. Seizures and enhanced cortical GABAergic inhibition in two mouse models of human autosomal dominant nocturnal frontal lobe epilepsy. *Proc Natl Acad Sci USA*. 2006;103:19152–19157.
6. Teper Y, Whyte D, Cahir E, et al. Nicotine-induced dystonic arousal complex in a mouse line harboring a human autosomal dominant nocturnal frontal lobe epilepsy mutation. *J Neurosci.* 2007:27:10128–10142.
7. Zhu G, Okada M, Yoshida S, et al. Rats harboring S284L Chrna4 mutation show attenuation of synaptic and extrasynaptic GABAergic transmission and exhibit the nocturnal frontal lobe epilepsy phenotype. *J Neurosci*. 2008;28:12465–12476.
8. Provini F, Plazzi G, Tinuper P, et al. Nocturnal frontal lobe epilepsy. A clinical and polygraphic overview of 100 consecutive cases. *Brain*. 1999;122:1017–1031.
9. Hirose S, Kurahashi H. Autosomal dominant nocturnal frontal lobe epilepsy (April 2010). In: Gene Reviews at GeneTests: Medical Genetics Information Resource [database online]. Copyright University of Washington, Seattle, 1997–2010. Available at http://www.genetests.org.
10. Phillips HA, Marini C, Scheffer IE, et al. A de novo mutation in sporadic nocturnal frontal lobe epilepsy. *Ann Neurol*. 2000;48:264–267.
11. Ito M, Kobayashi K, Fujii T, et al. Electroclinical picture of autosomal dominant nocturnal frontal lobe epilepsy in a Japanese family. *Epilepsia*. 2000;41:52–58.
12. Combi R, Dalprà L, Tenchini ML, et al. Autosomal dominant nocturnal frontal lobe epilepsy—a critical overview. *J Neurol*. 2004;251:923–934.
13. Cho YW, Motamedi GK, Laufenberg I, et al. A Korean kindred with autosomal dominant nocturnal frontal lobe epilepsy and mental retardation. *Arch Neurol*. 2003;60:1625–1632.
14. Provini F, Plazzi G, Montagna P, et al. The wide clinical spectrum of nocturnal frontal lobe epilepsy. *Sleep Med Rev*. 2000;4:375–386.
15. Oldani A, Zucconi M, Asselta R, et al. Autosomal dominant nocturnal frontal lobe epilepsy. A videopolysomnographic and genetic appraisal of 40 patients and delineation of the epileptic syndrome. *Brain*. 1998;121:205–223.
16. Oldani A, Zucconi M, Ferini-Strambi L, et al. Autosomal dominant nocturnal frontal lobe epilepsy: electroclinical picture. *Epilepsia*. 1996;37:964–76.
17. Marini C, Guerrini R. The role of the nicotinic acetylcholine receptors in sleep-related epilepsy. *Biochem Pharmacol*. 2007;74:1308–1314.
18. Lombroso CT. Pavor nocturnus of proven epileptic origin. *Epilepsia*. 2000;41:1221–1226.
19. Tinuper P, Cerullo A, Cirignotta F, et al. Nocturnal paroxysmal dystonia with short-lasting attacks: three cases with evidence for an epileptic frontal lobe origin of seizures. *Epilepsia*. 1990;31:549–556.
20. Hayman M, Scheffer IE, Chinvarun Y, et al. Autosomal dominant nocturnal frontal lobe epilepsy: demonstration of focal frontal onset and intrafamilial variation. *Neurology*. 1997;49:969–975.
21. El Helou J, Navarro V, Depienne C, et al. K-complex-induced seizures in autosomal dominant nocturnal frontal lobe epilepsy. *Clin Neurophysiol*. 2008;119:2201–2204.
22. Sáenz A, Galán J, Caloustian C, et al. Autosomal dominant nocturnal frontal lobe epilepsy in a Spanish family with a Ser252Phe mutation in the CHRNA4 gene. *Arch Neurol*. 1999;56:1004–1009.
23. Steinlein OK, Stoodt J, Mulley J, et al. Independent occurrence of the CHRNA4 Ser248Phe mutation in a Norwegian family with nocturnal frontal lobe epilepsy. *Epilepsia.* 2000;41:529–535.

24. Magnusson A, Stordal E, Brodtkorb E, et al. Schizophrenia, psychotic illness and other psychiatric symptoms in families with autosomal dominant nocturnal frontal lobe epilepsy caused by different mutations. *Psychiatr Genet*. 2003;13:91–95.
25. McLellan A, Phillips HA, Rittey C, et al. Phenotypic comparison of two Scottish families with mutations in different genes causing autosomal dominant nocturnal frontal lobe epilepsy. *Epilepsia*. 2003;44:613–617.
26. Hirose S, Iwata H, Akiyoshi H, et al. A novel mutation of CHRNA4 responsible for autosomal dominant nocturnal frontal lobe epilepsy. *Neurology*. 1999;53: 1749–1753.
27. Steinlein OK, Magnusson A, Stoodt J, et al. An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy. *Hum Mol Genet*. 1997;6:943–947.
28. Leniger T, Kananura C, Hufnagel A, et al. A new Chrna4 mutation with low penetrance in nocturnal frontal lobe epilepsy. *Epilepsia*. 2003;44:981–985.
29. Chen Y, Wu L, Fang Y, et al. A novel mutation of the nicotinic acetylcholine receptor gene CHRNA4 in sporadic nocturnal frontal lobe epilepsy. *Epilepsy Res*. 2009;83:152–156.
30. De Fusco M, Becchetti A, Patrignani A, et al. The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy. *Nat Genet*. 2000;26:275–276.
31. Hoda JC, Gu W, Friedli M, et al. Human nocturnal frontal lobe epilepsy: pharmacogenomic profiles of pathogenic nicotinic acetylcholine receptor beta-subunit mutations outside the ion channel pore. *Mol Pharmacol*. 2008;74:379–391.
32. Bertrand D, Elmslie F, Hughes E, et al. The CHRNB2 mutation I312M is associated with epilepsy and distinct memory deficits. *Neurobiol Dis*. 2005;20:799–804.
33. Aridon P, Marini C, Di Resta C, et al. Increased sensitivity of the neuronal nicotinic receptor alpha 2 subunit causes familial epilepsy with nocturnal wandering and ictal fear. *Am J Hum Genet*. 2006;79:342–350.
34. Gu W, Bertrand D, Steinlein OK. A major role of the nicotinic acetylcholine receptor gene CHRNA2 in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is unlikely. *Neurosci Lett*. 2007;422: 74–76.
35. Ottman R, Hirose S, Jain S, et al. Genetic testing in the epilepsies—report of the ILAE Genetics Commission. *Epilepsia*. 2010;51:655–670.
36. Dani JA, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol*. 2007;47:699–729.
37. Liu JZ, Tozzi F, Waterworth DM, et al. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. *Nat Genet*. 2010;42:436–440.
38. The Tobacco and Genetics Consortium. Genome-wide meta-analyses identify multiple loci associated with smoking behaviour. *Nat Genet*. 2010;42:441–447.
39. Thorgeirsson TE, Gudbjartsson DF, Surakka I, et al. Sequence variants at CHRNB3-CHRNA6 and CYP2A6 affect smoking behavior. *Nat Genet*. 2010;42:448–453.
40. Fehér A, Juhász A, Rimanóczy A, et al. Association between a genetic variant of the alpha-7 nicotinic acetylcholine receptor subunit and four types of dementia. *Dement Geriatr Cogn Disord*. 2009;28:56–62.
41. Carson R, Craig D, Hart D, et al. Genetic variation in the alpha 7 nicotinic acetylcholine receptor is associated with delusional symptoms in Alzheimer's disease. *Neuromol Med*. 2008;10:377–384.
42. De Luca V, Voineskos S, Wong G, et al. Genetic interaction between alpha4 and beta2 subunits of high affinity nicotinic receptor: analysis in schizophrenia. *Exp Brain Res*. 2006;174:292–296.
43. Leonard S, Gault J, Hopkins J, et al. Association of promoter variants in the alpha7 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia. *Arch Gen Psychiatry*. 2002;59: 1085–1096.
44. Jones IW, Wonnacott S. Why doesn't nicotinic ACh receptor immunoreactivity knock out? *Trends Neurosci*. 2005;28:343–345.
45. Moser N, Mechawar N, Jones I, et al. Evaluating the suitability of nicotinic acetylcholine receptor antibodies for standard immunodetection procedures. *J Neurochem*. 2007;102:479–492.
46. Gotti C, Moretti M, Gaimarri A, et al. Heterogeneity and complexity of native brain nicotinic receptors. *Biochem Pharmacol*. 2007;74:1102–1111.
47. Gotti C, Guiducci S, Tedesco V, et al. Nicotinic acetylcholine receptors in the mesolimbic pathway: primary role of ventral tegmental area alpha6beta2* receptors in mediating systemic nicotine effects on dopamine release, locomotion, and reinforcement. *J Neurosci*. 2010;30:5311–5325.
48. Vincler MA, Eisenach JC. Immunocytochemical localization of the alpha3, alpha4, alpha5, alpha7, beta2, beta3 and beta4 nicotinic acetylcholine receptor subunits in the locus coeruleus of the rat. *Brain Res*. 2003;974:25–36.
49. Quik M, Polonskaya Y, Gillespie A, et al. Localization of nicotinic receptor subunit mRNAs in monkey brain by in situ hybridization. *J Comp Neurol*. 2000;425: 58–69.
50. Matsushima N, Hirose S, Iwata H, et al. Mutation (Ser284Leu) of neuronal nicotinic acetylcholine receptor alpha 4 subunit associated with frontal lobe epilepsy causes faster desensitization of the rat receptor expressed in oocytes. *Epilepsy Res*. 2002;48:181–186.
51. Gambardella A, Annesi G, De Fusco M, et al. A new locus for autosomal dominant nocturnal frontal lobe epilepsy maps to chromosome 1. *Neurology*. 2000;55: 1467–1471.
52. Phillips HA, Favre I, Kirkpatrick M, et al. CHRNB2 is the second acetylcholine receptor subunit associated with autosomal dominant nocturnal frontal lobe epilepsy. *Am J Hum Genet*. 2001;68:225–231.
53. Moulard B, Picard F, le Hellard S, et al. Ion channel variation causes epilepsies. *Brain Res Brain Res Rev*. 2001;36:275–284.
54. Bertrand D, Picard F, Le Hellard S, et al. How mutations in the nAChRs can cause ADNFLE epilepsy. *Epilepsia*. 2002;43(suppl 5):112–122.
55. Rodrigues-Pinguet N, Jia L, Li M, et al. Five ADNFLE mutations reduce the Ca^{2+} dependence of the mammalian a4b2 acetylcholine response. *J Physiol* (*Lond*). 2003;550:11–26.
56. Kurahashi H, Wang JW, Ishii A, et al. Deletions involving both KCNQ2 and CHRNA4 present with benign familial neonatal seizures. *Neurology*. 2009;73(15): 1214–1217.
57. Picard F, Bruel D, Servent E, et al. In vivo PET study of the mutated nicotinic receptors using [18F]-A-85380 in patients with autosomal dominant nocturnal

frontal lobe epilepsy (ADNFLE). Clin Neurophysiol. 2009;120(9):e192–e193.
58. Taly A, Changeux JP. Functional organization and conformational dynamics of the nicotinic receptor: a plausible structural interpretation of myasthenic mutations. *Ann NY Acad Sci*. 2008;1132:42–52.
59. Sarter M, Hagan J, Dudchenko P. Behavioral screening for cognition enhancers: from indiscriminate to valid testing: part I. *Psychopharmacology* (*Berl*). 1992;107: 144–159.
60. Sarter M, Hagan J, Dudchenko P. Behavioral screening for cognition enhancers: from indiscriminate to valid testing: part II. *Psychopharmacology* (*Berl*). 1992;107:461–473.
61. McKinney WT Jr, Bunney WE Jr. Animal model of depression. I. Review of evidence: implications for research. *Arch Gen Psychiatry*. 1969;21:240–248.
62. Scheffer IE, Bhatia KP, Lopes-Cendes I, et al. Autosomal dominant nocturnal frontal lobe epilepsy. A distinctive clinical disorder. *Brain*. 1995;118:61–73.
63. Rozycka A, Skorupska E, Kostyrko A, et al. Evidence for S284L mutation of the CHRNA4 in a white family with autosomal dominant nocturnal frontal lobe epilepsy. *Epilepsia*. 2003;44:1113–1117.
64. Varadkar S, Duncan JS, Cross JH. Acetazolamide and autosomal dominant nocturnal frontal lobe epilepsy. *Epilepsia*. 2003;44:986–987.
65. Cho YW, Motamedi GK, Laufenberg I, et al. A Korean kindred with autosomal dominant nocturnal frontal lobe epilepsy and mental retardation. *Arch Neurol*. 2003;60:1625–1632.
66. Okada M, Zhu G, Yoshida S, et al. Validation criteria for genetic animal models of epilepsy model. *Epilepsy Seizure*. 2010;3:109–120.
67. Haxhiu MA, Mack SO, Wilson CG, et al. Sleep networks and the anatomic and physiologic connections with respiratory control. *Front Biosci*. 2003;8: 946–962.
68. Yang JJ, Wang YT, Cheng PC, et al. Cholinergic modulation of neuronal excitability in the rat suprachiasmatic nucleus. *J Neurophysiol*. 2010;103:1397–1409.
69. Cobb SR, Buhl EH, Halasy K, et al. Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature*. 1995;378:75–78.

Chapter 59

Gene Interactions and Modifiers in Epilepsy

Miriam H. Meisler
Janelle E. O'Brien

INTRODUCTION

During the past decade, there has been great progress in identifying the genetic basis for human seizure disorders, based largely on the application of research strategies for identifying the genes responsible for monogenic disorders.[1] Positional cloning has been used when large pedigrees with clear-cut dominant or recessive inheritance patterns are available. This involves linkage analysis with molecular markers throughout the genome to define a target chromosome region, followed by sequencing genes within the region to detect the causal mutation. The second approach has been to select a *candidate gene* based on its biological function or its known role in related disorders or animal models, and then to test for mutations by sequencing the gene in a group of unrelated individuals with epilepsy.

In spite of substantial progress, the genetic factors involved in most cases of human epilepsy are not known. One likely explanation is that mutations or variants in two or more genes, with interacting effects, are involved in many types of epilepsy. Identification of multiple

interacting genes is more difficult than identification of single genes, and this field is in an early stage of development. While indirect evidence suggests that multigenic causality may be very common in human epilepsy, few specific examples have been well documented. In this chapter, we describe recent evidence for gene interaction in human epilepsy and approaches to identifying genetic modifiers that influence disease in view of the unanticipated level of rare variation in protein coding sequences. Understanding the interaction between variants in an individual genome is likely to be a major focus of research in the coming years.

Many of the examples discussed here involve genes encoding voltage-gated sodium channels. The structure of this nine-member gene family and its role in epilepsy has recently been reviewed.[2] The most common known genetic cause of epilepsy is mutation of the neuronal sodium channel *SCN1A*. The role of this gene was first identified by positional cloning based on linkage analysis that mapped the epilepsy gene to chromosome 2q24 in two large pedigrees with generalized epilepsy with febrile seizures plus (GEFS+).[3,4] Based on our work with sodium channels and epilepsy in the mouse,[5] we sequenced *SCN1A* as a positional candidate gene in these two families and identified two causal missense mutations.[6] Subsequent functional analysis demonstrated biophysical defects associated with both mutated alleleles.[7] After the positional cloning of *SCN1A* in the GEFS+ families, it was sequenced as a candidate gene for Dravet syndrome, also known as *severe myoclonic epilepsy of infancy* (SMEI), a severe, progressive seizure syndrome that occurs as a sporadic trait in patients without a family history. Remarkably, sporadic mutations of *SCN1A* were identified in a majority of the Dravet syndrome patients tested.[8] Dravet syndrome and GEFS+ represent the extremes of a spectrum of *SCN1A*-associated disorders. Approximately 10% of GEFS+ is accounted for by heterozygous missense mutations of *SCN1A*, and >80% of patients with Dravet syndrome carry heterozygous mutations in *SCN1A* that include truncation mutations, deletions, and missense mutations. More than half of the mutations in Dravet syndrome cause loss of channel function,[9,10] demonstrating haploinsufficiency of *SCN1A*.Spontaneous seizures are also seen in heterozygous mouse models with inactivating mutations of *Scn1a*.[11,12] More than 700 different mutations of *SCN1A* have been identified in patients with Dravet syndrome; their molecular and clinical features are compiled in two recently developed databases.[13,14]

Analysis of mouse models provides an experimental system for identification of epilepsy modifier genes. When the same epilepsy mutation is bred onto multiple strains of inbred mice, the severity of the disorder often varies dramatically due to genetic differences between inbred strains. If the effect of any one modifier gene is sufficiently large, it may be identified by positional cloning in crosses between the inbred strains.[15] We have included examples of the use of mouse genetics to detect and analyze gene interaction in seizure disorders.

WITHIN-FAMILY HETEROGENEITY MAY REFLECT THE SEGREGATION OF GENETIC MODIFIERS

It is well known to clinicians that family members with identical genotypes at a disease locus may nonetheless exhibit very different clinical courses. One source of phenotypic heterogeneity may be the independent segregation of genetic variants at other loci, so-called *modifier genes*, that exacerbate or ameliorate the effect of the primary mutation.

Variability in Families with GEFS+

An example of intra-familial variation is provided by the GEFS+ pedigree in Fig. 59–1. Two categories of clinical phenotype are found in this pedigree. Seven affected individuals exhibited mild childhood febrile seizures with no progression, while five affected individuals had febrile seizures that progressed to adult epilepsy.[3] The sodium channel mutation *SCN1A*-Thr875Met was identified in all 12 affected individuals.[6] This type of intrafamily variation is suggestive of the segregation of a second variant at a modifier locus. If the pedigree is sufficiently large, both primary and modifier genes can be mapped.[16]

Mildly Affected Carriers in a Family with Dravet Syndrome

Deletion or inactivation of sodium channel *SCN1A* usually results in Dravet syndrome,

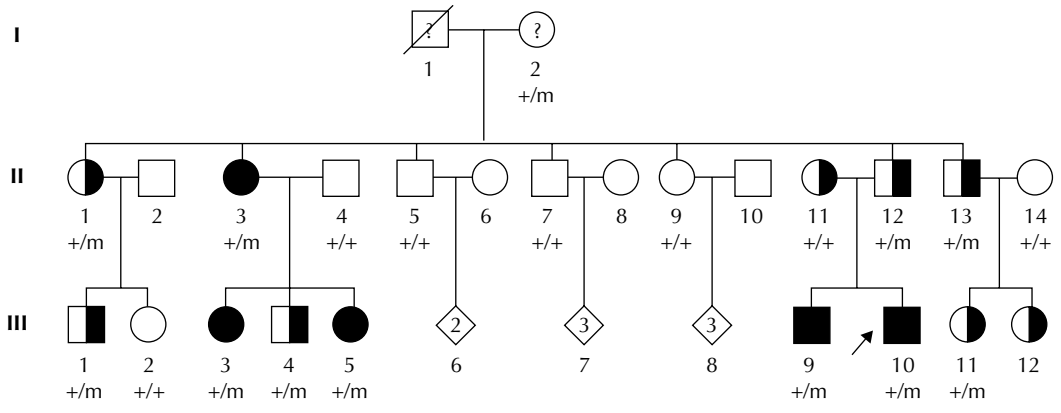

Figure 59–1. Two distinct phenotypes in affected heterozygous individuals from a GEFS+ family. The *SCN1A* mutation Thr875Met cosegregates with GEFS+; affected individuals exhibit febrile seizures only (half-filled symbols) or febrile seizures progressing to epilepsy (filled symbols). +, wild type; m, mutant; possible seizures not confirmed by relatives or medical records. Modified from ref. 5.

a haploinsufficiency syndrome that includes severe progressive seizures and impaired cognition.[10, 17] Most affected individuals are sporadic cases without affected family members, and are unable to live independently or transmit the defect. However, in 2010, Suls et al. described a four-generation Bulgarian family in which a complete deletion of the *SCN1A* gene was transmitted through three generations.[18] Two family members with the deletion exhibit typical progressive Dravet syndrome, while two others who transmitted the deletion have moderate epilepsy and are literate and living independently. The authors suggest that the differences in severity between family members may reflect the presence of one or more genetic modifiers.

Parental Mosaicism in One-Generation Families with Multiplex Dravet Syndrome

The transmission of Dravet syndrome from an unaffected parent to affected offspring has been observed in approximately 10% of cases. The parent in these families must carry the *SCN1A* mutation but does not exhibit disease. The possibility that these individuals carry a protective variant in a modifier gene has been considered. However, a different explanation emerged in a recent study describing genetic mosaicism in the transmitting parent.[19] To test for genetic mosaicism, a quantitative, allele-specific polymerase chain reaction (PCR) assay for the *SCN1A* mutation was carried out on DNA isolated from the parent's blood. The proportion of mutant *SCN1A* allele in different transmitting parents was found to vary from 0.04% to 85% of the dose expected in a normal heterozygote, which would be 50%. In one case, a reduced dosage was also observed in sperm. The most likely explanation is that the mutations arose de novo during the embryonic development of the parent, resulting in mosaicism of somatic and germline tissues. Assuming that expression of the mutant allele would also be reduced in the brain of mosaic individuals, that could account for the parents' mild clinical condition compared to that of their offspring. Mosaicism was demonstrated for 12 of the 19 cases of transmission of Dravet syndrome examined in this study.

LINKAGE EVIDENCE FOR DIGENIC INTERACTION IN HUMAN EPILEPSY

In rare families, linkage mapping for positional cloning identifies more than one chromosome region related to the epilepsy phenotype. These large families offer the potential for identification of specific pairs of interacting loci. Although the genes have not yet been identified, the linkage data strongly support a two-gene-interaction mechanism of epilepsy in these families.

Digenic Inheritance of Febrile Seizures with Temporal Lobe Epilepsy

Linkage analysis was carried out on a four-generation French family segregating febrile convulsions accompanied by temporal lobe epilepsy.[20] Family members included eight affected individuals with febrile seizures that progressed to afebrile epilepsy, one obligate unaffected carrier, and nine unaffected family members. A genome-wide scan using microsatellite markers demonstrated linkage to two chromosome regions, 1q25–31 and 18qter, with significant LOD scores of 2.3 and 3.0, respectively. All of the affected individuals carried the disease-related haplotype in both of these chromosome regions, and none inherited the disease-related haplotype at one region only, strongly indicating digenic inheritance with a requirement for the mutant genes at both loci. The same investigators studied another four-generation pedigree segregating febrile seizures in combination with temporal lobe epilepsy or childhood absence epilepsy.[21] In this family, the genome-wide linkage scan detected linkage signals on chromosomes 3p and 18p. As above, all patients with febrile seizures and epilepsy shared a common haplotype in both chromosome regions. (The lack of common linked regions in these two families is an indication of the degree of genetic heterogeneity underlying inherited epilepsy.)

Digenic Inheritance of Epilepsy in Families with Light Sensitivity and Myoclonic Epilepsy

Pinto and colleagues focused a genome-wide linkage study on 19 families with photosensitive epilepsy. The photoparoxysmal response (PPR) is defined by an abnormal electroencephalographic (EEG) response to intermittant photic stimulation. Pinto et al. studied 16 unrelated families with multiple affected members who demonstrated both PPR and epilepsy.[22] Two linkage signals of comparable strength were detected on chromosomes 7q32 and 16p13. A subsequent two-locus linkage analysis supported a multiplicative epistasis model in which each locus is necessary but not sufficient to generate the observed phenotypes.[23]

INTERACTING ION CHANNEL MUTATIONS IN MOUSE MODELS

Since the firing patterns of neurons directly reflect the overall identities and levels of their ion channel expression, it is intuitively obvious that the presence of multiple ion channel variants in the same neuron could have a combinatorial effect on firing properties. Genetic interaction between ion channel mutations would be among the most straightforward to interpret. Large-scale sequencing of 237 ion channel genes in several hundred epilepsy patients and controls revealed a comparable frequency of ion channel variants in the two groups.[23a] Studies in the mouse provide a system for testing the effects of combining two or more ion channel mutations, with the possibility of examining the effects on neuronal activity as well as seizure phenotypes. The examples described below provide proof of principle for the types of ion channel interactions that may also contribute to human epilepsy.

Mouse *Scn2a* and *Kcnq2*

Mutations in human sodium channel *SCN2A* and potassium channel *KCNQ2* can each cause inherited human epilepsy, but families with variants in both genes have not been described. To test their possible interaction, Kearney et al.[24] generated mice that were double heterozygotes for a mild mutation of *Scn2a* and a subclinical mutation of *Kcnq2*. The combination resulted in mice with severe seizures that died in status epilepticus within 3 weeks after birth. This dramatic example of gene interaction may be understood in terms of the functions of the two channels. The *Kcn2q* channel is part of a complex that produces a slowly inactivating potassium current that limits neuronal firing rates. Impaired *Kcn2q* activity would be expected to increase neuronal firing. The *Scn2a* mutation produced increased persistent current that also predisposes to a reduced threshold for neuronal firing. The presence of both mutant channels in the same cells would act in the same direction toward increased neuronal excitability. Subclinical variants of ion channels in the

human population may similarly interact in cases of apparently sporadic epilepsy.

Interaction between Mouse *Scn1a* and *Scn8a*

Mice heterozgyous for null mutations in *Scn1a* exhibit a spontaneous seizure syndrome that is a model of human Dravet syndrome.[11,12] The mechanism is thought to involve preferential reduction in the firing of inhibitory interneurons in the hippocampus. Martin et al.[25] generated mice carrying a null allele of *Scn1a* in combination with a missense mutation of *Scn8a* that reduces channel activity by shifting the voltage dependence of activation toward more positive voltages.[26] Interestingly, the double heterozygotes carrying both the *Scn1a* and *Scn8a* mutations were completely protected from seizures. The reduced activity of excitatory neurons due to the *Scn8a* mutation may compensate for the reduced activity of inhibitory interneurons due to the *Scn1a* mutation. This work predicts that mutations of human *SCN8A* could act as protective modifier variants in individuals inheriting a pathogenic allele of *SCN1A*. This prediction could be tested in families such as the one in Fig. 59–1, where the less severely affected individuals might carry a second mutation in *SCN8A*.

Mouse Calcium Channel *Cacna1a* and Potassium Channel *Kcna1*

The calcium channel *CACNA1A* and the potassium channel *KCNA1* (Kv1.1) are both localized in presynaptic terminals of neurons in the thalamus, neocortex, and hippocampus. The mouse mutant *tottering* carries a partial loss-of-function allele of *Cacna1a* that results in spike-wave absence seizures. The null mutation of *Kcna1* in the mouse results in juvenile lethality that appears to result from seizures. When these two mutations were combined in double homozygotes, survival was enhanced and seizures were suppressed.[27] The authors suggest that the increased excitability of nerve terminals lacking the potassium channel would be countered by the reduction in calcium signaling at the same terminals in the double mutant.

These three examples demonstrate that the predicted gene interactions between ion channels can be detected in vivo and can have striking effects on the clinical outcome, as summarized in Fig. 59–2. The combined effect may be either beneficial or deterimental, depending on the effects of each mutation at the cellular level. These experiments support the possibility that similar interactions will be discovered in human patients, and they suggest the value of screening for additional ion channel mutations in families with known channel mutations exhibiting clinical heterogeneity.

Human *SCN9A* as a Potential Modifier of Dravet Syndrome

SCN1A and *SCN9A* are adjacent sodium channel genes located on chromosome 2q24.[2] A mutation in *SCN9A* was recently identified in a large Utah pedigree with febrile seizures and no mutation of *SCN1A*, suggesting that *SCN9A* can also be responsible for seizures.[28] A follow-up study of 102 patients with Dravet syndrome identified 7 patients with mutations in both *SCN1A* and *SCN9A*[28] . The *SCN1A* mutations in the seven patients included one truncation mutation and three mutations in invariant splice site nucleotides, which should be sufficient to cause Dravet syndrome. The *SCN9A* mutations were missense mutations that changed amino acid residues that are evolutionarily conserved from chicken to human. The authors suggest that the *SCN9A* variants may modify the severity of Dravet syndrome in these patients with primary mutations in *SCN1A*.

INBRED STRAINS OF MICE PROVIDE ACCESS TO ADDITIONAL MODIFIERS OF EPILEPSY GENES

The inbred strains of mice were generated from wild populations during the past 100 years. Their genomes contain chromosome segments derived from two subspecies of mouse, *M. m. domesticus* and *M. m. musculus*. New mutations have accumulated in each strain during decades of laboratory breeding. The differences between any two inbred strains are

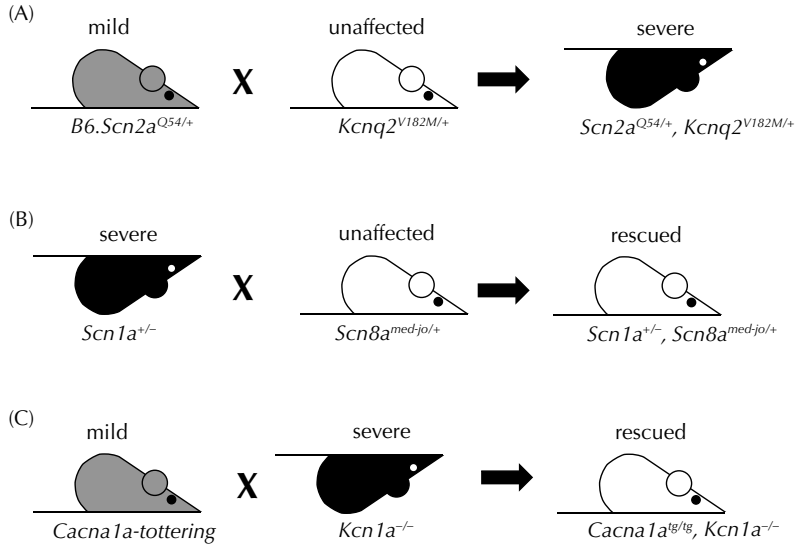

Figure 59–2. Digenic interactions between ion channel mutations in mouse models of epilepsy. **A**. Interaction between mutations in a sodium channel and a potassium channel. **B.** Interaction between mutations in two sodium channels. **C.** Interaction between mutations in a calcium channel and a potassium channel. Adapted from ref. 16.

roughly comparable to the differences between two humans. Inbred mouse strains provide an experimental opportunity to compare the effects of the same mutation in the context of different genome backgrounds, and thereby to recognize and identify modifier genes responsible for differences between strains. Interstrain variation can also be exploited to isolate genes that influence susceptibility to environmental factors and drugs, as indicated in Table 59–1.

Mouse Modifier Genes and Human Epilepsy

The conservation of basic mammalian neurobiology suggests that similar mutations would have similar effects on seizures in the human and the mouse. However, it is not known a priori whether an identified mouse modifier gene is represented by genetic variation in the human population. The practical impact of modifiers discovered in the mouse will depend on whether corresponding mutations have arisen during human history and are represented in modern human genomes.

Genetics of Complex Epilepsy

This chapter has focused on the detection of modifier mutations influencing seizure disorders whose primary cause is a single gene mutation with a large functional effect. In

Table 59–1 **Genetics of Complex Epilepsy**

Primary cause	Susceptible strains	Resistant strains	Genetic basis	Ref.
Scn1a - null	B6	129	Unknown	12
Scn2a - Q54	SJL	B6	Two candidates	29
Scn8a (ataxia)	B6	Others	*SCNM1*	15
Electroconvulsive	DBA,129	B6,C3H,SJL	Unknown	30
Electroconvulsive	DBA	B6	*KCNJ10*	31
Cocaine	A/J, SJL	B6	Unknown	32
Fluorethyl	DBA, C3H	B6	Unknown	33
Kainate	FVB/N	B6	Polygenic	34

polygenic or *complex* epilepsy, multiple susceptibility mutations with individually small effects combine to produce an epileptic phenotype. Polygenic inheritance is thought to be a major cause of common human epilepsies. Recent reviews address the subject of polygenic inheritance of epilepsy in the human and the mouse.[35,36]

FUTURE PROSPECTS FOR UNDERSTANDING GENE INTERACTION IN HUMAN EPILEPSY

The genetic basis for most cases of human epilepsy remains unknown, in spite of recent successes in identifying the roles of *SCN1A* and related ion channels. This situation is likely to change dramatically in the near future with the introduction of individual genome sequencing. Using inexpensive, high-throughput next-generation sequencing technology, >90% of the 180,000 exons in the human genome can be sequenced from individual samples. The first few exomes published in 2009 and 2010 revealed that every human carries approximately 500 rare amino acid sequence variants not previously described. A spectrum of variation extends from benign variants without functional consequences to mutations causing significant loss of function. By revealing *all* of their genetic variants, genome sequencing of epilepsy patients will accelerate the discovery of primary disease genes as well as genetic modifiers. The urgent challenge will then be to recognize the subset of amino acid substitutions that change the function of the encoded protein. Functional assays to distinguish between benign and pathogenic variants will be an increasingly important component of epilepsy research in order to interpret the abundant genetic information. Identification of additional epilepsy genes and their genetic modifiers will provide new targets for intervention and should lead to more effective treatments for seizure disorders.

DISCLOSURE STATEMENT

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REFERENCES

1. Kearney JA, Meisler, MH. Single gene mutations in inherited and sporadic epilepsy. In: Schwartzkroin P, ed. *Encyclopedia of Basic Epilepsy Research*. Elsevier: San Diego; 2009: 369–374.
2. Meisler MH, O'Brien JE, Sharkey LM. Sodium channel gene family: epilepsy mutations, gene interactions and modifier effects. *J Physiol*. 2010;588:1841–1848.
3. Baulac S, Gourfinkel-An I, Picard F, Rosenberg-Bourgin M, Prud'homme JF, Baulac M, Brice A, LeGuern E. A second locus for familial generalized epilepsy with febrile seizures plus maps to chromosome 2q21-q33. *Am J Hum Genet*. 1999;65:1078–1085.
4. Moulard B, Guipponi M, Chaigne D, Mouthon D, Buresi C, Malafosse A. Identification of a new locus for generalized epilepsy with febrile seizures plus (GEFS+) on chromosome 2q24-q33. *Am J Hum Genet*. 1999;65:1396–1400.
5. Kearney, J. A., Plummer, N. W., Smith, M. R., Kapur, J., Cummins, T. R., Waxman, S. G., Goldin, A. L. and Meisler, M. H. (2001) A gain-of-function mutation in the sodium channel gene Scn2a results in seizures and behavioral abnormalities, Neuroscience 102: 307–317.
6. Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. *Nat Genet*. 2000;24:343–345.
7. Spampanato J, Escayg A, Meisler MH, Goldin AL. Functional effects of two voltage-gated sodium channel mutations that cause generalized epilepsy with febrile seizures plus type 2. *J Neurosci*. 2001;21:7481–7490.
8. Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet*. 2001;68: 1327–1332.
9. Kearney JA, Wiste AK, Stephani U, Trudeau MM, Siegel A, Ramachandran Nair R, Elterman RD, Muhle H, Reinsdorf J, Shields WD, Meisler MH, Escayg A. Recurrent de novo mutations of SCN1A in severe myoclonic epilepsy of infancy. *Pediatr Neurol*. 2006;34:116–120.
10. Meisler MH, Kearney JA. Sodium channel mutations in epilepsy and other neurological disorders. *J Clin Invest*. 2005;115:2010–2017.
11. Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, Takeuchi T, Itohara S, Yanagawa Y, Obata K, Furuichi T, Hensch TK, Yamakawa K. Na(v)1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. *J Neurosci*. 2007;27:5903–5914.
12. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci*. 2006;9: 1142–1149.
13. Claes LR, Deprez L, Suls A, Baets J, Smets K, Van Dyck T, Deconinck T, Jordanova A, De Jonghe P. The SCN1A variant database: a novel research and diagnostic tool. *Hum Mutat*. 2009;30:E904–E920.

14. Lossin C. A catalog of SCN1A variants. *Brain Dev*. 2009;31:114–130.
15. Buchner DA, Trudeau M, Meisler MH. SCNM1, a putative RNA splicing factor that modifies disease severity in mice. *Science*. 2003;301:967–969.
16. Riazuddin S, Castelein CM, Ahmed ZM, Lalwani AK, Mastroianni MA, Naz S, Smith TN, Liburd NA, Friedman TB, Griffith AJ, Wilcox ER. Dominant modifier DFNM1 suppresses recessive deafness DFNB26. *Nat Genet*. 2000;26:431–434.
17. Catterall WA, Dib-Hajj S, Meisler MH, Pietrobon D. Inherited neuronal ion channelopathies: new windows on complex neurological diseases. *J Neurosci*. 2008;28: 11768–11777.
18. Suls A, Velizarova R, Yordanova I, Deprez L, Van Dyck T, Wauters J, Guergueltcheva V, Claes LR, Kremensky I, Jordanova A, De Jonghe P. Four generations of epilepsy caused by an inherited microdeletion of the SCN1A gene. *Neurology*. 2010;75:72–76.
19. Depienne C, Trouillard O, Gourfinkel-An I, Saint-Martin C, Bouteiller D, Graber D, Barthez-Carpentier MA, Gautier A, Villeneuve N, Dravet C, Livet MO, Rivier-Ringenbach C, Adam C, inherited SCN1A mutations causing Dravet syndrome. *J Med Genet*. 2010;47:404–410.
20. Baulac S, Picard F, Herman A, Feingold J, Genin E, Hirsch E, Prud'homme JF, Baulac M, Brice A, LeGuern E. Evidence for digenic inheritance in a family with both febrile convulsions and temporal lobe epilepsy implicating chromosomes 18qter and 1q25-q31. *Ann Neurol*. 2001;49:786792.
21. Nabbout R, Baulac S, Desguerre I, Bahi-Buisson N, Chiron C, Ruberg M, Dulac O, LeGuern E. New locus for febrile seizures with absence epilepsy on 3p and a possible modifier gene on 18p. *Neurology*. 2007;68: 1374–1381.
22. Pinto D, Westland B, de Haan GJ, Rudolf G, da Silva BM, Hirsch E, Lindhout D, Trenite DG, Koeleman BP. Genome-wide linkage scan of epilepsy-related photoparoxysmal electroencephalographic response: evidence for linkage on chromosomes 7q32 and 16p13. *Hum Mol Genet*. 2005;14:171–178.
23. Pinto D, Kasteleijn-Nolst Trenite DG, Cordell HJ, Mattheisen M, Strauch K, Lindhout D, Koeleman BP. Explorative two-locus linkage analysis suggests a multiplicative interaction between the 7q32 and 16p13 myoclonic seizures-related photosensitivity loci. *Genet Epidemiol*. 2007;31:42–50.
23a. Klassen T, Davis C, Goldman A, Burgess D, Chen T, Wheeler D, McPherson J, Bourquin T, Lewis L, Villasana D, Morgan M, Muzny D, Gibbs R, Noebels J. Exome sequencing of ion channel genes reveals complex profiles confounding personal risk assessment in epilepsy. *Cell*. 2011;145:1036–48.
24. Kearney JA, Yang Y, Beyer B, Bergren SK, Claes L, DeJonghe P, Frankel WN. Severe epilepsy resulting from genetic interaction between Scn2a and Kcnq2. *Hum Mol Genet*. 2006;15:1043–1048.
25. Martin MS, Tang B, Papale LA, Yu FH, Catterall WA, Escayg A. The voltage-gated sodium channel Scn8a is a genetic modifier of severe myoclonic epilepsy of infancy. *Hum Mol Genet*. 2007;16:2892–2899.
26. Kohrman DC, Smith MR, Goldin AL, Harris J, Meisler MH. A missense mutation in the sodium channel Scn8a is responsible for cerebellar ataxia in the mouse mutant jolting. *J Neurosci.* 1996;16:5993–5999.
27. Glasscock E, Qian J, Yoo JW, Noebels JL. Masking epilepsy by combining two epilepsy genes. *Nat Neurosci*. 2007;10:1554–1558.
28. Singh NA, Pappas C, Dahle EJ, Claes LR, Pruess TH, De Jonghe P, Thompson J, Dixon M, Gurnett C, Peiffer A, White HS, Filloux F, Leppert MF. A role of SCN9A in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. *PLoS Genet*. 2009;5:e1000649.
29. Bergren SK, Rutter ED, Kearney JA. Fine mapping of an epilepsy modifier gene on mouse chromosome 19. *Mamm Genome*. 2009;20:359–366.
30. Frankel WN, Taylor L, Beyer B, Tempel BL, White HS. Electroconvulsive thresholds of inbred mouse strains. *Genomics*. 2001;74:306–312.
31. Ferraro TN, Golden GT, Dahl JP, Smith GG, Schwebel CL, MacDonald R, Lohoff FW, Berrettini WH, Buono RJ. Analysis of a quantitative trait locus for seizure susceptibility in mice using bacterial artificial chromosome-mediated gene transfer. *Epilepsia*. 2007;48:1667–1677.
32. Golden GT, Ferraro TN, Smith GG, Snyder RL, Jones NL, Berrettini WH. Acute cocaine-induced seizures: differential sensitivity of six inbred mouse strains. *Neuropsychopharmacology*. 2001;24:291–299.
33. Papandrea D, Anderson TM, Herron BJ, Ferland RJ. Dissociation of seizure traits in inbred strains of mice using the flurothyl kindling model of epileptogenesis. *Exp Neurol*. 2009;215:60–68.
34. Schauwecker PE, Williams RW, Santos JB. Genetic control of sensitivity to hippocampal cell death induced by kainic acid: a quantitative trait loci analysis. *J Comp Neurol*. 2004;477:96–107.
35. Mulley JC, Scheffer IE, Harkin LA, Berkovic SF, Dibbens LM. Susceptibility genes for complex epilepsy. *Hum Mol Genet*. 2005;14 spec no. 2:R243–R249.
36. Frankel WN. Genetics of complex neurological disease: challenges and opportunities for modeling epilepsy in mice and rats. *Trends Genet*. 2009;25: 361–367.

Chapter 60

Rare Genetic Causes of Lissencephaly May Implicate Microtubule-Based Transport in the Pathogenesis of Cortical Dysplasias

Judy S. Liu
Christian R. Schubert
Christopher A. Walsh

INTRODUCTION

Disruption of early neural development can cause severe forms of mental retardation and epilepsy associated with defects in cortical structure, such as lissencephaly ("smooth brain"), a disorder resulting from abnormal neuronal migration. Of the six causative genes for classical lissencephaly, three—*LIS1*, *DCX*, and *TUBA1A*—encode for microtubule-related proteins, indicating the importance of this pathway for neuronal migration (Table 60–1). The lissencephaly 1 (*LIS1*) protein is an adaptor for dynein, a microtubule motor protein.[1] Doublecortin (*DCX*) encodes a microtubule-associated protein (MAP).[2,3] Finally, tubulin α1a (*TUBA1A*) is a gene that encodes an α-tubulin subunit that is enriched during brain development.[4]

These lissencephalic syndromes clinically all share a widespread disruption of lamination in the cerebral cortex (Fig. 60–1). Since all three genes appear to regulate microtubule-based transport, their functional relation and regulation during development is an area of active investigation with implications that may be

Table 60–1 **Lissencephaly Genetics**

Mode of Inheritance	Gene	Locus	Type	Pathway Defect
X-linked or autosomal dominant				
a. X-linked lissencephaly with abnormal genitalia	*ARX*	Xp22.1	Type 1	Transcriptional regulation
b. Isolated lissencephaly or subcortical band heterotopia	*DCX*	Xq22.3-q23	Type 1	MT-based transport
	TUBA1A	12q13.12	Type 1	MT-based transport
	LIS1	17p13.3	Type 1	MT-based transport
c. Miller-Dieker syndrome	*LIS1* + *YWHAE*	17p13.3	Type 1	MT-based transport
Autosomal recessive				
d. Lissencephaly with cerebellar hypoplasia group b	*RELN*	7q22.1	Type 1	Signaling
	VLDLR	9p24.2	Type 1	Signaling
e. Cobblestone lissencephaly				
Fukuyama congenital muscular dystrophy or Walker-Warburg syndrome	*FCMD*	9q31.2	Type 2	Matrix protein glycosylation
Muscle-eye-brain disease or Walker-Warburg syndrome	*FKRP*	19q13.32	Type 2	Matrix protein glycosylation
	POMT1	9q34.13	Type 2	Matrix protein glycosylation
	POMT2	14q24.3	Type 2	Matrix protein glycosylation
Muscle-eye-brain disease	*LARGE*	22q12.3	Type 2	Matrix protein glycosylation
	POMGnT1	1p34.1	Type 2	Matrix protein glycosylation

significant for a wider cohort of patients with focal cortical dysplasias. Cortical dysplasias are a common cause of refractory epilepsy and share some of the histological features of lissencephaly, including the dyslamination and abnormal neuronal morphology.[5] Disruption of microtubule-based pathways may lead to cortical dysplasias, and the causative genes for lissencephaly are a starting point for further investigation.

Normal cognitive function is dependent on proper brain development, including the coordination of multiple steps of neuronal development throughout gestation and beyond. Disruption in the early steps of neuronal development, including neuronal migration, can result in severe cognitive deficits and epilepsy. In the cortex, subsets of neurons may be differentially affected, including excitatory pyramidal neurons that migrate radially from the ventricular zone and inhibitory neurons that migrate tangentially from the lateral and medial ganglionic eminences (Fig. 60–2A,B). As a consequence, the normal lamination of the neurons in the cortex may be altered, leading to a failure of normal circuit formation and/or the establishment of abnormal circuits leading to cognitive dysfunction and epilepsy (Fig. 60–2C).

The phenotypic characteristics of brain malformations can be correlated with defects at certain stages in development. For example, cortical development is dependent on the proliferation of neural progenitors. When the number of neural progenitors is reduced, the result may be a brain with microcephaly ("small brain") that is otherwise normal in structure. Microcephaly can be caused by a variety of environmental factors including infection [e.g., TOxoplasmosis, Rubella, Cytomegalovirus, HErpes simplex, Syphilis (TORCHES)] or toxins (e.g., alcohol).[6,7] Microcephaly can be genetic, caused by mutations in genes that regulate cell division resulting in defects in the expansion of progenitors.[8] Furthermore, defects in genes that are important for multiple stages of development can result in a patient with a malformation with multiple features. Microcephaly can also occur in combination with a migration defect, such as microcephaly with pachygyria (Norman-Roberts syndrome),[9] so that the malformation appears to reflect the disruption of function of the gene throughout development.

Generally, disruptions in neuronal migration can cause a number of malformations. Lissencephaly ("smooth brain") and pachygyria

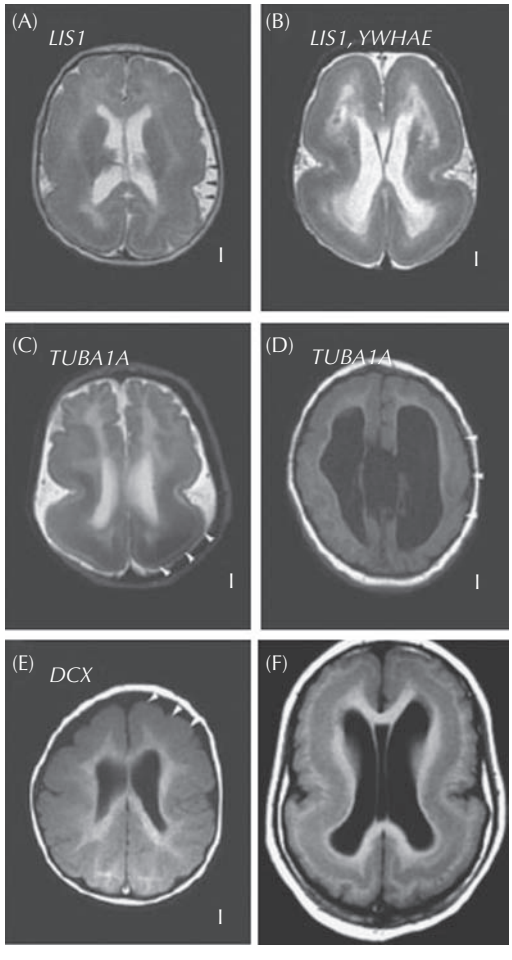

Figure 60–1. MRI of lissencephaly caused by microtubule pathway genes**.** High axial magnetic resonance imaging (MRI) scans of lissencephaly (LIS) associated with mutations in microtubule-related genes. Arrowheads on the axial images mark the most severely involved brain regions. **A.** Classic LIS gradient, posterior more severe than anterior (p > a), associated with an intragenic mutation of *LIS1*. **B.** Severe classic LIS due to deletion of 17p13.3 that results in loss of *LIS1*, *YWHAE*, and all of the intervening genes in a child with Miller-Dieker syndrome. **C.** Classic LIS with a p > a gradient caused by an intragenic mutation of *TUBA1A*. **D.** Moderate-severity LIS with cerebellar hypoplasia (LCH) with complete agenesis of the corpus callosum, large dysplastic midbrain and tectum, and severe cerebellar hypoplasia associated with another intragenic mutation of *TUBA1A*. **E.** Classic LIS gradient, anterior more severe than posterior (a > p), caused by an intragenic mutation of *DCX*. **F.** The MRI scan of a 15-year-old girl with a severe subcortical band heterotopia due to a *DCX* mutation. **A–E** adapted from *Epilepsia* 51: Dobyns, WB. The clinical patterns and molecular genetics of lissencephaly and subcortical band heterotopia, 5–9, Copyright (2010), with permission from Wiley. **F** reprinted from *Neurobiology of Disease*, 38(2): Guerrini R, Parrini E. Neuronal migration disorders, 154–66, Copyright (2010). With permission from Elsevier.

("few gyri") are malformations caused by a disorder of neuronal migration. Pachygyria and lissencephaly are often less and more severe manifestations of gene mutations causing type 1 lissencephaly including *LIS1* and *DCX* mutations. Other variants of lissencephaly that resemble type 1 have been identified, including those due to mutations in *ARX*,[10] *RELN*,[11] *VLDLR*,[12] and *TUBA1A*[13] (Table 60–1).

Type II lissencephaly, also called *cobblestone lissencephaly*, is associated with Walker-Warburg and Fukuyama muscular dystrophies and is caused by defects in the basement membrane that result from mutations in glycosyl transferase enzymes (Table 60–1). Again, mutations in additional genes involved in dystroglycan glycosylation have been observed.[14] Cobblestone lissencephaly is similar but not identical in appearance to polymicrogyria ("many small gyri"). This malformation can be regional, with variants that are perisylvian, or parietal, or predominantly bifrontal. The pathophysiology of polymicrogyria is not apparent and may be due to damage of a deeper layer or early-born neurons, resulting in overfolding of later-born or more superficial neurons, or it may be due to an abnormal expansion of the superficial layers.

Finally, defects in axonal growth and guidance can lead to the commonly observed white matter abnormalities, such as agenesis of the corpus callosum or enlarged ventricles. Interestingly, most causes of lissencephaly (non-cobblestone) are also known to be associated with defects in axon outgrowth,[15] thus implying a role for the causative genes in both stages of development and closely relating the molecular pathways regulating both neuronal migration and axon outgrowth. In addition, these defects in axonal growth are likely associated with abnormal connectivity and may very generally be associated with seizure pathogenesis, including that in patients with lissencephaly.

IMPLICATIONS OF MICROTUBULE ASSEMBLY AND MICROTUBULE-BASED TRANSPORT FOR NEURONAL DEVELOPMENT

Not only have these specific diseases described above been modeled and studied, but basic science has significantly informed the understanding

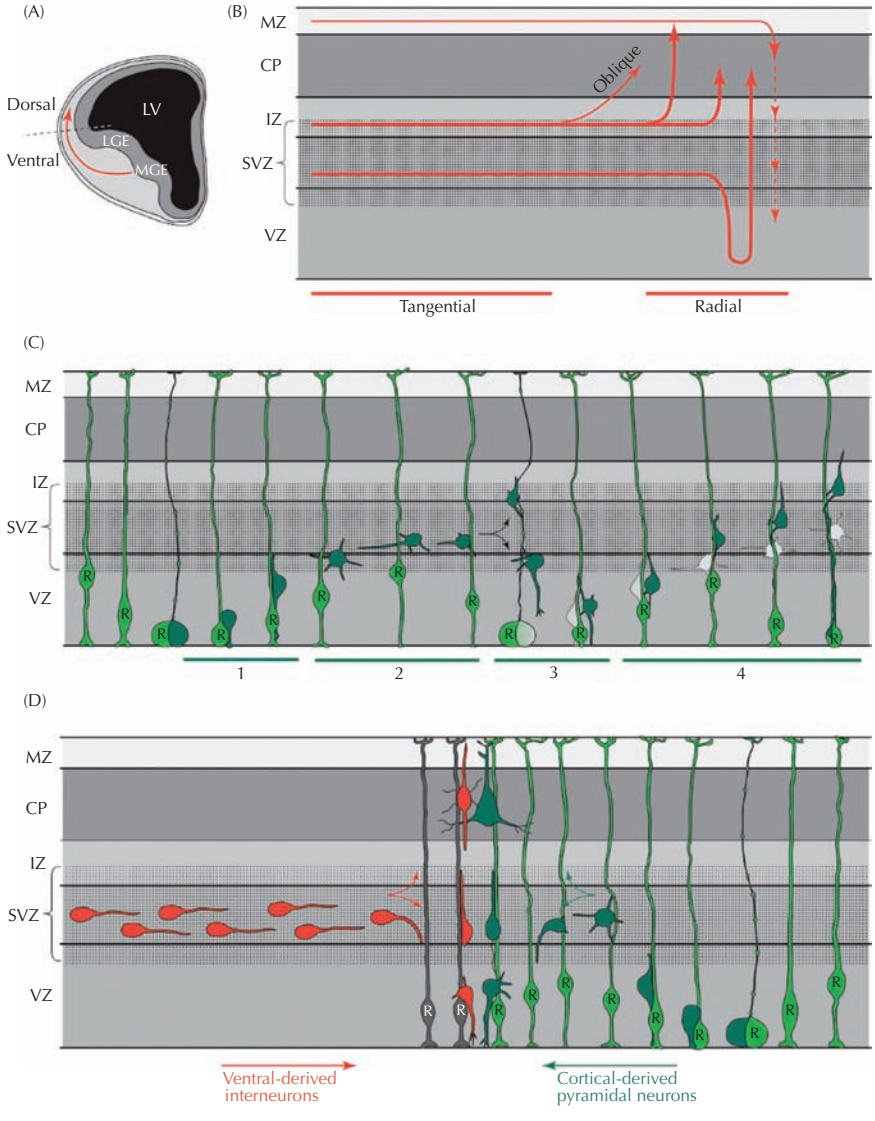

Figure 60–2. Neuronal migration. **A, B.** Cortical interneurons are derived from the ventral telencephalon and reach their final locations by migrating through specific phases. **A.** Origin of interneurons in the ventral telencephalon. Most cortical interneurons are generated in the medial ganglionic eminence (MGE) of the ventral telencephalon and migrate across the corticostriatal junction (broken line) to enter the dorsal telencephalon. **B.** Phases of interneuron migration within the dorsal telencephalon. Cortical interneurons arising in the ventral telencephalon migrate tangentially in the cortex and then change direction to enter the cortical plate (CP) by following a radial or an oblique path. The broken line indicates that some interneurons have been observed to descend radially into the CP and others to continue radially to deeper lamina. IZ, intermediate zone; LGE, lateral ganglionic eminence; LV, lateral ventricle; MZ, marginal zone; SVZ subventricular zone; VZ, ventricular zone. **C.** Cortical pyramidal neurons undergo distinct phases of locomotion migration. Phase one involves radial movement of pyramidal neurons (dark green) from the site of origin at the ventricular surface to the SVZ. In phase two, cells become multipolar, and their migration pauses in the lower IZ and the SVZ. Some neurons undergo phase three, which is characterized by retrograde motion toward the ventricle. Phase four is the final radial migration to the CP, guided by radial glial fibers. Radial glia (light green) remain mitotic, undergo interkinetic nuclear migration, and generate additional daughter cells (gray). R, radial glial cell. **D.** Migratory patterns of interneurons and pyramidal neurons converge in the dorsal cortex. This scheme depicts the apparent convergence of the migratory patterns of interneurons (red) and pyramidal cell movements (dark green) in the cortex. Subsets of both cell types display ventricle-directed migration followed by radial movement to the CP. Interneurons might migrate radially along unrelated adjacent radial glial cells (gray) to reach the CP. Reprinted from *Trends in Neurosciences*, 27(7), Arnold R. Kriegstein and Stephen C. Noctor, "Patterns of neuronal migration in the embryonic cortex," pages 392–399, Copyright (2004). With permission from Elsevier.

of human disease. Neuronal migration was studied extensively in the mouse even prior to identification of the causative genes for human syndromes. Prior to identification of *RELN* as a causative gene for lissencephaly, work on the reeler mouse identified striking neuronal migration defects.[16] The *Cdk5–/–* mouse also has similar, but not identical lamination defects, including the inverted cortical lamination,[17] and CDK5 is thought to be downstream of reelin signaling. In turn, the CDK5 protein is known to interact with both DCX and LIS1.[1,18] Thus, it has become apparent that the genes identified in mice with neuronal migration defects interact with human lissencephaly proteins, including the microtubule-related proteins, as well to define a neuronal migration signaling pathway (Fig. 60–3).

A key question is why the microtubule pathway is important and specific for neuronal migration. During development of pyramidal neurons, newly born neuroblasts undergo changes in morphology that correspond to different stages in neuronal migration[19] (Fig. 60–2B). The newly born neuroblast in the ventricular zone (VZ) passes into the subventricular zone (SVZ), where it can divide further into additional neurons or, alternatively, enter the intermediate zone (IZ). In the IZ, the neuroblast assumes a multipolar morphology, but as it migrates into the cortical plate, the neuron becomes bipolar, with a leading process that eventually becomes the dendrite and a trailing process that will become the axon (Fig. 60–2B). Transport of organelles and polarization of trafficking underlie many of these changes, and since cytoskeletal dynamics and transport regulate cell shape and structure, these pathways are critical for correct neuronal migration. Studies aimed at identifying and characterizing causative genes for neuronal migration disorders have emphasized the importance of microtubule function in human neural development (Table 60–1). *LIS1*, *DCX*, and *TUBA1A*

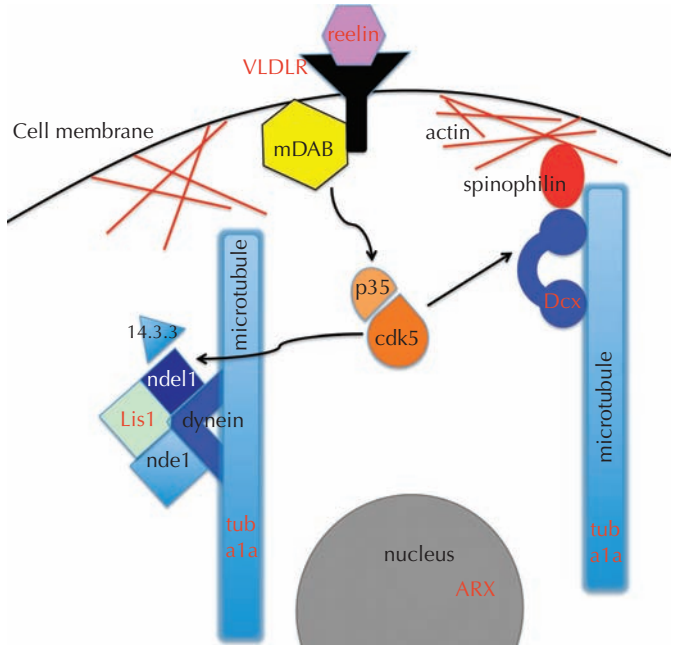

Figure 60–3. Neuronal migration signaling pathways. A simplified diagram describing the relationship between the known neuronal migration proteins in mouse and human is shown. Protein names encoded by causative genes for human lissencephaly are in red. Reelin is an extracellular ligand that binds, among other receptors, very-low-density lipoprotein receptor (VLDLR). Doublecortin (Dcx) is a microtubule binding protein; Lissencephaly 1 (Lis1) is an adaptor protein for the minus end motor, dynein, and Tuba1a is a tubulin isomer. Aristaless (Arx) is a transcription factor. Reelin signaling may activate the cdk5/p35 kinase via mDAB. Cdk5 regulates Dcx microtubule binding and assembly of the Lis1/dynein complex including NDEL1, NDE1, and 14-3-3ε. Dcx interacts with microtubules as well as actin via spinophilin and may be a mediator of actin/tubulin crosstalk.

encode proteins that are related to microtubule function and microtubule-based transport. The LIS1 protein is part of the dynein complex, a microtubule motor protein.[1] *DCX* encodes a MAP that has a role in regulating vesicle transport in developing neurites.[20] Finally, *TUBA1A* encodes an α-tubulin subunit that is enriched during brain development.[4] The other genes, *ARX*, *RELN*, and *VLDLR*, encode for a transcription factor and for the ligand and receptor of the very-low-density lipoprotein receptor system, respectively, that may define upstream or downstream events regulating neuronal migration (Fig. 60–3).

From these studies, it appears evident that the basic biology of microtubules and microtubule-based functions, including transport, is extremely important for proper neuronal development, such as neuronal migration (Fig. 60–4). Microtubules are hollow tube-shaped polymers assembled through polarized polymerization of tubulin heterodimers that are comprised of a variety of α- and β-tubulin isoforms. Tubulin subunits α and β are encoded by a family of genes that are structurally similar but have distinctive features important for specific cellular functions. *TUBA1A* is highly expressed in the nervous system during development and is a causative gene for lissencephaly.[13] Other tubulin genes are causative for different human syndromes, including tubulin β3 (*TUBB3*), an axonal-specific tubulin causing a syndrome, congenital fibrosis of the extraocular muscles (CFEOM3), that is also associated with cognitive defects and a neuropathy.[21]

Microtubule assembly is dependent on guanosine triphosphate (GTP), which binds to the soluble tubulin heterodimers and, upon hydrolysis, induces a conformational change that favors the polarized polymerization and elongation of the microtubule. Once assembled, however, microtubules are by no means static structures; not only can they be depolymerized, but they are moreover dynamically and developmentally tightly regulated so that their structure, polarization, and function are fine-tuned for the specific requirements of the cell. For example, some *TUBA1A* mutations block GTP binding, thereby preventing polymerization.[13]

In neurons, as in any other cell type, microtubule polarization results in clearly defined *plus* and *minus* ends, with elongation occurring exclusively at the plus end. In axons, the minus ends are usually oriented toward the cell body, whereas their elongating plus ends project toward the distal regions of the axon. In contrast, in dendrites, the polarity of microtubules is mixed, and the structural differences of microtubules in axons and dendrites likely reflect specific functional differences.[22] Microtubules in axons and dendrites can also be distinguished through their interacting proteins. Microtubule-associated proteins are specific for axons or dendrites and are often bound to microtubules in gradients along

(*continued from page 777*)

been described as a B-type lattice with a seam (long arrow, part A in the figure). A third tubulin isoform, γ-tubulin, functions as a template for the correct assembly of microtubules. On addition of a new dimer at the plus end, the catalytic domain of α-tubulin contacts the nucleotide exchangeable site (E site) of the previous β subunit and becomes ready for hydrolysis; the plus end generally has a minimum GTP cap of one tubulin layer that stabilizes the microtubule structure. When this GTP cap is stochastically lost, the protofilaments splay apart and the microtubule rapidly depolymerizes. During or soon after polymerization, the tubulin subunits hydrolyze their bound GTP and become nonexchangeable. Thus, the microtubule lattice is predominantly composed of GDP-tubulin, with depolymerization being characterized by the rapid loss of GDP-tubulin subunits and oligomers from the microtubule plus end. At the minus end, contact is made between the E site of the new dimer and the catalytic region of the last subunit at the end; therefore, no GTP cap should be present. The properties of microtubules depend on the tubulin isoforms they consist of—there are three α-tubulins (α1, α2, and α4) and five β-tubulins (βI, βII, βIII, βIVa, and βIVb)—and on how they have been altered by various forms of posttranslational modification, including tyrosination, detyrosination, acetylation, polyglutamylation, polyglycylation, phosphorylation, and palmitoylation. Except for tubulin tyrosine ligase, the enzyme that adds a tyrosine to nonassembled α-tubulin, most of the modifying enzymes act preferentially on tubulin subunits that are already incorporated into microtubules. Posttranslational modifications of tubulin subunits mark subpopulations of microtubules and selectively affect their functions. Although they are not directly involved in determining the dynamic properties of microtubules, posttranslational modifications of tubulin, such as the sequential tyrosination-detyrosination-acetylation, correlate well with the half-life and spatial distribution of microtubules. C. Axons have tau-bound microtubules of uniform orientation, whereas dendrites have microtubule-associated protein 2 (MAP2)-bound microtubules of mixed orientation. Dendrites also contain organelles that are not found in axons, such as rough endoplasmic reticulum, polyribosomes, and Golgi outposts. Reprinted with permission from Macmillan Publishers Ltd: *Nature Reviews Neuroscience* 10, 319–332, Copyright (2009).

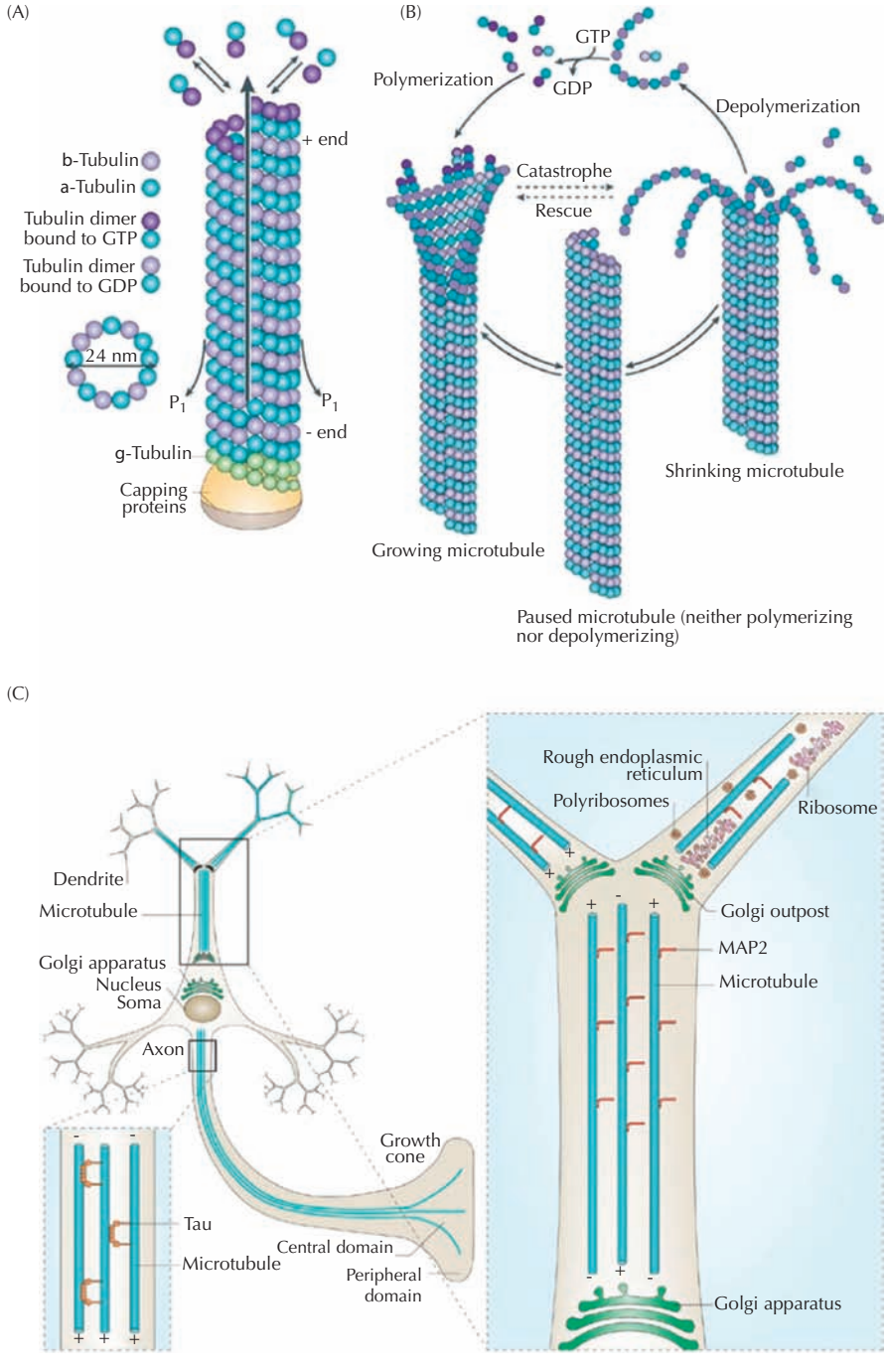

Figure 60–4. Microtubule regulation in neurons**. A, B.** Microtubules are noncovalent cytoskeletal polymers found in all eukaryotic cells that are involved in mitosis, cell motility, intracellular transport, secretion, and the maintenance of cell shape and cell polarization. They are polarized structures composed of α- and β-tubulin heterodimer subunits assembled into linear protofilaments. A single microtubule consists of 10–15 protofilaments (usually 13 in mammalian cells) that associate laterally to form a 24 nm wide hollow cylinder. The head-to-tail association of the heterodimers makes microtubules polar structures, and they have different polymerization rates at the two ends. In each protofilament, the heterodimers are oriented with their β-tubulin monomer pointing toward the faster-growing end (plus end) and their α-tubulin monomer exposed at the slower-growing end (minus end). The lateral interaction between subunits of adjacent protofilaments has

(*continues on page 776*)

neural processes,[23] serving several functions. For example, binding of MAPs provides structural stability to microtubules. In addition, MAPs can facilitate transport targeting and specificity by either blocking or enhancing motor interaction. Most MAPs compete with motors for binding sites on tubulin and thus are negative regulators of motor transport. DCX, however, does not competitively inhibit motor binding.[24,25] Moreover, DCX is a MAP that is regulated by phosphorylation by CDK5 and binds microtubules in a signal-dependent manner.[18] Our data show that DCX facilitates binding of some motors to microtubules. Posttranslational modifications of tubulin including acetylation and polyglutamylation, respectively can also confer stability and enhance motor function on microtubules.[26,27] And other modifications, such as tyrosination, interfere with motor function on microtubules.[27]

Thus, polarity, posttranslational modification, and MAP binding of microtubules are important for transport functions, but the actual transport is mediated by individual microtubule-based motors.[28] Motors are directed toward either the plus or minus ends of microtubules. For example, kinesin motors transport vesicles and organelles toward the plus ends of microtubules, and the single dynein motor transports cargo toward the minus end of the microtubule. In the axon this means that kinesins transport cargoes away from the cell body to distal parts of the axon, and dynein mediates transport back toward the cell body. While there is only 1 minus end motor, dynein, there are 45 kinesins that transport cargo toward the plus end of the microtubule. Thus, achieving cargo identification and coupling for the minus end motor, dynein, is considerably more complex than for the kinesins. The LIS1 protein actually is associated with dynein and is important as a regulator of dynein force generation.[29]

Thus, mutations in genes that have a major role in microtubule assembly and microtubule-based transport, including *LIS1*, *DCX*, and *TUBA1A*, can cause defects in neuronal migration as well as axon and dendrite outgrowth. In this chapter, describing three specific examples, we illustrate how rare genetic causes of lissencephaly may implicate microtubule-based transport in the pathogenesis of cortical dysplasias.

LIS1 IS THE CAUSATIVE GENE ASSOCIATED WITH MILLER-DIEKER LISSENCEPHALY SYNDROME

Haploinsufficiency of *LIS1* (also called *platelet-activating factor acetyl-hydrolase*) is known to cause classical lissencephaly, either in isolation (Fig. 60–1A) or as part of the Miller-Dieker syndrome of 17p13.3 deletion[30] (Fig. 60–1B). Miller-Dieker syndrome also includes facial dysmorphology, caused by haploinsufficiency of the other genes deleted in the interval. *YWHAE* encodes the protein 14–3-3ε, which is a modifier of Lis1.[31,32] Patients with *LIS1* mutations alone have lissencephaly, but are often found to have hypoplasia of the corpus callosum as well as enlarged ventricles, suggesting a role for LIS1 in neuronal migration as well as axon formation.[33] Supporting evidence is provided by the expression pattern of LIS1, which is enriched in neuronal progenitor cells in early development. Moreover, expression of LIS1 in later stages of development supports a wider role for LIS1 function outside of neuronal migration.

Functional characterization of LIS1 has been facilitated by the creation and characterization of an animal model. As predicted by human genetics, haploinsufficiency of *Lis1* results in structural defects in the brain, including dyslamination in the hippocampi.[34] Homozygosity of the mutation is lethal in early embryogenesis.[35] In addition to radial migration defects, tangential migration of interneurons is impaired.[36] However, most striking are the apparent defects in neurogenesis that were not emphasized in the initial clinical description of patients. In the mouse model, Lis1 was found to be critical for expansion of the neuronal progenitor pool through regulation of symmetric cell division. Decreased Lis1 is correlated with abnormal spindle pole orientation, a marker for the type of cell division—asymmetric to generate a postmitotic neuron or symmetric to generate two progenitors that can divide further.[37]

Mouse genetics and molecular biology have allowed the identification and characterization of proteins interacting with Lis1, most importantly dynein, Ndel1 and Nde1. Lis1 has also been found to interact with dynactin, another member of the dynein complex, and with the plus-end microtubule binding protein

CLIP-170, which may mediate the assembly of the complex.[38] These proteins have multiple functions during neuronal migration, including nuclear and centrosomal positioning, as well as neurite outgrowth.[39] It is unknown, however, if dynein transport in each of these contexts functions similarly, and likely the complexity of the dynein complex, including Lis1, facilitates specificity of function. Given dynein's role as the single motor protein responsible for minus-end microtubule transport, it is more likely that defects associated with Lis1-dynein transport are due to specific defects in the dynein cargo load. For example, neurite outgrowth problems may be due to defects in retrograde signaling, and neuronal migration and neurogenesis problems may result from disruption of nucleokinesis.[40,41]

Molecular studies have demonstrated a specific role for LIS1 in the regulation of dynein activity: LIS1 and NDE1 bind to dynein to enhance microtubule binding and prolong force production of the motor protein.[29] As a consequence, LIS1 has been shown to mediate dynein transport of relatively large and heavy cargos, such as the nucleus, while other small vesicular cargoes appear to be unaffected. Unfortunately, other aspects of LIS1 function, including its reported interaction with another lissencephaly gene, DCX, as well as its regulation by developmentally important kinases, which may explain aspects of the *LIS1* phenotype, including the seizures, remain uncharacterized to date.

DCX MUTATIONS CAUSE BOTH X-LINKED LISSENCEPHALY AND SUBCORTICAL BAND HETEROTOPIA

DCX, or doublecortin, is one of two genes that cause X-linked lissencephaly in affected males[42,43] (Table 60–1 and Fig. 60–1E). Women with *DCX* mutations classically have a migration disorder called *subcortical band heterotopia* (Fig. 60–1F). X-inactivation to achieve gene dosage compensation in females with *DCX* mutations results in cellular mosaicism: two populations of neurons occur with either the mutant gene or the normal gene. Affected mosaic females can have a range of neurodevelopmental phenotypes, including subcortical band heterotopia, a disorder in which DCX-deficient neurons arrest before reaching the cortical plate to form abnormal islands within the white matter with abnormal axons tracts rather than frank lissencephaly.[44] In addition, women with *DCX* mutations can exhibit a range of milder phenotypes, including nonsyndromic mental retardation or cryptogenic epilepsy without an overt neuronal heterotopia.[45] The degree of dysfunction and the severity of the phenotype are thought to be due to the skewing of X-inactivation of the X chromosome with the defective *DCX*.

The genetic mouse model of *Dcx* mutations significantly improved our understanding of the brain phenotype in the human condition. While the mutant mouse with a targeted deletion of *Dcx* does not have an overt cortical migration defect and is more mildly affected,[46] it has multiple other defects that have led to a closer examination of the human phenotype. For example, the *Dcx* mutant mouse exhibits disruption of lamination in the hippocampi and white matter defects.[46] These defects were subsequently also described in humans with *DCX* mutations.[47] In addition, defects in migration and morphology of GABAergic interneurons have been described, which may be a common factor in the pathogenesis of epilepsy in these disorders.

Interestingly, the female *Dcx* mutant mouse with a single undamaged copy of *Dcx* appears to be phenotypically normal.[20] Instead, short hairpin RNA interference (shRNAi) has been used in rats to model the formation and physiology of subcortical band heterotopia.[48] However, it has to be noted that the same experiment does not produce heterotopia in the mouse. The shRNAi is introduced by microinjection into the lateral ventricle of an embryonic rat and by application of an electrical pulse, the interference construct is transfected into neuronal progenitors that are adjacent to the ventricle. In these cells, the RNAi prevents the expression of *Dcx* during the migratory phase of neuronal development. Moreover, electrophysiological studies of these rats have shown a decrease of GABAergic tone in the cortex overlying the subcortical band, which is populated not only with Dcx-deficient excitatory neurons but also with normal GABAergic neurons.[49] These appear to be misdirected and unable to migrate to their normal positions in the cortex. Finally, this model has also been used to show

that reexpression of Dcx in the subcortical band neurons during adulthood initiates migration of these neurons into the cortex and further decreases the seizure threshold of the animals.

From animal models, it has become clear that Dcx is a member of a family of structurally related, functionally redundant proteins that have a role in seizure pathogenesis. In addition to the single mutant of *Dcx*, double mutants of *Dcx* and *Dclk1*[20,50] and *Dclk2*[51] have been published, showing much more severe structural effects in the double mutants than in the single mutants and proving overlapping roles for Dcx family members in development and beyond. All of these animals have been shown to have seizures (Deuel, Walsh, and Nobles, unpublished data), and the seizures in the *Dcx-/y; Dclk2–/–* mutant are thought to emanate from the hippocampus.[51] It is unknown whether seizures in humans are related to the severe temporal lobe dyslamination and how much the disruption of the microcircuitry in the neocortex contributes to seizure propagation in humans. Epilepsy surgery has not been used on lissencephaly patients; however, patients with subcortical band heterotopia have generally not had favorable outcomes after temporal lobectomy.[52]

The molecular role of DCX can be understood by its interaction with other proteins. Mutations in the tandem microtubule binding domains of DCX have been shown to abrogate microtubule binding and cause the neuronal migration defects.[53] In addition to microtubules, DCX is known to interact with spinophilin/neurabin II, an actin-binding protein, suggesting a role in actin/microtubule crosstalk, and human mutations disrupting this interaction also cause defects in neuronal migration.[54] Moreover, DCX has been shown to interact with the μ subunit of the clathrin adaptor complex,[55] which is involved in vesicle biogenesis from the Golgi complex and in endocytosis as part of clathrin-coated pits. Finally, we have identified a role for DCX in regulating microtubule-based transport.[20]

MUTATIONS IN α-TUBULIN CAUSE LISSENCEPHALY

Heterozygous missense mutations in *TUBA1A*, coding for an α-tubulin isoform that is highly expressed in developing neurons, cause a spectrum of cortical malformations that include lissencephaly and pachygyria. Affected individuals may also be microcephalic and have cortical malformations that range from agyria and posterior pachygyria in severe cases to perisylvian predominant pachygyria in the more common and less severe forms.[56] Findings from autopsies reveal abnormal cortical layering, hypoplastic and disorganized hippocampi, and clusters of heterotopic neurons interspersed within the white matter.

The *TUBA1A* phenotype (Fig. 60–1C,D) is somewhat distinct from those of *LIS1* and *DCX*, however. Patients with *TUBA1A* mutations have additional defects that are less commonly associated with *LIS1* and *DCX* mutations, including cerebellar and brainstem hypoplasia, as well as hypoplasia of the anterior limb of the internal capsule. This long tract finding appears to be extremely specific to *TUBA1A* mutations and is associated with dysmorphic basal ganglia that lack a clear separation between the caudate and putamen.[56] As with *LIS1* and *DCX* mutations, hypoplastic and disorganized white matter tracts suggest further disruption of axon growth and guidance beyond defects in cell migration. Thus, patients usually have severe neurological impairments, including mental retardation, spastic diplegia or tetraplegia, facial paralysis, and epilepsy.

In contrast to *DCX* and *LIS1* mutations, *TUBA1A* mutations have not been extensively modeled in mice. However, a mouse with a mutation in the GTP binding site on Tuba1a has been described, with abnormal hippocampal lamination but no overt migration disorder in the cortex.[13] This mouse histopathology appears very similar to that of the *Dcx* mutant mouse, and it is likely that on further examination, other abnormalities will be observed.

Mutations in genes that encode different α– and β-tubulin isoforms, including *TUBB2B*, *TUBA8*, and *TUBB3*, also cause other congenital neurological syndromes. Mutations in *TUBA8* and *TUBB2B* cause polymicrogyria with and without ocular hypoplasia, respectively.[4,57] These syndromes are associated with developmental delay and seizures. In contrast, mutations in *TUBB3* cause a defect in axon guidance; patients present with restrictions in eye movement, mild cognitive impairments, spasticity, and, later, polyneuropathy. Radiological findings reveal

hypoplastic oculomotor nerves, dysmorphic basal ganglia with or without internal capsule hypoplasia, and agenesis or hypoplasia of the corpus callosum and anterior commissure, but no cortical malformations. The *TUBB3* syndrome is thus not as severe as the other tubulin mutation syndromes in terms of central nervous system dysfunction; as a result, these patients rarely have seizures.[58]

Each of the tubulin syndromes described above can result from mutations in tubulin isoforms that inhibit the formation of microtubules by interfering with the levels of tubulin expression, folding, or function (i.e., GTP binding), yet some pathogenic mutations do not appear to have any discernible effects on these specific tubulin properties. It is thought, however, that these mutations cause the phenotypic defects by disrupting the binding of other proteins to microtubules, including motor proteins such as kinesins and dynein and/or MAPs such as DCX. For example, the R402H mutation in TUBA1A has been shown to lie directly in the groove where DCX is thought to bind to tubulin,[13] and DCX is implicated in the transport of presynaptic vesicles. In addition, TUBB3 mutations are known to disrupt the function of another kinesin motor protein, KIF21A.[58] These findings strongly suggest that the tubulinopathies may be best understood in terms of a motor defect.[59]

MICROTUBULE FUNCTION AND THE PATHOGENESIS OF SEIZURES

Defects in neuronal migration of either excitatory or inhibitory neurons result, broadly speaking, in abnormal neuronal connectivity and circuit formation, as axons are unable to find their normal postsynaptic targets. Defects in neuronal migration of excitatory neurons may result in the abnormal formation of circuits. The dyslamination of pyramidal neurons means that the GABAergic interneurons may not be able to form the correct connections. This alone may be enough to tilt the balance in cortical circuitry toward hyperexcitability. In fact, the use of RNAi in the rat model to knock down *Dcx* specifically in pyramidal neurons without affecting GABAergic neurons does result in abnormal migration of interneurons to the subcortical band. These animals have been shown to have decreased inhibition in the overlying regions of cortex.[49] However, in animal models with targeted mutations in Lis1 and Dcx, the interneurons are also affected since these proteins are expressed in every neuron.[60,61] And while interneuron migration is not totally normal, the inhibitory interneurons still successfully migrate into the cortex. However, it is unknown whether specific subsets of interneurons are preferentially affected in either *DCX*- or *LIS1*- mediated disorders.

In these migration disorders, however, there might be additional specific molecular defects in the axons themselves, and microtubule dysfunction in such axons may explain the clinical phenotypes observed through disruption of dynein- and kinesin-mediated transport processes and their specific cargoes. For example, the *LIS1* syndrome may result from failure of dynein-mediated nuclear translocation during migration. However, the continued absence of retrograde transport in these neurons following the developmental stages into maturity may affect signaling from distal regions of the axons and dendrites. Although the specific signaling pathways affected are unknown, they may be pathways that respond specifically to neuronal activity such as that of Cdk5, which is both regulated by activity and known to interact with Lis1. In comparison, *DCX* mutations may cause both a failure of migration and axon outgrowth through defects in anterograde vesicle transport in growing axons and dendrites. In fact, the defect in kinesin-medicated vesicle transport observed in DCX mutant neurons may have far-reaching effects, such as impairments of membrane addition, mislocalization of guidance receptors, and ultimately, mislocalization of ion channels.

CORTICAL MALFORMATIONS ARE AN IMPORTANT CAUSE OF PEDIATRIC EPILEPSY

Genetic causes of brain malformations have a clearly defined etiology and can be studied with animal models, yet they are relatively rare. In the pediatric population, however, other cortical malformations, especially focal cortical dysplasias (FCDs), are the underlying cause of a large percentage of first presentation with seizures (reviewed in ref. 62). Epilepsies resulting

from cortical malformations are often difficult to control, requiring multiple medications and surgeries. In addition, compared to other epilepsy patients, the societal cost for such patients is disproportionately greater in terms of both health care spending and comorbidity. However, advances in genetics and neuroimaging have resulted in significant improvements in our understanding of these disorders as well as expansion of our diagnostic capabilities, and we are poised to develop new treatment options for patients suffering from these disorders.

Focal cortical dysplasia is a heterogeneous disorder, which has a wide range of severity by histopathological appearance and thus an unknown (or poorly defined) etiology. The severity of FCD is graded and correlated with the radiological findings.[62,63] Pathological examination of magnetic resonance imaging (MRI)-negative focal epilepsies results in a diagnosis of a mild form of FCD (Type I FCD) in up to half of the patients who undergo surgery for resection of a seizure focus.[64] Type II FCD is more severe and makes up most of the cases, which are diagnosed presurgically by MRI. Thus, FCD appears to encompass a wide range of severity that may reflect multiple etiologies ranging from mild dyslamination to the more severe form with heterotopic neurons, abnormal giant neurons, and balloon glial cells.[63]

While giant neurons and balloon cells are not found in lissencephaly, changes associated with mild FCD (heterotopic neurons and abnormal neuronal polarity) are reminiscent of the histopathology seen in human lissencephaly. Studies of mild forms of cortical dysplasia demonstrate cellular defects similar to those seen in tissue from animal models of *Dcx* and *Lis1* lissencephaly, including the disruption of lamination and multipolar heterotopic neurons. Thus, the same pathways that are disrupted in genetic causes of lissencephaly may be important for the pathogenesis of cortical dysplasia, and it is reasonable to hypothesize that microtubule dysfunction may be a leading cause of cortical dysplasia.

In contrast to milder forms of FCD, severe forms, which are more readily diagnosed by imaging, include histopathological features that are also seen in tuberous sclerosis complex (TSC).[65] Both severe FCD (Type II) and TSC have, in addition to dyslamination, giant neurons and balloon cells (reactive glial cells). Thus, TSC pathway proteins, including mTOR, are other possible candidates for FCD pathogenesis. What remains unclear is whether mild FCD and severe FCD result from the extent of dysfunction of one particular pathway, or if they result from developmental disruption of separate pathways. It is not inconceivable that FCD is a heterogeneous disorder with several different causes, and that some subsets of FCD result from molecular pathway dysfunction in terms of severity and other subsets are caused by entirely different pathways. Finally, the pathways that cause FCD and lissencephaly may be related.

Recent success in the manipulation of these candidate pathways in FCD highlights the importance of understanding the pathogenesis of FCD. Manipulation of these pathways has been shown to alter epilepsy in non-FCD models: rapamycin, an mTOR inhibitor, has been successfully used for treating seizures in animal models of TSC.[66] In addition, a recent study has shown that reexpression of a lissencephaly gene, *DCX*, in heterotopic neurons of *adult* animals resulted in a decrease in the size of the heterotopia and a reduction in seizure threshold.[67] Thus, determining whether these pathways are involved in the pathogenesis of the different types of FCD may result in (1) development of an appropriate animal model and/or (2) targeting of seizure therapy. With the successful therapeutic intervention in the animal model, the possibility of helping patients with disorders stemming from disruption of the same molecular pathway becomes a real possibility, and it is critical to adequately characterize FCD to know whether this can be achieved.

This characterization of human FCD will be extremely challenging, with obstacles including sample collection, as well as the heterogeneity of the patient population in terms of age, gender, treatment, and genetic background. In addition, many of the candidate proteins are developmentally expressed, and human samples obtained are typically outside the window of expression. However, the availability of animal models in all stages of development may make detailed phenotypic assessment and correlation of molecular pathway defects in human FCD more feasible. As discussed above, mouse models of neuronal migration have been extensively characterized and can be compared with human FCD, as well as with the animal model for TSC. Thus, characterization of FCD can be

conducted with reference to the animal models of candidate molecular pathways. Furthermore, detailed characterization of the microcircuitry in both FCD and lissencephaly models may yield meaningful comparisons for understanding the development of hyperexcitability.

DISCLOSURE STATEMENT

The authors have no conflicts or disclosures regarding this work.

REFERENCES

1. Niethammer M, Smith DS, Ayala R, et al. NUDEL is a novel Cdk5 substrate that associates with LIS1 and cytoplasmic dynein. *Neuron*. 2000;28:697–711.
2. Gleeson JG, Lin PT, Flanagan LA, et al. Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. *Neuron*. 1999;23:257–271.
3. Francis F, Koulakoff A, Boucher D, et al. Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron*. 1999;23:247–256.
4. Jaglin XH, Poirier K, Saillour Y, et al. Mutations in the beta-tubulin gene TUBB2B result in asymmetrical polymicrogyria. *Nat Genet*. 2009;41:746–752.
5. Hadjivassiliou G, Martinian L, Squier W, et al. The application of cortical layer markers in the evaluation of cortical dysplasias in epilepsy. *Acta Neuropathol*. 2010;120:517–528.
6. Dahlgren L, Wilson RD. Prenatally diagnosed microcephaly: a review of etiologies. *Fetal Diagn Ther*. 2001;16:323–326.
7. Scheffer IE, Baraitser M, Brett EM. Severe microcephaly associated with congenital varicella infection. *Dev Med Child Neurol*. 1991;33:916–920.
8. Woods CG. Human microcephaly. *Curr Opin Neurobiol*. 2004;14:112–117.
9. Dobyns WB, Stratton RF, Greenberg F. Syndromes with lissencephaly. I: Miller-Dieker and Norman-Roberts syndromes and isolated lissencephaly. *Am J Med Genet*. 1984;18:509–526.
10. Kitamura K, Yanazawa M, Sugiyama N, et al. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet*. 2002;32:359–369.
11. Hong SE, Shugart YY, Huang DT, et al. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat Genet*. 2000;26:93–96.
12. Ozcelik T, Akarsu N, Uz E, et al. Mutations in the very low-density lipoprotein receptor VLDLR cause cerebellar hypoplasia and quadrupedal locomotion in humans. *Proc Natl Acad Sci USA*. 2008;105:4232–4236.
13. Keays DA, Tian G, Poirier K, et al. Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. *Cell*. 2007;128:45–57.
14. Clement E, Mercuri E, Godfrey C, et al. Brain involvement in muscular dystrophies with defective dystroglycan glycosylation. *Ann Neurol*. 2008;64:573–582.
15. Kara S, Jissendi-Tchofo P, Barkovich AJ. Developmental differences of the major forebrain sommissures in lissencephalies. *Am J Neuroradiol*. 2010;31:1602–7.
16. Caviness VS Jr. Neocortical histogenesis in normal and reeler mice: a developmental study based upon [3H]thymidine autoradiography. *Brain Res*. 1982;256:293–302.
17. Gilmore EC, Ohshima T, Goffinet AM, et al. Cyclin-dependent kinase 5-deficient mice demonstrate novel developmental arrest in cerebral cortex. *J Neurosci*. 1998;18:6370–6377.
18. Tanaka T, Serneo FF, Tseng HC, et al. Cdk5 phosphorylation of doublecortin ser297 regulates its effect on neuronal migration. *Neuron*. 2004;41:215–227.
19. Noctor SC, Martinez-Cerdeno V, Ivic L, et al. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat Neurosci*. 2004;7:136–144.
20. Deuel TA, Liu JS, Corbo JC, et al. Genetic interactions between doublecortin and doublecortin-like kinase in neuronal migration and axon outgrowth. *Neuron*. 2006;49:41–53.
21. Demer JL, Clark R, Tischfield MA, et al. Magnetic resonance imaging evidence of an asymmetrical endophenotype in congenital fibrosis of extraocular muscles type 3 resulting from TUBB3 mutations. *Invest Ophthalmol Vis Sci*. 2010;51:4600-4611
22. Baas PW, Slaughter T, Brown A, et al. Microtubule dynamics in axons and dendrites. *J Neurosci Res*. 1991;30:134–153.
23. Tint I, Jean D, Baas PW, et al. Doublecortin associates with microtubules preferentially in regions of the axon displaying actin-rich protrusive structures. *J Neurosci*. 2009;29:10995–11010.
24. Moores CA, Perderiset M, Kappeler C, et al. Distinct roles of doublecortin modulating the microtubule cytoskeleton. *EMBO J*. 2006;25:4448–4457.
25. Moores CA, Perderiset M, Francis F, et al. Mechanism of microtubule stabilization by doublecortin. *Mol Cell*. 2004;14: 833–839.
26. Ikegami K, Heier RL, Taruishi M, et al. Loss of alpha-tubulin polyglutamylation in ROSA22 mice is associated with abnormal targeting of KIF1A and modulated synaptic function. *Proc Natl Acad Sci USA*. 2007;104:3213–3218.
27. Konishi Y, Setou M. Tubulin tyrosination navigates the kinesin-1 motor domain to axons. *Nat Neurosci*. 2009;12:559–567.
28. Nakata T, Hirokawa N. Neuronal polarity and the kinesin superfamily proteins. *Sci STKE*. 2007;2007:pe6.
29. McKenney RJ, Vershinin M, Kunwar A, et al. LIS1 and NudE induce a persistent dynein force-producing state. *Cell*.141:304–314.
30. Reiner O, Carrozzo R, Shen Y, et al. Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats. *Nature*. 1993;364:717–721.
31. Lo Nigro C, Chong CS, Smith AC, et al. Point mutations and an intragenic deletion in LIS1, the lissencephaly causative gene in isolated lissencephaly sequence and Miller-Dieker syndrome. *Hum Mol Genet*. 1997;6:157–164.

32. Chong SS, Pack SD, Roschke AV, et al. A revision of the lissencephaly and Miller-Dieker syndrome critical regions in chromosome 17p13.3. *Hum Mol Genet*. 1997;6:147–155.
33. Saillour Y, Carion N, Quelin C, et al. LIS1-related isolated lissencephaly: spectrum of mutations and relationships with malformation severity. *Arch Neurol*. 2009;66:1007–1015.
34. Fleck MW, Hirotsune S, Gambello MJ, et al. Hippocampal abnormalities and enhanced excitability in a murine model of human lissencephaly. *J Neurosci*. 2000;20:2439–2450.
35. Hirotsune S, Fleck MW, Gambello MJ, et al. Graded reduction of Pafah1b1 (Lis1) activity results in neuronal migration defects and early embryonic lethality. *Nat Genet*. 1998;19:333–339.
36. Gopal PP, Simonet JC, Shapiro W, et al. Leading process branch instability in Lis1$^{+/-}$ nonradially migrating interneurons. *Cereb Cortex*. 2010;20:1497–1505.
37. Yingling J, Youn YH, Darling D, et al. Neuroepithelial stem cell proliferation requires LIS1 for precise spindle orientation and symmetric division. *Cell*. 2008;132:474–486.
38. Coquelle FM, Caspi M, Cordelieres FP, et al. LIS1, CLIP-170's key to the dynein/dynactin pathway. *Mol Cell Biol*. 2002;22:3089–3102.
39. Tsai JW, Bremner KH, Vallee RB. Dual subcellular roles for LIS1 and dynein in radial neuronal migration in live brain tissue. *Nat Neurosci*. 2007;10:970–979.
40. Stehman SA, Chen Y, McKenney RJ, et al. NudE and NudEL are required for mitotic progression and are involved in dynein recruitment to kinetochores. *J Cell Biol*. 2007;178:583–594.
41. Grabham PW, Seale GE, Bennecib M, et al. Cytoplasmic dynein and LIS1 are required for microtubule advance during growth cone remodeling and fast axonal outgrowth. *J Neurosci*. 2007;27:5823–5834.
42. Gleeson JG, Allen KM, Fox JW, et al. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. *Cell*. 1998;92:63–72.
43. des Portes V, Pinard JM, Billuart P, et al. A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. *Cell*. 1998;92:51–61.
44. Gleeson JG, Minnerath SR, Fox JW, et al. Characterization of mutations in the gene doublecortin in patients with double cortex syndrome. *Ann Neurol*. 1999;45:146–153.
45. Guerrini R, Moro F, Andermann E, et al. Nonsyndromic mental retardation and cryptogenic epilepsy in women with doublecortin gene mutations. *Ann Neurol*. 2003;54:30–37.
46. Corbo JC, Deuel TA, Long JM, et al. Doublecortin is required in mice for lamination of the hippocampus but not the neocortex. *J Neurosci*. 2002;22:7548–7557.
47. Kappeler C, Dhenain M, Phan Dinh Tuy F, et al. Magnetic resonance imaging and histological studies of corpus callosal and hippocampal abnormalities linked to doublecortin deficiency. *J Comp Neurol*. 2007;500:239–254.
48. Bai J, Ramos RL, Ackman JB, et al. RNAi reveals doublecortin is required for radial migration in rat neocortex. *Nat Neurosci*. 2003;6:1277–1283.
49. Ackman JB, Aniksztejn L, Crepel V, et al. Abnormal network activity in a targeted genetic model of human double cortex. *J Neurosci*. 2009;29:313–327.
50. Koizumi H, Tanaka T, Gleeson JG. Doublecortin-like kinase functions with doublecortin to mediate fiber tract decussation and neuronal migration. *Neuron*. 2006;49:55–66.
51. Kerjan G, Koizumi H, Han EB, et al. Mice lacking doublecortin and doublecortin-like kinase 2 display altered hippocampal neuronal maturation and spontaneous seizures. *Proc Natl Acad Sci USA*. 2009;106:6766–6771.
52. Bernasconi A, Martinez V, Rosa-Neto P, et al. Surgical resection for intractable epilepsy in "double cortex" syndrome yields inadequate results. *Epilepsia*. 2001;42:1124–1129.
53. Taylor KR, Holzer AK, Bazan JF, et al. Patient mutations in doublecortin define a repeated tubulin-binding domain. *J Biol Chem*. 2000;275:34442–34450.
54. Bielas SL, Serneo FF, Chechlacz M, et al. Spinophilin facilitates dephosphorylation of doublecortin by PP1 to mediate microtubule bundling at the axonal wrist. *Cell*. 2007;129:579–591.
55. Friocourt G, Chafey P, Billuart P, et al. Doublecortin interacts with mu subunits of clathrin adaptor complexes in the developing nervous system. *Mol Cell Neurosci*. 2001;18:307–319.
56. Bahi-Buisson N, Poirier K, Boddaert N, et al. Refinement of cortical dysgeneses spectrum associated with TUBA1A mutations. *J Med Genet*. 2008;45:647–653.
57. Abdollahi MR, Morrison E, Sirey T, et al. Mutation of the variant alpha-tubulin TUBA8 results in polymicrogyria with optic nerve hypoplasia. *Am J Hum Genet*. 2009;85:737–744.
58. Tischfield MA, Baris HN, Wu C, et al. Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. *Cell*. 2010;140:74–87.
59. Tischfield MA, Engle EC. Distinct alpha- and beta-tubulin isotypes are required for the positioning, differentiation and survival of neurons: new support for the "multi-tubulin" hypothesis. *Biosci Rep*. 2010; 30:319–330.
60. Friocourt G, Liu JS, Antypa M, et al. Both doublecortin and doublecortin-like kinase play a role in cortical interneuron migration. *J Neurosci*. 2007;27:3875–3883.
61. McManus MF, Nasrallah IM, Pancoast MM, et al. Lis1 is necessary for normal non-radial migration of inhibitory interneurons. *Am J Pathol*. 2004;165:775–784.
62. Lerner JT, Salamon N, Hauptman JS, et al. Assessment and surgical outcomes for mild type I and severe type II cortical dysplasia: a critical review and the UCLA experience. *Epilepsia*. 2009;50:1310–1335.
63. Palmini A, Najm I, Avanzini G, et al. Terminology and classification of the cortical dysplasias. *Neurology*. 2004;62:S2–S8.
64. Porter BE, Judkins AR, Clancy RR, et al. Dysplasia: a common finding in intractable pediatric temporal lobe epilepsy. *Neurology*. 2003;61:365–368.

65. Cepeda C, Andre VM, Yamazaki I, et al. Comparative study of cellular and synaptic abnormalities in brain tissue samples from pediatric tuberous sclerosis complex and cortical dysplasia type II. *Epilepsia*. 51(suppl 3): 160–165.
66. Zeng LH, Xu L, Gutmann DH, et al. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol*. 2008;63:444–453.
67. Manent JB, Wang Y, Chang Y, et al. Dcx reexpression reduces subcortical band heterotopia and seizure threshold in an animal model of neuronal migration disorder. *Nat Med.* 2009;15: 84–90.

Chapter 61

The Generation of Cortical Interneurons

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INTRODUCTION

Epilepsy can simplistically be conceived of as a disorder of uncontrolled brain excitation; thus, disrupting the excitation/inhibition (E/I) balance of neural transmission can predispose the brain to seizures. In the pallium (cortex and hippocampus), E/I balance is mediated largely by the relative activity of two classes of neurons, glutamatergic pyramidal cells and gamma-aminobutyric acid (GABA)-containing (GABAergic) local circuit neurons, also known as *interneurons*. In the adult cortex, pyramidal cells are excitatory, whereas GABAergic interneurons are typically inhibitory. In addition to epilepsy, disruption of E/I balance is linked to several neuropsychiatric disorders.[1–4] Furthermore, genes associated with several of these disorders regulate cortical interneuron development.[5–8] Thus, to understand the underlying causes of many forms of epilepsy, particularly in childhood, it is important to understand the genetic and cellular mechanisms that control the development of cortical interneurons. This is the focus of this chapter.

Cortical excitatory projection neurons are generated by the pallial neuroepithelium.[9] By contrast, most, if not all, pallial interneurons are generated by subpallial progenitors, at least in nonprimate mammals.[10–13] The initial observations leading to this understanding came from analysis of *Dlx2* expression in mice.[14] *Dlx2* is a homeodomain transcription factor that is expressed in most subpallial progenitors and in many immature interneurons that tangentially migrate to the pallium. Vital dye migration assays have identified a robust tangential subpallial-to-pallial migration in the prenatal mouse brain.[15–17] Mice lacking *Dlx1* and *Dlx2* expression have a severe block in interneuron migration that results in a four-fold reduction in neocortical interneurons.[16,18]

Ongoing studies are aimed at elucidating the precise regions within the subpallium that generate these cells and the mechanisms that regulate their specification, migration, and differentiation into distinct interneuron subtypes in pallial structures (e.g., cortex, hippocampus, parts of the amygdala and olfactory bulb) and in subpallial structures (e.g., striatum).

The subpallium consists of four major subdivisions that have distinct molecular and morphological features: the lateral ganglionic eminence (LGE), medial ganglionic eminence (MGE), septum (SE), and preoptic area (POA).[6,19] Sulci approximate the transitions between most of these domains at early embryonic stages; however, the caudal part of the LGE and MGE lack a clear morphological demarcation, leading this region to be called the *caudal ganglionic eminence* (CGE), even though it has distinct LGE and MGE components. Efforts are underway to identify subdivisions within the progenitor domains of the LGE, MGE, SE, and POA. One approach has used transcription factor expression patterns.[6]

It is uncertain how many interneuron subtypes exist due to the broad diversity of their morphology, connectivity, physiology, and molecular properties. For instance, in the hippocampus, it is estimated that there are at least 21 different functional types.[20] However, for this review, we have adopted a conservative grouping of GABAergic interneurons into four major classes: (1) fast spiking, PV-containing basket and chandelier cells; (2) somatostatin (SST)-containing interneurons, which typically display intrinsic burst spiking or adapting non-fast-spiking electrophysiological profiles, and many have long axons that extend into layer I; (3) non-fast-spiking and rapidly adapting interneurons with bipolar or double-bouquet morphologies, which frequently express calretinin (CR) and/or vasointestinal peptide (VIP); and (4) rapidly adapting interneurons with multipolar morphologies that express neuropeptide Y (NPY) and/or Reelin, but not SST (Fig. 61–1).

Recent progress on the origin of interneurons suggests that these different classes of cells originate from three main sources in the developing subpallium—the MGE, CGE, and POA—and reach the cortex following different migratory routes (Fig. 61–2). Here we review our current view of this process, which is largely based on studies in the mouse. The origin of some populations of GABAergic interneurons in the developing pallium of monkeys and human embryos will not be addressed in this chapter, as this topic has recently been reviewed elsewhere.[21] Also, we will not review the literature concerning the contribution of LGE-derived interneurons to the olfactory bulb.

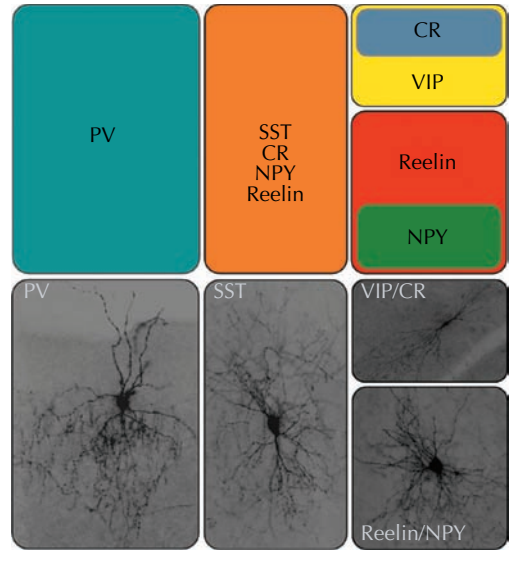

Figure 61–1. Four major groups of cortical interneurons can be distinguished in the mouse neocortex. (1) Fast spiking, PV-containing basket and chandelier cells. To date, there are no other markers that subdivide this large population of interneurons. (2) Somatostatin-containing interneurons, which typically display intrinsic-burst spiking or adapting non-fast spiking electrophysiological profiles. Many of these neurons have the morphology of Martinotti cells. This population of SST-containing cells is very heterogeneous and includes several classes of interneurons that may also express Reelin, CR, and/or NPY. (3) Rapidly adapting interneurons with bipolar or double-bouquet morphologies. These cells very frequently express VIP, and many of them also contain CR. (4) Rapidly adapting interneurons with multipolar morphologies. Most of these cells express Reelin but not SST, and many also express NPY.

THE MEDIAL GANGLIONIC EMINENCE GENERATES THE MAJORITY OF CORTICAL INTERNEURONS

The MGE is the origin of approximately 50%–60% of the population of cortical interneurons in the mouse.[13,22,23] The MGE gives rise

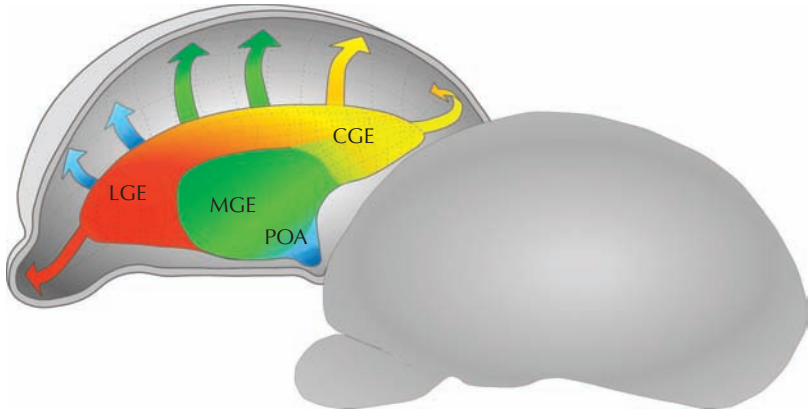

Figure 61–2. Cortical interneurons are born in the subpallium and migrate tangentially to the cortex. The schema represents an E13.5 embryo brain hemisection. The arrows show representative migratory routes. Preoptic area-derived interneurons (blue) tend to invade the cortex through its rostral region, while CGE-derived interneurons (yellow) reach the cortex primarily by its caudal pole. The lateral ganglionic eminence (red) is a major source for many olfactory bulb interneurons, although they are also produced in other regions. For simplicity, the septum and the thalamus are not depicted in the schema. ob, olfactory bulb; NCx, neocortex; PCx, paleocortex.

to most PV-containing and SST-containing interneurons (Fig. 61–3). This latter group is heterogeneous in their electrophysiological properties, morphologies, and expression of Reelin, NPY, and/or CR.[24,25]

Early studies of MGE-derived interneurons were based on the analysis of mice lacking the transcription factor *Nkx2-1*.[26] The analysis of *Nkx2-1* mutants revealed that this transcription factor is required for the generation of more than half of the GABAergic cells populating the cortex.[26] In neonatal mice, these include SST^+, NPY^+, and $calbindin^+$ interneurons.[23] However, because the *Nkx2-1* mutant dies on the day of birth due to multiple organ defects, additional work has been necessary to define the identity of the mature interneurons that depend on *Nkx2-1* function.

Subsequent studies have focused on the functions of *Nkx2-1* and other transcription factors in the development of distinct interneuron subtypes. For example, acquisition of the fast-spiking characteristics and expression of PV are regulated by the actions of the *Nkx2-1*, *Dlx5*, *Dlx6*, *Lhx6*, and *Sox6* transcription factors.[27–32]

In vitro experiments[33,34] and in vivo transplantation analyses[6,22,34–36] have revealed that the large majority of cortical interneurons derived from the MGE are PV-containing (~65%), while the remaining cells express SST (~35%). These studies have recently been confirmed by genetic fate-mapping studies that took advantage of the existence of genes with patterns of expression that are largely confined to the MGE, such as *Nkx2-1* and *Lhx6*,[37,38] as well as by the analysis of null or conditional mutants for these genes.[27,29]

A question that remains open is to what extent progenitor cells that give rise to PV- and SST-containing interneurons are spatially segregated within the MGE. The analysis of the expression pattern of dozens of transcription factors within the ventricular zone of the MGE have led to the proposal that this region may consist of up to five distinct progenitor domains, designated pMGE1 to pMGE5, which have been hypothesized to give rise to different classes of neurons.[6] Consistently, several lines of evidence suggest that the dorsal (pMGE1-2) and ventral (pMGE3-5) regions of the MGE have a tendency to preferentially give rise to SST- and PV-containing interneurons, respectively.[6,34,37] Furthermore, *Gli1* and *Nkx6-2* are expressed in the dorsal MGE.[6,39] Cre fate mapping with *Nkx6-2* provides strong evidence that the dorsal MGE generates "Martinotti" cortical interneurons (SST^+ and CR^+).[37,40] Mice lacking *Nkx6-2* or *Gli1* (in conjunction with *Gli2*) have cortical interneuron defects.[39,40]

Furthermore, recent fate-mapping analyses have suggested that the progenitor cells giving rise to PV-containing GABAergic neurons populating the basal ganglia might be

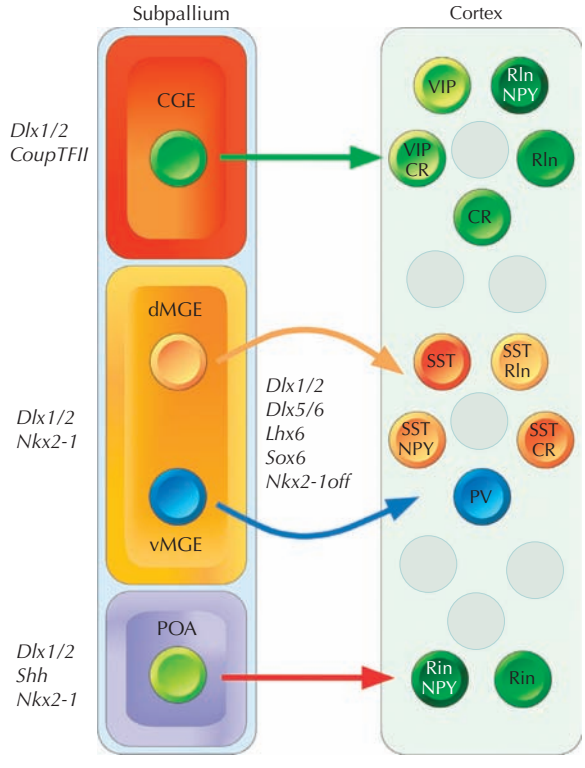

Figure 61–3. Cortical interneuron diversity largely emerges from spatially segregated progenitor cells with distinct transcriptional profiles. The schema shows the main sources of cortical interneurons—CGE, MGE, and POA—which contain progenitor cells that can be distinguished by their expression of transcription factors and other proteins. Thus, CGE cells express both *Dlx1/2* and *Couptf2*, MGE cells express *Dlx1/2* and *Nkx2-1*, and POA cells express *Dlx1/2*, *Nkx2-1*, and *Shh*. Furthermore, each of these regions seems to contain distinct progenitor domains (not shown in the schema), characterized by the expression of different transcription factors.[6] Each progenitor region produces a particular group of interneurons, although some interneuron classes may emerge from different progenitor domains. This is the case of multipolar Reelin/NPY-containing interneurons, which derive from both CGE and POA. It is possible, however, that these cells derived from a progenitor domain that bridges both structures and that is characterized by the expression of *Couptf2*.[59] The transcriptional mechanisms involved in the generation of MGE-derived interneurons are best elucidated. Thus, downregulation of *Nkx2-1* and expression of *Lhx6* and *Sox6* are necessary for the proper specification of MGE-derived interneurons. Not illustrated: the *Ascl1*, *Arx*, and *Dlx* transcription factors are required for the differentiation and migration of interneurons derived from the subpallium; *Gsx2* function is required in the CGE.

also spatially segregated from those producing PV-containing GABAergic interneurons for the cortex.[41,42] Thus, while pMGE5 seems to produce primarily PV-containing GABAergic neurons in the globus pallidus and other GABAergic neurons of the subpallium, it seems to produce very few PV-containing cortical interneurons (although these are the predominant interneuron subtype generated from this region). In turn, very few globus pallidus neurons seem to be generated from pMGE3-4, which therefore may primarily generate cortical interneurons. In sum, although progenitor domains in the telencephalon do not seem to segregate as sharply as in the spinal cord, increasing evidence suggests that the generation of distinct classes of GABAergic interneurons in the subpallium is tightly linked to the existence of distinct classes of progenitor cells (Fig. 61–3).

The mechanisms that differentially specify the generation of PV- and SST-containing interneurons are beginning to be elucidated. As mentioned above, the generation of both types of interneurons requires the maintenance of *Nkx2-1* expression in MGE progenitors, a process that involves Shh signaling.[43] Interestingly, the level of Shh signaling induced in MGE progenitors seems to dictate the type of interneuron produced, as is the case for the

spinal cord.[44] Thus, high levels of Shh signaling favor the generation of SST-containing neurons at the expense of PV-containing neurons.[45] This is consistent with previous findings that reported high levels of Shh effectors, such as *Gli1*, *Gli2*, or *Hhip1*, in the dorsal MGE.[34] What is paradoxical in this system is that the highest level of Shh activation within the ventral telencephalon occurs in the dorsal MGE, far away from the ventricular zone source of the signal in pMGE5 and the POA. This is in sharp contrast with the situation in the spinal cord. The answer to this paradox may be that the dorsal MGE receives Shh signals from Shh^+ neurons in the MGE mantle zone.[46] The fate of the large majority of PV- and SST-containing interneurons depends on *Lhx6*, a direct target of *Nkx2-1*.[47] In the absence of *Lhx6*, MGE-derived interneurons reach the pallium, but most of them fail to express PV or SST, whereas there is an increase in NPY^+ interneurons.[27,29] In addition, *Lhx6*-deficient interneurons have problems allocating into their appropriate target layers in the cortex, suggesting that genes downstream of this transcription factor regulate this process, such as *CXCR4*, *CXCR7*, and *ErbB4*.[29] Interestingly, a small population of GABAergic interneurons continues to express PV and SST in the cortex of *Lhx6* mutants,[27,29] which suggests that some of these interneurons are generated outside the MGE (see below).

Recent studies have begun to identify transcription factor genes that act downstream and/or in parallel with *Nkx2-1* and *Lhx6* in the specification and early differentiation of MGE-derived interneurons: these include *Lhx8*(7), *Sox6*, *Dlx5&6*, and *Arx*. *Lhx8*(7) is coexpressed with *Lhx6* in immature MGE cells and, like *Lhx6*, is downstream of *Nkx2-1*.[26,48] Cells that maintain *Lhx8*(7) expression do not become cortical interneurons; rather, they become basal telencephalic neurons, including globus pallidus GABAergic neurons and cholinergic interneurons of the striatum.[48,49] While *Lhx8*(7) mutants have normal cortical interneurons, *Lhx6/8* double mutants have a severe defect of MGE interneuron production and migration that is more severe than that of *Lhx6* mutants.[46]

The Sry-related HMG-box-containing transcription factor *Sox6* is expressed by most, if not all, MGE-derived immature and mature cortical interneurons. *Lhx6* function is required to maintain *Sox6* expression in migrating interneurons but not in MGE progenitors.[31,46] *Sox6* null and conditional mutant mice lack PV^+ and have reduced numbers of SST^+ interneurons.[30,31]

The *Arx* and *Dlx* homeobox genes will be discussed more extensively below in the section on the CGE. However, *Arx* and the *Dlx1,2,5&6* genes are expressed in most subpallial progenitors, including the MGE, and many of its postmitotic neuronal products.[50,51] For instance, *Dlx5* is expressed in mature PV^+ interneurons.[32] There is evidence, based on transplantation experiments, that loss of *Dlx5 or Dlx5&6* functions results in a reduction in the number of PV^+ neurons and a defect in their dendritic architeture.[32] Furthermore, $Dlx5/6^{+/-}$ mice have epilepsy and a defect in their gamma rhythm, implying an abnormality in PV^+ neuron function.[32]

Temporal regulation of interneuron neuronogenesis has an important role in defining the eventual laminar position of neurons in the neocortex.[52–55] Although the mechanisms underlying this process are unclear,[56,57] recent genetic fate-mapping analyses have shown that some types of MGE-derived neurons are preferentially generated at specific times during neurogenesis,[58] which may explain their relatively restricted laminar distribution in the cortex.

THE CAUDAL GANGLIONIC EMINENCE PRODUCES DISTINCT INTERNEURON SUBTYPES

The CGE produces 30%–40% of all cortical interneurons. Fate mapping the contribution of the CGE to the complement of cortical interneurons has been problematic because of the difficulties in consistently defining this region. Thus, while *Nkx2-1* has been a key gene for the identification of the MGE and its derivatives, the definition of the CGE has been largely based on anatomical references, which complicates the comparison between different studies. The similarities in gene expression patterns between the LGE and the CGE led to the suggestion that the CGE may indeed contains a caudal extension of the LGE progenitor domains.[6,13,50,51] Although this may actually be the case for some of the LGE

progenitor domains (in particular for pLGE3, which likely originates most GABAergic projection neurons populating the striatum and amygdala), recent studies have shown that the CGE contains progenitor domains with unique molecular profiles implying the existence of distinct CGE progenitor domains,[51,59,60] some of which are contiuous with LGE and MGE domains (compare Fig. 2A in ref. 59 with Fig. 9 in ref. 6). The *Couptf1* and *Couptf2* transcription factors are enriched in progenitor cells within the CGE, and experimental evidence suggests that *Couptf2* promotes the migration of CGE-derived interneurons to the cortex.[59]

1,1′-dihexadecyl-3,3,3′-tetramethylindocarbocyanine perchlorate (DiI) labeling studies provided evidence that interneurons are tangentially migrating through the LGE and CGE on their way to the cortex and that there are more robust migrations coming through the CGE.[16,18] However, these studies could not eliminate the possibility that some of the label was applied to MGE-derived cells. The first direct evidence supporting the origin of cortical interneurons in the CGE came from in utero transplantation studies.[54] These early observations were subsequently confirmed and extended by other studies, both in vitro and in vivo, which have demonstrated that the CGE is the main origin of interneurons with bipolar and double-bouquet morphologies, many of which express CR (but not SST) and/or VIP.[22,33] The inherent difficulty of delineating the entire population of CGE progenitors, along with the possibility that some MGE-derived cells migrate through the CGE, have complicated the precise identification of the entire complement of interneurons produced in the CGE. A recent fate-mapping study, however, has taken advantage of a *Mash1-CreER* driver line that was preferentially expressed in the LGE and CGE to report the existence of an additional population of CGE-derived interneurons that express Reelin but not SST,[25] have a multipolar morphology, and have the electrophysiological features of rapidly adapting interneurons.

The mechanisms underlying the specification of CGE-derived interneurons are beginning to be understood. The *Gsx1* and *Gsx2* (*Gsh*) homeobox transcription factor genes are required for specification of the LGE and CGE. *Gsx2* conditional loss-of-function and gain-of-function analyses demonstrate its role in generating CR^+ bipolar interneurons.[45]

Gsx2 is upstream of *Acsl1* (*Mash1*), a basic helix-loop-helix (bHLH) transcription factor. While gain-of-function analysis (ectopic expression in the cortex) shows that *Acsl1* can function in regional or at least cell type specification,[61] loss-of-function analysis primarily shows *Acsl1*'s role in the regulation of differentiation. *Ascl1*$^{-/-}$ mutants exhibit (1) loss of early-born neurons and (2) premature differentiation of secondary progenitor (SVZ)-type cells that express the *Dlx* family of homeobox genes.[62,63] *Ascl1* mutants have reduced cortical interneurons, probably due to defects in the LGE/CGE and MGE.[62] *Ascl1* is required to express Delta1, a Notch ligand, and thus, through Notch signaling, it represses differentiation.[62–64]

The *Dlx* genes are downstream of both *Gsx2* and *Ascl1*.[61,65–67] There are four *Dlx* genes that are expressed in the developing forebrain: *Dlx1, Dlx2, Dlx5*, and *Dlx6*.[68] *Dlx1&2* and *Dlx5&6* form bigene clusters that are coordinately regulated by intragenic and extragenic enhancers.[69,70] Expression of the four *Dlx* genes follows a temporal sequence: *Dlx2, Dlx1, Dlx5*, and *Dlx6*.[71,72] *Dlx1&2* are coexpressed with subsets of *Gsx2* and *Ascl1* progenitor cells; loss of *Dlx1&2* function results in maintenance of *Gsx2* and *Ascl1* expression and failure to express *Dlx5&6*.[16,50,63] Furthermore, in the *Dx1&2*$^{-/-}$ mutant, the dorsal LGE/CGE is misspecified, expressing markers of the ventral cortex, such as *Id2*.[50,51] Extensive analysis has been performed in mapping the expression of nearly 100 transcription factors in the *Dlx1&2*$^{-/-}$ mutants.[50,51] This work identified *Dlx*-dependent and *Dlx*-independent pathways of basal ganglia development.

The dorsal LGE/CGE is particularly dependent on the *Dlx*-dependent pathway, where it promotes the expression of several other transcription factors, including *Arx, Atbf1, Brn4, Dlx5, Dlx6, Er81, Esrg, Meis1, Meis2, Oct6, Pbx1, Six3, Sp8, Tshz1*, and *Vax1*. The *Dlx*-independent pathway is more robust in the ventral LGE and septum, and includes transcription factors such as *Ascl1, COUP-TFI, Gsx2, Foxg1, Hes5, Lhx2, Otx2, Pax6, Sall3, Sox11*, and *Sp9*. Furthermore, *Dlx1&2* repress the expression of transcription factors that ordinarily are not expressed in the LGE, including

Gbx1 and *Otp*, or are weakly expressed in the LGE, such as *Gsx1*. This analysis sets the stage for a systematic analysis of the transcription factor hierarchy that regulates basal ganglia development. For instance, it predicts that *Dlx1&2* and *Ascl1* regulate distinct processes; as such, compound mutants should have a more severe phenotype. Indeed, basal ganglia patterning and differentiation are rudimentary in *Dlx1&2;Ascl1* triple mutants.[50,51]

Ascl1 and the *Dlx* genes are also expressed in, and regulate the development of, the MGE. The MGE is hypoplastic in the *Ascl1* mutants[62] and has migration defects in the *Dlx1&2* mutants.[16,18] The migration defects in the *Dlx1&2* mutants appear to reflect alterations in cell shape and survival.[73] The mutant cells prematurally extend neurites, and overexpression of the *Pak3* kinase appears to contribute to this phenotype.[73] However, *Dlx1&2* mutant cells exhibit many other molecular defects that are likely to contribute to their migration defect, including reduced expression of the *CXCR4* and *CXCR7* cytokine receptor genes.[50,51]

Unlike most *Dlx* mutants, *Dlx1*$^{-/-}$ mutants are viable postnatally. In the adult cortex, *Dlx1* expression is not detected in PV^{+} interneurons (unlike that of *Dlx5, Lhx6*, and *Sox6)* but is prominent in other interneuron subtypes, including CR^{+}.[36] *Dlx1* mutants have a selective postnatal loss of dendrite-innervating interneurons, including those expressing CR, SST, NPY, and reelin.[36]

As noted above, the *Dlx* genes have a high position in the transcription factor hierarchy that directs development of ganglionic eminence derivatives. For instance, *Arx* expression is strongly downregulated in *Dlx1/2*$^{-/-}$ mutants.[74] The *Arx* homeobox transcription factor is essential for MGE differentiation, including the expression of *Lhx8*(7) and *Gbx1*[75]; in its absence, there is a block in the migration of cells out of the MGE and a deficit in cortical interneurons.[75,76] Conditional mutation of *Arx* in the ganglionic eminences results in mice with reduced cortical interneurons and epilepsy that resembles that seen in humans with *Arx* mutations.[77]

The mechanisms controlling the final allocation of CGE-derived interneurons into specific layers of the cortex may be different from those regulating the distribution of MGE-derived cells, since CGE-derived interneurons tend to occupy superficial layers of the cortex independently of their time of neurogenesis.[25] Nevertheless, most CGE-derived interneurons are produced at relatively late stages of neurogenesis in the subpallium (i.e., at around E15.5), and neurons born at this stage in both the MGE and CGE primarily colonize superficial layers of the cortex.[25]

THE PREOPTIC AREA GENERATES SEVERAL CLASSES OF CORTICAL INTERNEURONS

Recent work indicates that a proportion of interneurons may derive from the embryonic POA[78] (Fig. 61–3), which, like the MGE, expresses *Nkx2-1*. In contrast, many of the cells that emerge from this structure, at least from its ventral domain (pPOA2), do not seem to express *Lhx6*.[6] To identify interneuron derivates of the POA,[78] investigators used a *Cre* line expressed from *Nkx5-1*, which is expressed in a small population of POA cells, but not in the MGE or in any other embryonic telencephalic structure. Fate mapping this population with *Nkx5-1-Cre* revealed that the POA is the origin of a small population of multipolar GABAergic cells with an electrophysiological profile of rapidly adapting interneurons.[78] Interestingly, these cells express NPY and/or Reelin (D. Gelman and O. Marín, unpublished observations), but none of the other markers of cortical interneurons, such as PV, SST, CR, or VIP.[78] As such, these cells closely resemble those recently identified to derive from the CGE,[25] suggesting that both the POA and the CGE may contribute to this population of cortical interneurons. The POA *Nkx5-1* lineage may contribute up to 4% of the entire population of cortical GABAergic interneurons. Furthermore, recent experiments show that the progeny of an additional distinct group of POA progenitor cells that are characterized by the expression of the transcription factor Dbx1 produce a small but highly diverse population of interneurons that primarily populate deep layers of the cortex. These results indicate that the POA is the origin of nearly 10% of the total population of cortical GABAergic interneurons.[79]

OVERVIEW

Most, if not all, mouse pallial interneurons are derived from three progenitor regions in the embryonic subpallium: MGE, CGE, and POA (Fig. 61–3). While there is controversy about this in the human, there is strong evidence that the ganglionic eminences are fundamental sources for pallial interneurons in all vertebrates. Development of these regions is regulated by multiple transcription factors. *Arx, Ascl1*, and *Dlx1,2,5&6* have roles in all of these regions, whereas MGE development is regulated by *Nkx2-1, Lhx6, Lhx8*, and *Sox6*, and CGE development is regulated by *Gsx2*. Future studies should aim at elucidating the molecular mechanisms downstream of these transcription factors that regulate cell fate specification and differentiation of specific interneuron subtypes. Furthermore, because many of the transcription factors that regulate early interneuron development are expressed in mature interneurons (e.g., *Arx, Dlx1,2,5&6, Lhx6, and Sox6*), it is likely that they have roles in controlling interneuron function and/or survival, such as *Dlx1*.[36] As interneuron defects that could contribute to epilepsy include abnormalities in their production, migration, differentiation, function, and survival, the mechanisms gleaned from basic studies should provide insights into the molecular, cellular, and histological underpinnings of epileptogenesis.

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REFERENCES

1. Rubenstein JLR, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav*. 2003;2:255–267.
2. Dani VS, Chang Q, Maffei A, Turrigiano GG, Jaenisch R, Nelson SB. Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc Natl Acad Sci USA*. 2005;102:12560–12565.
3. Levitt P. Disruption of interneuron development. *Epilepsia*. 2005;46:22–28.
4. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci*. 2005;6: 312–324.
5. Erbel-Sieler C, Dudley C, Zhou Y, Wu X, Estill SJ, Han T, Diaz-Arrastia R, Brunskill EW, Potter SS, McKnight SL. Behavioral and regulatory abnormalities in mice deficient in the NPAS1 and NPAS3 transcription factors. *Proc Natl Acad Sci USA*. 2004;101: 13648–13653.
6. Flames N, Pla R, Gelman DM, Rubenstein JLR, Puelles L, Marin O. Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. *J Neurosci*. 2007;27: 9682–9695.
7. Fazzari P, Paternain A, Valiente M, Pla R, Luján R, Lloyd K, Lerma J, Marín O, Rico B. Control of cortical GABAergic circuitry development by Nrg1/ErbB4 signalling. *Nature*. 2010;464(7293):1376–1380.
8. Wen L, Lu YS, Zhu XH, Li XM, Woo RS, Chen YJ, Yin DM, Lai C, Terry AV, Vazdarjanova A, Xiong WC, Mei L. Neuregulin 1 regulates pyramidal neuron activity via ErbB4 in parvalbumin-positive interneurons. *Proc Natl Acad Sci USA*. 2010;107:1211–1216.
9. Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JL, Jones KR. Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J Neurosci*. 2002;22:6309–6314.
10. Flames N, Marin O. Developmental mechanisms underlying the generation of cortical interneuron diversity. *Neuron*. 2005;5:46(3):377–381.
11. Marin O, Rubenstein JLR. A long, remarkable journey: cellular and molecular mechanisms of tangential migration in the telencephalon. *Nat Neurosci Rev*. 2001;2:780–790.
12. Marín O, Rubenstein JLR. Cell migration in the forebrain. *Annu Rev Neurosci*. 2003;26:441–483.
13. Wonders CP, Anderson SA. The origin and specification of cortical interneurons. *Nat Rev Neurosci*. 2006;7: 687–696.
14. Porteus M, Bulfone A, Liu J, Puelles L, Lo L, Rubenstein J. DLX-2, MASH-1, and MAP-2 expression and bromodeoxyuridine incorporation define molecularly distinct cell populations in the embryonic mouse forebrain. *J Neurosci*. 1994;14:6370–6383.
15. De Carlos JA, Lopez-Mascaraque L, Valverde F. Dynamics of cell migration from the lateral ganglionic eminence in the rat. *J Neurosci*. 1996;16:6146–6156.
16. Anderson SA, Eisenstat DD, Shi L, Rubenstein JLR. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science*. 1997;278: 474–476.
17. Tamamaki N, Fujimori KE, Takauji R. Origin and route of tangentially migrating neurons in the developing

neocortical intermediate zone. *J Neurosci*. 1997;17: 8313–8323.
18. Anderson SA, Marín O, Horn C, Jennings K, Rubenstein JL. Distinct cortical migrations from the medial and lateral ganglionic eminences. *Development.* 2001;128:353–363.
19. Puelles L, Rubenstein JLR. Forebrain gene expression domains and the evolving prosomeric model. *TINS*. 2003;26:469–476.
20. Klausberger T, Somogyi P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science.* 2008;321:53–57.
21. Jones EG. The origins of cortical interneurons: mouse versus monkey and human. *Cereb Cortex*, 2009;19: 1953–1956.
22. Butt SJB, Fuccillo M, Nery S, Noctor S, Kriegstein A, Corbin JG, Fishell G. The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron*. 2005;48:591–604.
23. Pleasure SJ, Anderson S, Hevner R, Bagri A, Marin O, Lowenstein DH, Rubenstein JLR. Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. *Neuron*. 2000;28:727–740.
24. Xu X, Roby KD, Callaway EM. Mouse cortical inhibitory neuron type that coexpresses somatostatin and calretinin. *J Comp Neurol*. 2006;499:144–160.
25. Miyoshi G, Hjerling-Leffler J, Karayannis T, Sousa VH, Butt SJB, Battiste J, Johnson JE, Machold RP, Fishell G. Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. *J Neurosci*. 2010;30:1582–1594.
26. Sussel L, Marin O, Kimura S, Rubenstein JL. Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development.* 1999;126: 3359–3370.
27. Liodis P, Denaxa M, Grigoriou M, Akufo-Addo C, Yanagawa Y, Pachnis V. Lhx6 activity is required for the normal migration and specification of cortical interneuron subtypes. *J Neurosci*. 2007;27:3078–3089.
28. Butt SJB, Sousa VH, Fuccillo MV, Hjerling-Leffler J, Miyoshi G, Kimura S, Fishell G. The requirement of Nkx2–1 in the temporal specification of cortical interneuron subtypes. *Neuron*. 2008;59:722–732.
29. Zhao Y, Flandin P, Long JE, Cuesta MD, Westphal H, Rubenstein JLR. Distinct molecular pathways for development of telencephalic interneuron subtypes revealed through analysis of Lhx6 mutants. *J Comp Neurol*. 2008;510:79–99.
30. Azim E, Jabaudon D, Fame RM, Macklis JD. SOX6 controls dorsal progenitor identity and interneuron diversity during neocortical development. *Nat Neurosci.* 2009;12:1238–1247.
31. Batista-Brito R, Rossignol E, Hjerling-Leffler J, Denaxa M, Wegner M, Lefebvre V, Pachnis V, Fishell G. The cell-intrinsic requirement of Sox6 for cortical interneuron development. *Neuron*. 2009;63:466–481.
32. Wang Y, Dye CA, Sohal V, Long JE, Estrada RC, Roztocil T, Lufkin T, Deisseroth K, Baraban SC, Rubenstein JLR. Dlx5 and Dlx6 regulate the development of parvalbumin-expressing cortical interneurons. *J Neurosci*. 2010;30:5334–5345.
33. Xu Q, Cobos I, De La Cruz E, Rubenstein JL, Anderson SA. Origins of cortical interneuron subtypes. *J Neurosci*. 2004;24:2612–2622.
34. Wonders CP, Taylor L, Welagen J, Mbata IC, Xiang JZ, Anderson SA. A spatial bias for the origins of interneuron subgroups within the medial ganglionic eminence. *Dev Biol*. 2008;314:127–136.
35. Wichterle H, Turnbull DH, Nery S, Fishell G, Alvarez-Buylla A. In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. *Development.* 2001;128: 3759–3771.
36. Cobos I, Calcagnotto ME, Vilaythong AJ, Thwin MT, Noebels JL, Baraban SC, Rubenstein JLR. Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. *Nat Neurosci*. 2005;8:1059–1068.
37. Fogarty M, Grist M, Gelman D, Marin O, Pachnis V, Kessaris N. Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex. *J Neurosci*. 2007;27: 10935–10946.
38. Xu Q, Tam M, Anderson SA. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. *J Comp Neurol*. 2008;506:16–29.
39. Yu W, Wang Y, McDonnell K, Stephen D, Bai CB. Patterning of ventral telencephalon requires positive function of Gli transcription factors. *Dev Biol.* 2009;334:264–275.
40. Sousa VH, Miyoshi G, Hjerling-Leffler J, Karayannis T, Fishell G. Characterization of Nkx6–2-derived neocortical interneuron lineages. *Cereb Cortex.* 2009; 19(suppl 1):i1–i10.
41. Nóbrega-Pereira S, Kessaris N, Du T, Kimura S, Anderson SA, Marín O. Postmitotic Nkx2–1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. *Neuron*. 2008;59: 733–745.
42. Flandin P, Kimura S, Rubenstein JLR. The progenitor zone of the ventral medial ganglionic eminence requires Nkx2–1 to generate most of the globus pallidus but few neocortical interneurons. *J Neurosci*. 2010;30:2812–2823.
43. Xu Q, Wonder, CP, Anderson SA. Sonic hedgehog maintains the identity of cortical interneuron progenitors in the ventral telencephalon. *Development*. 2005;132: 4987–4998.
44. Jessell TM. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat Rev Genet*. 2000;1:20–29.
45. Xu Q, Guo L, Moore H, Waclaw RR, Campbell K, Anderson SA. Sonic hedgehog signaling confers ventral telencephalic progenitors with distinct cortical interneuron fates. *Neuron*. 2010;65:328–340.
46. Flandin P, Zhou Y, Vog V, Jeong J, Long JE, Potter GB, Westphal H, Rubenstein JLR. Lhx6 and Lhx8 coordinately induce neuronal expression of Shh that controls the generation of interneuron progenitors. *Neuron*. 2011;70(5):939–950.
47. Du T, Xu Q, Ocbina PJ, Anderson SA. NKX2.1 specifies cortical interneuron fate by activating Lhx6. *Development.* 2008;135:1559–1567.
48. Zhao Y, Marín O, Hermesz E, Powell A, Flames N, Palkovits M, Rubenstein JL, Westphal H. The LIM-homeobox gene Lhx8 is required for the development

of many cholinergic neurons in the mouse forebrain. *Proc Natl Acad Sci USA*. 2003;100:9005–9010.
49. Fragkouli A, Hearn C, Errington M, Cooke S, Grigoriou M, Bliss T, Stylianopoulou F, Pachnis V. Loss of forebrain cholinergic neurons and impairment in spatial learning and memory in LHX7-deficient mice. *Eur J Neurosci.* 2005;21:2923–2938.
50. Long JE, Cobos I, Potter GB, Rubenstein JLR. Dlx1 and Mash1 transcription factors control MGE and CGE patterning and differentiation through parallel and overlapping pathways. *Cereb Cortex.* 2009;19: i96–i106.
51. Long JE, Swan C, Liang WS, Cobos I, Potter GB, Rubenstein JL. Dlx1&2 and Mash1 transcription factors control striatal patterning and differentiation through parallel and overlapping pathways. *J Comp Neurol*. 2009;512:556–572.
52. Miller MW. Cogeneration of retrogradely labeled corticocortical projection and GABA-immunoreactive local circuit neurons in cerebral cortex. *Brain Res.* 1985;355:187–192.
53. Fairén A, Cobas A, Fonseca M. Times of generation of glutamic acid decarboxylase immunoreactive neurons in mouse somatosensory cortex. *J Comp Neurol.* 1986;251:67–83.
54. Nery S, Fishell G, Corbin JG. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. *Nat Neurosci.* 2002;5:1279–1287.
55. Valcanis H, Tan SS. Layer Specification of transplanted interneurons in developing mouse neocortex. *J Neurosci*. 2003;23:5113–5122.
56. Hammond V, So E, Gunnersen J, Valcanis H, Kalloniatis M, Tan SS. Layer positioning of late-born cortical interneurons is dependent on Reelin but not p35 signaling. *J Neurosci*, 2006;26:1646–1655.
57. Pla R, Borrell V, Flames N, Marin O. Layer acquisition by cortical GABAergic interneurons ss independent of Reelin signaling. *J Neurosci*. 2006;26:6924–6934.
58. Miyoshi G, Butt SJB, Takebayashi H, Fishell G. Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic Olig2-expressing precursors. *J Neurosci*. 2007;27:7786–7798.
59. Kanatani S, Yozu M, Tabata H, Nakajima K. COUP-TFII is preferentially expressed in the caudal ganglionic eminence and is involved in the caudal migratory stream. *J Neurosci*. 2008;28:13582–13591.
60. Willi-Monnerat S, Migliavacca E, Surdez D, Delorenzi M, Luthi-Carter R, Terskikh AV. Comprehensive spatiotemporal transcriptomic analyses of the ganglionic eminences demonstrate the uniqueness of its caudal subdivision. *Mol Cell Neurosci*. 2008;37: 845–856.
61. Fode C, Ma Q, Casarosa S, Ang SL, Anderson DJ, Guillemot F. A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. *Genes Dev.* 2000;14:67–80.
62. Casarosa S, Fode C, Guillemot F. Mash1 regulates neurogenesis in the ventral telencephalon. *Development*. 1999;126:525–534.
63. Yun K, Fischman S, Johnson J, Hrabe de Angelis M, Weinmaster G, Rubenstein JL. Modulation of the notch signaling by Mash1 and Dlx1/2 regulates sequential specification and differentiation of progenitor cell types in the subcortical telencephalon. *Development.* 2002;129:5029–5040.
64. Horton S, Meredith A, Richardson JA, Johnson JE. Correct coordination of neuronal differentiation events in ventral forebrain requires the bHLH factor MASH1. *Mol Cell Neurosci*. 1999;14:355–369.
65. Toresson H, Potter SS, Campbell K. Genetic control of dorsal-ventral identity in the telencephalon: opposing roles for Pax6 and Gsh2. *Development.* 2000;127: 4361–4371.
66. Yun K, Potter S, Rubenstein JLR. Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. *Development.* 2001;128:193–205.
67. Waclaw RR, Wang B, Pei Z, Ehrman LA, Campbell K. Distinct temporal requirements for the homeobox gene Gsx2 in specifying striatal and olfactory bulb neuronal fates. *Neuron.* 2009;63:451–465.
68. Panganiban G, Rubenstein JLR. Developmental functions of the Distal-less (Dlx) homeobox genes (review). *Development*. 2002;129:4371–4386.
69. Zerucha TS, Stuhmer T, Park BK, Long Q, Yu G, Hatch G, Gambarotta A, Schultz J, Rubenstein JLR, Ekker M. A highly conserved enhancer in the Dlx5/Dlx6 intergenic region is the site of cross-regulatory interactions between Dlx genes in the embryonic forebrain. *J Neurosci.* 2000;20:709–721.
70. Ghanem N, Yu M, Long J, Hatch G, Rubenstein JL, Ekker M. Distinct cis-regulatory elements from the Dlx1/Dlx2 locus mark different progenitor cell populations in the ganglionic eminences and different subtypes of adult cortical interneurons. *J Neurosci*. 2007;27:5012–5022.
71. Liu JK, Ghattas I, Liu S, Chen S, Rubenstein JLR. The Dlx genes encode DNA-binding proteins that are expressed in an overlapping and sequential pattern during basal ganglia differentiation. *Dev Dyn*. 1997;210:498–512.
72. Eisenstat DD, Liu JK, Mione M, Zhong W, Yu G, Anderson SA, Ghattas I, Puelles L, Rubenstein JLR. DLX-1, DLX-2 and DLX-5 expression define distinct stages of basal forebrain differentiation. *J Comp Neurol*. 1999;414:217–237.
73. Cobos I, Borello U, Rubenstein JLR. Dlx transcription factors promote migration through repression of axon and dendrite growth. *Neuron*. 2007;54:873–888.
74. Cobos I, Broccoli V, Rubenstein JLR. The vertebrate ortholog of Aristaless is regulated by Dlx genes in the developing forebrain. *J Comp Neurol.* 2005a;483:292–303.
75. Colombo E, Collombat P, Colasante G, Bianchi M, Long J, Mansouri A, Rubenstein JLR, Broccoli V. Inactivation of Arx, the murine ortholog of the X-linked lissencephaly with ambiguous genitalia gene, leads to severe disorganization of the ventral telencephalon with impaired neuronal migration and differentiation. *J Neurosci.* 2007;27:4786–4798.
76. Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, Omichi K, Suzuki R, Kato-Fukui Y, Kamiirisa K, Matsuo M, Kamijo S, Kasahara M, Yoshioka H, Ogata T, Fukuda T, Kondo I, Kato M, Dobyns WB, Yokoyama M, Morohashi K. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet*. 2002;32:359–369.
77. Marsh E, Fulp C, Gomez E, Nasrallah I, Minarcik J, Sudi J, Christian SL, Mancini G, Labosky P, Dobyns W,

Brooks-Kayal A, Golden JA. Targeted loss of Arx results in a developmental epilepsy mouse model and recapitulates the human phenotype in heterozygous females. *Brain.* 2009;132:1563–1576.
78. Gelman DM, Martini FJ, Nobrega-Pereira S, Pierani A, Kessaris N, Marin O. The embryonic preoptic area is a novel source of cortical GABAergic interneurons. *J Neurosci*. 2009;29:9380–9389.
79 Gelman DM, Griveau A, Dehorter N, Teissier A, Varela C, Pla R, Pierani A, Marín, O. A wide diversity of cortical GABAergic interneurons derives from the embryonic preoptic area. *J Neurosci*. 2011;31:16570–16580.

Chapter 62

Genes in Infantile Epileptic Encephalopathies

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INTRODUCTION

Epileptic encephalopathies (EEs) are conditions in which cognitive, sensory, and/or motor function deterioration results mainly from epileptic activity.[1] This epileptic activity, which can be frequent and severe, as in Dravet syndrome (DS), or continuous, subcontinuous, or abundant in the interictal period, as in Landau-Kleffner or Lennox-Gastaut syndrome, is believed to have a deleterious impact on the developing and mature brain, interfering with cognitive functions at different times of life.[2]

Epileptic encephalopathies have numerous etiologies, which differ from one to another.[3] For example, various causes were identified in West syndrome, including damage and malformations of the brain and, more rarely, an inborn error of metabolism. However, at least one-third are cryptogenic, without any identifiable underlying cause.[2] When the more frequent etiologies have been excluded by extensive biological and neuroimaging examinations, the possibility of a genetic cause must be addressed. In contrast, DS is the archetype of the genetically determined EE, with a mutation identified in 60%–80% of patients. In this context, genetic testing has to be provided in order to confirm the clinical diagnosis and offer genetic counseling.

Patients with EE are mainly sporadic cases, making the differentiation between an acquired and a genetic cause a challenge. In rare cases, however, the recurrence of the same phenotype in a family strongly supports the involvement of a mutated gene. In addition, because of the high phenotypic variability of genetic diseases, EE can occur in a familial context of epilepsy. Indeed, 10% of patients with DS have a familial history of epilepsy or febrile convulsions.

In this chapter, we will focus on the genes responsible for infantile EEs as the main component of the clinical picture and not on those included in complex phenotypes encountered in metabolic diseases or in contiguity syndromes due to large chromosomal abnormalities. Indeed, the association of EE with a dysmorphy or extraneurological symptoms should lead to specific metabolic or cytogenetic investigations. In the first part of this chapter, we will review the genes involved in early infantile epileptic encephalopathies (EIEEs). Although the mutations in these genes are rare, their screening has to be integrated into a rational diagnostic strategy. It is particularly important to rule out genetic etiologies since there is a risk of the disease recurring in the family. In this review, we will focus on DS as an example of a genetically determined infantile EE to emphasize the difficulties and pitfalls that might be encountered in such diseases.

THE MONOGENIC FORMS OF EIEES

Geneticists have long known that a fraction of sporadic cases of patients with developmental defects could be due to a mutation in a single gene (monogenic forms). All modes of inheritance can be hypothesized, each of them being associated with particular features (Fig. 62–1). Three of them have already been reported in EIEEs: autosomal dominant (mainly de novo mutations), autosomal recessive, and X-linked.

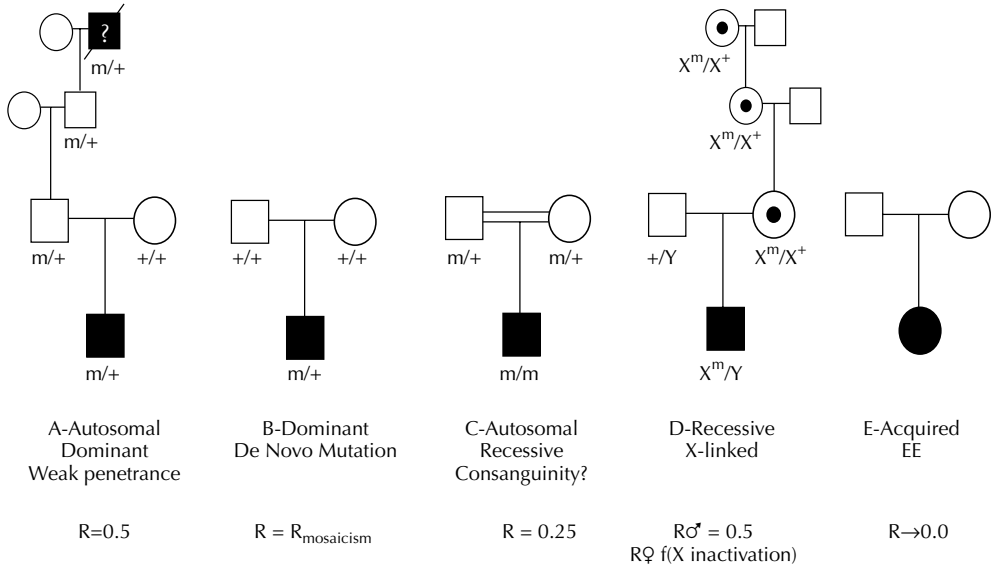

Figure 62–1. Different genetic conditions leading to isolated cases (example of an affected male). m: mutated copy of the gene; +: normal copy of the gene; X: X chromosome; Y: Y chromosome; R: risk of recurrence in the sibling. **A.** *Autosomal dominant inheritance* with weak penetrance: mutation carriers are asymptomatic over two generations, and the familial memory concerning the great-grandfather's phenotype is imprecise. **B.** *De novo mutation*: the mutation occurred in the spermatozoid or oocyte giving birth to the patient and is, therefore, absent from the parents' cells. This dominant mutation can affect a gene on the autosome (autosomal dominant) or the X chromosome (dominant X-linked). The risk of recurrence is generally low but not null: there is the risk of mosaicism (see the section "DS in a Familial Context of Epilepsy"). **C.** *Autosomal recessive inheritance*: this mode of inheritance is suspected when parents are related (see the text). **D.** *X-recessive inheritance*: hemizygous males are affected, and carrier females are mainly asymptomatic (pointed circle) or sometimes (about 10%) weakly or mildly affected. **E.** *Acquired EE:* In many EEs, this condition is the most frequent; the risk of recurrence is nearly null if the identified cause is avoided for future pregnancies.

Table 62–1 **Main Etiologies of Epilepileptic Encephalopathies (EEs)**

Epileptic Encephalopathies	Main Etiologies
Neonatal encephalopathies with suppression-bursts EEG pattern: – Ohtahara syndrome – Early myoclonic encephalopathies	*Brain damages*: malformations, perinatal anoxo-ischemic event *Metabolic diseases:* pyridoxine or pyridoxine-phosphate-dependent convulsions; hyperglycemia with ketosis; sulfate oxydase deficit; mitochondriopathies. *Genetic causes: ARX* (♂), *CG1*, and *STXBP1* genes. *Cryptogenic.*
– West syndrome – Lennox-Gastaut syndrome	*Brain damages:* acquired in the pre-, peri-, or postnatal period (60%–90% of cases): malformations, vascular insult, head trauma or infection. *Metabolic diseases:* mitochondriopathies, pyruvate dehydrogenase deficit, Menkes disease, phenylketonuria. *Genetic causes:* Down syndrome (21 trisomy); *STK9* (♀>>>♂), deletion 1p36, inv-dup chromosome 15 *Cryptogenic.*
EEs with continuous spike-waves in slow sleep Continuous spike-waves in slow sleep and related disorders (Landau-Kleffner syndrome, acquired frontal syndrome, and acquired opercular syndrome)	*Brain damages:* vascular insult (porencephalic cavity), leukomalacia, polymicrogyria, cortical dysplasia. *Mainly cryptogenic*
Migrating partial epilepsy	*Cryptogenic*
Dravet syndrome (severe myoclonic epilepsy of infancy)	*Genetic causes:* – *SCN1A* (de novo mutations and inherited mutations in a familial context of generalized epilepsy with febrile seizures plus [GEFS+]) – *PCDH19* (♀) (de novo mutations and inherited mutations in a familial context of epilepsy and mental retardation limited to females [EFMR]) – *GABRG2* (in a familial context of GEFS+)
Myoclono-astatic epilepsy with a poor outcome	*Genetic causes:* – *SCN1A* and *GABRG2*, both in a familial context of GEFS+ *Cryptogenic*
Rasmussen encephalitis	*Immune mechanisms suspected*

A genetic classification exists for genetically determined EIEE (OMIM: Online Mendelian Inheritance in Man: http://www.ncbi.nlm.nih.gov/omim): EIEE1 (OMIM 3008350) is part of the phenotypic spectrum of disorders caused by mutation in the *ARX* gene comprising a nearly continuous series of developmental disorders; EIEE2 (OMIM 300672), an X-linked disorder caused by mutation in the *CDKL5/STK9* gene (OMIM 300203); EIEE3 (MIM 609304), caused by mutation in the *CG1/SLC25A22* gene (OMIM 609302); EIEE4 (OMIM 612164), caused by mutation in the *STXBP1* gene (OMIM 602926); and EIEE5 (MIM 613477), caused by mutation in the *SPTAN1* gene (OMIM 182810). This genetic classification differs from the clinical classification of EEs based on type of seizures, age at onset, evolution, and prognosis[3] (Table 62–1). The articulation of clinical and genetic classifications will be a challenge in the coming years. The main problem is that the number of mutations identified in each gene is relatively small and is usually associated with a large phenotypic spectrum, making positive phenotype–genotype correlations difficult.

Dominant Mutations Responsible for EIEE Mostly Occur de Novo

THE DOMINANT MUTATIONS OF STXBP1 RESPONSIBLE FOR EIEE4

Dominant mutations of the *STXPB1* (also called *MUNC18-1*) gene, which is located on

chromosome 9q34.11, can cause sporadic EE. In autosomal dominant diseases, weak penetrance (the proportion of affected individuals among mutation carriers) can explain why parents are not affected, one of them being an asymptomatic carrier. However, for deleterious phenotypes like EEs, most sporadic cases are due to de novo mutations. In this case, it is hypothesized that mutations, which are eliminated from the population by their negative selective effect on patients' reproduction, are counterbalanced by the occurrence of neomutations. The mutation generally occurs in a gamete that will be involved later on in fecundation, the corresponding zygote being affected since the mutation is dominant. This is often the case for EE associated with mutations in *STXBP1*. The *STXBP1* gene contains 20 exons and encodes the syntaxin-binding protein 1. Forming a complex with the *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein syntaxin1, the STXBP1 protein might mediate synaptic vesicle fusion.[4] In accordance with this hypothesis, deletion of Munc18-1 (the paralog of *STXBP1*) in mice renders the brain synaptically silent, identifying Munc18-1 (*STXBP1*) as the currently most upstream essential protein in neurotransmitter release.[5] Saitsu et al.[6] first described mutations in this gene in 5 unrelated cases among 13 patients with EIEE with suppression-burst (Ohtahara syndrome) for whom the main causes of EEIE were excluded. Missense mutations, premature termination codons (nonsense mutations) and deletions were reported, leading to the conclusion that the disease is likely due to a haploinsufficiency (loss of function of the mutated copy of the gene) of *STXBP1*.[6,7] Early infantile epileptic encephalopathy 4 is most often associated with neurological signs such as mental retardation, which can be profound, and spastic paraplegia. The rarity of reports on mutations in this gene has to be underlined and raises the question of whether its systematic screening is indicated for patients with mental retardation and EIEE with suppression-burst.

SPTAN1, A NEWLY IDENTIFIED GENE RESPONSIBLE FOR SPORADIC EIEE5

Four patients presenting with early-onset EE with poor attention, severe hypomyelination, and reduction in cerebral white matter (EIEE5; 613477) were reported in 2008. On electroencephalography (EEG), one patient presented with suppression-burst and the other three had hypsarrhythmia. The authors stated that this phenotype could be considered a new clinical condition associated with early-onset West syndrome (WS).[8,9] A de novo 9q33.3-q34.11 microdeletion encompassing the *STXBP1* gene has been detected in one of these patients. Since no mutations of *STXBP1* were found in two of the remaining three patients (one was unavailable) , the authors suspected that another gene within the deletion might contribute to this severe phenotype. *SPTAN1* encoding alpha-II spectrin, which is involved in cell proliferation via arrest at cell cycle phase G1 and in myelination in zebrafish,[10] was localized in the deletion. In the two tested subjects, an in-frame 3 bp deletion and a 6 bp duplication in *SPTAN*, likely resulting in the synthesis of an abnormal protein, were identified at the initial nucleation site of the alpha/beta spectrin heterodimer.[9] *SPTAN1* was further screened in six unrelated individuals with WS and hypomyelination, but no mutations were found. In vitro functional expression studies suggested a dominant-negative effect of the two identified mutations on spectrin heterodimer stability, as well as perturbation of the axon initial segment.

GC1/SLC25A22, a Gene Responsible for Rare Autosomal Recessive (AR) EIEE with a Suppression-Burst Pattern

In Europe, the vast majority of families with an AR disease have a single affected child because of the small number of siblings. However, for rare diseases, consanguinity has to be searched for since it increases the probability of encountering two mutated copies of a gene in the same individual. This was the case for recessive mutations of *GC1* (Glutamate carrier 1; also called *SLC25A22* for solute carrier family 25) identified in patients with EIEE3 (EIEE3; OMIM 6093304). This gene (OMIM 609304) was first mapped on chromosome 11p15.5 in a consanguineous Arab Muslim family from Jerusalem. Patients presented with neonatal intractable

seizures associated with a suppression-burst EEG pattern and hypotonia. Brain atrophy was later diagnosed on a computed tomography (CT) scan (at about 3 years of age).[11] The missense mutation p.Pro206Leu was subsequently identified at the homozygous state in the affected members of this family in the *GC1/SLC25A2* gene, which was located within the candidate genomic region. A second mutation, p.Gly236Trp (G236W), which confirmed the responsibility of the *GC1* gene in this EE, was more recently identified in an Algerian boy also born to related parents with a severe encephalopathy and without any psychomotor acquisition.[12] *GC1/SLC25A22* encodes one of the two mitochondrial glutamate/H^+ symporters, the other being *SLC25A18*.[13] In vitro functional expression assays of G236W-mutated *GC1* cDNA in *Escherichia coli* showed that the mutant protein was correctly inserted into the liposomal membrane but had no functional transport activity, confirming the deleterious effects of the mutation on the protein function.[12]

X-Linked EEs

X-linked heredity is characterized by a difference of phenotype related to gender. The recessive X-linked mode of inheritance is most often characterized by a familial context, with affected males being born to asymptomatic female carriers. The transmission of the mutation by females over several generations leads to isolated affected males. For severe diseases, sporadic cases can also occur by de novo mutation, such as in Duchenne muscular dystrophy, in which one-third of affected males are sporadic cases due to de novo mutations. In dominant X-linked inheritance, heterozygous females can be affected, but generally less severely, in terms of age at onset or prognosis, compared to hemizygous males (i.e., with a single mutated copy of the gene). In some diseases, the presence of the mutations at the hemizygous state in males is even lethal, with the disease consequently being restricted to females. There is also an intermediate condition in which only a small fraction of affected males are born. They usually have a profoundly deleterious disease with a very poor prognosis. All these possibilities will be illustrated in the following examples of X-linked EE.

MUTATIONS IN CDKL5/STK9 CAUSE EE, PREFERENTIALLY IN FEMALES

The *CDKL5/STK9* gene was first incriminated in two unrelated girls with infantile spasm syndrome (ISSX2; OMIM 300672) associated with de novo balanced X-autosome translocations, t(X;7)(p22.3;p15) and t(X;6)(p22.3;q14), respectively. In both cases, the genomic rearrangements disrupted the *CDKL5/STK9* gene.[14] Although *CDKL5/STK9* was submitted to X-inactivation in normal female somatic cells, the expressed protein was not functional in the two patients because of a preferential inactivation of the normal X.[14] Indeed, the inactivation of the translocated X might have diffused to the translocated autosome fragment, leading to a monosomy of the corresponding genomic region, which is lethal.

Mutations in *CDKL5* were later on identified in female patients with atypical Rett syndrome.[15] These patients clearly had some features of Rett syndrome, such as deceleration of head growth, stereotypies, and hand apraxia. In contrast, some signs were absent, such as a period of nearly normal development followed by regression with loss of acquired fine finger skill in early childhood and intensive eye communication, and the characteristic evolution of Rett syndrome on EEG. In the mouse brain, Weaving et al. showed that Cdkl5 expression overlapped with that of Mecp2 (OMIM 300005), which is mutated in the classic Rett syndrome,[16] leading Tao et al. to postulate that these two genes may be involved in a common pathogenic pathway.[15]

The characteristics of the epilepsy associated with *CDKL5* mutations were defined by Bahi-Buisson et al., who showed in a retrospective study of 12 female patients with *CDKL5* mutations that the epilepsy course had three successive stages: Stage I: early epilepsy (onset at 1–10 weeks) with a normal interictal EEG despite frequent convulsive seizures; Stage II: EE with infantile spasms and hypsarrhythmia; stage III: at the age of examination (3–19 years), seven patients were seizure free and six had developed refractory epilepsy with tonic seizures and myoclonia.[17] Moreover, early epilepsy with a normal interictal EEG (stage I)

and severe hypotonia seem to be key clinical features in identifying patients with *CDKL5* mutations.[18]

EES AS PART OF THE PHENOTYPIC SPECTRUM ASSOCIATED WITH ARX MUTATIONS

Mutations in *ARX* were first identified in patients with three different phenotypes, including X-linked lissencephaly with abnormal genitalia (XLAG; OMIM 300215),[19] nonsyndromic X-linked mental retardation (MRX),[20] and X-linked infantile spasms (ISSX; OMIM 308350).[21] The phenotypic spectrum associated with *ARX* mutations includes severe EEs with or without brain malformations. Shoubridge et al. recently reviewed families with *ARX* mutations reported in the literature.[22] Among the 97 families identified, Ohtahara syndrome, X-linked myoclonic epilepsy, and X-linked infantile spasms (WS) were reported in 1, 1, and 12 families, respectively. Other syndromes including seizures were also identified, including Partington syndrome (intellectual disability with dystonic movements, ataxia, and seizures), mental retardation with tonic seizures with dystonia, and infantile epileptic-dyskinetic encephalopathy, in 7, 1, and 4 families, respectively.

The *ARX* gene, which contains five coding exons (GenBank: NM_139058.2: OMIM 300382), maps to Xp22 and encodes a 562 amino acid mature protein, which is expressed predominantly in the fetal and adult brain, testis, skeletal muscle, and pancreas.[23–25] ARX is a paired-type homeobox protein that contains four polyalanine (PolyA) tracts, a homeodomain, and a conserved C-terminal aristaless domain. Male *ARX*-deficient mice have abnormal differentiation and deficient tangential migration of GABAergic (gamma-aminobutyric acid) interneurons in the ganglionic eminence, neocortex, and hippocampus and abnormal testicular differentiation.[19]

All types of mutations—missense, nonsense, insertions, and deletions—have been reported in *ARX*. It is interesting to note that a phenotype–genotype correlation has recently emerged:[22,26] nonmalformative syndromes including EE[27] are mostly due to expansions of polyalanine tracts located in the N-terminal fragment of the protein, whereas syndromes with brain malformations are mostly associated with mutations leading to a premature termination codon (PTC) (nonsense or splice mutations) or deletions encompassing several exons. These mutations lead to a loss of function by haploinsufficiency since the mRNAs with PTC are mainly destroyed in the cells by the nonsense-mediated mRNA decay (NMD) system.[28] Very recently, Kato et al. reported two families, comprising six males with Ohtahara syndrome in two generations, in which frameshift mutations in the terminal exon of the *ARX* gene, Ala524fsX534 and Glu536fsX672, were identified. It is noticeable that the PTC mutations localized in the last exon of genes escape the NMD system and most often cause the synthesis of a truncated protein. Interestingly, two patients developed WS, and one of these later developed Lennox-Gastaut syndrome. The authors concluded that the analysis of *ARX* should be considered in sporadic or familial male patients with Ohtahara syndrome.[29]

SRPX2 IS RESPONSIBLE FOR ROLANDIC SEIZURES WITH SPEECH APRAXIA AND MENTAL RETARDATION

Roll et al. reported a three-generation French family with oral and speech dyspraxia (OSP), rolandic seizures (RS), and mental retardation. Eight individuals had OSP, RS, and mental retardation and three had only OSP and mental retardation without seizures. Epileptic patients had the EEG hallmark of RS (centrotemporal spikes that tend to occur in clusters and are strongly activated during sleep) during the active phase of the epileptic seizures.[30] A whole-genome scan was performed, and the gene was mapped to chromosome Xq. In the 20 cM large candidate interval, a missense mutation, N327S, was identified in the *SRPX2* gene. SRPX2 is a secreted sushi-repeat-containing protein expressed in neurons of the human adult brain, including the rolandic area. The disease-causing mutation resulted in gain of glycosylation of the secreted mutant protein.[30] Roll at al. also described a 12-year-old boy who presented with focal seizures beginning with numbness in the fingers of the right hand and leg and a sudden fall. Neurological examination revealed clonus at both knees and generalized hyperreflexia with an equivocal right plantar response. Neuropsychological examination at age 15 years showed low average to average intellect with weakness in mathematical ability.

The EEG showed left centrotemporal epileptiform activity with a rolandic horizontal dipole. The magnetic resonance imaging (MRI) scan showed bilateral posterior perisylvian polymicrogyria (OMIM 300388), more severe on the left and extending back to involve the parieto-occipital regions. Two of the boy's maternal aunts had mild mental retardation, whereas his mother and another aunt were of normal intelligence. In this family, a second mutation was identified within the first sushi domain of *SRPX2*. These data suggest that *SRPX2* may play a role in the development and/or function of the perisylvian region, which is critical for language and cognitive development. Interestingly, the orthologous *Srpx2* gene is not expressed during murine embryogenesis, suggesting that this gene is involved in the development and functioning of language areas in human cortex.[30]

A GENETICALLY DETERMINED INFANTILE EE: DRAVET SYNDROME (OR SEVERE MYOCLONIC EPILEPSY OF INFANCY)

In contrast to the previously described EE, a large proportion of patients with DS have mutations in known genes, leading to their systematic testing if the clinical context is suggestive.

Clinical Context

Dravet syndrome (DS), previously called *severe myoclonic epilepsy of infancy* (SMEI), is an intractable epileptic syndrome characterized by onset of seizures during the first year of life in a child with normal psychomotor development and no brain damage (the MRI scan is normal at onset). Seizures are clonic or tonic-clonic and may be generalized or unilateral, with either side of the body being involved. They are mainly febrile and often prolonged, resulting in convulsive status epilepticus. Subsequently, children present with myoclonic jerks, absences, and focal seizures. Psychomotor development is delayed from the second year of life, and ataxia may appear. Although these seizures are severe, the interictal EEGs remain free of spikes during the first years of the disorder. However, generalized spike waves with photosensitivity and focal abnormalities occur later on.[31]

De Novo Mutations in *SCN1A* Are the Main Cause of DS

A common genetic predisposition between DS and febrile seizures was first suggested by Benlounis et al..[32] Furthermore, Singh et al. considered DS to be a severe phenotype of the generalized epilepsy with febrile seizures plus (GEFS+) familial context, particularly because of the presence of patients with DS in families with GEFS+ (OMIM 604233).[33] Generalized epilepsy with febrile seizures plus is a variable autosomal dominant epileptic condition that also associates febrile and afebrile seizures. Affected family members present with phenotypes ranging from isolated febrile seizures to various idiopathic generalized epilepsy subtypes (epilepsy with grand mal seizures, childhood absence, or juvenile myoclonic epilepsy) or can remain asymptomatic. The outcome is usually benign, and patients are sensitive to classical antiepileptic treatments.[34] However, family members can occasionally experience focal seizures, be severely affected and/or pharmacoresistant, and even present with DS.[33] This common genetic background was confirmed by Claes et al., who identified seven de novo mutations in *SCN1A*, which had previously been involved in GEFS+,[35] in seven sporadic cases of DS.[36] The vast majority (six of seven) of these mutations led to a premature termination codon.[36] More recent studies have confirmed the high frequency of mutations of *SCN1A* in sporadic DS.[37–42]

DIFFERENT TYPES OF MUTATION IN SCN1A CAUSE DS

All types of mutations have been identified in the coding sequence of the *SCN1A* gene in patients with DS: missense, nonsense, and splice-site mutations, small deletions and insertions. The mutations are located all along the *SCN1A* gene. Missense mutations are the most common mutation type identified (about 40%).[42] The main remaining mutation

types (nonsense, splice-site, and frameshift mutations) introduce PTCs into the mRNA, which are probably recognized and degraded via the NMD system. This hypothesis is compatible with the effects of the other mutation types detected, including alteration of the initiation codon and the microdeletions that delete essential regions of the proteins, introduce PTC, or delete the whole gene in the heterozygous state. Indeed, during the past few years, many techniques have been developed in order to detect genomic rearrangements such as deletions or duplications: quantitative PCR (QPCR),[43] multiplex ligation-dependent probe amplification (MLPA),[44] and microarrays.[45] The analysis of series of patients without point mutations with these methods has identified several heterozygous rearrangements in *SCN1A*, variable in length and breakpoints and ranging from a single exon to the whole gene.[42,46,47] Deletion of the whole gene was the most common rearrangement found, supporting haploinsufficiency as the main molecular mechanism responsible for DS. Analysis of the parents showed that most deletions occurred de novo. The nearby genes comprised in the deletion, including other genes encoding voltage-gated sodium channels (*SCN7A* and *SCN9A*), differed among patients, without evident variability of the phenotype.[42] However, patients with very large deletions could have consistent dysmorphic features, including ear abnormalities, microcephaly, micrognathia, and brachysyndactyly, likely related to the size of the deletion and to deletions of genes other than *SCN1A*.[48]

In spite of many functional studies, it remains unclear how missense mutations can cause a clinical phenotype indistinguishable from that of PTC mutations or whole-gene deletion and why some missense mutations are associated with a severe phenotype, such as DS, and others with pharmaco-sensitive epilepsies, as in GEFS+ families. Kanai et al. suggested a preferential location of the missense mutations leading to DS in the S5 and S6 segments and the S5–S6 intracellular boucle, which together form the "pore" of the Nav1.1 channel.21.[49] Depienne et al. reported that most missense. mutations causing DS affected highly conserved amino acids located in ion-transport sequences and resulted in chemically dissimilar changes in amino acid classes. However, these mutations were not preferentially located in S5–S6 segments, in contrast to the previous report[42] (Fig. 62–2).

The proportion of patients with *SCN1A* mutations is highly variable in reported studies, ranging from 33%[39,50] to 80%–100%.[40,51,52] These discrepancies may be due to the sizes of the series, the methods of screening, and use of different clinical criteria to define DS. Supporting this hypothesis, the proportion of positive patients is higher in DS using strict criteria than in epileptic syndromes closely related to DS including intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC) or borderline SMEI (SMEB).[51,53] Considering only the patients with typical DS, approximately 20% of them do not have mutations in *SCN1A*, even when microrearrangements have been excluded.[41,42] In addition,

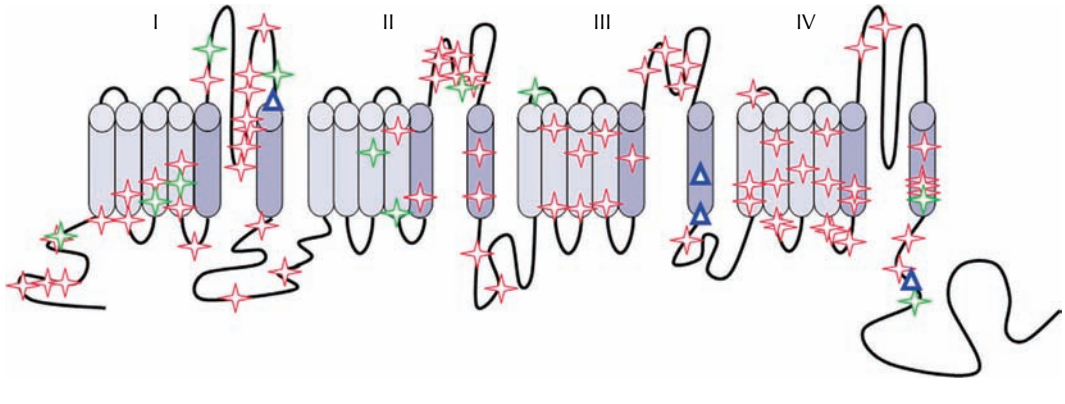

Figure 62–2. Schematic representation of the missense mutations and in-frame deletions in the Nav1.1 protein. Each star represents a missense mutation. Red stars: mutations identified in a single patient; green stars: recurrent mutations; blue triangles: inframe deletions. From ref. 42.

recent studies have established that rare pathogenic mutations in *SCN1A*, some of which are de novo, can also be identified in other infantile EEs, such as cryptogenic generalized epilepsies, cryptogenic focal epilepsies, or infantile spasms.[50,53,54] This extension of the clinical spectrum related to *SCN1A* has called into question the initial concept of DS, because neither clinical nor genetic criteria are sufficient to delimit accurately the various syndromes.

DS IN A FAMILIAL CONTEXT OF EPILEPSY

Few patients with DS have a parent or another relative with a milder epileptic phenotype. Depienne et al. showed that mutations inherited from an asymptomatic or mildly affected parent were identified in 10% of the DS patients. Mosaicism (the mutation is present only in a fraction of germinal [germinal mosaicism] or nongerminal [somatic mosaicism] cells) was the main event associated with inherited *SCN1A* mutations in DS patients (about 70%). Parental mosaicism in DS was not a rare situation since it was found in at least 7% of families with an *SCN1A* mutation. When the level of somatic mosaicism was high, the parent could present with seizures, although he or she was less severely affected than his or her child who carried the mutation in all cells. The clinical status of the mosaic parent appeared to be somehow correlated with the amount of the mutation in his or her blood cells, although this correlation was not strict.[55]

Nevertheless, mosaicism is not the only situation accounting for inherited mutations, as DS can also be encountered in the context of GEFS+ families. In that case, *SCN1A* missense mutations segregating in the family are associated with wide phenotypic variability, with DS at the severe end of the spectrum.[33] Distinguishing mosaicism from de novo constitutional mutations or other situations is of particular concern in disorders that are frequently sporadic, such as DS, in order to give appropriate genetic counseling.[56] The risk of recurrence is, therefore, not null when the mutation is apparently de novo, which should be taken into account for genetic counseling.

A question that remains is whether *SCN1A* neomutations causing DS act through the same pathophysiological mechanisms as mutations found in GEFS+. Basically, mutations associated with clear loss of function are always associated with DS, except in one family recently described by Suls et al., in which a microdeletion of *SCN1A* segregated with a very variable phenotype in a four-generation family.[57] In mosaic patients, the mutations with a loss-of-function effect can be associated with milder phenotypes reminiscent of those seen in GEFS+ if present in only some neurons. Conversely, missense mutations found in GEFS+ are generally associated with mild epileptic phenotypes but can occasionally cause DS. From a mechanistic point of view, it is likely that the pathophysiological pathways are different: de novo mutations would be sufficient to cause DS, whereas missense mutations associated with GEFS+ would not, and additional genetic or nongenetic factors would be necessary to cause DS in the latter case.

DS-Like Syndrome in Females Associated with Mutations in the *PCDH19* Gene

At least 20% of patients with DS are negative for mutations or rearrangements in *SCN1A*. In addition, rare mutations in *GABRG2* have been described in cases of DS belonging to GEFS+ families[58] but were not demonstrated in 29 sporadic cases.[38] These findings suggest that DS could be genetically heterogeneous (i.e., other genes could be involved in DS).

MUTATIONS IN PCDH19 MAINLY AFFECT FEMALES WITH A DS-LIKE SYNDROME

In order to identify new genes responsible for the disorder in *SCN1A* mutation-negative patients, Depienne et al. screened 41 patients with DS for microrearrangements with high-density single nucleotide polymorphism (SNP) microarrays. Interestingly, a hemizygous deletion on chromosome Xq22.1, encompassing the *PCDH19* gene, was identified in one male patient. To confirm that *PCDH19* was responsible for a DS-like syndrome, its coding region was sequenced in 73 additional *SCN1A* mutation-negative patients. Different point mutations (four missense and five truncating mutations) were identified in 11 unrelated female patients (15%). The spectrum

of mutations includes nonsense mutations and small deletions/insertions introducing a frameshift, as well as missense mutations affecting highly conserved amino acids in the protein, predominantly in the extracellular domain, which is presumably involved in cell–cell interaction. These mutations are therefore predicted to result in a loss of function of the mutated allele.[59]

Protocadherin 19 is a 1148 amino acid transmembrane protein belonging to the protocadherin delta2 subclass of the cadherin superfamily, which is highly expressed in neural tissues and at different developmental stages.[60–63] The precise functions of the protein remain unknown. However, delta protocadherins were reported to mediate cell–cell adhesion in vitro and cell sorting in vivo, and could regulate the establishment of neuronal connections during brain development.[64,65]

Patients with *PCDH19* mutations could present with clinical features similar to those of patients with *SCN1A* mutations, including the association of early febrile and afebrile seizures, seizures occurring in clusters, developmental and language delays, behavioral disturbances, and cognitive regression. There were, however, slight but constant differences in the evolution of epilepsy, including fewer polymorphic seizures (in particular, rare myoclonic jerks and atypical absences) in patients with *PCDH19* mutations. These results show that *PCDH19* plays a major role in epileptic encephalopathies, with a clinical spectrum overlapping that of DS.[59]

PCDH19-LINKED EPILEPSIES: AN UNUSUAL MODE OF INHERITANCE

Mutations in *PCDH19* were first reported to cause epilepsy and mental retardation limited to females (EFMR). The clinical features of EFMR, unlike those of DS, are highly variable, even in members of the same family: onset of seizures occurs between 6 and 36 months, and affected females present with a combination of febrile and afebrile seizures of various types and a variable degree of psychomotor delay and cognitive impairment, ranging from mild to severe mental retardation.[66] Dibbens et al. reported *PCDH19* mutations in six large families and one small family with two affected sib pairs.[60] All the patients were family members who were, for the most part, already adults at the time of examination, and appeared socially integrated in that most of them were married and had children.

PCDH19-related EE therefore mainly affects females. In a large series of *PCDH19* mutation-positive index cases in whom inheritance could be assessed, half of the mutations occurred de novo and half were inherited from fathers who were healthy, had no cognitive impairment, and had never had febrile seizures or epilepsy[59] . This heredity is very different from the X-linked mode of inheritance encountered for *ARX* or *CDKL5* mutations since, only the heterozygous females with *PCDH19* mutation are affected, whereas the hemizygous males are asymptomatic and spared (Fig. 62–3).

Several mechanisms have been suggested to account for the unusual mode of inheritance observed in *PCDH19*-linked epilepsy, one of which is cellular interference, a mechanism reminiscent of metabolic interference.[60,67,68] This concept postulates that random inactivation of one X chromosome in mutated females generates tissue mosaicism (i.e., coexistence of *PCDH19*-positive and *PCDH19*-negative cells), which would be pathogenic by altering cell–cell interactions; normal individuals and mutated males, who are homogeneous for *PCDH19*-positive or *PCDH19*-negative cells, respectively, would not develop the disease (Fig. 62–4). The identification of an affected male who was mosaic for the *PCDH19* deletion in his fibroblasts, and therefore had *PCDH19*-positive and *PCDH19*-negative cells in this tissue, strongly supports the hypothesis of cellular interference as the main pathogenic mechanism associated with *PCDH19* mutations.[59]

PROSPECTS AND CONCLUSION

Identification of a Mutation Leads to Genetic Counseling

In the vast majority of EEs, genetic causes are very rare. However, after the most common, nongenetic causes are excluded, it is crucial to perform appropriate genetic analyses, depending on the detailed phenotype of the patient, even if they are time-consuming and costly, since the identification of a mutation raises the possibility of a risk of recurrence in the family. A scrupulous reconstruction of the family

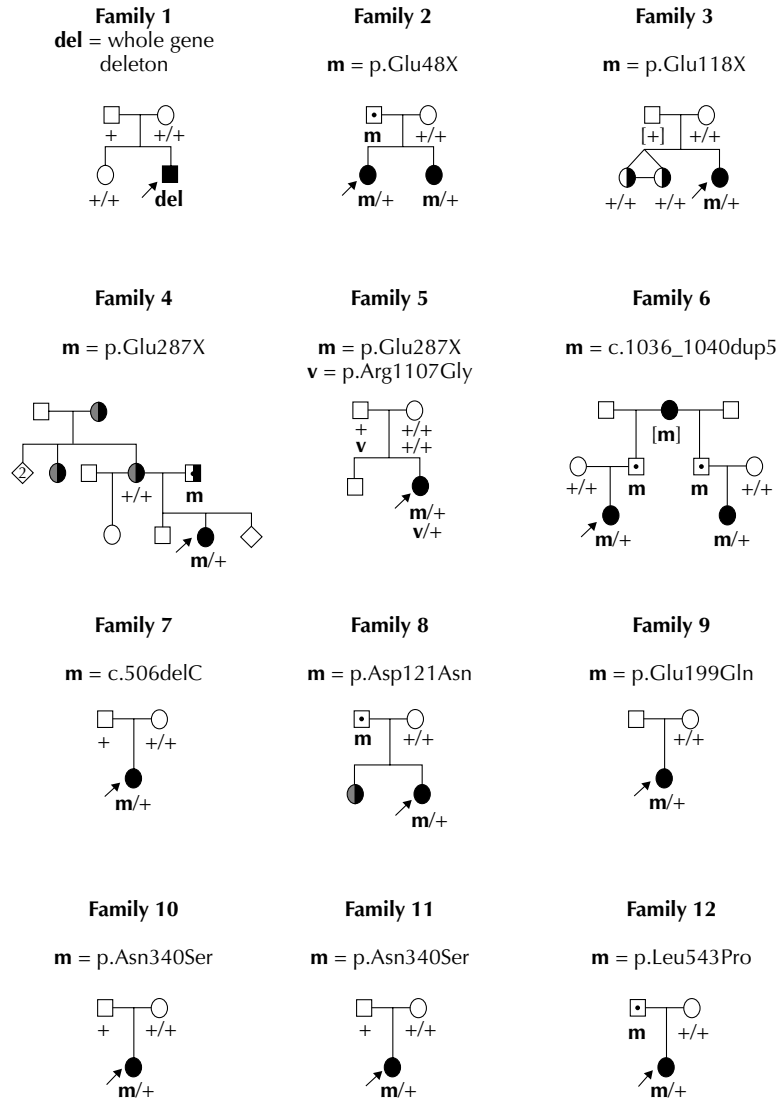

Figure 62–3. Segregation analysis of the *PCDH19* deletion and point mutations in 12 families. del/+, m/+ and v/+ denote individuals heterozygous for the deletion, mutation, or variant, respectively; +/+ denotes individuals carrying homozygous wild-type alleles. Squares represent males, circles females; filled black symbols: patients diagnosed as having DS; right black half: cognitive delay or impairment; left gray half: adolescence-onset idiopathic epilepsy. Dots in the middle of the squares indicate unaffected mutation carriers. The arrows indicate the index cases. From ref. 59.

history has to be made and a pedigree drawn in order to determine the most probable mode of inheritance.

For AR diseases like EIEE3 associated with *GC1* mutations, recurrence in the family is high (25%) and genetic counseling should be provided. The mutation(s) must first be identified in the index case. If the parents are related, we would expect to identify a mutation at the homozygous state. The analysis of the parents' DNA is important to determine whether the mutation is on both copies of the gene in the affected child. However, in some rare cases, only one parent carries the identified mutation, the other having a deletion encompassing the exon bearing the mutation in his or her spouse. If the parents are not related, patients most often have two different mutations in the *GC1* gene; they are then called *compound heterozygotes*. The study of the parents' DNA

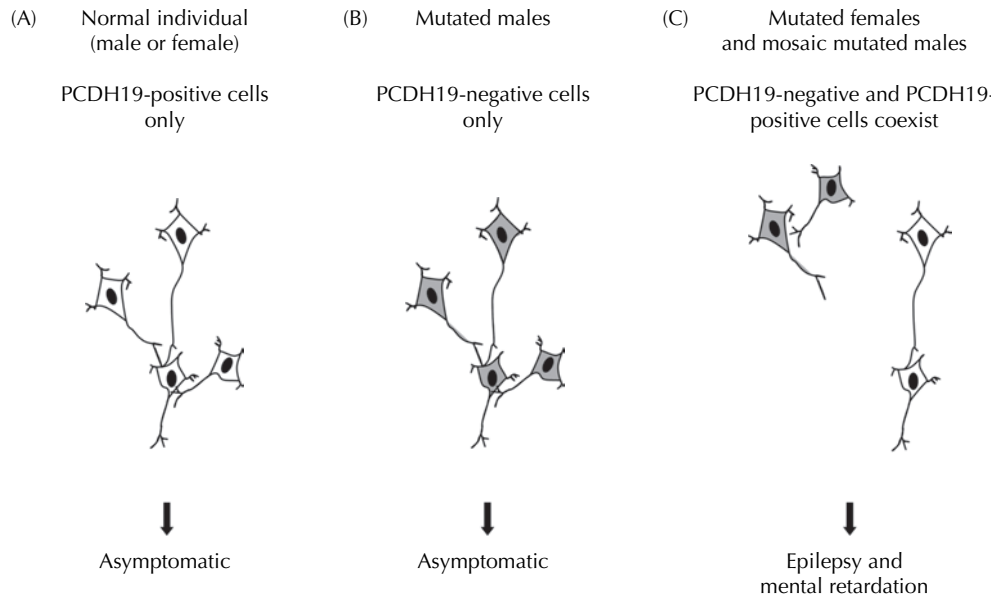

Figure 62–4. Schematic illustration of the cellular interference mechanism associated with *PCDH19* mutations. **A.** In normal individuals characterized by a homogeneous population of *PCDH19*-positive cells, neurons are able to form normal neuronal networks. **B.** In male patients with a *PCDH19* mutation, hemizygosity leads to a homogeneous population of *PCDH19*-negative cells; in this condition, neurons preserve the ability to form normal neuronal networks. This hypothesis implies that there is a compensatory mechanism with the expression of other protocadherins permitting the formation of functional neuronal networks. **C.** In heterozygous mutated females, random X inactivation leads to a functional mosaicism with the coexistence of two *PCDH19*-positive and *PCDH19*-negative cell populations. These two cell populations cause divergent cell sorting and migration (due to attractive or repulsive interactions) and lead to abnormal neuronal networks. Somatic mosaicism in mutated males gives rise to the same pathological situation. The precise mechanisms by which the neuronal networks are altered are still unknown. From ref. 59.

is also used to exclude the possibility that both mutations are on the same copy of the gene. This needs to be verified before a prenatal diagnosis can be proposed.

Several genetically determined EEs are due to de novo mutations, including DS associated with mutations in *SCN1A* or *PCDH19* and early infantile EE due to mutations in *CDKL5/STK9* or *STXBP1*. Genetic counseling in these cases is a very delicate issue since the possibility of germinal mosaicism has to be taken into account. Germinal mosaicism may be suspected in families in certain contexts: (1) the mutation can be detected at a low level in the blood cells' DNA of one parent (the mutation can be detected by direct sequencing when its amount is greater than 20% in DNA from blood cells) or (2) at least two children are affected and carry the same mutation, even if the mutation is not detected in the parent's blood. Importantly, germinal mosaicism or somatic mosaicism undetectable from the blood may be missed in the absence of a second affected child, which means that the possibility of mosaicism should be consider for every sporadic de novo case. The frequency of mosaicism in DS was found to be 7% in a large cohort of patients with *SCN1A* mutations, but this might only be the tip of the iceberg.[55] The risk of recurrence is therefore not null and is difficult to evaluate when the mutation is apparently de novo, which raises the question of prenatal diagnosis in some cases.

The last condition most often concerns autosomal dominant diseases with phenotype variability and is illustrated by the occurrence of DS in families with GEFS+. In this case, the parent with a mild and benign phenotype transmits a *SCN1A* missense mutation to his or her child, who then presents with severe, intractable epilepsy and mental retardation. The occurrence of a DS phenotype has to be related to this mutation but its severity is likely determined by other, as yet unidentified factors, often called *modifying factors*, which can be environmental or genetic. Until these

factors can be identified, the question of the recurrence of this severe phenotype must remain open.

Recent Strategies to Identify New Genes Responsible for EEs

We are only just beginning to appreciate the importance of genetic EE and the consequences of the identification of a mutation in a family in terms of diagnosis and genetic counseling. From a fundamental point of view, the identification of a new gene leads to the deciphering of a new pathological mechanism and may open new therapeutic avenues. Advances in human molecular genetics are revealing the great genetic heterogeneity of infantile EEs and the great variability of their pathological pathways. Moreover, one may suspect that the genes already identified represent only a small fraction of the genes involved in these disorders that can be considered as development disorders. Identifying these genes remains challenging. Nevertheless, the recent technological leap due to the development of next-generation sequencing, allowing the sequencing of the whole genome or coding sequences (exome) of a genome, opens new perspectives not only for monogenic but also polygenic forms of EEs.

IDENTIFYING NEW GENES RESPONSIBLE FOR MONOGENIC FORMS OF EES

Identifying genes in sporadic EEs is difficult since the classical genetic strategies based on the study of large families are not possible. However, alternative genetic approaches are available; they are various and are related to the context of the disease:

1. As for *ARX*, *CDKL5/STK9*, and *SRPX2*, EE can be part of a complex phenotype with or without brain abnormalities. The strategy of screening the responsible gene in series of patients with isolated EE of the same type can be fruitful. Some patients with isolated defined EEs, even if they were rare, had mutations in these genes.
2. The study of GEFS+ and DS highlights the possibility of a gene identification strategy based on the detailed description of syndromes: the occurrence of DS in families with GEFS+ and the high sensitivity to fever in both conditions led to DS being considered as part of the GEFS+ phenotypic spectrum. Claes et al.[36] therefore tested *SCN1A*, which had first been incriminated in GEFS+, as a candidate gene in sporadic patients with DS and identified the first de novo mutations of *SCN1A*.
3. Genomic rearrangements have been identified in most of the genes known to be responsible for infantile EE. Comparative genomic hybridization (CGH) and SNP microarrays have enabled the entire genome to be screened at various resolutions. The detection of a de novo rearrangement in a patient points to a candidate region in which the causative gene is localized. The candidate region often contains several genes, and the involvement of one particular gene needs to be confirmed by the identification of point mutations located in the coding region of this gene in patients with the same phenotype. As we saw in previous sections, this strategy was applied to identify the *SPTAN1* and *CDKL5/STK9* genes. When the rearrangement is inherited from an asymptomatic parent, its causality in the patient's phenotype is more difficult to prove, as it may correspond to a benign copy number variant (CNV) or copy number polymorphism (CNP). However, it cannot be excluded that a recessive mutation in a gene of the region has been transmitted on the undeleted remaining copy by the other parent. Association of an inherited CNV with a phenotype in more than one patient would argue in favor of such an autosomal recessive pathology and lead to the sequencing of candidate genes in the regions concerned.

IDENTIFYING SUSCEPTIBILITY GENES IN POLYGENIC-DETERMINED EES

Since the vast majority of cases of EE are sporadic, the most frequent inheritance of EE is usually thought of as being complex or multifactorial, resulting from the interaction between several genes (susceptibility genes)

and environmental factors. The identification of these genes requires nonparametric analyses (genetic analyses that do not depend on parameters of monogenic diseases as the mode of inheritance, penetrance, rate of phenocopies, etc.). For these analyses, three conditions are needed: (1) a large number of affected individuals (at least several hundred) and a similar number of controls well matched for age, sex, and ethnic background; (2) previous exclusion of monogenic forms; and (3) low genetic heterogeneity of the population (great genetic heterogeneity could mask or dilute the effect of individual susceptibility genes). These conditions are difficult to obtain in EEs, which are rare diseases affecting children (with the problem of constituting a control group) with well-known etiological heterogeneity (Table 62–1). However, such a strategy has already been used, in which Landau-Kleffner syndrome, continuous spike-and-waves during sleep syndrome, benign childhood epilepsy with centrotemporal spikes, and benign rolandic epilepsy were considered to be part of a single continuous spectrum of disorders.[69] The centrotemporal spikes (CTS) were considered to be an endophenotype determined by a common set of susceptibility genes, the different phenotypes being determined by other susceptibility genes and/or environmental factors. A genome-wide study showed a linkage of the CTS endophenotype to 11p13, especially with polymorphic markers in the *ELP4* (Elongator Protein Complex 4) gene.[70] The yeast Elp4 protein is one of the six subunits of *Elongator*. The *Elongator* complex is involved in transcription of several genes that regulate the actin cytoskeleton, cell motility, and migration. These functions are crucial in the nervous system for nerve cell growth cone motility, axon outgrowth and guidance, neuritogenesis, and neuronal migration during development.[71,72] This gene is a good candidate-by-function. It remains to identify functional variants in the vicinity of the *ELP4* gene to confirm its genetic involvement. This strategy could be applied to other clinical or EEG traits.

In conclusion, in the past 10 years, a great amount of genetic data has accumulated, updating our knowledge of the etiologies and pathophysiological mechanisms of infantile EEs. We can expect this chapter to become incomplete in the very next future and hope that all of this basic science will soon open new therapeutic avenues to cure these devastating disorders.

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REFERENCES

1. Dulac O. Epileptic encephalopathy. *Epilepsia.* 2001; 42(suppl 3):23–26.
2. Nabbout R, Dulac O. Epileptic encephalopathies: a brief overview. *J Clin Neurophysiol.* 2003;20(6):393–397.
3. Guerrini R. Epilepsy in children. *Lancet.* 2006; 367(9509):499–524.
4. Gerber SH, Rah JC, Min SW, et al. Conformational switch of syntaxin-1 controls synaptic vesicle fusion. *Science.* 2008;321(5895):1507–1510.
5. Verhage M, Maia AS, Plomp JJ, et al. Synaptic assembly of the brain in the absence of neurotransmitter secretion. *Science.* 2000;287(5454):864–869.
6. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet.* 2008;40(6):782–788.
7. Hamdan FF, Piton A, Gauthier J, et al. De novo STXBP1 mutations in mental retardation and nonsyndromic epilepsy. *Ann Neurol.* 2009;65(6):748–753.
8. Tohyama J, Akasaka N, Osaka H, et al. Early onset West syndrome with cerebral hypomyelination and reduced cerebral white matter. *Brain Dev.* 2008;30(5): 349–355.
9. Saitsu H, Tohyama J, Kumada T, et al. Dominant-negative mutations in alpha-II spectrin cause West syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delay. *Am J Hum Genet.* 2010;86(6):881–891.
10. Metral S, Machnicka B, Bigot S, Colin Y, Dhermy D, Lecomte MC. AlphaII-spectrin is critical for cell adhesion and cell cycle. *J Biol Chem.* 2009;284(4): 2409–2418.
11. Molinari F, Raas-Rothschild A, Rio M, et al. Impaired mitochondrial glutamate transport in autosomal recessive neonatal myoclonic epilepsy. *Am J Hum Genet.* 2005;76(2):334–339.
12. Molinari F, Kaminska A, Fiermonte G, et al. Mutations in the mitochondrial glutamate carrier SLC25A22 in neonatal epileptic encephalopathy with suppression bursts. *Clin Genet.* 2009;76(2):188–194.
13. Fiermonte G, Palmieri L, Todisco S, Agrimi G, Palmieri F, Walker JE. Identification of the mitochondrial glutamate transporter. Bacterial expression, reconstitution, functional characterization, and tissue distribution of two human isoforms. *J Biol Chem.* 2002;277(22):19289–19294.
14. Kalscheuer VM, Tao J, Donnelly A, et al. Disruption of the serine/threonine kinase 9 gene causes severe X-linked infantile spasms and mental retardation. *Am J Hum Genet.* 2003;72(6):1401–1411.

15. Tao J, Van Esch H, Hagedorn-Greiwe M, et al. Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5/STK9) gene are associated with severe neurodevelopmental retardation. *Am J Hum Genet.* 2004;75(6):1149–1154.
16. Weaving LS, Christodoulou J, Williamson SL, et al. Mutations of CDKL5 cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. *Am J Hum Genet.* 2004;75(6):1079–1093.
17. Bahi-Buisson N, Kaminska A, Boddaert N, et al. The three stages of epilepsy in patients with CDKL5 mutations. *Epilepsia.* 2008;49(6):1027–1037.
18. Bahi-Buisson N, Nectoux J, Rosas-Vargas H, et al. Key clinical features to identify girls with CDKL5 mutations. *Brain.* 2008;131(pt 10):2647–2661.
19. Kitamura K, Yanazawa M, Sugiyama N, et al. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet.* 2002;32(3): 359–369.
20. Bienvenu T, Poirier K, Friocourt G, et al. ARX, a novel Prd-class-homeobox gene highly expressed in the telencephalon, is mutated in X-linked mental retardation. *Hum Mol Genet.* 2002;11(8):981–991.
21. Stromme P, Mangelsdorf ME, Shaw MA, et al. Mutations in the human ortholog of Aristaless cause X-linked mental retardation and epilepsy. *Nat Genet.* 2002;30(4):441–445.
22. Shoubridge C, Fullston T, Gecz J. ARX spectrum disorders: making inroads into the molecular pathology. *Hum Mutat.* 2010;31(8):889–900.
23. Miura H, Yanazawa M, Kato K, Kitamura K. Expression of a novel aristaless related homeobox gene "Arx" in the vertebrate telencephalon, diencephalon and floor plate. *Mech Dev.* 1997;65(1–2):99–109.
24. Collombat P, Mansouri A, Hecksher-Sorensen J, et al. Opposing actions of Arx and Pax4 in endocrine pancreas development. *Genes Dev.* 2003;17(20):2591–2603.
25. Gecz J, Cloosterman D, Partington M. ARX: a gene for all seasons. *Curr Opin Genet Dev.* 2006;16(3): 308–316.
26. Kato M, Saitoh S, Kamei A, et al. A longer polyalanine expansion mutation in the ARX gene causes early infantile epileptic encephalopathy with suppression-burst pattern (Ohtahara syndrome). *Am J Hum Genet.* 2007;81(2):361–366.
27. Guerrini R, Moro F, Kato M, et al. Expansion of the first PolyA tract of ARX causes infantile spasms and status dystonicus. *Neurology.* 2007;69(5):427–433.
28. Stalder L, Muhlemann O. The meaning of nonsense. *Trends Cell Biol.* 2008;18(7):315–321.
29. Kato M, Koyama N, Ohta M, Miura K, Hayasaka K. Frameshift mutations of the ARX gene in familial Ohtahara syndrome. *Epilepsia.* 2010;51(9):1679–1684.
30. Roll P, Rudolf G, Pereira S, et al. SRPX2 mutations in disorders of language cortex and cognition. *Hum Mol Genet.* 2006;15(7):1195–1207.
31. Dravet C, Catani C, Bureau M, Roger J. Partial epilepsies in infancy: a study of 40 cases. *Epilepsia.* 1989;30(6):807–812.
32. Benlounis A, Nabbout R, Feingold J, et al. Genetic predisposition to severe myoclonic epilepsy in infancy. *Epilepsia.* 2001;42(2):204–209.
33. Singh R, Andermann E, Whitehouse WP, et al. Severe myoclonic epilepsy of infancy: extended spectrum of GEFS+? *Epilepsia.* 2001;42(7):837–844.
34. Scheffer IE, Berkovic SF. Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes. *Brain.* 1997;120(pt 3): 479–490.
35. Escayg A, Goldin AL. Sodium channel SCN1A and epilepsy: mutations and mechanisms. *Epilepsia.* 2010;51(9): 1650–1658.
36. Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet.* 2001;68(6): 1327–1332.
37. Sugawara T, Mazaki-Miyazaki E, Fukushima K, et al. Frequent mutations of SCN1A in severe myoclonic epilepsy in infancy. *Neurology.* 2002;58(7): 1122–1124.
38. Ohmori I, Ouchida M, Ohtsuka Y, Oka E, Shimizu K. Significant correlation of the SCN1A mutations and severe myoclonic epilepsy in infancy. *Biochem Biophys Res Commun.* 2002;295(1):17–23.
39. Nabbout R, Gennaro E, Dalla Bernardina B, et al. Spectrum of SCN1A mutations in severe myoclonic epilepsy of infancy. *Neurology.* 2003;60(12):1961–1967.
40. Claes L, Ceulemans B, Audenaert D, et al. De novo SCN1A mutations are a major cause of severe myoclonic epilepsy of infancy. *Hum Mutat.* 2003;21(6):615–621.
41. Marini C, Mei D, Temudo T, et al. Idiopathic epilepsies with seizures precipitated by fever and SCN1A abnormalities. *Epilepsia.* 2007;48(9):1678–1685.
42. Depienne C, Trouillard O, Saint-Martin C, et al. Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333 patients. *J Med Genet.* 2009;46(3):183–191.
43. Laccone F, Junemann I, Whatley S, et al. Large deletions of the MECP2 gene detected by gene dosage analysis in patients with Rett syndrome. *Hum Mutat.* 2004;23(3):234–244.
44. Montagna M, Dalla Palma M, Menin C, et al. Genomic rearrangements account for more than one-third of the BRCA1 mutations in northern Italian breast/ovarian cancer families. *Hum Mol Genet.* 2003;12(9): 1055–1061.
45. Bignell GR, Huang J, Greshock J, et al. High-resolution analysis of DNA copy number using oligonucleotide microarrays. *Genome Res.* 2004;14(2):287–295.
46. Suls A, Claeys KG, Goossens D, et al. Microdeletions involving the SCN1A gene may be common in SCN1A-mutation-negative SMEI patients. *Hum Mutat.* 2006;27(9):914–920.
47. Marini C, Scheffer IE, Nabbout R, et al. SCN1A duplications and deletions detected in Dravet syndrome: implications for molecular diagnosis. *Epilepsia.* 2009;50(7):1670–1678.
48. Davidsson J, Collin A, Olsson ME, Lundgren J, Soller M. Deletion of the SCN gene cluster on 2q24.4 is associated with severe epilepsy: an array-based genotype–phenotype correlation and a comprehensive review of previously published cases. *Epilepsy Res.* 2008;81(1):69–79.
49. Kanai K, Hirose S, Oguni H, et al. Effect of localization of missense mutations in SCN1A on epilepsy phenotype severity. *Neurology.* 2004;63(2):329–334.
50. Wallace RH, Hodgson BL, Grinton BE, et al. Sodium channel alpha1-subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms. *Neurology.* 2003;61(6):765–769.

51. Fujiwara T. Clinical spectrum of mutations in SCN1A gene: severe myoclonic epilepsy in infancy and related epilepsies. *Epilepsy Res.* 2006;70(suppl 1):S223–S230.
52. Fukuma G, Oguni H, Shirasaka Y, et al. Mutations of neuronal voltage-gated Na^+ channel alpha 1 subunit gene SCN1A in core severe myoclonic epilepsy in infancy (SMEI) and in borderline SMEI (SMEB). *Epilepsia.* 2004;45(2):140–148.
53. Harkin LA, McMahon JM, Iona X, et al. The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain.* 2007;130(pt 3):843–852.
54. Zucca C, Redaelli F, Epifanio R, et al. Cryptogenic epileptic syndromes related to SCN1A: twelve novel mutations identified. *Arch Neurol.* 2008;65(4):489–494.
55. Depienne C, Trouillard O, Gourfinkel-An I, et al. Mechanisms for variable expressivity of inherited SCN1A mutations causing Dravet syndrome. *J Med Genet.* 2010;47(6):404–410.
56. Youssoufian H, Pyeritz RE. Mechanisms and consequences of somatic mosaicism in humans. *Nat Rev Genet.* 2002;3(10):748–758.
57. Suls A, Velizarova R, Yordanova I, et al. Four generations of epilepsy caused by an inherited microdeletion of the SCN1A gene. *Neurology.* 2010;75(1):72–76.
58. Harkin LA, Bowser DN, Dibbens LM, et al. Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am J Hum Genet.* 2002;70(2):530–536.
59. Depienne C, Bouteiller D, Keren B, et al. Sporadic infantile epileptic encephalopathy caused by mutations in PCDH19 resembles Dravet syndrome but mainly affects females. *PLoS Genet.* 2009;5(2):e1000381.
60. Dibbens LM, Tarpey PS, Hynes K, et al. X-linked protocadherin 19 mutations cause female-limited epilepsy and cognitive impairment. *Nat Genet.* 2008;40(6):776–781.
61. Hynes K, Tarpey P, Dibbens LM, et al. Epilepsy and mental retardation limited to females with PCDH19 mutations can present de novo or in single generation families. *J Med Genet.* 2010;47(3):211–216.
62. Gaitan Y, Bouchard M. Expression of the delta-protocadherin gene Pcdh19 in the developing mouse embryo. *Gene Expr Patterns.* 2006;6(8):893–899.
63. Wolverton T, Lalande M. Identification and characterization of three members of a novel subclass of protocadherins. *Genomics.* 2001;76(1–3):66–72.
64. Junghans D, Haas IG, Kemler R. Mammalian cadherins and protocadherins: about cell death, synapses and processing. *Curr Opin Cell Biol.* 2005;17(5):446–452.
65. Redies C, Vanhalst K, Roy F. Delta-protocadherins: unique structures and functions. *Cell Mol Life Sci.* 2005;62(23):2840–2852.
66. Scheffer IE, Turner SJ, Dibbens LM, et al. Epilepsy and mental retardation limited to females: an under-recognized disorder. *Brain.* 2008;131(pt 4):918–927.
67. Wieland I, Jakubiczka S, Muschke P, et al. Mutations of the ephrin-B1 gene cause craniofrontonasal syndrome. *Am J Hum Genet.* 2004;74(6):1209–1215.
68. Johnson WG. Metabolic interference and the + - heterozygote. A hypothetical form of simple inheritance which is neither dominant nor recessive. *Am J Hum Genet.* 1980;32(3):374–386.
69. Rudolf G, Valenti MP, Hirsch E, Szepetowski P. From rolandic epilepsy to continuous spike-and-waves during sleep and Landau-Kleffner syndromes: insights into possible genetic factors. *Epilepsia.* 2009;50(suppl 7):25–28.
70. Strug LJ, Clarke T, Chiang T, et al. Centrotemporal sharp wave EEG trait in rolandic epilepsy maps to Elongator Protein Complex 4 (ELP4). *Eur J Hum Genet.* 2009;17(9):1171–1181.
71. Esberg A, Huang B, Johansson MJ, Bystrom AS. Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. *Mol Cell.* 2006;24(1):139–148.
72. Close P, Hawkes N, Cornez I, et al. Transcription impairment and cell migration defects in elongator-depleted cells: implication for familial dysautonomia. *Mol Cell.* 2006;22(4):521–531.

Chapter 63

Developing Models of Aristaless-Related Homeobox Mutations

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INTRODUCTION

Mutations in the Aristaless-related homeobox gene (*ARX*) have been causally linked to a variety of neurological conditions, particularly infantile spasms syndrome. *ARX* is a developmentally regulated homeobox transcription factor with expression both in the ganglionic eminence and in the cortical ventricular zone early in development.[1] Postnatally, the expression pattern is restricted to GABAergic (gamma-aminobutyric acid) neurons in the cortex and basal ganglia. During development, *ARX* functions primarily as a transcriptional repressor[2]: modulating migration and fate specification of interneurons and controlling ventricular zone proliferation. How loss of function of *ARX* leads to an epilepsy phenotype is poorly understood. Three genetically modified mice lines have been generated[3–5] to address this issue. These models each develop epilepsy, and all have changes in interneuron subtype patterns strongly implicating alterations of interneuron development as a cause of epilepsy. Analysis of these models will both further the molecular understanding of the function of *ARX* and allow dissection of the pathophysiological properties of the *ARX*-related epilepsies. This chapter will review the current knowledge of the function of *Arx*, the *Arx* mouse models, and discuss how these models can lead to a better understanding of the role of interneuron loss in the development of epilepsy during early childhood.

During the first few years of life, there exists a spectrum of malignant epileptic disorders. These early epileptic encephalopathies often proceed from one clinical syndrome to another. The named syndromes include early infantile epileptic encephalopathy (Otahara syndrome), severe myoclonic epilepsy of infancy (Dravet syndrome), infantile spasms (West syndrome), Lennox-Gastaut syndrome, and a few others (for review, see ref. 6, as well as various

chapters in this book). Up to the last few years, the etiology of these conditions was unknown for many patients. Recently, a number of genes have been linked to early epileptic encephalopathies, and often a single gene has been found to be responsible for multiple conditions. *ARX* is, to date, one of the genes most commonly associated with these conditions. A sizable literature has accumulated describing mutations in *ARX* that have been reported to cause a variety of neurological disorders, all with epilepsy as a major symptom. *ARX* was first associated with human disease by three groups in 2002.[7–9] The initial descriptions were of three different disorders: X-linked lissencephaly with ambiguous genitalia (XLAG), X-linked mental retardation (XLMR) with epilepsy and spasticity, and X-linked infantile spasms syndrome (ISSX). The finding of a single gene having significant clinical and genetic heterogeneity has led to a considerable amount of research, both clinical and basic science, on this gene. In this chapter, we will introduce the clinical and molecular details of *ARX*, then discuss the molecular mechanisms of the gene and the rodent models of *ARX*-related disease, and end with a discussion of the role of *ARX* in the pathophysiology of the early epileptic encephalopathies.

ARX IN CLINICAL NEUROLOGY

Mutations in the gene *ARX* have been primarily described in boys and generally result in a clinical phenotype with intractable epilepsy as a predominant feature. The mutations can be broadly classified as those resulting in brain malformations and those without brain malformations. The brain malformation syndromes include XLAG,[8,10] Proud syndrome (agenesis of the corpus callosum and ambiguous genitalia),[11] and hydranencephaly with abnormal genitalia[12] (OMIM 300215). The *ARX* disorders without brain malformations include XLMR,[7,9] West syndrome/ISSX,[8,13] Partington syndrome (mental retardation with hand dystonia)[14–16] (OMIM 309510), X-linked myoclonic epilepsy with spasticity and intellectual development (XMESID),[17,18] and Ohtahara/early infantile epileptic encephalopathy syndrome (EIEE),[19–21] as well as other cases with epilepsy, intellectual disability, and dystonia/spasticity as part of the syndrome.[22,23] A number of patients with *ARX* mutations, both with and without clear brain malformations, have also been observed to have cysts within the basal ganglia.[4,12,13] Though not a clear malformation, this is a finding that may be typical in patients with *ARX* mutations.

In females, the only central nervous system (CNS) malformation currently described is agenesis of the corpus callosum (ACC) with or without mental retardation (MR) and seizures.[4,12] Females have also been reported to have infantile spasms, epilepsy, and varying degrees of cognitive dysfunction without clear brain malformation.[4,12,24,25] All of these conditions have been linked to a large number of mutations in the gene. From this large group of mutations and conditions, a number of authors have debated the existence of a clear genotype–phenotype correlation.[12,26,27]

Ostensibly, a clear genotype–phenotype correlation exists between the malformation phenotypes and the nonmalformation phenotypes. Mutations leading to loss of protein function (deletions, nonsense, frameshift, or splice site mutations) or to changes in the homeodomain (for details, see below) lead to malformation syndromes, while missense mutations or expansions of the polyalanine tracts lead to epilepsy phenotypes with normal brain magnetic resonance imaging (MRI) scans.[12] However, this genotype–phenotype correlation breaks down when one looks at the severity of disease in the nonmalformation phenotypes and females.[26] For example, the most common mutation reported (45% of all mutations), the expansion of the second polyalanine tract from 12 to 20 alanines, has been associated with a spectrum of phenotypes ranging from mild MR and seizures to the severe phenotype of Ohtahara syndrome, suggesting greater genotype–phenotype heterogeneity. Another condition in which the genotype–phenotype correlation is less clear is in females with *ARX* mutations. There are mother-daughter and mother-aunt pairs in which one is normal and the other has cognitive disabilities and epilepsy,[4] though this may be due to differences in X-inactivation. Overall, *ARX* mutations result in a spectrum of disorders all with epilepsy as a major phenotype. A degree of genotype–phenotype correlation exists, but other factors, such as familial modifiers or environmental mediators, must exist to explain the variability that does occur.

MOLECULAR BIOLOGY OF *ARX*

What Is *ARX*?

ARX encodes a transcription factor with a role in cortical development. Designated *ARX* in humans and *Arx* in mice, the gene codes for a paired class homeodomain protein and is the vertebrate ortholog to the *Drosophila aristaless* (*al*) gene. *ARX* resides at Xp22.11 and produces a 2.8 kb mRNA with significant homology between human and mouse (89.3% sequence and 95% amino acid homology). The gene encodes a 562 amino acid protein, localized to the nucleus, that functions by binding DNA.[7,28,29] *Arx* is expressed in the developing hypothalamus, thalamus, basal ganglia, and cerebral cortex beginning at embryonic day 8 (E8). In the forebrain, *Arx* is expressed in the anterior neural plate at E8.[7,30] By E10.5, expression is clearly observed in the ventricular zone (VZ) of the dorsal cortex and begins to be seen in the mantle zone of the emerging ganglionic eminences (GE).[30] Ventral telencephalic *Arx* expression is clearly present in the mantle zone of the GE at E12.5.[30] By E14.5 *Arx* protein can be observed in the dorsal VZ, in the intermediate zone (IZ) of the GE, and in scattered cells in the marginal zone (MZ) and dorsal IZ and subventricular zone (SVZ)[7,30,31] (Fig. 63–1A). The ventral expression, at E14.5, has settled into the SVZ of the GE, primarily in proliferating neuroblasts,[30] as determined by colabeling with bromodeoxyuridine (BrdU) staining. *Arx* remains expressed as the cells leave the SVZ to migrate tangentially into the cortex.[8,30,32] The ventral and migratory expression of *Arx* has almost complete overlap with Gad1 (glutamic acid decarboxylase 1) expression.[30] This expression pattern remains until shortly after birth, when the dorsal VZ and GE are no longer present and *Arx* expression in the forebrain is observed only in scattered cells in the cortex and basal ganglia (Fig. 63–1B,C). Colabeling experiments with different interneuron markers have revealed that more than 75% of all interneurons are *Arx* positive.[29–31,33,34] *Arx* is also expressed in the developing pancreas, testis, and muscle. Data from the pancreas suggest an important role of *Arx* in fate determination.[35–37] The role of *ARX* in the brain appears to be more complicated than in the pancreas.

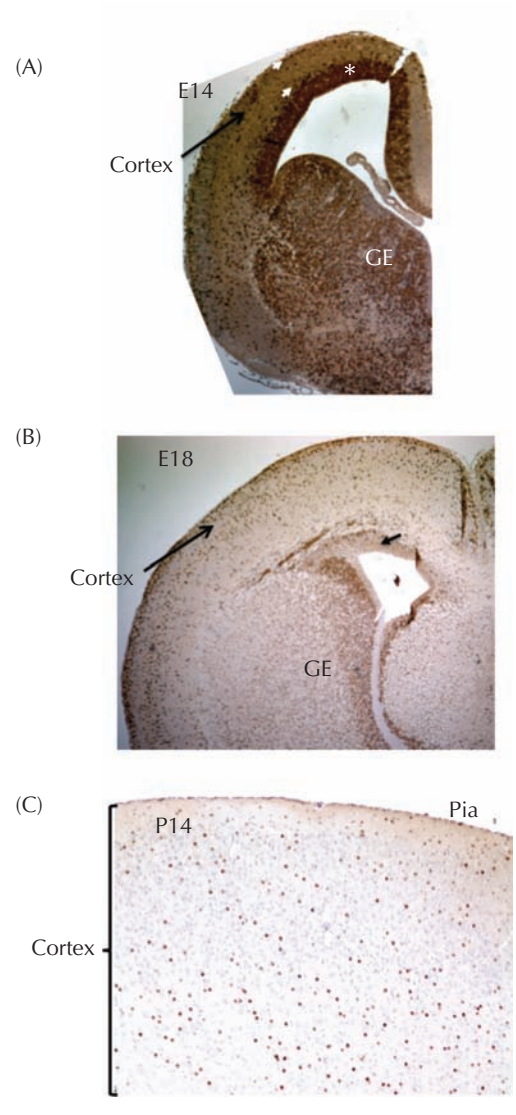

Figure 63–1. Ontogeny of *Arx* protein staining in dorsal neocortex of a wild-type mouse. E14.5, E18.5, and P14 coronal sections are presented. **A.** E14.5 coronal section (4x) with *Arx* staining (dark brown) in the GE and in the dorsal VZ (asterisk). Two trails of migrating *Arx*-positive cells are present in the SVZ/IZ and the MZ (two white arrowheads). **B.** At E18.5, the *Arx* staining is essentially gone from the dorsal VZ (arrow), but it remains in the GE and in cells scattered throughout the cortex. A 4x image is presented. **C.** By postnatal time (P14), staining is present only in scattered cells throughout the cortex. The lighter blue stain is a hematoxylin counterstain for nuclei (magnification 10x). E, embryonic; P, postnatal; GE, ganglionic eminence. All three sections are paraffin-embedded, paraformaldehyde-fixed 6 µm sections. All staining was performed as previously described using an *Arx* antibody (kindly provided by K. Kitamura) and the Vectastain ABC kit as a secondary antibody.[2]

The functional role of *Arx* in the telencephalon is an active area of research. Recent studies suggest that *Arx* is primarily a transcriptional repressor[2,38] and, through this mechanism, has important roles in pallial progenitor cell proliferation, nonradial cell migration of interneurons from the ganglionic eminence, and basal ganglia development.[8,32]

Arx as a Transcriptional Repressor

The Aristaless homeodomain protein in *Drosophila* is a paired related homeobox protein, distinguished by containing a paired homeodomain region, a C-terminal aristaless domain, and an N-terminal octapeptide/engrailed domain.[29,39] The aristaless domain is known to act primarily as a repressor,[40] and the octapeptide domain is believed to be a potent transcriptional repressor.[41] Based on the findings in *Arx* homologues, the transcriptional activity of murine *Arx* has been investigated and also been found to be primarily repressive in nature.[2,38,42] Using a Gal4-reporter assay, the engrailed/octapeptide domain, a region near the C terminus, and the fourth polyalanine tract were found to mediate transcriptional repression.[38,42] The transcriptional repression of the engrailed domain was determined to act via the transducin-like enhancer (TLE) proteins,[38] which are known cofactors for engrailed domain-containing proteins. Indeed, in the same reporter system, introduction of mutations believed to cause XLMR and ISSX reduced repression of the reporter construct[38,42] by decreasing the binding to the TLE elements. *Arx* also functions as a transcriptional promoter, though this is not believed to be as prominent an action of the transcription factor.[2,38] As the data fairly well establish *Arx* as primarily an inhibitor of transcriptional programs, two questions remain: which gene networks are affected, and how does this repression alter normal cortical development?

Attempting to answer these questions, two labs performed mouse whole genome transcriptional arrays followed by bioinformatic analyses to begin to elucidate the downstream targets of *Arx*.[2,43] In these experiments, the ventral telenecephalon, including the GE, developing basal ganglion, and hypothalamic regions were dissected away from the cortex, and their RNA was isolated and hybridized to Affymetrix whole genome transcriptional arrays. Both studies demonstrated that the majority of differentially expressed genes were upregulated in the knockout animals. Many of these were genes previously not (or minimally) expressed in the ventral forebrain, suggesting that *Arx* plays an important role in regionalization and fate determination by suppressing the expression of other transcription factors. The loss of *Arx* also resulted in the decreased expression of a subset of differentially expressed genes, presumably ones that are upregulated by Arx. Some of these latter genes appear to be important in the control of migration and proliferation.[2,43] Together, these experiments confirmed the function of *Arx* as primarily a repressor and began to define the downstream targets of *Arx*-related transcriptional networks that are important in ventral forebrain development.

Role in Interneuron Development

The above studies suggest that *Arx* plays an important role in ventral forebrain development. This is likely for basal ganglia formation, interneuron production, migration, and specification, as well as cholinergic neuronal specification. There is much interest in the interneuron phenotype because of the intractable epilepsy present in most patients with *ARX* mutations. With one of the original three clinical descriptions, a germline knockout of *Arx* was developed and experiments on these animals showed a clear alteration in interneuronal development.[8] The knockout mouse data and other subsequent experiments have confirmed the important role of *Arx* in this developmental process. The expression pattern data described above (e.g., see Fig. 63–1) further corroborate that *Arx* is essential for normal interneuron development.[8,9,30,31] How *Arx* guides interneuronal development is less well understood.

A number of different transcription factors have important roles during interneuron development (for review, see refs. 44 and 45). Early (E8–9) Mash1 expression sets ventral forebrain identity,[46] and subsequent expression of Dlx1/2 is vital for interneuronal fate determination.[47,48] Subsequently, ventral regionalization occurs by the sequential expression of various transcription factors, including Nkx2.1 and Lhx6.1. Accumulating evidence is beginning to place

Arx within this transcriptional network, with *Arx* as a direct target of Dlx1/2[34] by acting through an enhancer element over 12 kB downstream from the end of the coding region. Activation of *Arx* by Dlx1/2 is thought to mediate the Dlx-dependent interneuron migration but not specification of the GABAergic cell fate.[34,49] Loss of *Arx* increases the expression of Gbx1, Lhx6, Dlx2, Nkx2., Magel2, Efb3, and Lmo3 into ventral regions with minimal previous expression[2,32,34] but decreases the expression of Cxcr4 and Bmper.[34] From the results of these experiments, the effect of proper *Arx* expression is the correct specification of ventral areas into regions producing interneurons and subsets of basal ganglia neurons and the appropriate expression of proteins necessary for interneuronal migration (*Cxcr4* and *Ebf3*). The exact mechanisms need to be further elucidated, but a framework for understanding how *Arx* disrupts interneuronal development is now present.

Role in Cortical VZ Development

In contrast to the emerging evidence defining a mechanism of *Arx* action in interneuronal development, much less is known about the role in dorsal cortex during development. *Arx* expression is first observed in the dorsal VZ at E12.5, but by birth (P0), most VZ progenitor cells have matured and have entered the cortex, with resultant loss of *Arx* expression. As stated in the previous sections, all postnatal expression of *Arx* is in interneurons and not in the excitatory cells that are derived from the dorsal VZ. *Arx* expression in the dorsal VZ is under a presumed regulatory element in an ultraconserved region in the 3.5 kB downstream of the *Arx* coding region. The function of *Arx* in the VZ is believed to be in controlling proliferation, and several pieces of evidence support this role. First, humans with *ARX* mutations can develop a form of lissencephaly, with only three thin layers and thickened white matter, suggesting a proliferative defect. Second, germline knockout mice have a thin cortex and a small forebrain.[5,8] Third, the expression in the VZ apparently terminates when the cells enter the SVZ or IZ. Finally, in utero electroporation experiments altering *Arx* levels in the VZ (by a shRNA- or *Arx*-overexpressing vector) described changes in cell cycle time and in the number of cells proliferating in the VZ.[49] These authors showed a lack of cell death, but they could not differentiate between *Arx* involvement in cell cycle timing and proliferation or neuronal differentiation, but they concluded that the role is in cell proliferation and cell cycle timing.[49] Though *Arx* is likely involved in cell proliferation by controlling cell cycle dynamics, the downstream targets for this role and its location within a gene regulatory network in the dorsal cortex are not known. Research into this important area of *Arx* function is ongoing.

Role of Expansion Mutations

A unique feature of *Arx* is the presence of four alanine repeats within the gene. A number of genes are known to contain polyalanine tracts, with the majority acting as transcription factors or having nuclear localization signals in the protein.[50] Polyalanine repeats, unlike the more common/well-known polyglutamine repeats, are shorter, averaging less than 20 alanines, more stable (lacking genetic anticipation), and presumed to cause disease by either protein misfolding or altering the nuclear localization.[50,51] Nine diseases are associated with expansions in these alanine tracts, eight of which are transcription factors and result in developmental disorders similar to those caused by *Arx*. These results led to a series of experiments to determine the effect of different expansions on *Arx* function.[52,53] Expansions in the first polyalanine tract from 16 to 23 alanines resulted in protein aggregations, with two labs finding these protein inclusions in different cellular locations. Our lab reported the presence of intranuclear inclusions,[52] while another lab described cytoplasmic aggregations.[53] Both labs used overexpression experiments in cell culture. It is not clear why the two studies showed a difference in location of the protein aggregates, as both used similar cell lines and culture methods, suggesting that subtle differences in methodology can lead to alterations in protein processing. Importantly, to understand the functional implication of the inclusions, our lab showed increased cell death in HEK cells and both partial reduction in the number of cells with inclusions and partial rescue of the cell death response by overexpressing the heat shock protein HSP-70.[52] Mechanistically, the polyalanine expansion may induce inclusions and

cellular aggregations, not by altering the binding of *Arx* with one potential nuclear import protein, IPO13, but rather due to changes in protein–protein interactions.[53] Finally, the expansions were found to occur in vivo after in utero electroporation.[52] However, inclusions were not observed in the two expansion mouse models that have been recently generated.[3,5] In one expanded mouse model, alterations were reported in cellular localization of the *Arx* protein,[3] partially consistent with some of the cell culture experiments described above.[53]

Recent work has begun to address the issue of mislocalization of *Arx*, though not with the expanded mutation. A few studies have addressed this issue by producing full-length *Arx* cDNA constructs with mutations/disruptions in the nuclear localization sequence (NLS) of *Arx*.[54,55] *Arx* contains at least three NLSs, which are found in the homeodomain or in the 3′ region, and alterations in these sequences cause nuclear or perinuclear protein aggregations. The transport of *Arx* into the nucleus is protein dependent particularly the importin 13 (IPO13)[55] and importin β.[54] The possibility that many *ARX* mutations, both expansions and point mutations, alter ARX binding, inhibiting nuclear import, was tested. Both groups found that the change in cellular localization occurs without affecting binding to nuclear import proteins,[54,55] suggesting that they are purely mistargeted, not altered in protein binding. This work is limited, as there could be alterations in the binding to other *Arx* cofactors, either for nuclear import or DNA binding, and these may lead to the cellular aggregations or nuclear inclusions. Of note, in a single patient with XLAG not due to expansion of the polyalanine tract, no inclusions or aggregations were noted on histopathological exam.[10] This implies that in the more severe mutations, there may be alterations in protein processing and, hence, no aggregations. Further research is needed to fully understand how the protein expansion leads to the *Arx* clinical phenotypes.

ARX MODELS

Since the first description of *Arx*-related disorders, a number of *Arx* mouse models have been developed.[3–5,8,36] These models have led to different insights into the disorders. The first mouse was made by homologous recombination of a lacZ-containing vector into exon 2, producing an *Arx* allele with a premature stop codon[8] and resulting in a truncated protein. These mice died at P0–P2 but were found to have many of the features of XLAG in humans, including a poorly formed small cortex, absent corpus collosum, abnormal tangential migration and differentiation of interneurons, and abnormalities in the male genitalia.[8] There was a severe alteration in interneuron migration, with a delay in onset and loss of all migrating interneurons except for those along the SVZ.[8] These authors also uncovered changes in basal ganglia cell populations. Because of the early lethality of the mice, no behavioral or electrophysiological studies could be performed. Shortly afterward, Collombat and colleagues created a mouse line with targeted knockout of exon 1 and 2 producing a mutant protein with the loss of the first 360 N-terminal amino acids.[36] These mice are born but die by P2, and are smaller and appear dehydrated.[36] The mice were first developed to study the role of *Arx* in pancreatic development, but subsequently they have been used to study both the downstream targets of *Arx* and the alterations in interneurons, basal ganglia, and cholinergic neurons, as described in the preceding sections.[30,32,34,43] As these mice also had perinatal lethality, attributable to loss of glucagon-producing alpha cells, no phenotypic or physiological analysis could be performed. The functional shortcomings of these two mice lines were corrected with a series of five *Arx* model mice lines that were published in 2009.

The latter *Arx* models took two approaches to developing the mice. The first approach, from our lab, used the Cre-Lox system to stop expression of *Arx* in cells expressing Dlx5/6, a transcription factor that is expressed in the majority of developing interneurons within the GE.[4] The Dlx5/6 promoter was chosen to drive the Cre recombinase, as loss of interneurons in patients with *Arx* mutations is believed to be a major contributor to the epilepsy and infantile spasms phenotype.[56] We were, therefore, attempting to prove the hypothesis that interneuron dysfunction is central to the epilepsy phenotype in various brain malformations. Indeed, the conditional knockout (CKO) mice appear to give credence to this view. The adult CKO male mice had faster, higher-voltage electroencephalographic (EEG) tracings with

multifocal spikes reminiscent of a hypsarrhythmic pattern seen in infants.[4] The male mice develop convulsive seizures at P14, the earliest time we were able to assess accurately for the presence of electrographic seizures. The male CKO mice, which live past P14, continue to have seizures and develop an infantile spasms-like seizure type. The development of the spasm phenotype was found more often in the adult animals, most likely due to differences in maturity and neuroanatomy between mice and humans. In addition, we found that approximately half of the female animals also developed seizures at P14 that persisted into adulthood. The loss (or partial loss) of *Arx* reduced the number of calbindin-positive interneurons.[4] Currently, we are determining if there are other interneuronal changes, as well as the developmental time course of this loss. Our approach contrasts with the method of the two other laboratories that recently developed *Arx* models.

The approach taken by these labs was to generate mice with known human mutations associated with disease.[3,5] The Noebels' lab developed a knockin *Arx* mouse with an expansion of the first polyalanine tract, increasing the repeat size from 16 to 23 amino acids.[3] These authors found the animals to have possible behavioral spasms at 7–11 days of age, electroclinical spasms at 14–21 days of age, and then the development of other seizure types (behavioral arrest and motoric seizures) as the animals matured.[3] Behavioral tests on these animals revealed deficits in anxiety, fear conditioning, and social behaviors. Notably, the animals were less anxious and had diminished retention of a conditioned stimulus compared to wild-type mice, suggesting more of an anxiety phenotype rather than diminished ability to learn.[3] The pathological alteration of the *Arx* expansion was regional loss of a population of *Arx*-expressing interneurons. A 50% reduction of *Arx* was found in the hilus of the hippocampus and striatum, and a 68% decrease was discovered in somatosensory and motor cortex, but no change was found in parietal cortex.[3] The authors reported a change in protein localization from nuclear to cytoplasmic, but this contrasts with the other *Arx* expanded mouse, which showed no inclusions or alteration of protein localization.[5] The partial loss of *Arx*-positive interneurons was due primarily to loss of calbindin-positive cells, similar to the changes found in our Dlx5/6 conditional knockout mouse line. There was no loss of neuropeptide Y (NPY) or calretinin-positive cells in the cortex. Finally, the authors reported a loss of calbindin, NPY, and cholinergic neurons in the striatum.[3] Overall, the features of this mouse model were similar to those of the *Arx* CKO model.

The third group, in the Kitamura laboratory, generated three mouse lines. Two were knockins of known pathogenic mutations, both disrupting the homeodomain, but one mutation was associated with the XLAG phenotype and the other with the XLMR phenotype.[5] The third line derived by this group was an expansion of seven alanines to the first polyalanine tract,[5] a line similar to that made by the Noebels' lab. The XLAG mutation mouse line was similar in phenotype to the germline knockout. This mouse was perinatal lethal and had smaller brains, severely diminished numbers of interneurons in the cortex, and a large loss of cholinergic neurons in the striatum.[5] The other two lines were viable and recapitulated aspects of the XLMR or ISSX phenotypes. These two lines both developed spontaneous seizures but of differing severities. Only 10% of XLMR point mutation mice were observed to have a seizure, and no seizures were captured on video EEG in the three mice that were recorded. In contrast, 70% of the expanded mice developed behavior seizures, and all (three of three) mice with EEG recordings developed seizures but no interictal EEG abnormalities.[5] Like the Noebels' lab expansion mice, the two lines were tested for behavioral deficits. Both lines were observed to have impaired motor coordination and increased anxiety. They were also found to learn poorly in both a passive avoidance task and a radial arm task, which tests hippocampal spatial memory.[5] The differences in anxiety and motor phenotypes between the Kitamura and Noebels' expanded mice could be due to the different background strains, C57BL/6J × 129Sv/SvEvBrd mice[3] versus pure C57BL/6J mice,[5] or to other differences with strain generation, suggesting that in humans, genetic background and environmental features may modify the phenotypic expression in patients with the expansion mutation. This is indeed what other authors have proposed for the variability of phenotypes observed in patients with expansions of the first and second polyalanine tracts.[26]

Like the other conditional lines, the two viable lines from the Kitamura lab also had deficiencies in interneuron and striatal neurons. In these animals, interneurons began to migrate at the appropriate time (E12.5) and migrated normally, but reduced numbers of interneurons entered the cortex. Although the total number of interneurons was reduced in postnatal mice, these authors did not find an interneuron subtype-specific difference in either the XLMR or ISSX mutations.[5] This contrasts with what was found in the basal ganglia of these mice. In the striatum, the XLAG mutation resulted in a loss of both radial and tangential migration, whereas the two less severe mutations only altered tangential migration of interneurons into the basal ganglia. Finally, there was a loss of cholinergic neurons in the cortex and basal ganglia, but it was greater in the striatum.[5] Overall, these five mouse lines have a number of similarities but also a few differences. These lines can now be used to attempt to understand how *Arx* alters normal cortical circuitry and how these changed neuronal networks result in an epilepsy phenotype and varying degrees of cognitive dysfunction.

ARX as Disease Mediator

Mutations in *Arx* result in a variety of phenotypes. Two aspects of *Arx* function described in the sections above have both research and clinical implications for the study of *ARX*-related disorders. The first relates to *ARX*'s role in interneuron development. A number of genes can lead to brain malformations, which can be focal or diffuse. In many cases, these malformations present later in life when the child is evaluated for learning issues or for a first seizure. Even in the most severe malformations, such as in lissencephaly, there is variability in the severity of the cognitive disability and the intractable nature of the epilepsy that occurs. In *ARX*-mediated malformations, and in particular, XLAG, the disease is particularly severe. The children often make no developmental progress and have seizures that are not responsive to any treatment. The severity of this disorder, compared to those resulting from other genes that cause lissencephaly or other malformations, has been attributed to the specific loss of interneurons.[56] Kato and Dobyns have used *ARX*-related disorders to designate a new term for a class of neurological diseases called *interneuronopathies*.[56] Other interneuronopathies could be uncovered by screening patients with similar clinical phenotypes for genes either upstream or downstream from *Arx* in the gene regulatory network.

The second aspect of *ARX* activity to have clinical implications is the finding that *Arx* has seemingly very different roles in two regions of the developing forebrain. The fact that *Arx* may control proliferation in the dorsal VZ, but control migration or fate specification in the ventral forebrain, means that altering *Arx* activity throughout the entire brain could have multiple unintended consequences. Therefore, developing a better understanding of how *Arx* functions, and where in the gene regulatory networks of telencephalon development *Arx* acts, is vital to be able to generate therapeutic interventions for patients with *ARX* mutations. In addition, greater understanding of the temporal and spatial regulation of *Arx* expression and function is necessary if future therapeutics are to be designed.

FUTURE DIRECTIONS IN THE STUDY OF *ARX*

Over the last decade, *Arx* has been linked to over 10 overlapping clinical disorders, and a great deal of information regarding the expression pattern, molecular function, and role in cortical development has been generated. This work has answered many questions but inevitably has raised many more. Further study is necessary to understand how different mutations lead to the spectrum of disorders and whether genetic background effect, environment, or changes in the protein due to the mutations are the cause of such variability. Next, a full categorization of the *Arx* involved gene networks is needed, as well as an understanding of how the gene would have differential effects in the ventral and dorsal telecephalon. Another area for future investigation is the role of *Arx* in the postnatal brain. Lastly, consideration of how alteration in interneurons leads to a seizure or cognitive phenotype is warranted, and the four mouse lines are available to study the pathophysiological changes that occur. The next decade will likely yield the answers to many of these questions.

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The authors have nothing to disclose regarding this research.

REFERENCES

1. Cobos I, Broccoli V, Rubenstein JL. The vertebrate ortholog of Aristaless is regulated by Dlx genes in the developing forebrain. *J Comp Neurol*. 2005;483(3): 292–303.
2. Fulp CT, Cho G, Marsh ED, Nasrallah IM, Labowski PA, Golden JA. Identification of Arx transcriptional targets in the developing basal forebrain. *Hum Mol Genet*. 2008;17(23):3740–3760.
3. Price MG, Yoo JW, Burgess DL, Deng F, Hrachovy RA, Frost JD Jr, Noebels JL. A triplet repeat expansion genetic mouse model of infantile spasms syndrome, Arx(GCG)10+7, with interneuronopathy, spasms in infancy, persistent seizures, and adult cognitive and behavioral impairment. *J Neurosci*. 2009;29(27): 8752–8763.
4. Marsh E, Fulp C, Gomez E, Nasrallah I, Minarcik J, Sudi J, Christian SL, Mancini G, Labosky P, Dobyns W, Brooks-Kayal A, Golden JA. Targeted loss of Arx results in a developmental epilepsy mouse model and recapitulates the human phenotype in heterozygous females. *Brain*. 2009;132(Pt 6):1563–1576.
5. Kitamura K, Itou Y, Yanazawa M, Ohsawa M, Suzuki-Migishima R, Umeki Y, Hohjoh H, Yanagawa Y, Shinba T, Itoh M, Nakamura K, Goto Y. Three human ARX mutations cause the lissencephaly-like and mental retardation with epilepsy-like pleiotropic phenotypes in mice. *Hum Mol Genet*. 2009;18(19):3708–3724.
6. Korff CM, Nordli DR Jr. Epilepsy syndromes in infancy. *Pediatr Neurol*. 2006;34(4):253–263.
7. Bienvenu T, Poirier K, Friocourt G, Bahi N, Beaumont D, Fauchereau F, Ben Jeema L, Zemni R, Vinet MC, Francis F, Couvert P, Gomot M, Moraine C, van Bokhoven H, Kalscheuer V, Frints S, Gecz J, Ohzaki K, Chaabouni H, Fryns JP, Desportes V, Beldjord C, Chelly J. ARX, a novel Prd-class-homeobox gene highly expressed in the telencephalon, is mutated in X-linked mental retardation. *Hum Mol Genet*. 2002;11(8): 981–991.
8. Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, Omichi K, Suzuki R, Kato-Fukui Y, Kamiirisa K, Matsuo M, Kamijo S, Kasahara M, Yoshioka H, Ogata T, Fukuda T, Kondo I, Kato M, Dobyns WB, Yokoyama M, Morohashi K. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet*. 2002;32(3):359–369.
9. Stromme P, Mangelsdorf ME, Shaw MA, Lower KM, Lewis SM, Bruyere H, Lutcherath V, Gedeon AK, Wallace RH, Scheffer IE, Turner G, Partington M, Frints SG, Fryns JP, Sutherland GR, Mulley JC, Gecz J. Mutations in the human ortholog of Aristaless cause X-linked mental retardation and epilepsy. *Nat Genet*. 2002;30(4):441–445.
10. Bonneau D, Toutain A, Laquerriere A, Marret S, Saugier-Veber P, Barthez MA, Radi S, Biran-Mucignat V, Rodriguez D, Gelot A. X-linked lissencephaly with absent corpus callosum and ambiguous genitalia (XLAG): clinical, magnetic resonance imaging, and neuropathological findings. *Ann Neurol*. 2002;51(3):340–349.
11. Proud VK, Levine C, Carpenter NJ. New X-linked syndrome with seizures, acquired micrencephaly, and agenesis of the corpus callosum. *Am J Med Genet*. 1992;43(1–2):458–466.
12. Kato M, Das S, Petras K, Kitamura K, Morohashi K, Abuelo DN, Barr M, Bonneau D, Brady AF, Carpenter NJ, Cipero KL, Frisone F, Fukuda T, Guerrini R, Iida E, Itoh M, Lewanda AF, Nanba Y, Oka A, Proud VK, Saugier-Veber P, Schelley SL, Selicorni A, Shaner R, Silengo M, Stewart F, Sugiyama N, Toyama J, Toutain A, Vargas AL, Yanazawa M, Zackai EH, Dobyns WB. Mutations of ARX are associated with striking pleiotropy and consistent genotype–phenotype correlation. *Hum Mutat*. 2004;23(2):147–159.
13. Guerrini R, Moro F, Kato M, Barkovich AJ, Shiihara T, McShane MA, Hurst J, Loi M, Tohyama J, Norci V, Hayasaka K, Kang UJ, Das S, Dobyns WB. Expansion of the first PolyA tract of ARX causes infantile spasms and status dystonicus. *Neurology*. 2007;69(5):427–433.
14. Demos MK, Fullston T, Partington MW, Gecz J, Gibson WT. Clinical study of two brothers with a novel 33 bp duplication in the ARX gene. *Am J Med Genet A*. 2009;149A(7):1482–1486.
15. Partington MW, Mulley JC, Sutherland GR, Hockey A, Thode A, Turner G. X-linked mental retardation with dystonic movements of the hands. *Am J Med Genet*. 1988;30(1–2):251–262.
16. Turner G, Partington M, Kerr B, Mangelsdorf M, Gecz J. Variable expression of mental retardation, autism, seizures, and dystonic hand movements in two families with an identical ARX gene mutation. *Am J Med Genet*. 2002;112(4):405–411.
17. Scheffer IE, Wallace RH, Phillips FL, Hewson P, Reardon K, Parasivam G, Stromme P, Berkovic SF, Gecz J, Mulley JC. X-linked myoclonic epilepsy with spasticity and intellectual disability: mutation in the homeobox gene ARX. *Neurology*. 2002;59(3): 348–356.
18. Stromme P, Mangelsdorf ME, Scheffer IE, Gecz J. Infantile spasms, dystonia, and other X-linked phenotypes caused by mutations in Aristaless related homeobox gene, ARX. *Brain Dev*. 2002;24(5):266–268.
19. Kato M, Saitoh S, Kamei A, Shiraishi H, Ueda Y, Akasaka M, Tohyama J, Akasaka N, Hayasaka K. A longer polyalanine expansion mutation in the ARX gene causes early infantile epileptic encephalopathy with suppression-burst pattern (Ohtahara syndrome). *Am J Hum Genet*. 2007;81(2):361–366.
20. Kato M, Koyama N, Ohta M, Miura K, Hayasaka K. Frameshift mutations of the ARX gene in familial Ohtahara syndrome. *Epilepsia*. 2010;51(9): 1679–1684.
21. Absoud M, Parr JR, Halliday D, Pretorius P, Zaiwalla Z, Jayawant S. A novel ARX phenotype: rapid neurodegeneration with Ohtahara syndrome and a dyskinetic movement disorder. *Dev Med Child Neurol*. 2010;52(3):305–307.
22. Poirier K, Eisermann M, Caubel I, Kaminska A, Peudonnier S, Boddaert N, Saillour Y, Dulac O, Souville I, Beldjord C, Lascelles K, Plouin P, Chelly J,

Bahi-Buisson N. Combination of infantile spasms, non-epileptic seizures and complex movement disorder: a new case of ARX-related epilepsy. *Epilepsy Res*. 2008;80(2–3):224–228.
23. Shinozaki Y, Osawa M, Sakuma H, Komaki H, Nakagawa E, Sugai K, Sasaki M, Goto Y. Expansion of the first polyalanine tract of the ARX gene in a boy presenting with generalized dystonia in the absence of infantile spasms. *Brain Dev*. 2009;31(6):469–472.
24. Okazaki S, Ohsawa M, Kuki I, Kawawaki H, Koriyama T, Ri S, Ichiba H, Hai E, Inoue T, Nakamura H, Goto Y, Tomiwa K, Yamano T, Kitamura K, Itoh M. Aristaless-related homeobox gene disruption leads to abnormal distribution of GABAergic interneurons in human neocortex: evidence based on a case of X-linked lissencephaly with abnormal genitalia (XLAG). *Acta Neuropathol*. 2008;116(4):453–462.
25. Wallerstein R, Sugalski R, Cohn L, Jawetz R, Friez M. Expansion of the ARX spectrum. *Clin Neurol Neurosurg*. 2008;110(6):631–634.
26. Friocourt GParnavelas JG. Mutations in ARX result in several defects involving GABAergic neurons. *Front Cell Neurosci*. 2010;4:1–11.
27. Friocourt G, Poirier K, Rakic S, Parnavelas JG, Chelly J. The role of ARX in cortical development. *Eur J Neurosci*. 2006;23(4):869–876.
28. Galliot B, de Vargas C, Miller D. Evolution of homeobox genes: Q50 Paired-like genes founded the Paired class. *Dev Genes Evol*. 1999;209(3):186–197.
29. Miura H, Yanazawa M, Kato K, Kitamura K. Expression of a novel aristaless related homeobox gene "Arx" in the vertebrate telencephalon, diencephalon and floor plate. *Mech Dev*. 1997;65(1–2):99–109.
30. Colombo E, Galli R, Cossu G, Gecz J, Broccoli V. Mouse orthologue of ARX, a gene mutated in several X-linked forms of mental retardation and epilepsy, is a marker of adult neural stem cells and forebrain GABAergic neurons. *Dev Dyn*. 2004;231(3):631–639.
31. Poirier K, Van Esch H, Friocourt G, Saillour Y, Bahi N, Backer S, Souil E, Castelnau-Ptakhine L, Beldjord C, Francis F, Bienvenu T, Chelly J. Neuroanatomical distribution of ARX in brain and its localisation in GABAergic neurons. *Brain Res Mol Brain Res*. 2004;122(1):35–46.
32. Colombo E, Collombat P, Colasante G, Bianchi M, Long J, Mansouri A, Rubenstein JL, Broccoli V. Inactivation of Arx, the murine ortholog of the X-linked lissencephaly with ambiguous genitalia gene, leads to severe disorganization of the ventral telencephalon with impaired neuronal migration and differentiation. *J Neurosci*. 2007;27(17):4786–4798.
33. Kitamura K, Miura H, Yanazawa M, Miyashita T, Kato K. Expression patterns of Brx1 (Rieg gene), Sonic hedgehog, Nkx2.2, Dlx1 and Arx during zona limitans intrathalamica and embryonic ventral lateral geniculate nuclear formation. *Mech Dev*. 1997;67(1):83–96.
34. Colasante G, Collombat P, Raimondi V, Bonanomi D, Ferrai C, Maira M, Yoshikawa K, Mansouri A, Valtorta F, Rubenstein JL, Broccoli V. Arx is a direct target of Dlx2 and thereby contributes to the tangential migration of GABAergic interneurons. *J Neurosci*. 2008;28(42):10674–10686.
35. Collombat P, Hecksher-Sorensen J, Broccoli V, Krull J, Ponte I, Mundiger T, Smith J, Gruss P, Serup P, Mansouri A. The simultaneous loss of Arx and Pax4 genes promotes a somatostatin-producing cell fate specification at the expense of the alpha- and beta-cell lineages in the mouse endocrine pancreas. *Development*. 2005;132(13):2969–2980.
36. Collombat P, Mansouri A, Hecksher-Sorensen J, Serup P, Krull J, Gradwohl G, Gruss P. Opposing actions of Arx and Pax4 in endocrine pancreas development. *Genes Dev*. 2003;17(20):2591–2603.
37. Hancock AS, Du A, Liu J, Miller M, May CL. Glucagon deficiency reduces hepatic glucose production and improves glucose tolerance in adult mice. *Mol Endocrinol*. 2010;24(8):1605–1614.
38. McKenzie O, Ponte I, Mangelsdorf M, Finnis M, Colasante G, Shoubridge C, Stifani S, Gecz J, Broccoli V. Aristaless-related homeobox gene, the gene responsible for West syndrome and related disorders, is a Groucho/transducin-like enhancer of split dependent transcriptional repressor. *Neuroscience*. 2007;146(1): 236–247.
39. Meijlink F, Beverdam A, Brouwer A, Oosterveen TC, Berge DT. Vertebrate aristaless-related genes. *Int J Dev Biol*. 1999;43(7):651–663.
40. Hudson R, Taniguchi-Sidle A, Boras K, Wiggan O, Hamel PA. Alx-4, a transcriptional activator whose expression is restricted to sites of epithelial-mesenchymal interactions. *Dev Dyn*. 1998;213(2): 159–169.
41. Smith ST, Jaynes JB. A conserved region of engrailed, shared among all en-, gsc-, Nk1-, Nk2- and msh-class homeoproteins, mediates active transcriptional repression in vivo. *Development*. 1996;122(10): 3141–3150.
42. Fullenkamp ANEl, Hodiri HM. The function of the Aristaless-related homeobox (Arx) gene product as a transcriptional repressor is diminished by mutations associated with X-linked mental retardation (XLMR). *Biochem Biophys Res Commun*. 2008;377(1):73–78.
43. Colasante G, Sessa A, Crispi S, Calogero R, Mansouri A, Collombat P, Broccoli V. Arx acts as a regional key selector gene in the ventral telencephalon mainly through its transcriptional repression activity. *Dev Biol*. 2009;334(1):59–71.
44. Batista-Brito R, Fishell G. The developmental integration of cortical interneurons into a functional network. *Curr Top Dev Biol*. 2009;87:81–118.
45. Marin O, Rubenstein JL. A long, remarkable journey: tangential migration in the telencephalon. *Nat Rev Neurosci*. 2001;2(11):780–790.
46. Rallu M, Corbin JG, Fishell G. Parsing the prosencephalon. *Nat Rev Neurosci*. 2002;3(12):943–951.
47. Anderson SA, Eisenstat DD, Shi L, Rubenstein JL. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science*. 1997; 278(5337):474–476.
48. Anderson SA, Qiu M, Bulfone A, Eisenstat DD, Meneses J, Pedersen R, Rubenstein JL. Mutations of the homeobox genes Dlx-1 and Dlx-2 disrupt the striatal subventricular zone and differentiation of late born striatal neurons. *Neuron*. 1997;19(1):27–37.
49. Friocourt G, Kanatani S, Tabata H, Yozu M, Takahashi T, Antypa M, Raguenes O, Chelly J, Ferec C, Nakajima K, Parnavelas JG. Cell-autonomous roles of ARX in cell proliferation and neuronal migration during corticogenesis. *J Neurosci*. 2008;28(22):5794–5805.
50. Albrecht A, Mundlos S. The other trinucleotide repeat: polyalanine expansion disorders. *Curr Opin Genet Dev*. 2005;15(3):285–293.

51. Messaed C, Rouleau GA. Molecular mechanisms underlying polyalanine diseases. *Neurobiol Dis*. 2009;34(3):397–405.
52. Nasrallah IM, Minarcik JC, Golden JA. A polyalanine tract expansion in Arx forms intranuclear inclusions and results in increased cell death. *J Cell Biol*. 2004;167(3):411–416.
53. Shoubridge C, Cloosterman D, Parkinson-Lawerence E, Brooks D, Gecz J. Molecular pathology of expanded polyalanine tract mutations in the Aristaless-related homeobox gene. *Genomics*. 2007;90(1):59–71.
54. Lin W, Ye W, Cai L, Meng X, Ke G, Huang C, Peng Z, Yu Y, Golden JA, Tartakoff AM, Tao T. The roles of multiple importins for nuclear import of murine aristaless-related homeobox protein. *J Biol Chem*. 2009;284(30):20428–20439.
55. Shoubridge C, Tan MH, Fullston T, Cloosterman D, Coman D, McGillivray G, Mancini GM, Kleefstra T, Gecz J. Mutations in the nuclear localization sequence of the Aristaless related homeobox; sequestration of mutant ARX with IPO13 disrupts normal subcellular distribution of the transcription factor and retards cell division. Pathogenetics, 2010;3:1–15.
56. Kato M, Dobyns WB. X-linked lissencephaly with abnormal genitalia as a tangential migration disorder causing intractable epilepsy: proposal for a new term, "interneuronopathy." *J Child Neurol*. 2005;20(4): 392–397.

Chapter 64

Haploinsufficiency of *STXBP1* and Ohtahara Syndrome

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INTRODUCTION

Ohtahara syndrome (OS), also known as *early infantile epileptic encephalopathy with suppression-burst*, is one of the most severe and earliest forms of epilepsy.[1] It is characterized by early onset of tonic seizures, seizure intractability, characteristic suppression-burst patterns on the electroencephalogram (EEG), and a poor outcome with severe psychomotor retardation.[2,3] Brain malformations, such as cerebral dysgenesis or hemimegalencephaly, are often associated with OS, but cryptogenic or idiopathic OS is found in a subset of OS patients, in whom genetic aberrations might be involved.[4] Although mutations of the *ARX* gene have been found in several male patients with OS,[5–8] the genetic causes are unexplained in most cryptogenic OS cases. We have recently found de novo mutations in *STXBP1* (encoding syntaxin binding protein 1, also known as MUNC18-1) in individuals with cryptogenic OS.[9] Here we present all the mutations in *STXBP1* found to date in OS patients, as well as some evidence of mutations leading to haploinsufficiency.

OHTAHARA SYNDROME

Ohtahara syndrome was first reported as the earliest form of age-dependent epileptic syndromes by Ohtahara et al.[1] It is characterized by early onset of intractable tonic spasms, characteristic suppression-burst patterns on interictal EEG, and a poor outcome with severe psychomotor retardation.[2,3] According to a previous report,[4] all patients have seizure onset within the first 3 months, with the majority

(75%) in the first month. Tonic spasms were observed in all patients. One-third to one-half of patients also had partial seizures, such as erratic focal motor seizures and hemiconvulsions, or asymmetric tonic seizures; however, myoclonic seizures were rare. Hemiconvulsions, tonic seizures, or clonic seizures precede the onset of tonic spasms by 1 to several weeks to in 37.5% of OS patients.[4] Brain malformations, such as cerebral dysgenesis, hemimegalencephaly, porencephaly, and Aicardi syndrome, are often associated with OS, but a significant proportion of patients (31% to 50% of OS cases) remain etiologically unexplained.[2,4] Suppression-burst patterns on interictal EEG consisting of high-voltage activity alternating with nearly flat suppression phases are observed when the patient is both awake and asleep.

Early myoclonic encephalopathy (EME) is another epileptic syndrome showing suppression-burst patterns on EEG.[10] The prevailing initial seizure type is a main difference between OS and EME: tonic seizures in OS and myoclonic seizures in EME.[2,3] However, OS and EME have common features, and it is often difficult to distinguish between them. Homozygous missense mutations of the *SLC25A22* (mitochondrial glutamate carrier 1) gene have been recently found in EME individuals in consanguineous familes.[11,12] Age-dependent evolution is a characteristic feature of both OS and EME: approximately 75% and 40% of OS and EME cases, respectively, transit to West syndrome (WS), usually 3–4 months afterward.[2,3] West syndrome is characterized by tonic spasms with clustering, arrest of psychomotor development, and hypsarrhythmia on EEG. Such transitions suggest a common pathophysiology between OS and WS or between EME and WS. Consistent with this hypothesis, specific mutations of the *ARX* (*aristaless*-related homeobox) gene at Xp22.13, have been recently found in male OS and WS cases.[5–8,13–15]

DE NOVO *STXBP1* MUTATIONS CAUSE OS

Identification of *STXBP1* Mutations in Patients with OS

Through BAC array-based comparative genomic hybridization analysis of patients associated with mental retardation (MR), we found a microdeletion at 9q33.3-q34.11 in a female patient with OS.[9] As the microdeletion occurred de novo, we assumed that a gene within the deletion was responsible for OS. Among the genes mapped within the deletion, the gene encoding syntaxin binding protein 1 (STXBP1) was of particular interest because mouse *Stxbp1* has been shown to be essential for synaptic vesicle release[16] and is specifically expressed in the brains of rodents and humans.[17,18] We therefore analyzed *STXBP1* in 13 unrelated patients with OS. Four heterozygous missense mutations were found at evolutionarily conserved amino acids in three males and one female (Fig. 64–1 and Table 64–1). Three mutations were confirmed as de novo events (paternal DNA was unavailable for one remaining mutation).

STXBP1 Mutation Is a Major Genetic Cause of OS

To delineate the clinical spectrum of patients with *STXBP1* mutations, *STXBP1* was further analyzed in 29 and 54 cases of cryptogenic OS and WS, respectively.[19] No brain malformations were found in any of the cases. Seven novel heterozygous mutations were found in nine OS cases (the same mutation was found in three cases), but none in WS cases (six males and three females; Fig. 64–1 and Table 64–1). The mutations included one missense, one splicing, two frameshift, and three nonsense mutations. A recurrent missense mutation (c.1217G>A, p.R406H) occurred at an evolutionarily conserved amino acid (Fig. 64–1). All the mutations occurred de novo. Collectively, *STXBP1* aberrations account for about one-third of individuals with OS (14 out of 43). These data showed that *STXBP1* mutations are a major genetic cause of cryptogenic OS, but they are not a genetic cause of WS in our Japanese cohort.

Clinical Features of Patients with *STXBP1* Deletion/Mutations

Clinical information from 14 individuals with confirmed *STXBP1* deletion/mutations is summarized in Table 64–1. These persons showed

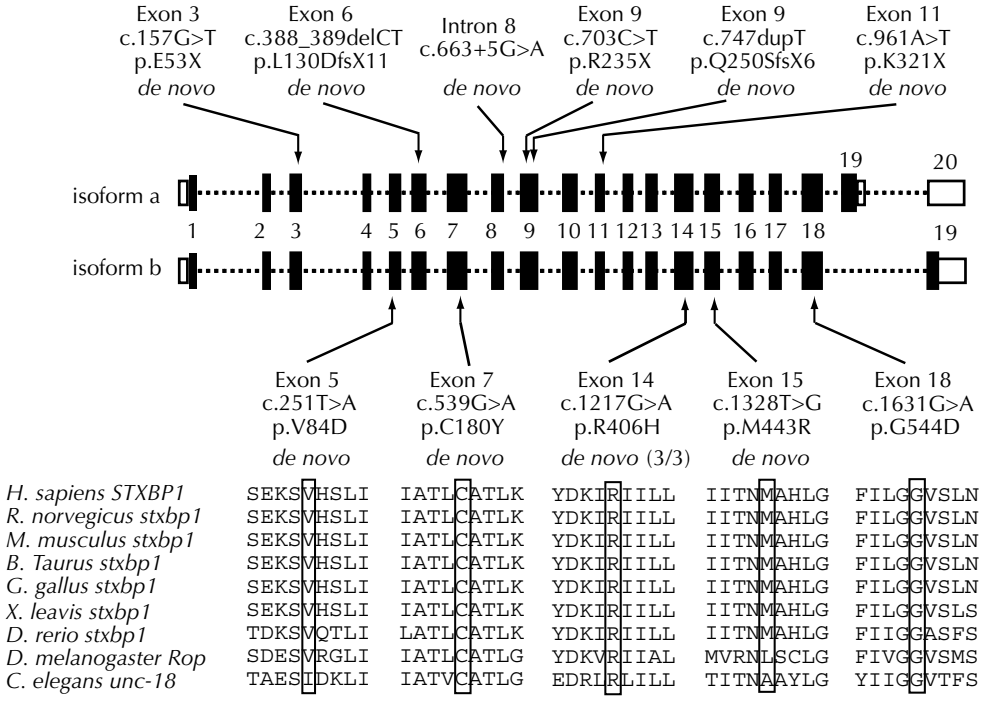

Figure 64–1. Summary of *STXBP1* mutations found in Japanese individuals with OS. Schematic representation of STXBP1, consisting of at most 20 exons (the UTR and the coding region are open and filled rectangles, respectively). There are two isoforms, a (GenBank accession number, NM_003165) with exon 19, and b (NM_001032221) without exon 19 of isoform a. Locations of mutations are indicated by arrows. Eleven different mutations are presented: missense mutations are indicated below the gene scheme, and the other types of mutations are indicated above the gene. Ten mutations in 12 subjects occurred de novo. All missense mutations occurred at evolutionarily conserved amino acids. CLUSTALW (http://align.genome.jp/) was used to align homologs of different species. Adapted from refs. 9 and 19.

distinctive features of OS, such as early-onset seizures including epileptic spasms, suppression-burst pattern on EEG, transition to WS after a few to several months, and severe developmental delay. Epileptic spasms were preceded by other seizure types, including partial seizures in 11 subjects. Transition to WS was observed in 11 subjects with OS. Although seizures were intractable in nine subjects, five subjects responded to medication, such as thyrotropin-releasing hormone (TRH) injection, adrenocorticotropic hormone (ACTH) injection, vitamin B_6, high-dose phenobarbital, and valproic acid. All subjects demonstrated severe psychomotor developmental delay. Brain magnetic resonance imaging (MRI) showed no structural anomalies or hippocampal anomalies but did show some atrophy (Fig. 64–2A). Suppression-burst on interictal EEG was observed in both awake and asleep states (Fig. 64–2B). We gained several insights into the phenotype of *STXBP1* aberrations. Firstly, the age at onset of epileptic spasms is later in subjects with *STXBP1* aberrations than in the 16 original subjects reported by Yamatogi and Ohtahara.[4] Only 27% of the subjects (4/15) in our series had onset of OS within 1 month compared to 75% (12/16) in the series of Yamatogi and Ohtahara. As subjects with *STXBP1* aberrations showed no structural anomalies on brain MRI examination, the onset of epileptic seizures might be affected by associated structural brain abnormalities, which are commonly seen in other reports of OS. Secondly, myoclonic seizures, which are thought to be rarely observed in OS, were occasionally observed (3/14). Myoclonic seizures are the main ictal symptom of EME. These three subjects can be diagnosed as having EME when myoclonic seizures dominate. Thus, *STXBP1* might also be causative for EME, implying a genetic linkage between OS and EME. Another infrequent but interesting finding is that one patient (no. 5) developed vigorous chorea-ballismus

Table 64–1 **Summary of Clinical Features of Subjects with STXBP1 Deletion/Mutations**

Case # Mutation	Initial Symptoms	Onset of Spasms	Transition from Spasms to Other Seizures	Response to Therapy
#1 Deletion	Tonic seizure and oral automatism	2 m	Generalized clonic seizure at 29 m	Seizure free from 5 m after TRH injection
#2 c.1631G>A	Blinking	10 d	No	Seizure free from 3 m
#3 c.539G>A	Tonic seizure with blinking	3 m	No	Intractable, daily
#4 c.1328T>G	Upward gazing and tonic seizure	2 m	Partial seizure at 8 m	Intractable, hourly TRH injection was temporally effective
#5 c.251T>A	Spasms and tonic-clonic seizure	2 m	No	Intractable, daily
#6 c.1217G>A	Generalized convulsions	3 w	No	Intractable, hourly
#7 c.1217G>A	GTCS with upward eye gazing	2 m	Myoclonic seizure at 48 d	Intractable, daily
#8 c.1217G>A	Partial seizures (right hemiconvulsion)	2 m	Tonic seizure to myoclonic seizure	Intractable, daily
#9 c.157G>T	Spasms	2 d	Versive seizure after hypoxia at 2 y	Intractable, daily
#10 c.388_389del	Secondary generalized seizures	2 m	CPS	Seizure free after ACTH or VPA with KBr
#11 c.663+5G>A	Blinking to tonic seizures	1 m	Tonic seizure	Seizure free with VB6 for spasms and ACTH for WS
#12 c.703C>T	Spasms in cluster	1 m	No	Seizure free from 6 m after high-dose PB
#13 c.747dup	Clonic convulsion	31 d	Partial seizure and myoclonic seizures	Intractable, hourly
#14 c.961A>T	Partial seizures	3 w	Partial seizure	Intractable, daily

GTCS, generalized tonic-clonic seizures; CPS, complex partial seizure; TRH, thyrotropin-releasing hormone; ACTH, adrenocorticotropic hormone; VPA, valproic acid; KBr, potassium bromide; VB6, vitamin B_6; PB, phenobarbital; d, day(s); w, week; m, month(s); y, year(s); 0 w, 0 to 6 days; 0 m, 0 to 3 weeks.

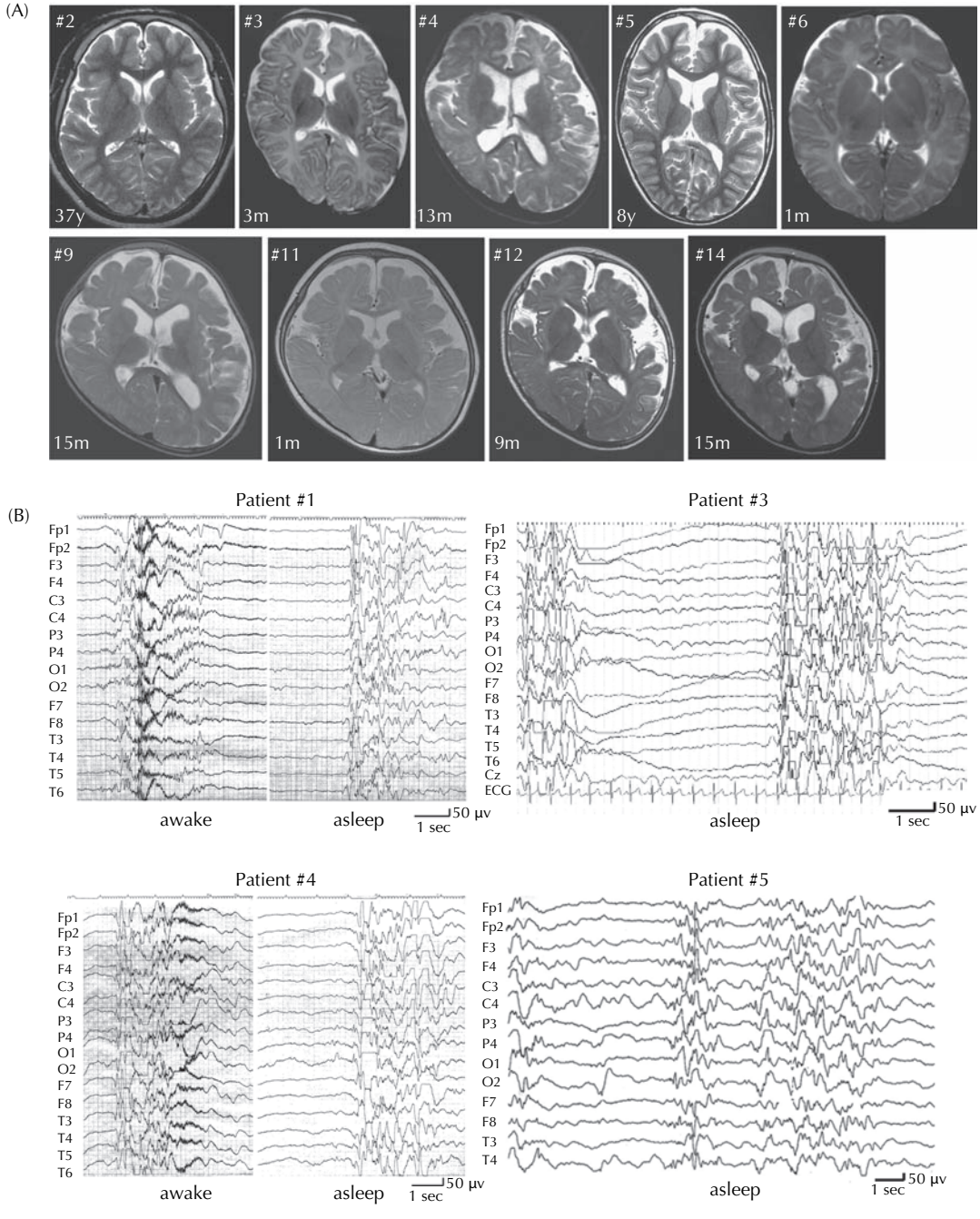

Figure 64–2. Brain MRI scan and EEG of OS patients with *STXBP1* aberrations**. A.** Brain MRI (T2-weighted axial) image through the basal ganglia shows normal brain structure in patients with *STXBP1* mutations. Patient IDs and developmental stages are indicated. Mild dilatation of lateral ventricles is observed in patients #4, #9, #11, and #14, but none shows brain malformation. y, year(s); m, month(s). **B.** Suppression-burst on interictal EEG of patients 1 (at age 2 months), 3 (at 3 months), 4 (at 2 months), and 5 (at 3 months). High-voltage bursts alternating with almost flat suppression phases at an approximately regular rate in both awake and asleep states. Adapted from refs. 9 and 19.

subsequent to OS, suggesting that mutations of *STXBP1* could affect the basal ganglia.[9,20] In terms of the genotype–phenotype relationship, we found no difference in clinical data between seven subjects with missense mutations and seven subjects with microdeletions, premature termination codons, or splicing mutations. This finding is supported by our experimental data that demonstrated both missense mutations and a splicing mutation resulted in haploinsufficiency of *STXBP1*: degradation of STXBP1 proteins containing missense mutations and nonsense-mediated mRNA decay (NMD) associated with aberrantly spliced mRNAs (see below).

MOLECULAR EVIDENCE OF *STXBP1* HAPLOINSUFFICIENCY

Mutant STXBP1 Proteins Are Unstable

All five missense mutations lead to amino acid replacements in the hydrophobic core of STXBP1 and are considered to destabilize the folding architecture. This is especially true for the three mutants (p.V84D, p.G544D, and p.M443R) that have replaced the wild-type (WT) residues with charged residues, which would be predicted to severely disrupt the conformation of STXBP1.[9] In fact, circular dichroism (CD) spectra showed that the helical content of the C180Y mutant is lower (39%) than that of the WT (43%), suggesting that the mutation destabilized the secondary structure of STXBP1[9] (Fig. 64–3A). Moreover, CD melting experiments revealed that the melting temperature (T_m) of the C180Y mutant was about 11 degrees lower than that of the WT (Fig. 64–3B), indicating that the C180Y mutant is much more unstable than the WT. The regulation of synaptic vesicle release by Stxbp1 is mediated in part by binding to Syntaxin-1A as well as directly to the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, which mediates fusion of vesicles with the target membrane.[21,22] Binding of a mutant protein (p.C180Y) to syntaxin-1A was also significantly impaired, even at 4°C in vitro.[9] Together with the fact that the T_m of the C180Y mutant is close to the physiological temperature of the human body, it is less likely that its functional activity could be retained in the human brain. Other STXBP1 mutants (p.V84D, p.G544D, and p.M443R) tend to form aggregates, and thus sufficient protein for biophysical analyses could not be obtained.

Degradation of Mutant *STXBP1* Proteins

Transient expression of mutant STXBP1 proteins in Neuroblastoma 2A (N2A) cells showed further evidence of *STXBP1* haploinsufficiency. The WT EGFP-STXBP1 was expressed in the cytosolic compartment, but not in the nucleus or plasma membrane, similar to endogenous expression.[9,23] However, in approximately 20% of cells expressing mutant EGFP-STXBP1 (p.V84D, p.C180Y, p.M443R, and p.G544D), intense clusters of fluorescence signals were observed, likely representing protein aggregation.[9] The other 80% of cells showed a diffuse cytosolic protein distribution similar to that expressing the WT, but the signal intensity was much weaker, implying possible protein degradation. Protein degradation of mutant STXBP1 proteins was also confirmed by immunoblotting using an anti-Munc18 antibody[19] (Fig. 64–3C). These experiments suggested that mutant STXBP1 proteins would be aggregated or degraded in neurons, both leading to loss of STXBP1 function.

Degradation of *STXBP1* mRNA with Abnormal Splicing

The splicing, frameshift, and nonsense mutations would produce a premature stop codon; therefore, these mutant mRNAs are likely to be degraded by NMD.[24,25] In fact, NMD associated with abnormal splicing was demonstrated in lymphoblastoid cells derived from a patient harboring a c.663+5G>A mutation. Polymerase chain reaction (PCR) primers designed to amplify exons 7 to 10 amplified a single band (338 bp), corresponding to the WT *STXBP1* allele, using a cDNA template from a control lymphoblastoid cell lines (LCL; Fig. 64–3D). By contrast, a smaller band was detected from the patient's cDNA, in which exon 8 of *STXBP1* was skipped (Fig. 64–3D), resulting in the insertion of nine new amino

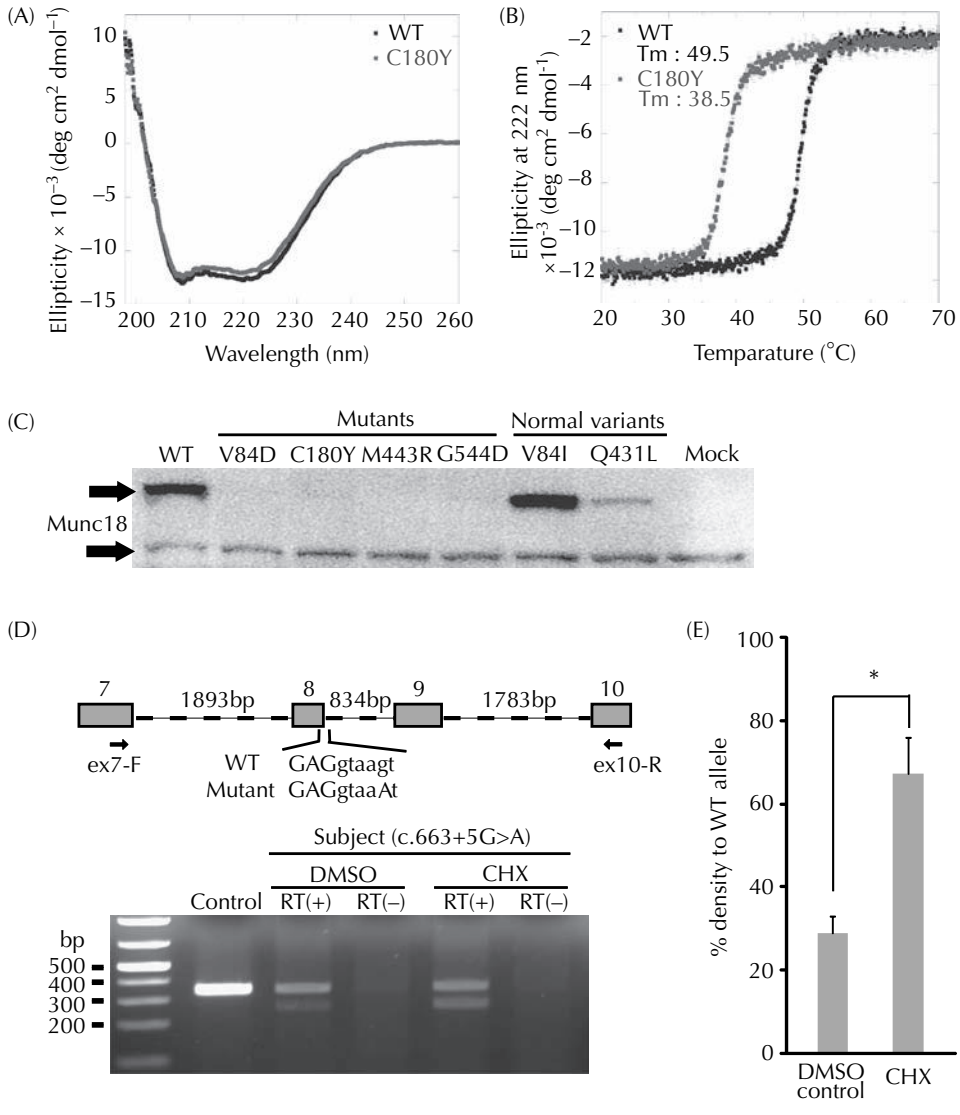

Figure 64–3. Characterization of mutant STXBP1 proteins and mRNAs with aberrant splicing. **A.** Circular dicroism spectra of the WT and C180Y-mutated STXBP1. The C180Y mutation is found to induce a subtle decrease in the helical contents of the STXBP1 structure by comparing the peaks of both proteins at 222 nm, where a large negative ellipticity value indicates a high helical content of the protein. **B.** Circular dicroic melting curves of the STXBP1 WT and C180Y proteins. Values of ellipticity at 222 nm are measured to monitor the thermal unfolding of the proteins. The C180Y mutant became unfolded at a lower temperature compared to the WT. Each dot represents the average of three repeated experiments, with standard deviations depicted as error bars. **C.** Immunoblot analysis of mutant STXBP1 proteins using a monoclonal anti-Munc18 antibody. Upper and lower bands represent EGFP-STXBP1 and endogenous STXBP1 proteins, respectively. Expression of four mutant STXBP1 proteins was not detected, while the WT and two normal variants could be detected. **D.** Top: Schematic representation of the genomic structure from exons 7 to 10 of *STXBP1*. Exons, introns, and primers are shown by boxes, dashed lines, and arrows, respectively. Sequences of exons and introns are presented in uppercase and lowercase, respectively. The mutation in intron 8 is highlighted in bold. Bottom: Reverse transcriptase-PCR analysis of patient 11 with a c.663+5G>A mutation and a normal control. Two PCR products were detected from the patient's cDNA: the upper one is the WT transcript and the lower one is the mutant. Only a single WT amplicon was detected in the control. The mutant amplicon was significantly increased by 30 μM cycloheximide (CHX) treatment compared to dimethyl sulfoxide (DMSO) treatment as a vehicle control. RT (+): with reverse transcriptase, RT (–): without reverse transcriptase as a negative control. **E.** Quantitative analysis of the NMD inhibition by CHX based on the data shown in **D**. **P* = 0.0023 by unpaired Student's *t*-test, two tailed. Averages of three repeated experiments are shown with error bars (s.d). Adapted from refs. 9 and 19.

acids followed by a premature stop codon at position 203. Moreover, the intensity ratio of the mutant compared to the normal band was increased by up to 67% after treatment with 30 μM cycloheximide, which inhibits NMD, compared to a ratio of 29% in the untreated condition (Fig. 64–3D,E). These facts suggest that the mutant mRNA possessing a premature stop codon suffered from degradation by NMD in neurons, resulting in haploinsufficiency.

Considering the degradation of STXBP1 proteins with missense mutations, NMD of mRNAs with premature stop codons and the effects of deletion of STXBP1, we conclude that haploinsufficiency of *STXBP1* causes OS.

HOW WOULD HAPLOINSUFFICIENCY OF *STXBP1* LEAD TO OS?

Impairment of Synaptic Vesicle Release

STXBP1 (*MUNC18-1*) is a member of the evolutionarily conserved Sec1/Munc-18 gene family that acts at specific steps of intracellular membrane transport.[26,27] In mammalian exocytosis, the vesicular SNARE protein, VAMP2 (also known as *synaptobrevin2*), and the target membrane SNARE proteins, Syntaxin-1 and SNAP25, constitute the core fusion machinery that bring two membranes into close apposition to fuse.[22,28] An *Stxbp1* null mutation led to complete loss of neurotransmitter secretion from synaptic vesicles throughout development in mice, though seizures have never been described.[16] Thus, *STXBP1* very likely plays a central role in synaptic vesicle release in coordination with SNARE proteins. The association of mutations of *STXBP1* with OS implies that perturbation of synaptic vesicle release forms part of the genetic basis of epilepsy. To date, the majority of genes associated with epilepsy syndromes are ion channel genes.[29] Synapsin I is a synaptic vesicle protein thought to regulate the kinetics of neurotransmitter release during priming of synaptic vesicles, and a mutation has been identified in a family with X-linked epilepsy and learning difficulties.[30] *STXBP1* is the second synaptic vesicle gene shown to be involved in epilepsy, and this finding will encourage further research into regulation of synaptic vesicle release and its involvement in seizures and related disorders.

Possible Interneuropathy

In *Stxbp1* heterozygous knockout mice, no seizures have been reported, and whole-cell recordings of autaptic glutamatergic or GABAergic (GABA: gamma-aminobutyric acid) neurons showed excitatory and inhibitory postsynaptic currents similar to those of WT littermate neurons upon single depolarizations.[31] However, with repeated stimulation, *Stxbp1*$^{+/-}$ neurons showed impaired synaptic function due to the reduced size and replenishment rate of readily releasable vesicles,[31] suggesting that heterozygous deletion of *Stxbp1* indeed affected synaptic function in mice. Interestingly, the reduction of readily releasable vesicles was more evident in GABAergic neurons than in glutamatergic neurons.[31] It has been reported that *Arx* is expressed in GABAergic interneurons and that *Arx* controls their genesis, migration, and differentiation, as *Arx* knockout mice showed a deficit of GABAergic interneurons.[32] Moreover, neuropathological examination of three patients with X-linked lissencephaly with absent corpus callosum and ambiguous genitalia, caused by *ARX* mutations, has suggested a loss of interneurons.[33] If haploinsufficiency of *STXBP1* affected GABAergic interneurons more severely than glutamatergic neurons in humans, as in mice, a defect in the GABAergic system could be postulated as a common pathophysiology among OS patients with *ARX* or *STXBP1* mutations. Ohtahara syndrome might be designated as a continuum of interneuronopathies.[34,35]

Cell Death of the Brainstem

As brain malformations are often associated with individuals with OS,[2,3] it could be speculated that *STXBP1* mutations would lead to abnormal brain structures directly related to the seizure phenotype of OS. However, we did not observe structural brain anomalies in any of the 14 OS patients with *STXBP1* defects. This is consistent with the findings that mice deleted for *Stxbp1* have normal brain architec-

ture. *Stxbp1* null mice, however, showed extensive cell death of mature neurons in lower brain areas, such as the brainstem; the lower brainstem was almost completely lost by embryonic day 18.[16] This is consistent with the suggestion that tonic seizures in OS might originate from subcortical structures, including the brainstem. Thus, in addition to the impaired synaptic vesicle release, it is possible that *STXBP1* haploinsufficiency leads to OS through microscopically impaired neuronal cell death in lower brain areas.

FUTURE CHALLENGES

Expansion of the Clinical Spectrum of *STXBP1* Mutations

Although OS is the core phenotype of *STXBP1* defects in our Japanese cohort (one-third of OS cases harbored *STXBP1* mutations), Hamdan et al. recently reported that two de novo *STXBP1* mutations, c.1162C>T (p.R388X) and c.169+1G>A, were identified in 2 out of 95 individuals with MR and nonsyndromic epilepsy.[36] According to their report, the two patients never showed the tonic seizures or infantile spasms associated with OS and WS, respectively. The onset of first seizures was at 6 weeks and 2 years of age, respectively. In addition, characteristic EEG patterns, such as suppression-burst or hypsarrhythmia, were never observed in these patients. Thus, the finding by Hamdan et al. indicated that *STXBP1* defects could cause a wide spectrum of clinical epileptic disorders in association with severe MR. Given that defects in synaptic dysfunction have also been implicated in many common neurodevelopmental disorders, such as MR, autism, and schizophrenia,[37,38] the possible involvement of *STXBP1* mutations in such common neurodevelopmental disorders is of interest. Elucidation of the molecular basis of synaptic vesicle processing disturbed by *STXBP1* mutations will allow us to understand not only the pathophysiology of infantile epilepsy, but also many neuropsychiatric conditions that present beyond childhood. The contribution of *STXBP1* mutations to EME also should be clarified, because myoclonic seizures, the characteristic feature of EME, are occasionally observed in 3/14 patients with *STXBP1* mutations.

Animal Model

An animal model is necessary to elucidate the pathophysiology of epilepsy caused by *STXBP1* mutations, including age dependency of seizure type and EEG pattern, and to test potential therapies directed specifically at OS and subsequent WS. The effect of gene dosage alterations of *STXBP1/Stxbp1* might vary between humans and mice: humans might be more susceptible than mice; thus, loss of function of one allele could cause seizures in humans but not in mice. Although it would be challenging to manipulate the gene dosage of *Stxbp1*—for example, in combination with a hypomorphic allele and a null allele—to the level at which mutant mice show a seizure phenotype, the establishment of an animal model will greatly benefit our understanding of the mechanisms of seizures in relation to impaired synaptic function.

DISCLOSURE STATEMENT

None of the authors have financial interests related to this work.

REFERENCES

1. Ohtahara S, Ishida T, Oka E, Yamatogi Y, Inoue H, Karita S, Ohtsuka Y. [On the specific age dependent epileptic syndrome: the early-infantile epileptic encephalopathy with suppression-burst.]. *No to Hattatsu*. 1976;8:270–279.
2. Djukic A, Lado FA, Shinnar S, Moshe SL. Are early myoclonic encephalopathy (EME) and the Ohtahara syndrome (EIEE) independent of each other? *Epilepsy Res*. 2006;70(suppl 1):S68–S76.
3. Ohtahara S, Yamatogi Y. Ohtahara syndrome: with special reference to its developmental aspects for differentiating from early myoclonic encephalopathy. *Epilepsy Res*. 2006;70(suppl 1):S58–S67.
4. Yamatogi Y, Ohtahara S. Early-infantile epileptic encephalopathy with suppression-bursts, Ohtahara syndrome; its overview referring to our 16 cases. *Brain Dev*. 2002;24:13–23.
5. Kato M, Saitoh S, Kamei A, Shiraishi H, Ueda Y, Akasaka M, Tohyama J, Akasaka N, Hayasaka K. A longer polyalanine expansion mutation in the ARX gene causes early infantile epileptic encephalopathy with suppression-burst pattern (Ohtahara syndrome). *Am J Hum Genet*. 2007;81:361–366.
6. Fullston T, Brueton L, Willis T, Philip S, MacPherson L, Finnis M, Gecz J, Morton J. Ohtahara syndrome in a family with an ARX protein truncation

mutation (c.81C>G/p.Y27X). *Eur J Hum Genet*. 2010;18:157–162.
7. Absoud M, Parr JR, Halliday D, Pretorius P, Zaiwalla Z, Jayawant S. A novel ARX phenotype: rapid neurodegeneration with Ohtahara syndrome and a dyskinetic movement disorder. *Dev Med Child Neurol*. 2009;3: 305–307.
8. Kato M, Koyama N, Ohta M, Miura K, Hayasaka K. Frameshift mutations of the ARX gene in familial Ohtahara syndrome. *Epilepsia*. 2010;51:1679–1684.
9. Saitsu H, Kato M, Mizuguchi T, Hamada K, Osaka H, Tohyama J, Uruno K, Kumada S, Nishiyama K, Nishimura A, Okada I, Yoshimura Y, Hirai S, Kumada T, Hayasaka K, Fukuda A, Ogata K, Matsumoto N. De novo mutations in the gene encoding STXBP1 (MUNC18–1) cause early infantile epileptic encephalopathy. *Nat Genet*. 2008;40:782–788.
10. Engel J Jr. Report of the ILAE classification core group. *Epilepsia*. 2006;47:1558–1568.
11. Molinari F, Raas-Rothschild A, Rio M, Fiermonte G, Encha-Razavi F, Palmieri L, Palmieri F, Ben-Neriah Z, Kadhom N, Vekemans M, Attie-Bitach T, Munnich A, Rustin P, Colleaux L. Impaired mitochondrial glutamate transport in autosomal recessive neonatal myoclonic epilepsy. *Am J Hum Genet*. 2005;76:334–339.
12. Molinari F, Kaminska A, Fiermonte G, Boddaert N, Raas-Rothschild A, Plouin P, Palmieri L, Brunelle F, Palmieri F, Dulac O, Munnich A, Colleaux L. Mutations in the mitochondrial glutamate carrier SLC25A22 in neonatal epileptic encephalopathy with suppression bursts. *Clin Genet*. 2009;76:188–194.
13. Kato M, Das S, Petras K, Sawaishi Y, Dobyns WB. Polyalanine expansion of ARX associated with cryptogenic West syndrome. *Neurology*. 2003;61:267–268.
14. Guerrini R, Moro F, Kato M, Barkovich AJ, Shiihara T, McShane MA, Hurst J, Loi M, Tohyama J, Norci V, Hayasaka K, Kang UJ, Das S, Dobyns WB. Expansion of the first PolyA tract of ARX causes infantile spasms and status dystonicus. *Neurology*. 2007;69:427–433.
15. Stromme P, Mangelsdorf ME, Shaw MA, Lower KM, Lewis SM, Bruyere H, Lutcherath V, Gedeon AK, Wallace RH, Scheffer IE, Turner G, Partington M, Frints SG, Fryns JP, Sutherland GR, Mulley JC, Gecz J. Mutations in the human ortholog of Aristaless cause X-linked mental retardation and epilepsy. *Nat Genet*. 2002;30:441–445.
16. Verhage M, Maia AS, Plomp JJ, Brussaard AB, Heeroma JH, Vermeer H, Toonen RF, Hammer RE, van den Berg TK, Missler M, Geuze HJ, Sudhof TC. Synaptic assembly of the brain in the absence of neurotransmitter secretion. *Science*. 2000;287:864–869.
17. Garcia EP, Gatti E, Butler M, Burton J, De Camilli P. A rat brain Sec1 homologue related to Rop and UNC18 interacts with syntaxin. *Proc Natl Acad Sci USA*. 1994;91:2003–2007.
18. Kalidas S, Santosh V, Shareef MM, Shankar SK, Christopher R, Shetty KT. Expression of p67 (Munc-18) in adult human brain and neuroectodermal tumors of human central nervous system. *Acta Neuropathol*. 2000;99:191–198.
19. Saitsu H, Kato M, Okada I, Kenji O, Higuchi T, Hoshino H, Kubota M, Arai H, Kimura S, Sudo A, Miyama S, Takami Y, Watanabe T, Nishimura A, Nishiyama K, Miyake N, Wada T, Osaka H, Kondo N, Hayasaka K, Matsumoto N. *STXBP1* mutations in early infantile epileptic encephalopathy with suppression-burst pattern. *Epilepsia*. 2010;51: 2397–2405.
20. Kanazawa K, Kumada S, Kato M, Saitsu H, Kurihara E, Matsumoto N. Choreo–ballistic movements in a case carrying a missense mutation in syntaxin binding protein 1 gene. *Mov Disord*. 2010;25:2265–2267.
21. Dulubova I, Khvotchev M, Liu S, Huryeva I, Sudhof TC, Rizo J. Munc18–1 binds directly to the neuronal SNARE complex. *Proc Natl Acad Sci USA*. 2007;104:2697–2702.
22. Toonen RF, Verhage M. Munc18–1 in secretion: lonely Munc joins SNARE team and takes control. *Trends Neurosci*. 2007;30:564–572.
23. Rickman C, Medine CN, Bergmann A, Duncan RR. Functionally and spatially distinct modes of munc18-syntaxin 1 interaction. *J Biol Chem*. 2007;282: 12097–12103.
24. Shyu AB, Wilkinson MF, van Hoof A. Messenger RNA regulation: to translate or to degrade. *EMBO J*. 2008;27:471–481.
25. Maquat LE, Kinniburgh AJ, Rachmilewitz EA, Ross J. Unstable beta-globin mRNA in mRNA-deficient β° thalassemia. Cell. 1981;27:543–553.
26. Sudhof TC. The synaptic vesicle cycle. *Annu Rev Neurosci*. 2004;27:509–547.
27. Weimer RM, Richmond JE. Synaptic vesicle docking: a putative role for the Munc18/Sec1 protein family. *Curr Top Dev Biol*. 2005;65:83–113.
28. Rizo J, Rosenmund C. Synaptic vesicle fusion. *Nat Struct Mol Biol*. 2008;15:665–674.
29. Gurnett CA, Hedera P. New ideas in epilepsy genetics: novel epilepsy genes, copy number alterations, and gene regulation. *Arch Neurol*. 2007;64:324–328.
30. Garcia CC, Blair HJ, Seager M, Coulthard A, Tennant S, Buddles M, Curtis A, Goodship JA. Identification of a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. *J Med Genet*. 2004;41: 183–186.
31. Toonen RF, Wierda K, Sons MS, de Wit H, Cornelisse LN, Brussaard A, Plomp JJ, Verhage M. Munc18–1 expression levels control synapse recovery by regulating readily releasable pool size. *Proc Natl Acad Sci USA*. 2006;103:18332–18337.
32. Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, Omichi K, Suzuki R, Kato-Fukui Y, Kamiirisa K, Matsuo M, Kamijo S, Kasahara M, Yoshioka H, Ogata T, Fukuda T, Kondo I, Kato M, Dobyns WB, Yokoyama M, Morohashi K. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet*. 2002;32: 359–369.
33. Bonneau D, Toutain A, Laquerriere A, Marret S, Saugier-Veber P, Barthez MA, Radi S, Biran-Mucignat V, Rodriguez D, Gelot A. X-linked lissencephaly with absent corpus callosum and ambiguous genitalia (XLAG): clinical, magnetic resonance imaging, and neuropathological findings. *Ann Neurol*. 2002;51:340–349.
34. Kato M. A new paradigm for West syndrome based on molecular and cell biology. *Epilepsy Res*. 2006; 70(suppl 1):S87–S95.
35. Kato M, Dobyns WB. X-linked lissencephaly with abnormal genitalia as a tangential migration disorder causing intractable epilepsy: proposal for

a new term, "interneuronopathy." *J Child Neurol*. 2005;20:392–397.
36. Hamdan FF, Piton A, Gauthier J, Lortie A, Dubeau F, Dobrzeniecka S, Spiegelman D, Noreau A, Pellerin S, Cote M, Henrion E, Fombonne E, Mottron L, Marineau C, Drapeau P, Lafreniere RG, Lacaille JC, Rouleau GA, Michaud JL. De novo STXBP1 mutations in mental retardation and nonsyndromic epilepsy. *Ann Neurol*. 2009;65:748–753.
37. Guilmatre A, Dubourg C, Mosca AL, Legallic S, Goldenberg A, Drouin-Garraud V, Layet V, Rosier A, Briault S, Bonnet-Brilhault F, Laumonnier F, Odent S, Le Vacon G, Joly-Helas G, David V, Bendavid C, Pinoit JM, Henry C, Impallomeni C, Germano E, Tortorella G, Di Rosa G, Barthelemy C, Andres C, Faivre L, Frebourg T, Saugier Veber P, Campion D. Recurrent rearrangements in synaptic and neurodevelopmental genes and shared biologic pathways in schizophrenia, autism, and mental retardation. *Arch Gen Psychiatry*. 2009;66:947–956.
38. Ramocki MB, Zoghbi HY. Failure of neuronal homeostasis results in common neuropsychiatric phenotypes. *Nature*. 2008;455:912–918.

Chapter 65

mTOR and Epileptogenesis in Developmental Brain Malformations

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INTRODUCTION

Malformations of cortical development (MCDs) are among the most common causes of epilepsy. While a wide variety of types and classifications of MCDs exists,[1] a subset of focal cortical malformations (FCMs), including tuberous sclerosis complex (TSC), focal cortical dysplasia, ganglioglioma, and hemimegalencephaly, is associated with an especially high incidence of epilepsy and other neurological deficits, such as cognitive dysfunction and autism.[2,3] Epilepsy related to these focal developmental brain malformations is often refractory to medical therapy. Even in patients whose seizures are well controlled with medications, currently available drugs are only symptomatic treatments that help suppress seizures; they have not been demonstrated to have antiepileptogenic or disease-modifying properties in preventing or altering the long-term prognosis of epilepsy. Although epilepsy surgery may eliminate seizures in some medically intractable cases, many patients are not good candidates for surgery or continue to have seizures despite surgical intervention. Thus, novel therapeutic strategies are needed to reduce the burden of seizures and other neurological symptoms caused by MCDs or, ideally, to prevent the development of epilepsy in the first place.

Tuberous sclerosis complex (TSC) is often viewed as a prototypical FCM associated with epilepsy, providing a detailed understanding of the clinical features, the pathological substrates, and now the molecular-genetic pathophysiology of this disease.[4,5] Up to 90% of patients with TSC have epilepsy, most of whom are refractory to seizure medications.[6] The cortical tuber is the pathological hallmark of TSC and is strongly correlated with the neurological manifestations including seizures, cognitive disability, and autism. Cortical tubers are characterized by a focal loss of normal cortical organization or lamination and the presence of a spectrum of abnormal dysmorphic or cytomegalic cell types. Although the mechanisms causing epilepsy in TSC are incompletely understood, a number of cellular and molecular abnormalities have been identified in both tuber and peri-tuber tissue from TSC patients that likely promote epileptogenesis and other neurological deficits in TSC, such as molecular

alterations in neurotransmitter receptors and ion channels, abnormalities in cellular growth and proliferation, and disrupted neuronal circuits (for review, see ref. 3). In addition to TSC, other FCMs, such as focal cortical dysplasia, gangliooglioma, and hemimegalencephaly, exhibit clinical, pathological, and molecular features very similar to those of TSC, which may indicate that common mechanisms of lesion formation and epileptogenesis are shared by these disorders.[2]

Recent exciting scientific discoveries have provided particularly revealing insights into the molecular pathophysiology of TSC and related FCM. In particular, abnormal signaling in the mammalian target of rapamycin (mTOR) pathway occurs in several of these focal brain malformations and may serve as a common trigger for epileptogenesis in these disorders. Furthermore, with the advent of drugs, such as rapamycin, that specifically inhibit the mTOR pathway, the use of mTOR inhibitors may represent a potent therapy for not only treating, but potentially also preventing, epilepsy in TSC and related FCMs. In this chapter, we will review the evidence, from both animal models and clinical studies, that a spectrum of FCM primarily represents disorders of mTOR signaling, or "mTORopathies",[2] that mediate epileptogenesis and other neurological deficits. Several recent comprehensive reviews have addressed the role of mTOR signaling in developmental brain disorders and in the generation of seizures.[7–9]

ANIMAL MODELS OF mTOROPATHIES: FROM YEAST TO MICE

The mammalian target of rapamycin proteins were first discovered in yeast in 1991, identified by genetic screening as the target of the potent immunosuppressant drug, rapamycin.[10] Subsequent studies have found highly conserved TOR analogs in other species, including *Caenorabdis elegans*, *Drosophila*, and mammals. mTOR and its analogs are relatively ubiquitous serine-threonine protein kinases and have been implicated in a large variety of cellular functions, including cellular growth, proliferation, survival/death, and metabolism.[11] On the biochemical level, mTOR integrates input from a number of upstream signaling pathways involved in controlling growth and metabolic requirements, such as the insulin-like growth factor/phosphatidylinositol-3-kinase (PI3K)/Akt and adenosine monophosphate (AMP)-kinase pathways (Fig. 65–1). In turn, two mTOR protein complexes, mTORC1 and mTORC2, modulate a number of downstream proteins, such as p70S6 kinase, ribosomal S6, and 4EBP1 that directly mediate protein synthesis and translation. Recent studies have identified many more mTORC1 substrates that significantly expand the potential cellular repertoire of mTOR signaling. In the brain, mTOR has been implicated in specific functions related to axonal and dendritic growth, synaptic plasticity, and other processes involved in normal brain development.

A potential link between the mTOR pathway and developmental brain malformations was first suggested by the discovery of the function of the genes causing TSC. Two genes, *TSC1* and *TSC2*, that are responsible for TSC and encode the proteins, TSC1 (hamartin) and TSC2 (tuberin), respectively. Importantly, TSC1 and TSC2 bind together and act as a complex to inhibit the mTOR signaling pathway, thus normally preventing excessive cellular growth and proliferation.[12] In human TSC, mutation of either the *TSC1* or *TSC2* gene leads to variable loss of TSC1 or TSC2 function and corresponding hyperactivation of the mTOR pathway. Dysregulated mTOR signaling can certainly explain many of the abnormal cellular and pathological features seen in TSC, such as cytomegalic cells and cellular proliferation, contributing to hamartoma or tumor generation. In addition, given mTOR's role in regulating synaptic plasticity and other fundamental neuronal properties, abnormal mTOR signaling may also contribute to mechanisms of epileptogenesis in TSC.[13]

The involvement of abnormal mTOR signaling in developmental brain anomalies related to epilepsy and other neurological deficits have been investigated in a number of animal models of TSC and related disorders. Several knockout mouse models of TSC, involving inactivation of the *Tsc1* or *Tsc2* gene in neurons, glia, or both, mimic many of the pathological features of TSC, such as neuronal hypertrophy, heterotopic neurons, abnormal myelination, and astrocytosis.[14–17] In addition, these models exhibit some of the cardinal neurological symptoms of TSC, including epilepsy and, in some instances, learning deficits. The role of mTOR

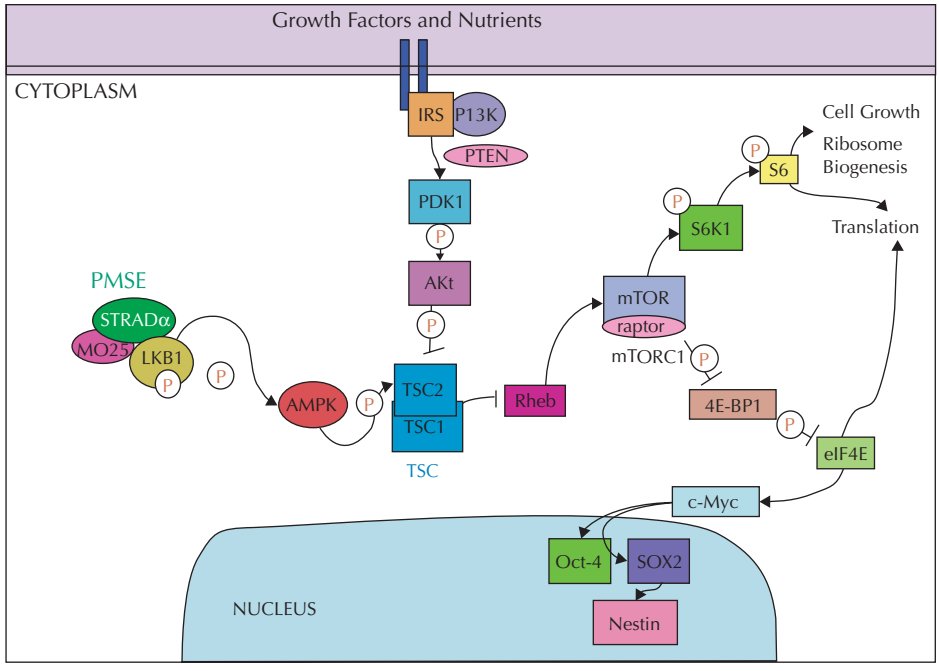

Figure 65–1. The mTOR pathway. Note the key regulatory points, including TSC1, TSC2, PTEN, and LKB1:STRAD. Each provides inhibitory control of mTOR signaling in response to nutrient, growth factor, or energy cues. AMPK, 5' adenosine monophosphate-activated protein kinase; eIF4E, elongation initiation factor 4E; IRS, insulin receptor substrate; mTOR, mammalian target of rapamycin; PDK, phosphoinositide-dependent kinase-1; PI3K, phosphoinositide-3 kinase; PMSE, polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome; PTEN, phosphatase and tensin homolog on chromosome 10; Rheb, Ras homolog enriched in brain; S6, ribosomal protein S6; S6K, ribosomal S6 kinase; TSC1, tuberous sclerosis complex 1 protein; TSC2, tuberous sclerosis complex 2 protein; 4E-BP1, elongation factor 4E binding protein 1.

in mediating most of the neuropathological and neurological symptoms in these TSC mouse models has been established by the reversal of these features by rapamycin.[17–19]

The specific role of the mTOR pathway in causing epileptogenesis has been studied most extensively in a mouse model with conditional inactivation of the *Tsc1* gene in cells expressing glial-fibrillary acidic protein (GFAP), which primarily constitute glia.[14] These *Tsc1*^{GFAP} conditional knockout (CKO) mice appear behaviorally normal for the first few weeks of life but then develop seizures at around 1 month of age, which become progressively more frequent over the following couple of months. Most mice die prematurely, sometimes in an episode of status epilepticus, by 3 months of age. The progressive epilepsy is directly correlated with pathological changes in the brains of *Tsc1*^{GFAP}CKO mice, including megalencephaly, astrogliosis, and neuronal disorganization, especially involving dispersion of the pyramidal cell layers in hippocampus.[14] In addition, molecular and functional changes in astrocytes, such as altered expression of glutamate transporter, potassium channels, and gap junctions, predispose to neuronal hyperexcitability and seizures in *Tsc1*^{GFAP}CKO mice.[20–22] Mammalian TOR signaling is abnormally increased in the brains of *Tsc1*^{GFAP}CKO mice and triggers most of these pathological and molecular changes, as they are mostly reversed by rapamycin.[18] Furthermore, rapamycin administration at an early age that precedes onset of epilepsy can completely prevent the development of seizures in *Tsc1*^{GFAP}CKO mice, indicating an antiepileptogenic effect of mTOR inhibition in this model[18] (Fig. 65–2). Rapamycin treatment after the onset of epilepsy also decreases seizures in already symptomatic *Tsc1*^{GFAP}CKO mice. Notably, however, after rapamycin is discontinued, seizures recur, indicating that continued inhibition of mTOR is necessary to maintain antiepileptogenic efficacy.

In addition to epilepsy, abnormal mTOR signaling has been implicated in causing learning deficits in animal models of TSC. Mice with a heterozygous mutation in the *Tsc2* gene exhibit

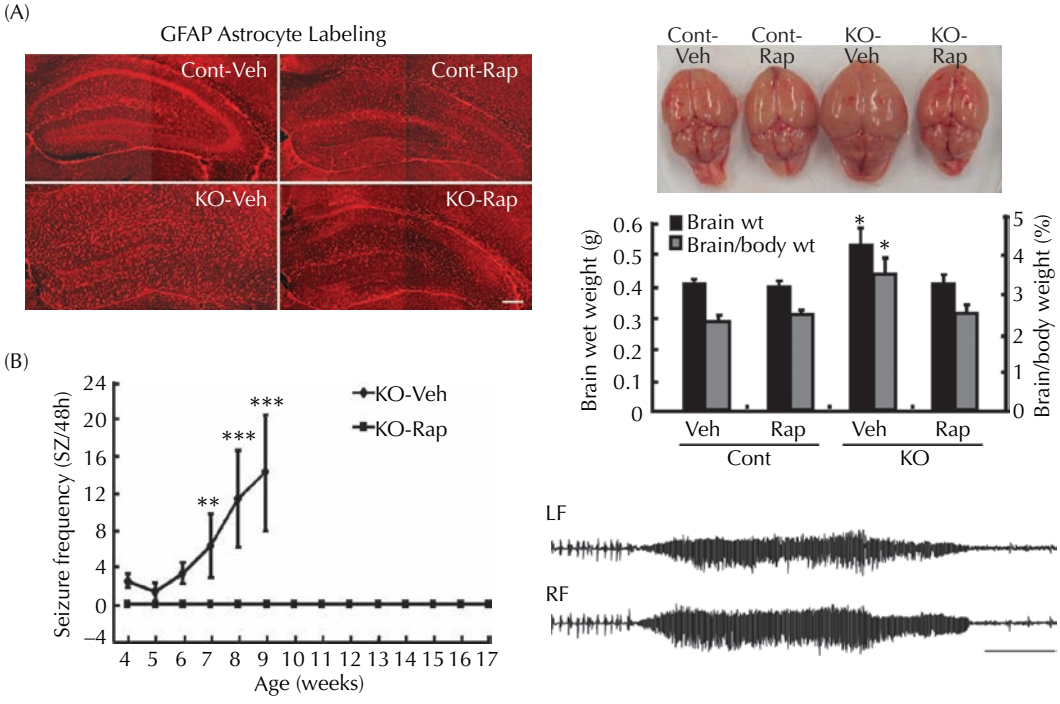

Figure 65–2. The neurological phenotype of a mouse model of TSC is dependent on mTOR and is reversed by mTOR inhibition. **A.** *Tsc1*^{GFAP}CKO mice, involving conditional inactivation of the *Tsc1* gene predominantly in glial cells, exhibit abnormal glial proliferation, as reflected by GFAP staining (left), and a corresponding megencephaly that results from glial proliferation (right). Rapamycin prevents the emergence of the glial proliferation and megencephaly in the *Tsc1*^{GFAP}CKO mice. **B.** *Tsc1*^{GFAP}CKO mice develop seizures (typical ictal EEG trace on the right) at around 4–5 weeks of age, which become progressively more frequent over the following month, with most mice dying prematurely by 2–3 months of age. Rapamycin treatment initiated at 2 weeks of age prior to the onset of seizures blocks the development of epilepsy in these mice. Cont-Veh, control mice treated with vehicle; Cont-Rap, control mice treated with rapamycin; KO-Veh, Tsc1GFAPCKO mice treated with vehicle; KO-rapamycin, Tsc1GFAPCKO mice treated with rapamycin. From ref. 15.

deficits in hippocampal learning, such as spatial learning tasks, in the absence of obvious neuropathological abnormalities or seizures.[17] Rapamycin reverses these learning deficits in these *Tsc2* mutant mice. Remarkably, the beneficial effects of rapamycin are observed after brief treatment of adult mice with already established learning deficits, suggesting that mTOR inhibition could be a potential utility for TSC patients with existing cognitive disabilities.

The role of abnormal mTOR signaling in causing neurological manifestations in animal models of TSC is well established, and the potential benefits of mTOR inhibitors for epilepsy and other neurological deficits are starting to be investigated in TSC patients.[23,24] Interestingly, similar principles and applications of mTOR involvement may be operational in other related developmental or genetic epilepsies. The *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) gene encodes the PTEN protein, which is an inhibitor of the PI3K/Akt pathway upstream from mTOR. Thus, *PTEN* mutations also lead to mTOR hyperactivation and produce several human hamartoma syndromes, including Cowden disease, Lhermitte-Duclos disease, and Proteus syndrome. Mice with *Pten* inactivation in the brain have a number of similarities to models of TSC and have been proposed to represent an animal model of cortical dysplasia.[25] As in the TSC mouse models, rapamycin decreases seizures and associated cellular and pathological abnormalities in the brains of these *Pten*-deficient mice.[25,26] In addition, rapamycin remarkably corrects social deficits in neuronal-specific *Pten* knockout mice suggestive of autistic features.[24] Thus, these findings suggest that mTOR dysregulation may be relevant to neurological manifestations in a variety of animal models of cortical malformations

and that mTOR inhibition could be a potential treatment for a spectrum of TORopathies causing epilepsy and learning deficits.

mTOR ACTIVATION IN HUMAN DEVELOPMENTAL BRAIN DISORDERS

Over the past 6 years, there has been mounting evidence that several distinct FCMs, including tubers in TSC, focal cortical dysplasia (FCD) type IIB (FCDIIB), hemimegalencephaly (HME), and ganglioglioma (GG) may be linked by aberrant activation of the mTOR cascade and constitute a spectrum of disorders known as m*TORopathies.*[2,28–32] The identification of enhanced mTOR cascade activation in human FCM has provided new insight into the pathogenesis of these brain disorders that are variably linked with intractable epilepsy, cognitive disability, and autism. Recently, a new disorder known as polyhydramnios, megalencephaly, symptomatic epilepsy (PMSE) syndrome was identified among the Old Order Mennonite population in Lancaster, Pennsylvania, that results from mutations in the *STRADA* gene[33] and that is characterized by severe epilepsy, profound cognitive disability, and FCM. Interestingly, *STRADA* encodes the novel mTOR regulatory protein, STRADα, and in fact, there is robust evidence of mTOR activation in postmortem brain tissue from PMSE patients.[33,34] Mutations in *PTEN*, which negatively modulates insulin growth factor (IGF), PI3K, PDK, and mTOR signaling, are found in a subset of patients with macrocephaly and autism.[35] Interestingly, the profile of mTOR activation also can be used to distinguish type II FCD from type I FCD, suggesting that mTOR hyperactivation is specific to some but not all FCMs[36] (Fig. 65–3). Thus, mTORopathies encompass a wide range of both rare and common FCMs associated with intractable epilepsy, cognitive disability, and autism that are characterized by abnormal brain architecture. These findings make the compelling case that mTOR inhibition could provide a plausible therapeutic approach to the treatment of neurological features of mTORopathies.

The first studies to demonstrate that the mTOR pathway was activated in FCM assayed the phosphorylation profiles of several downstream mTOR effectors, such as ribosomal S6, S6Kinase, and 4E-BP1 proteins in cortical tubers

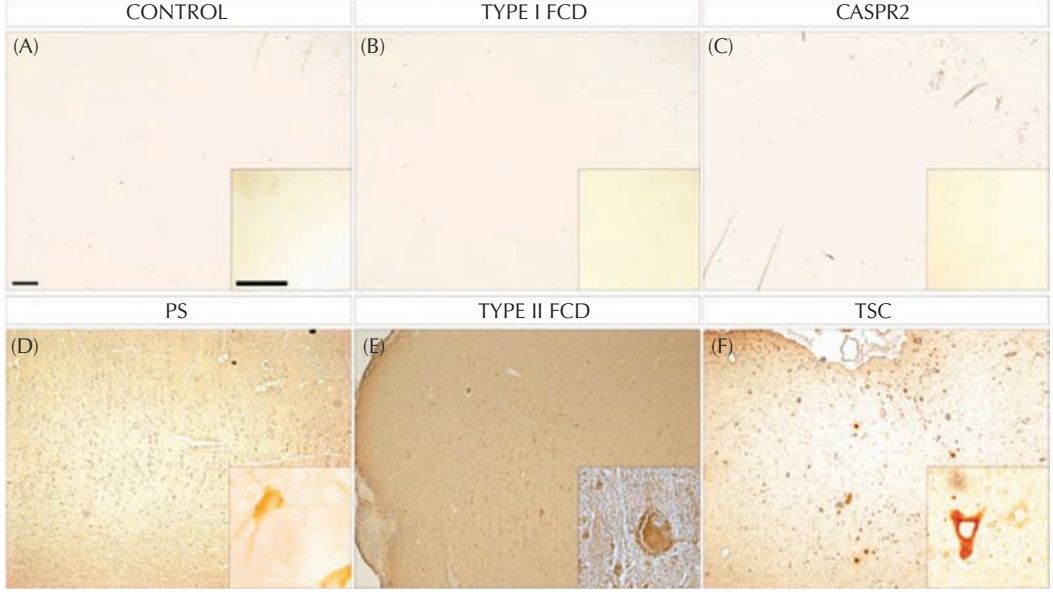

Figure 65–3. A–F. The phosphorylation profile of ribosomal protein S6 distinguishes control tissue and type I FCD from type II FCDs. The CASPR2 mutation is associated with type I FCD. PS, PMSE syndrome. Note the very low levels of phospho-S6 in controls and type I FCD but enhanced levels in type II FCDs.

and FCDs.[28,29] Because the cellular composition of tubers and FCDIIB is similar (e.g., cortical laminar disorganization, cytomegalic cells, and gliosis), it was postulated that there would be a similar activation profile of the mTOR cascade in both malformations. These reports showed that in fact there was cell-specific activation of mTOR in tubers and FCDIIB, evidenced by phosphorylation of ribosomal S6 protein (Ser 235/236) that was most robust in cytomegalic cell types such as balloon cells and large, dysmorphic neurons. These observations were replicated and extended by several other labs.[30] From a pathophysiological perspective, these findings were logical since central known biological roles of mTOR and its downstream target proteins include regulation of cell polarity, establishment and maintenance of cell size, and modulation of neuronal migration. In fact, loss of mTOR inhibition, that is, constitutive mTOR *activation,* leads to disproportionate enhancement of somatic and nuclear size in vitro in cells lacking, for example, Tsc1, Tsc2, or Pten. Subsequent studies demonstrated aberrant phosphorylation of S6K and S6 in balloon cells in HME[28] and atypical ganglion cells found in GG[32] and suggested that the shared pathological characteristics of tubers, FCDIIB, HME, and GG were linked to a core abnormality in mTOR signaling. These findings were relevant because for the first time, a possible pathogenic mechanism for FCDIIB, HME, and GG, sporadic malformations with no known cause, could be reasonably postulated. These data, plus work from preclinical models discussed previously, suggested that hyperactivation of mTOR signaling leads to a marked increase in cell size and invited the compelling and exciting possibility that mTOR inhibition could alter the cellular and clinical phenotypic features of TSC, FCDIIB, HME, and GG.

The evolving role of aberrant mTOR signaling in developmental brain diseases was further supported by the identification of mutations in *STRADA* in PMSE, a devastating autosomal recessive neurodevelopmental disorder characterized by FCM. Analysis of PMSE brain tissue revealed numerous heterotopic cells in the subcortical white matter, as well as cytomegalic cells akin to balloon cells in FCDIIB. Patients with PMSE exhibit macrocephaly, intractable epilepsy, and severe cognitive disability. The causative gene mutation results in loss of function in the STRADα protein, which comprises the heteromeric complex including the kinase LKB1 and its other binding partner, MO25 (see ref. 34 for review). STRADα is a pseudokinase that binds to and activates LKB1 by autophosphorylation. In addition, while LKB1 is mainly found in the nucleus, coexpression of STRADα with LKB1 results in relocalization of both binding partners to the cytoplasm. Cytoplasmic localization of LKB1 has been previously shown to be necessary for LKB1 function—for example, kinase activity toward its downstream targets, such as AMP kinase (AMPK), which becomes activated upon LKB1-mediated phosphorylation of its regulatory activation loop (Thr172). Phosphorylation of TSC2 (Thr1227 and Ser1345) by AMPK leads to direct activation of the heterodimeric TSC2:TSC1 complex. Thus, loss of STRADα function in PMSE leads to loss of AMPK-mediated activation of TSC2 and results in mTOR activation.

The detection of enhanced mTOR pathway signaling provided a rational mechanistic explanation for many of the histopathological similarities between these lesions, including enhanced cell size, cell dysmorphism, and altered cortical lamination. Enhanced cell size may result in part from S6K-mediated phosphorylation of ribosomal protein S6, a component of the 40S ribosomal subunit, and enhanced protein translation, although the precise mechanism leading to enhanced neuronal cell growth is unknown. There is ample evidence in neural and nonneural cell types that altered mTOR signaling leads to changes in actin cytoskeletal arrangement and activation of important structural proteins, such as Rac1 and cdc42, that could lead to changes in cell size and altered cell polarity.[38] It is unclear at what developmental epoch these changes take effect, but in view of the focal nature of FCDIIB, mTOR activation must occur fairly late in cortical development in a restricted number of cells, whereas because HME is an expansive hemispheric malformation, it could be postulated that the activation of mTOR occurs very early in brain development.

A central issue that relates to the pathogenesis of FCM is defining the phenotype of the cells in each lesion subtype. For example, balloon cells in FCDIIB express both glial (glial fibrillary acidic protein [GFAP]) and neuronal (TuJI, NeuN) protein markers, either independently or in combination, suggesting either a mixed lineage, or disordered lineage specification, or

aberrant protein expression.[39] Perhaps more challenging is that in tubers, HME, FCDIIB, and GG, there is robust expression of proteins such as nestin, vimentin, CD133, and Mcm2 that are characteristically expressed in immature cells or even neural stem cells.[40–45] Indeed, proteins such as CD133 and Mcm2 are normally found in stem cells during G1 phase, thus raising the fascinating possibility that balloon cells may be a type of neural stem cell. Recent evidence suggests that balloon cells can be cultured in vitro[36,46] and demonstrates that there is persistent activation of mTOR complex 1 (mTORC1) in vitro. A recent study has shown that proteins that are typically expressed in neural progenitor cells may be linked to mTOR1, such as c-Myc, sex-determining region Y-box 2 (SOX2), and Octamer-4 (Oct-4).[36] SOX2 regulates nestin expression by binding to its enhancer domain, and sustained Oct-4 expression promotes differentiation of nestin-positive neural precursors. Interestingly, differential expression of several stem cell marker proteins such as SOX2, Oct-4, c-Myc, the winged-helix transcription factor forkhead box G1 (FOXG1), Kruppel-like factor 4 (KLF4), Nanog, and sex-determining region Y-box 3 (SOX3) can distinguish between type II and type I FCDs.[36] These findings demonstrate that enhanced mTOR signaling in FCM may explain the profile of protein expression and phenotypic lineage in these cells.

Because single-gene defects associated with TSC and PMSE are linked to aberrant mTOR signaling, the mTOR pathway also provided a rational pathway to examine for candidate mutations in HME, GG, and FCDIIB, which typically occur sporadically without any known familial link or heritability. The prevailing view is that sporadic FCDIIB and HME result from a somatic mutational event that occurs during corticogenesis. A deletion in 15q11–13 has been reported in a single case of sporadic HME and syndromic HME has been reported in association with mutations in *PTEN*,[47] and mutations in LKB1 or B-RAF have been reported in GG.[48] However, the myriad possible genes that could account for these malformations makes identification of a causative mutation a daunting task. In addition, the detection of small or single base pair mutations that are distinct from single nucleotide polymorphisms may be challenging. The phosphorylation profile of the downstream mTOR effectors S6K and S6 provides evidence that the activation of mTOR signaling is enhanced in FCM. However, the mechanism leading to a cortical malformation is unknown e.g., mutation in enhancer or negative regulatory element, post-translational modification, epigenetic silencing, or external/environmental event. To address this question, Schicke and colleagues performed a valuable comparison between FCDII and TSC by examining activated phosphoproteins upstream in the mTOR cascade to determine where activation might be first initiated.[49] These investigators found that the cascade activation was initiated at TSC1:TSC2 in TSC, whereas there was an upstream signaling divergence point at PDK1 that was phosphorylated in FCD but not in TSC. These data suggest that while mTOR activation is initiated by direct loss of inhibitory mTOR control by TSC1:TSC2 in TSC, cascade activation may occur from a more upstream or parallel entry point in FCDIIB. This is currently unknown for GG or HME, although B-RAF mutations may lead to enhanced mTOR signaling. Thus, since HME, GG, and FCDIIB are histopathologically similar to TSC, and since there is a similar profile of mTOR activation in all four lesion types, it seems plausible that candidate gene mutations could be found within the mTOR pathway, such as a loss-of-function mutation in an mTOR inhibitor or a gain-of-function mutation in an mTOR activator. Thus far, however, attempts to screen for mutations in mTORC1 regulatory elements such as *TSC1*, *TSC2*, and *PTEN* have not revealed mutations in DNA extracted from these malformations. One complicating factor is that the heterogeneous pathology of each lesion may suggest that the somatic mutations are confined to particular cell types and, thus, single-cell genetic analysis may be warranted.

A pivotal question is how mTOR activation in FCM can lead to seizures and intractable epilepsy. As discussed above, altered expression of glutamate transporter, potassium channels, and gap junctions in astrocytes in the *Tsc1*^{GFAP}CKO mice are associated with neuronal hyperexcitability and seizures, although the precise mechanisms are unknown. In addition, there is clear evidence that in animal knockout strains, rapamycin can prevent the development of recurrent seizures, although whether this occurs by a reversal of histopathological features or by pharmacological changes in synaptic activity, neurotransmitter release or uptake, or

neuromodulation is not yet known. Analysis of these mechanisms in resected human tissue is challenging, though not impossible, and much work still is needed. Current data suggest that dysmorphic neurons may be important culprits in seizure generation in FCD, whereas balloon cells may be electrically silent.[50] There is clear evidence that expression of excitatory amino acid and $GABA_A$ receptor subunits is altered in TSC, FCD, HME, and GG, but how these changes culminate in recurrent and often intractable seizures is unknown.[51,52] From a molecular perspective, while much has been learned over the past 5 years about upstream control of mTOR activation, there still remains much to understand about the downstream effects of mTOR in neurons and a putative role in hyperexcitability. For example, mTOR governs cellular protein translation via its role in regulation of ribosomal protein synthesis and contributes to the transcription of a variety of genes via modulation of several transcription factors, including c-myc and STAT3. Each of these proteins regulates, in turn, numerous target proteins that are part of the overall mTOR activation response. Thus, it is likely that one or more of these downstream mTOR effectors play an important role in the establishment of cells size or epileptogenesis. Further investigation to define how downstream mTOR effectors can be targeted therapeutically could identify new compounds that, for example, are more effective or less toxic than rapamycin or its analogs.

SUMMARY AND FUTURE COURSE

Studies of both animal models and human brain specimens strongly implicate abnormal mTOR signaling in the pathophysiology of FCM. From a therapeutic standpoint, these findings suggest the exciting possibility that mTOR inhibitors may represent potent and specific treatments for the neurological manifestations of these disorders, including epilepsy. However, despite significant preclinical data, there is currently only anecdotal evidence that rapamycin is efficacious for seizures in human patients with TSC or other TORopathies. In one case report, a 10-year-old girl with TSC experienced a reduction in her seizures with systemic rapamycin therapy,[24] and in clinical trials of rapamycin for astrocytoma and renal tumor growth, some TSC patients have been reported to have decreased seizure frequency as a secondary finding[23]; however, no controlled clinical studies exist testing the benefit of rapamycin for epilepsy in TSC. Furthermore, there have been no reports of early rapamycin treatment preventing or slowing the development or progression of seizures in high-risk, presymptomatic TSC patients before the onset of epilepsy. If rapamycin is shown to be beneficial for seizures, either as antiepileptogenic or symptomatic therapy, several important clinical questions will need to be addressed. First, should rapamycin be used for TSC patients with neurological symptoms only or prophylactically in all TSC patients? The preclinical data suggest that appropriately timed rapamycin therapy could prevent seizures altogether, that is, serve as an antiepileptogenic modification. A particularly tantalizing hope is that rapamycin might yield a highly targeted therapy for infantile spasms in TSC. Thus, should rapamycin be given to TSC infants prophylactically? Once started, how long should rapamycin treatment be continued? Published clinical studies suggest that the benefits of rapapmycin in TSC may be limited to the duration of therapy; thus, one concern is seizure recurrence after cessation of mTOR inhibition. If rapamycin therapy is to be lifelong, then the long-term side effects, such as aphthous oral ulcers, hyperlipidemia, and an enhanced risk of infection, must be anticipated and effectively managed. Particular caution may be warranted with the use of rapamycin in children, given the potential role of mTOR in synaptic plasticity and learning.

There are other critical issues that need to be addressed regarding mTOR cascade activation in TORopathies. First, to date, there have been no reliable biomarker studies to determine whether mTOR pathway activation is a systemic or localized phenomenon in, for example, TSC and FCD. For example, previous studies in the renal transplant literature have demonstrated that changes in the phosphorylation status of p70S6 kinase is a better predictor of rapamycin effect in preventing rejection than a serum rapamycin level.[53] The detection of serum biomarkers for TSC or other TORopathies might provide predictive, prognostic, or diagnostic assays that could be related to seizure onset, seizures recurrence, infantile spasms, or autism. In fact, these markers could result

in improved disease surveillance or treatment protocols. Second, there has been little investigation of how mTOR signaling may differ or vary across individual TSC or FCD patients. One such scenario might be that greater disease severity correlates with increased levels of systemic or organ-specific mTOR activation. Finally, rapamycin therapy could lead to a paradoxical enhancement of mTOR signaling via feedback interactions with Akt and other upstream modulators.[54] Thus, new strategies to modify mTOR signaling may need to be considered as clinical data are analyzed.

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The authors have no conflicts to disclose.

REFERENCES

1. Barkovich AJ, Kuzniecky RI, Jackson GD, Guerrini R, Dobyns WB. A developmental and genetic classification for malformations of cortical development. *Neurology*. 2005;65:1873–1887.
2. Crino PB. Focal brain malformations: a spectrum of disorders along the mTOR cascade. *Novartis Found Symp*. 2007;288:260–272.
3. Wong M. Mechanisms of epileptogenesis in tuberous sclerosis complex and related malformations of cortical development with abnormal glioneuronal proliferation. *Epilepsia.* 2008;49:8–21.
4. Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. *N Engl J Med.* 2006;355: 1345–1356.
5. Holmes GL, Stafstrom CE, and the Tuberous Sclerosis Study Group. Tuberous sclerosis complex and epilepsy: recent developments and future challenges. *Epilepsia.* 2007;48:617–630.
6. Chu-Shore CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia*. 2010;1(7):1236–41.
7. Crino PB. mTOR: A pathogenic signaling pathway in developmental brain malformations. *Trends Mol Med*. 2011, September 2. [Epub ahead of print.]
8. Wong M. Mammalian target of rapamycin (mTOR) inhibition as a potential antiepileptogenic therapy: From tuberous sclerosis to common acquired epilepsies. *Epilepsia.* 2010;51(1):27–36.
9. Ehninger D, Silva AJ. Rapamycin for treating tuberous sclerosis and autism spectrum disorders. *Trends Mol Med*. 2011;17(2):78–87.
10. Heitman J, Mowa NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*. 1991;253:905–909.
11. Sandsmark DK, Pelletier C, Weber JD, Gutmann DH. Mammalian target of rapamycin: master regulator of cell growth in the nervous system. *Histol Histopathol*. 2007;22:895–903.
12. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J*. 2008;412:179–190.
13. Wong M. Mammalian target of rapamycin (mTOR) inhibition as potential antiepileptogenic therapy: from tuberous sclerosis to common acquired epilepsies. *Epilepsia.* 2010;51:27–36.
14. Uhlmann EJ, Wong M, Baldwin RL, Bajenaru ML, Onda H, Kwiatkowski DJ, Yamada KA, Gutmann DH. Astrocyte-specific TSC1 conditional knockout mice exhibit abnormal neuronal organization and seizures. *Ann Neurol*. 2002;52:285–296.
15. Meikle L, Talos DM, Onda H, Pollizzi K, Rotenberg A, Sahin M, Jensen FE, Kwiatkowski DJ. A mouse model of tuberous sclerosis: neuronal loss of Tsc1 causes dysplastic and ectopic neurons, reduced myelination, seizure activity, and limited survival. *J Neurosci*. 2007;27:5546–5558.
16. Way SW, McKenna J 3rd, Mietzsch U, Reith RM, Wu HC, Gambello MJ. Loss of Tsc2 in radial glia models the brain pathology of tuberous sclerosis complex in the mouse. *Hum Mol Genet*. 2009;18:1252–1265.
17. Ehninger D, Han S, Shiyansky C, Zhou Y, Li W, Kwaitkowski DJ, Ramesh V, Silva AJ. Reversal of learning deficits in a Tsc2+/- mouse model of tuberous sclerosis. *Nat Med.* 2008;14:843–848.
18. Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol*. 2008;63:444–453.
19. Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, Kwiatkowski DJ. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci.* 2008;28:5422–5432.
20. Wong M, Ess KE, Uhlmann EJ, Jansen LA, Li W, Crino PB, Mennerick S, Yamada KA, Gutmann DH. Impaired astrocyte glutamate transport in a mouse epilepsy model of tuberous sclerosis complex. *Ann Neurol*. 2003;54:251–256.
21. Jansen LA, Uhlmann EJ, Crino PB, Gutmann DH, Wong M. Epileptogenesis and reduced inward rectifier potassium current in tuberous sclerosis complex-1 deficient astrocytes. *Epilepsia*. 2005;46: 1871–1880.
22. Xu L, Zeng LH, Wong M. Impaired astrocyte gap junction coupling and potassium buffering in a mouse model of tuberous sclerosis complex. *Neurobiol Dis.* 2009;34:291–299.
23. Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, Wilson KA, Byars A, Sahmoud T, Franz DN. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med*. 2010;363:1801–1811.

24. Muncy J, Butler IJ, Koenig MK. Rapamycin reduces seizure frequency in tuberous sclerosis complex. *J Child Neurol*. 2009;24:477.
25. Ljungberg MC, Sunnen CN, Lugo JN, Anderson AE, D'Arcangelo G. Rapamycin suppresses seizures and neuronal hypertrophy in a mouse model of cortical dysplasia. *Dis Model Mech*. 2009;2:389–398.
26. Kwon CH, Zhu X, Zhang J, Baker SJ. mTOR is required for hypertrophy of Pten-deficient neuronal soma in vivo. *Proc Natl Acad Sci USA*. 2003;100:12923–12928.
27. Zhou J, Blundell J, Ogawa S, Kwon CH, Zhang W, Sinton C, Powell CM, Parada LF. Pharmacological inhibition of mTORC1 suppresses anatomical, cellular, and behavioral abnormalities in neural-specific Pten knock-out mice. *J Neurosci.* 2009;29:1773–1783.
28. Baybis M, Yu J, Lee A, Golden JA, Weiner H, McKhann II G, Aronica, E, Crino PB. Activation of the mTOR cascade distinguishes cortical tubers from focal cortical dysplasia. *Ann Neurol.* 2004;56:478–487.
29. Miyata H, Chiang AC, Vinters HV. Insulin signaling pathways in cortical dysplasia and TSC-tubers: tissue microarray analysis. *Ann Neurol.* 2004;56:510–519.
30. Ljungberg MC, Bhattacharjee MB, Lu Y, Armstrong DL, Yoshor D, Swann JW, Sheldon M, D'Arcangelo G. Activation of mammalian target of rapamycin in cytomegalic neurons of human cortical dysplasia. *Ann Neurol*. 2006;60:420–429.
31. Aronica E, Boer K, Baybis M, Yu J, Crino P. Co-expression of cyclin D1 and phosphorylated ribosomal S6 proteins in hemimegalencephaly. *Acta Neuropathol* (*Berl*). 114:287–293.
32. Samadani U, Judkins A, Akpalu A, Aronica A, Crino PB. Differential gene expression in neurons and astrocytes in ganglioglioma. *Epilepsia*. 2007;48:646–653
33. Puffenberger E, Strauss KA, Ramsey KE, Craig DW, Stephan DA, Robinson DL, Hendrickson CL, Ramsay DA, Siu V, Heuer GG, Crino PB, Morton DH. Syndromic cortical dysplasia caused by a homozygous 7 kilobase deletion in LYK5. *Brain*. 2007;130: 1929–1941.
34. Orlova KA, Parker WE, Heuer GG, Tsai V, Yoon J, Baybis M, Fenning RS, Strauss K, Crino PB. STRADa deficiency results in aberrant mTORC1 signaling during corticogenesis. *J Clin Invest*. 2010;120:1591–1602.
35. Butler MG, Dasouki MJ, Zhou XP, Talebizadeh Z, Brown M, Takahashi TN, Miles JH, Wang CH, Stratton R, Pilarski R, Eng C. Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. *J Med Genet*. 2005;42:318–321.
36. Orlova KA, Tsai V, Baybis M, Heuer GG, Sisodiya S, Thom M, Strauss K, Aronica E, Storm PB, Crino PB. Early progenitor cell marker expression distinguishes type II from type I focal cortical dysplasias. *J Neuropathol Exp Neurol.* 2010;69(8):850–863.
37. Orlova KA, Crino PB. The tuberous sclerosis complex. *Ann NY Acad Sci.* 2010;1184:87–105.
38. Astrinidis A, Cash TP, Hunter DS, Walker CL, Chernoff J, Henske EP. Tuberin, the tuberous sclerosis complex 2 tumor suppressor gene product, regulates Rho activation, cell adhesion and migration. *Oncogene*. 2002;21:8470–8476.
39. Mizoguchi M, Iwaki T, Morioka T, Fukui M, Tateishi J. Abnormal cytoarchitecture of cortical dysplasia verified by immunohistochemistry. *J Clin Neuropathol*. 1998;17:100–109.
40. Taylor JP, Sater R, French J, Baltuch G, Crino PB. Transcription of intermediate filament genes is enhanced in focal cortical dysplasia. *Acta Neuropathol*. 2001;102:141–148.
41. Ying Z, Gonzalez-Martinez J, Tilelli C, Bingman W, Najm I. Expression of neural stem cell surface marker CD133 in balloon cells of human focal cortical dysplasia. *Epilepsia*. 2005;46:1716–1723.
42. Crino PB, Trojanowski JQ, Eberwine J. Internexin, MAP1B, and nestin in cortical dysplasia as markers of developmental maturity. *Acta Neuropathol*. 1997;93: 619–627.
43. Thom M, Martinian L, Sisodiya SM, Cross JH, Williams G, Stoeber K, Harkness W, Harding BN. Mcm2 labelling of balloon cells in focal cortical dysplasia. *Neuropathol Appl Neurobiol*. 2005;31: 580–588.
44. Mizuguchi M, Yamanouchi H, Becker LE, Itoh M, Takashima S. Doublecortin immunoreactivity in giant cells of tuberous sclerosis and focal cortical dysplasia. *Acta Neuropathol*. 2002;104:418–424.
45. Lamparello P, Baybis M, Pollard J, Hol E, Eisenstat D, Aronica E, Crino PB. Developmental lineage of cell types in cortical dysplasia with balloon cells. *Brain*. 2007;130:2267–2276.
46. Yasin SA, Latak K, Becherini F, Ganapathi A, Miller K, Campos O, Picker SR, Bier N, Smith M, Thom M, Anderson G, Helen Cross J, Harkness W, Harding B, Jacques TS. Balloon cells in human cortical dysplasia and tuberous sclerosis: isolation of a pathological progenitor-like cell. *Acta Neuropathol*. 2010;120: 85–96.
47. Baybis M, Aronica E, Nathanson KL, Crino PB. Deletion of 15q11.2–15q13.1 in isolated human hemimegalencephaly. *Acta Neuropathol*. 2009;118: 821–823.
48. Resta N, Lauriola L, Puca A, Susca FC, Albanese A, Sabatino G, Di Giacomo MC, Gessi M, Guanti G. Ganglioglioma arising in a Peutz-Jeghers patient: a case report with molecular implications. *Acta Neuropa*thol. 2006;112:106–111.
49. Schicke V, Majores M, Engels G, Hartmann W, Elger CE, Schramm J, Schoch S, Becker AJ. Differential Pi3K-pathway activation in cortical tubers and focal cortical dysplasias with balloon cells. *Brain Pathol*. 2007;17:165–173.
50. Cepeda C, André VM, Vinters HV, Levine MS, Mathern GW. Are cytomegalic neurons and balloon cells generators of epileptic activity in pediatric cortical dysplasia? *Epilepsia.* 2005;46(suppl 5):82–88.
51. White R, Hua Y, Lynch DR, Scheitauer B, Crino PB. Differential transcription of neurotransmitter receptor subunits and uptake sites in giant cells and dysplastic neurons in cortical tubers. *Ann Neurol*. 2001;49:67–78.
52. Talos DM, Kwiatkowski DJ, Cordero K, Black PM, Jensen FE. Cell-specific alterations of glutamate receptor expression in tuberous sclerosis complex cortical tubers. *Ann Neurol*. 2008;63:454–465.
53. Hartmann B, Schmid G, Graeb C, Bruns CJ, Fischereder M, Jauch KW, Heeschen C, Guba M. Biochemical monitoring of mTOR inhibitor-based immunosuppression following kidney transplantation: a novel approach for tailored immunosuppressive therapy. *Kidney Int*. 2005;68:2593–2598.
54. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard s2008;412:179–190.

Chapter 66

Major Susceptibility Genes for Common Idiopathic Epilepsies

ELP4 in Rolandic Epilepsy and *BRD2* in Juvenile Myoclonic Epilepsy

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INTRODUCTION

Clinical observations confirm that the idiopathic epilepsies are neurodevelopmental disorders strongly influenced by genetic factors. Genetic epidemiological studies prove major genetic influences on two common forms of idiopathic epilepsy to be discussed here: rolandic epilepsy (RE) and juvenile myoclonic epilepsy (JME). The common forms of these idiopathic syndromes have a complex genetic inheritance, and this fact complicates the task of finding and elucidating the susceptibility genes as well as proving their pathogenic role. Genetic heterogeneity and phenotype definition are much more serious factors in studying common complex epilepsies than they are in the study of densely affected epilepsy pedigrees showing Mendelian inheritance. In complex disorders, several genes and sometimes environmental factors are believed to contribute to disease etiology; as a result, proving a causative role for any *one* gene, in a genetic model involving multiple genes, can present a challenge.

In this chapter, we first review the methodological issues associated with analyzing complex genetic disorders and then explain the

strategy that we have used in investigating RE and JME. Genome-wide linkage analyses and fine mapping have resulted in the identification of susceptibility genes *ELP4* in RE and *BRD2* in JME. We discuss the evidence in favor of a pathogenic role for each gene and the further steps that are required to establish causality.

THE IDIOPATHIC EPILEPSIES AS NEURODEVELOPMENTAL DISORDERS

Determining the etiology of the common idiopathic epilepsies is a difficult challenge. Although there have been several discoveries of genes involved in rare Mendelian variants of idiopathic epilepsies, for example in autosomal dominant juvenile myoclonic epilepsy[1] and benign familial infantile convulsions (BFIC),[2] mutations in these genes have not been demonstrated in the common forms of epilepsy that show complex, rather than simple Mendelian, inheritance. Further, the phenomenon of heterogeneity, in which similar phenotypes have different genetic etiologies, means that data thought to reflect a single disease may actually refer to diseases of different etiologies. In contrast to the rare Mendelian forms of epilepsy, the common forms have a complex inheritance in which susceptibility likely results from the combination of the interaction of a number of genes, more likely oligogenic (few genes of major effect) rather than polygenic (many genes, each of equal small effect), and there is no evidence for an environmental influence.[3] Many of the gene mutations identified in rare epilepsies are located in ion channel or neurotransmitter subunits, giving rise to the term *channelopathy*, but there is evidence that susceptibility genes influencing common idiopathic epilepsies may be involved in pathways related to neurodevelopment (rather than ion channels).

The hypothesis for a neurodevelopmental origin of common epilepsies derives from three lines of evidence—natural history, neuropathology, and neuroimaging—and is further supported by experimental data outlined later in this chapter. There is increasing recognition of premorbid symptoms in RE. Children with RE are at 2.5 times higher odds of developing a speech sound disorder than the general population and 5.8 times higher odds of developing dyslexia.[4] Speech sound disorder presents in the first 2 years of life, long before the median onset age for seizures, and dyslexia is also recognized at or soon after school entry, usually prior to or around the time of the first seizure. In RE families, these symptoms were reported as epilepsy-associated, but it is evident that their occurrence precedes the onset of seizures, and therefore their sequential onset may indicate a neurodevelopmental pathogenesis of RE. Furthermore, the presence of these same symptoms in relatives of RE patients (who themselves do not have epilepsy) suggests an underlying genetic etiology for these traits related to, but partly independent of, RE itself.[4]

Similar claims for a premorbid or comorbid diathesis have been made for a distinct frontal lobe personality in JME,[5] although this has been debated.[6,7] However, a growing number of neuroimaging studies suggest that JME shows focal brain structural abnormalities[8] associated with specific neuropsychological impairments, and similar findings are emerging from studies of childhood absence epilepsy.[9] Detailed morphometric neuropathological studies in idiopathic generalized epilepsy (IGE) suggest an abnormally increased number of neurons in the molecular layer of the cortex,[10] also described as *microdysgenesis*. These findings have proven hard to replicate owing to the scarcity of biological material. Nonetheless, the pertinence of brain structural abnormalities is further supported by the JME mouse model, discussed below. Taken together, these various lines of evidence suggest that the pathogenesis of idiopathic epilepsies is related to subtle deviations in the normal postnatal development of the brain.

METHODOLOGICAL ISSUES AND EXPERIMENTAL STRATEGIES IN COMMON EPILEPSIES

The experimental approaches available in common epilepsies are somewhat different than those in Mendelian disorders because, in most common epilepsies, there are usually not many affected people in a single kindred. Thus, a single family of a person with a common type of epilepsy does not contain sufficient information

for a genetic study. One way to overcome this limitation is to collect many families with the same phenotype; each family then contributes some genetic information. However, using this strategy, genetic heterogeneity becomes a critical confounder in both genetic association and linkage studies,[11] because signals from different genetic forms of disease may "cancel each other out." An obvious solution would be to separate out possibly different genetic forms on the basis of phenotypic differences. Sometimes, though, there are no obvious phenotypic features to distinguish different genetic forms. Nonetheless, what are often considered minor differences can be exploited to limit potential genetic heterogeneity. For example, generalized tonic-clonic seizures (GTCS) that occur on awakening may be inherited differently from GTCS that occur at random times of the day,[12,13] These studies show that some apparently minor distinguishing features can be feasibly used to stratify datasets to guide gene searches.

Genetic heterogeneity may occur within or between different subpopulations. For example, evidence for linkage to *EJM1* in JME is limited to European origin families and is not found in Hispanic origin or African-American families.[14–17] Another JME locus at 6p12-p11 was found in families from Belize and Mexico.[18] In a third example, GTCS are linked to markers on chromosome 10 in families from India, but no such linkage peak exists in corresponding European datasets.[19] This diversity of findings in genomic geography is increasingly recognized as the norm in international studies of complex disease and likely reflects differing genetic selection pressures on subpopulations in different environments. In epilepsy, the origins of genetic heterogeneity also reflect the multiple genes that contribute to the neural circuits that regulate cortical excitability.

A second complementary approach to overcome genetic heterogeneity is to divide the overall phenotype into simpler traits and then genetically analyze those traits. The success of this approach depends on stringent phenotyping. For example, in IGE, not only do probands have different types of seizures (e.g., absence, generalized tonic-clonic, myoclonic), but also clinically affected family members often have seizure types different from those seen in the proband.[20] By parsing the epilepsy syndrome into separate seizure types, different loci have been identified that lead to shared or distinct influences on the different seizure types in IGE.[21] Thus, *malic enyme 2* (*ME2*) has been identified as a major susceptibility gene common to all forms of IGE,[22] while myoclonic seizures have been mapped to *bromodomain 2* (*BRD2*).[23] Similarly, in RE, probands have combinations of neurodevelopmental traits including speech dyspraxia and dyslexia, while family members without seizures may be affected by the same disorders and/or exhibit centrotemporal sharp waves (CTS) on their electroencephalograms (EEGs). Thus, instead of conducting genetic analysis on a family in which only one person has epilepsy, it is possible to increase the power of genetic analysis by analyzing traits that segregate in more than one family member. Using this strategy, a 4–6 Hz spike-and-wave EEG trait seen in unaffected family members of JME proband families was found, like JME itself, to be influenced by the chromosome 6p21 locus,[15,24] and possibly photosensitivity as well.[25,26] We discovered linkage to the 11p13 region for CTS[27] and showed that speech dyspraxia and CTS share linkage at the same locus.[28] Loci for dyslexia in RE appear to be distinct from those for CTS or speech dyspraxia in RE,[29] adding complexity to the genetic model of the overall phenotype.

Below we review the evidence for the role of *ELP4* in RE and that of *BRD2* in JME.

ELP4 AND RE

Segregation Analysis

Segregation analysis is a method that allows one to test hypotheses about different modes of inheritance. It only has the power to exclude tested modes of inheritance, not to prove conclusively that one mode is correct, and it is often complicated by methodological errors in ascertainment and analysis. Although some attempts were made to estimate the inheritance of CTS and of RE in the past, they were affected by such methodological errors. We designed our study to avoid such errors: we collected 23 probands with RE through unambiguous single ascertainment. Thirty siblings of RE probands in the age range 4–16 years underwent sleep-deprived EEG; observations from those who remained awake were

omitted. Centrotemporal sharp waves were rated as present or absent by two independent observers blinded to the study's hypothesis and the subjects' identities. Twenty-three siblings showed evidence of sleep in their EEG recordings. Eleven of the 23 recordings demonstrated CTS, yielding a corrected segregation ratio of 0.48 (95% CI: 0.27–0.69). The male-to-female ratio of CTS affectedness was approximately equal. The segregation ratio of CTS in RE families is therefore consistent with a highly penetrant autosomal dominant inheritance with an equal sex ratio. Autosomal recessive and X-linked inheritance can be rejected.

Linkage Analysis

Linkage analysis is a method for localizing major effect genes to within several million base pairs. Linkage can be used either to test if a chromosomal region containing a suspected gene is related to disease expression or to do a genome-wide search for disease-related loci. We conducted genome-wide linkage analysis of the CTS trait in 38 U.S. families singly ascertained through a RE proband. In 11 of the families, one additional sibling was known to carry the CTS trait, but the CTS status of individuals younger than 4 years or older than 16 years was unknown because of its age-limited expression. Only markers on chromosome 11 yielded two-point logarithm of the odds (LOD) scores exceeding 3.0. Markers in the region of chromosomal band 11p13 provided strong and compelling evidence for linkage to CTS, with a maximum two-point LOD score of 4.01 and a multipoint LOD score of 4.30. Both European and non-European ancestry families contributed proportionally to the LOD score. Maximization of the LOD score in this region of 11p most often occurred at 95% penetrance, consistent with predictions from segregation analysis. We did not observe significant evidence of linkage at markers previously reported for CTS at 15q14,[30] nor for a rare recessive variant of RE at 16p12–11.2,[31] nor for X-linked rolandic seizures and cognitive deficit.[32] Similarly, we did not find evidence of linkage to 11p13 in an autosomal dominant variant of RE with speech dyspraxia and cognitive impairment.[33]

Association Analysis

Association analysis can show that having a specific allele (variant) at a locus correlates with an increased risk for disease. We designated a 13 cM linkage region at 11p13, encompassing the area in which LOD scores >2.0, as our region of interest for fine mapping. We then tested for association of CTS with single nucleotide polymorphism (SNP) markers distributed across genes in this region. We initially used a *discovery* dataset that included 68 cases and 187 controls group matched for ancestry and gender; 38 of these cases were included in the original linkage screen. In addition to case-control analysis, we used family-based analysis to guard against the potential for positive confounding due to population stratification. We took a pure likelihood approach to the statistical analysis of linkage and association.[34] We then typed additional SNPs around genes that showed compelling evidence of association in the preliminary analysis. In a second, independent, *replication* case-control dataset, we typed a subset of the SNPs in our region of interest. The replication set included 40 RE cases and 120 controls from western Canada; the two datasets were then jointly analyzed.

We discovered a significant association with SNPs in *ELP4* introns 9, 6, and 5, with estimated odds ratios of 1.80–2.04 at these markers (Fig. 66–1). These associations were demonstrated in both family-based analysis and case-control analysis. Joint analysis of the U.S. and Canadian datasets provided confirmation of these associations; the maximum likelihood ratio for association at intron 9 was 589:1 (Fig. 66–2). All associated markers are in linkage disequilibrium with each other (Fig. 66–3), so it is unlikely that they are exerting independent effects. However, none of the associated SNPs are predicted to have deleterious functional consequences, and we therefore resequenced this genomic region to search for a causative variant.

Genomic Resequencing

We resequenced the coding portions, the exon-intron boundaries, and the 50 kb upstream region of the *ELP4* gene in 40 RE probands from the discovery set. The 274 kb *ELP4* gene

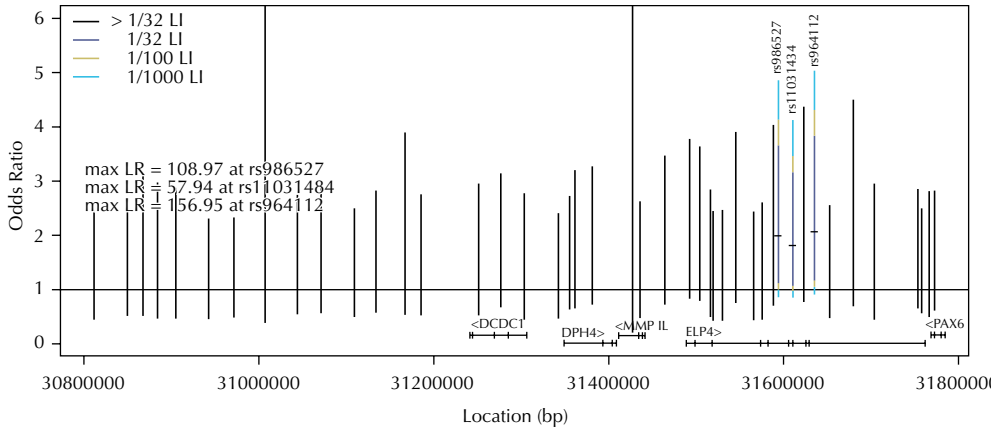

Figure 66–1. Pure likelihood plot of association evidence in a discovery set and in joint analysis of datasets (Fig. 66–2). This pure likelihood analysis plots the odds ratio (OR) on the *y*-axis and the base-pair position on the *x*-axis. Each vertical line represents a likelihood interval (LI) for the OR at a given SNP. The OR 1⁄4 1 line is plotted as a solid black horizontal line for reference. LIs in color are denoted as SNPs of interest, whereas a gray line indicates that the SNP is not of interest because the 1/32 LI for that SNP covers the OR 1⁄4 1 line. The small horizontal tick on each LI is the maximum likelihood estimator for the OR. The portion of the colored LI that covers the OR 1⁄4 1 horizontal line indicates the strength of the association information at that SNP. In particular, if the navy blue portion is above the OR 1⁄4 1 line while the yellow portion of the LI covers the OR 1⁄4 1 line, then the LOD evidence at that SNP is between 1.5 and 2 (i.e., the 1/32 LI does not include the OR 1⁄4 1 value, but the 1/100 LI does); similarly, if both the yellow and navy blue portions are above the OR 1⁄4 1 line but the turquoise portion covers the line, then the LOD evidence is between 2 and 3 (i.e., the 1/100 LI does not include OR 1⁄4 1 as a plausible value but the 1/1000 LI does). The further the colored line is above the OR 1⁄4 1 line, the stronger the association evidence. The max LR for each SNP in color is also provided as text in the plot, providing evidence not only of whether the LOD evidence is between 2 and 3, but also the exact value of the max LR.

is transcribed into a 1584 bp mRNA consisting of 12 exons, a 35 bp 5′untranslated region (5′-UTR), and a 257 bp 3′-UTR. Alternative transcripts have been reported that include or exclude the last two exons. Three previously reported SNP variants were found in these 40 individuals, but the distribution of these SNPs was not different from that found in controls or reference databases.

Summary of Evidence for *ELP4* as a Susceptibility Gene for CTS

Obviously, CTS is a mandatory component of the overall RE phenotype. The sum of our analyses points to CTS as a highly penetrant autosomal dominant trait. We have very strong evidence of a single genome-wide locus at 11p13 influencing the CTS trait. Association evidence unambiguously points to an association with markers in the *ELP4* gene, but not with markers in the *DCDC1*, *DPH4*, *IMMP1L*, or *PAX6* genes under the 11p13 linkage peak. This association evidence is present in family-based analysis, making population stratification an unlikely explanation for the finding, and was also replicated in an independent dataset. However, no obvious coding mutation has been discovered in the *ELP4* gene. There are several possible explanations for this that are yet to be fully explored. First, coding changes are not common in complex diseases, in contrast to the simple loss- or gain-of-function coding mutations found in Mendelian diseases. Hence, the sought-after variant may reside in a regulatory or conserved region of the gene; second, some regulatory regions may be distant from the gene that they regulate, so the causative variant may not be in *ELP4*; third, it is possible that the causative variant lies in another part of the 11p13 linkage region and that the association with *ELP4* represents long-range linkage disequilibrium with the true variant. The search for the causative variant will continue; meanwhile, a coherent evaluation of the evidence should take account of the biological function of potential candidate genes. The relevant information about the role of *Elongator* in the nervous system is presented below.

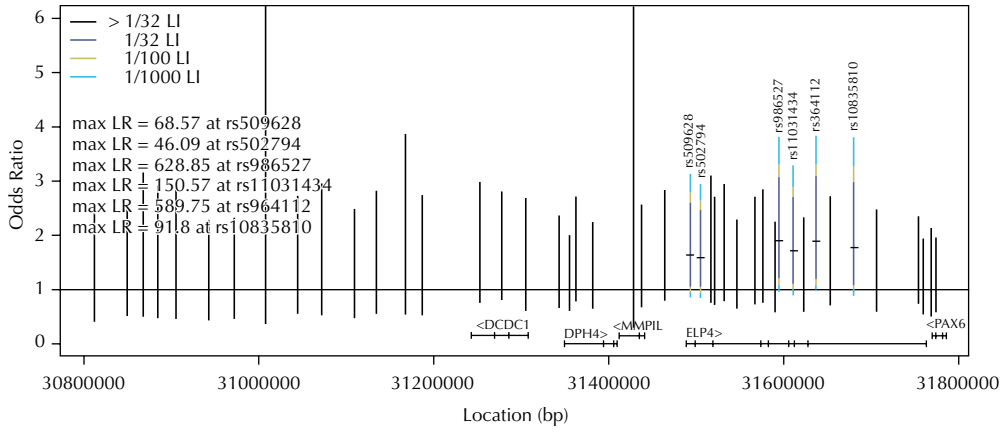

Figure 66–2. Joint analysis of discovery (U.S.) and replication (Canadian) datasets.

Elongator Biology

There is increasing evidence that impairment of the *Elongator* complex may be involved in several different neurological disorders, and this topic has recently been extensively reviewed[35] and is excerpted below. *Elongator* is composed of six subunits (*ELP1–ELP6*). *ELP1* is the main scaffold protein, whereas *ELP3* is the main enzymatic subunit that has histone acetyltransferase activity.[36] *Elongator* is believed to play an important role in the transcriptional elongation of some genes,[37,38] a hypothesis further supported by the observation of transcriptional defects in multiple genes in *ELP1*-depleted cells and mouse embryos.[37,39]

As well as a nuclear function associated with RNA polymerase II and histone H3 acetylation, there is evidence that the *Elongator* complex has a role in tRNA modification in the cytoplasm. Cytoplasmic *ELP1* colocalizes with filamin A in membrane ruffles, and a defective actin cytoskeleton has been reported in *ELP1*-depleted cells, possibly explaining the cell motility defects.[40] Thus, it appears that *Elongator* critically regulates the migration of multiple cell types.

In humans, mutations of the *ELP1* gene on chromosome 9 cause familial dysautonomia (FD) or Riley-Day syndrome.[41] *ELP1* levels are very low in brain tissues of FD patients, and brain neuroimaging abnormalities are

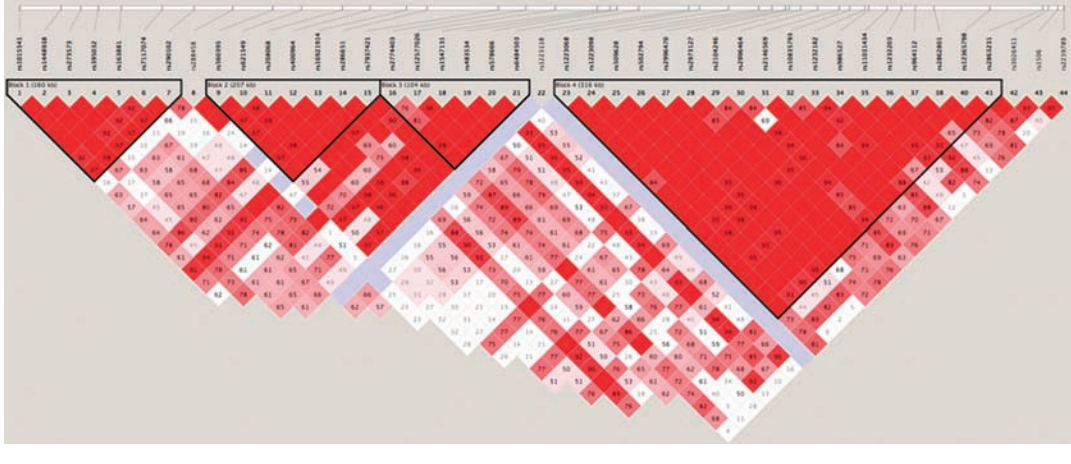

Figure 66–3. Linkage disequilibrium map in the 11p13 region from SNP association data. Block 4 spans the *DPH4, IMMP1L, ELP4,* and *PAX6* genes.

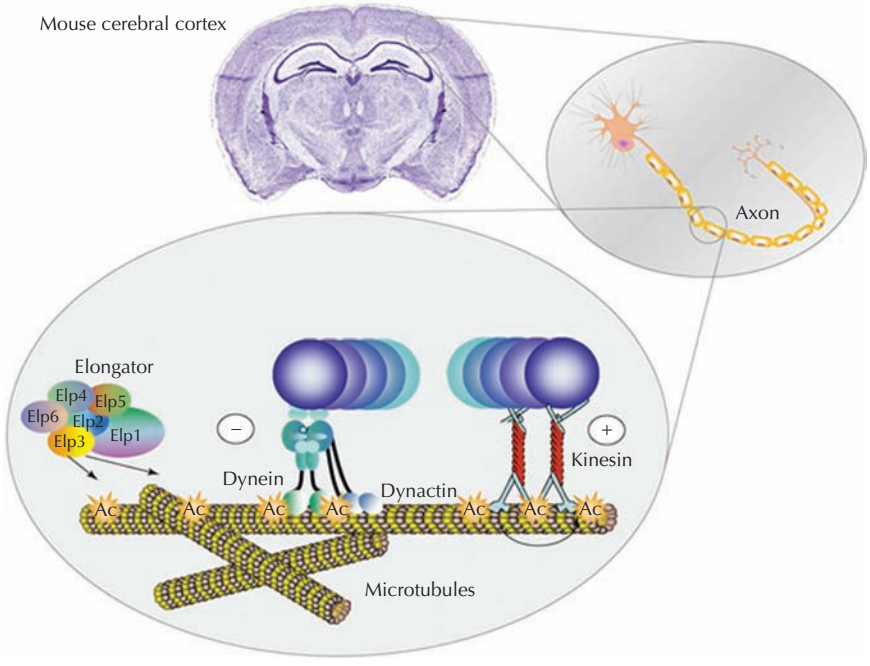

Figure 66–4. A putative role for *Elongator* in the microtubule-dependent transport of cargoes required for the development and/or survival of neurons. *Elongator* catalyzes a-tubulin acetylation via Elp3, a modification that promotes the anchoring of molecular motors and underlies microtubule-dependent trafficking. Two molecular motors anchored to microtubules are illustrated, namely, kinesin and the dynein/dynactin complex, which are involved in the anterograde or retrograde transport of various cargoes (blue circles), respectively. From ref. 35.

reported,[42] as well as EEG abnormalities and seizures.[43] *Elongator* plays an important role in the migration and branching of cerebral cortical projection neurons by promoting the acetylation of alpha-tubulin[44] (Fig. 66–4). Association of *ELP3* variants has been shown with sporadic Amyotrophic Lateral Sclerosis (ALS),[45] and it has been speculated that *Elongator* contributes to the microtubule-dependent trafficking of anti-apoptosis cues in central and peripheral neurons.[35] We speculate that *ELP4* mutations might also partially abrogate *Elongator* function in a regionally and temporally specific manner that results in disruption of neural networks involved in vocal tract function, and with a resulting imbalance of excitatory and inhibitory circuits. We expect the story to become clearer in the near future.

BRD2 AND JME

In discussing our work examining *BRD2* as a gene related to the expression of JME, we must explain why we examined *BRD2* as opposed to any other gene, especially since it had no obvious relationship to our preconceptions about the origins of idiopathic epilepsy in humans. First, it came to our attention when we chose to study JME because the phenotype appeared easy to define; the awakening myoclonic jerks are almost pathognomonic for the condition (when there are no exclusionary symptoms). Second, we could make use of the interictal 4–6 Hz spike-and-wave seen in almost all JME patients as the phenotype because this trait is also seen with increased frequency in clinically unaffected family members.[46,47] This meant that we might be able to use the presence of the EEG trait as the phenotype for the linkage analysis instead of the epilepsy itself, which proved to be a useful approach,[15,47] an approach later confirmed when it led to the discovery that *ELP4* is related to the centrotemporal spikes of RE.[48]

Segregation Analysis

A segregation analysis of JME was able to exclude simple Mendelian modes of inheritance,

even when the 4–6 Hz spike-and-wave trait was used as the phenotype of interest (clinically unaffected family members with the trait were included as "affected").[49] However, the segregation analysis showed that the best fit to the data was a model of inheritance involving two genes, one showing dominant and one showing recessive inheritance. Models in which both loci were dominant or both were recessive did not fit the data as well. Interestingly, two loci later identified as contributing to JME supported that mode of inheritance: linkage analysis identified a dominantly inherited locus at chromosome 6p21, which was eventually identified as *BRD2*, and one on chromosome 18, *ME2*, that indicated recessive inheritance.

Linkage and Association Analysis

Our initial linkage scan revealed the presence of a JME-related locus on chromosome 6p21, a finding confirmed in the dataset of Janz.[14,15] Later, after an entirely new and larger dataset was collected, we were not only able to confirm the linkage again, but the discovery of two families with recombinants allowed us to narrow down the region containing the gene to between the HLA-DQ and HLA-DP loci. Furthermore, the advance in genetic technology allowed us to discover an association of JME, first with a microsatellite marker[17] and later with SNP haplotypes in *BRD2*.[23] Both of these results supported *BRD2* as the gene influencing JME expression. Subsequently, we demonstrated a strong association of *BRD2* variants with JME, with an odds ratio of 6.45.[23]

There are two important points to emphasize. First, these results started with a highly specific phenotype, then used linkage data from entire families to find a locus containing a disease-related gene, and then used association analysis to identify the gene within that locus. No genes were excluded from examination on the basis that they did not fit a preconceived epilepsy mechanism. Second, in the absence of any idea of what the mechanism might be, and confronted with a candidate gene of unknown function and containing no epilepsy-related exonic mutations after sequencing, the problem remained (and remains) one of proving that the gene we found is the gene we are looking for. Up to this point, all the evidence is statistical. In the absence of any idea of how a so-called transcription factor element could lead to a common form of epilepsy, we needed some biological evidence that *BRD2* was somehow involved in expression. This led to our development of the *Brd2* knockout mouse.

Brd2 Knockout Mouse

Our *Brd2* knockout mouse was created using the gene-trap technique,[50] and embryos were raised on a B57Bl/CJ background. We made several observations about mice homozygous for the knockout ($Brd2^{-/-}$):

1. $Brd2^{-/-}$ embryos do not survive past the 13th gestational day (E13).
2. $Brd2^{-/-}$ embryos are considerably smaller than their wild-type $brd2^{+/+}$ or $brd2^{+/-}$ littermates. Figure 66–5 presents a homozygote embryo and a wild-type embryo at E11, showing the much smaller size of the homozygote.
3. The neural tube of the homozygote is not closed, as the arrow in Fig. 66–5 shows.
4. The most obvious effect of a lack of *brd2* during development is on the mouse nervous system. Figure 66–6 shows a wild-type and a $brd2^{-/-}$ mouse in cross section at E11. Note that the brain and spinal cord of the homozygote are severely dysmorphic, with brain development apparently spatially disorganized. Note also how other organs, while smaller than those in the wild type, appear structurally intact.
5. Confirming the apparently major effect of *brd2* mostly on nervous system development, a *brd2* probe showed that *brd2* is expressed mostly in developing nervous tissue. Figure 66–7 shows a wild-type mouse (left) with the densest areas of stain, indicating the greatest expression of *Brd2*, being the nervous system. The $Brd2^{-/-}$ mouse (right) shows no *Brd2* expression.

Thus, from the studies of the $Brd2^{-/-}$ mouse, we can conclude that *Brd2* is not only essential for life, it is a major determinant of brain development and its expression mostly in the brain of developing embryos suggests a critical role, at E13 and earlier, in the nervous system. This immediately raises the question: what is

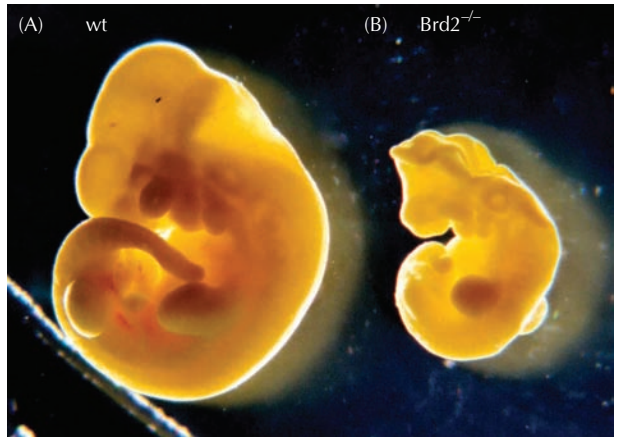

Figure 66–5. Photographs of wild-type (*Brd2*$^{-/-}$, left) and *Brd2*$^{-/-}$ (right) embryos at embryonic day (E) 9.5 of gestation. The mutant embryo is smaller, and the developing neural tube is unfused (arrow). From ref. 50.

the effect of having a deficit, not an absence of, *Brd2*?

The *Brd2*$^{+/-}$ Heterozygote

By all outward appearances, the heterozygote is normal and behaves grossly normally. The question is: are the mice more sensitive to seizures, and is their brain structure normal? We determined that *Brd2*$^{+/-}$ mice are more susceptible to chemically induced seizures than are wild-type mice. We initially used pentylenetetrazole to test seizure susceptibility. The heterozygotes were much more sensitive to the pentylenetetrazole than the wild-type mice, as shown in Fig. 66–8.[51]

These results were confirmed using the seizure-inducing ether, flurothyl (Velisek et al., unpublished data); we also showed that female heterozygote mice are much more sensitive to tonic-clonic seizures than male mice, a finding

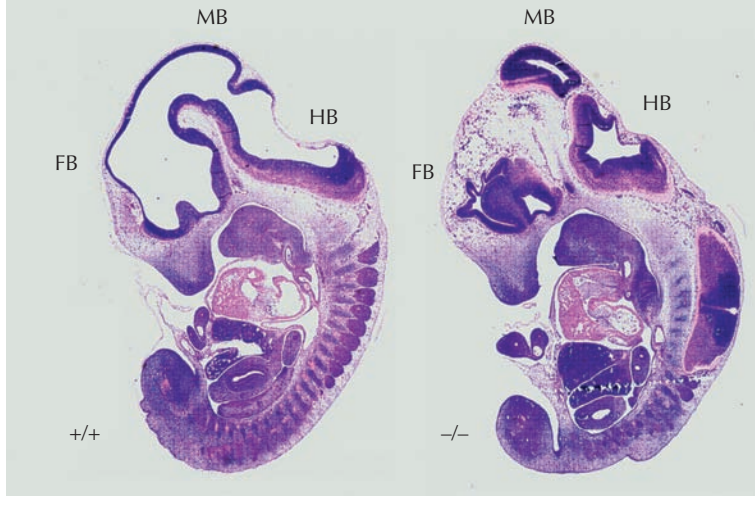

Figure 66–6. Sagittal sections of E11.5 embryos of wild-type and mutant embryos. Grossly normal-appearing heart, lung, and so on, are seen in the mutant, but the brain of the mutant is aberrant. FB, forebrain; MB, midbrain; HB, hindbrain. From ref. 50.

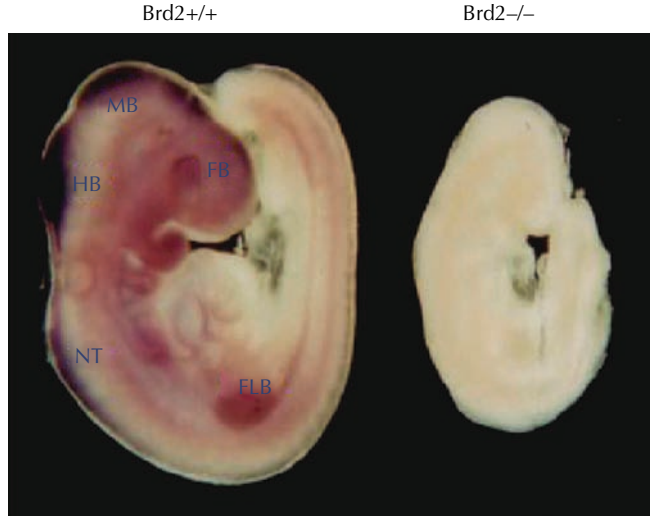

Figure 66–7. Whole-mount in situ hybridization of wild-type and *Brd2* mutant E9.5 embryos using digoxygenin-labeled *Brd2* RNA probes. *Brd2* mRNA is detected in the wild-type developing neural system but is absent in the mutant. FB, forebrain; FLB, forelimb bud; MB, midbrain; HB, hindbrain; NT, neural tube. From ref. 50.

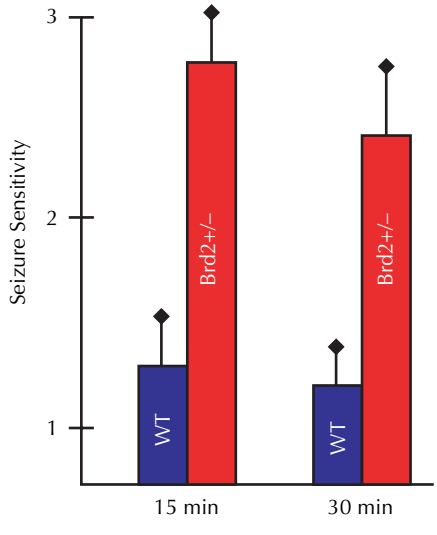

Figure 66–8. Seizure sensitivity to pentylenetetrazol in wild-type and *Brd2*$^{+/-}$ mice. Seizure intensity was scored blind to genotype by four independent observers on a scale of 0–3, where 0 = no change in muscle tone or activity, 1 = mild tremor, 2 = moderate seizure, and 3 = severe seizure. Three of five heterozygotes died following severe convulsion, but all five wild-type animals completely recovered.

perhaps echoing a result found by Pal et al.,[20] in which human females had up to a 12-fold increased risk of seizures in JME families compared to males.

We also found that the brain structures of the *Brd2*$^{+/-}$ mice were subtly abnormal. In the frontal lobes there was up to a 50% deficit of parvalbumin staining interneurons compared to the wild-type mice (Fig. 66–9).

These results were also later confirmed and expanded.[52] Almost all brain areas examined showed varying levels of GABAergic neuron deficit. Taken together with early ultrastructural studies in postmortem human IGE brains,[10] as well as with recent volumetric studies in JME,[8] the corpus of evidence supports a neurodevelopmental origin for common IGE.

The implications of these findings could have a profound effect on our view of the origins of IGE. They imply that, for JME, if not for other IGEs, part of the neural substrate for the epilepsy is a deficit of inhibitory neurons caused by a subtle insufficiency of *BRD2* for JME. This deficit leads to underproduction or abnormal migrations or early apoptosis of such neurons, a deficit that later will lead to epilepsy in the presence of some other, probably subtle, abnormality caused by a second gene or genes.

These results are compelling evidence that *BRD2* is, in fact, the *EJM1* locus on chromosome 6p21. However, mice are not the same as humans. In the absence of human biological material to test the hypothesis that there are fewer GABAergic inhibitory neurons in the brains of JME patients, we must continue to investigate the effects of *Brd2* in mice to discover a definitive connection that will allow us to predict the risk for JME. Identifying the

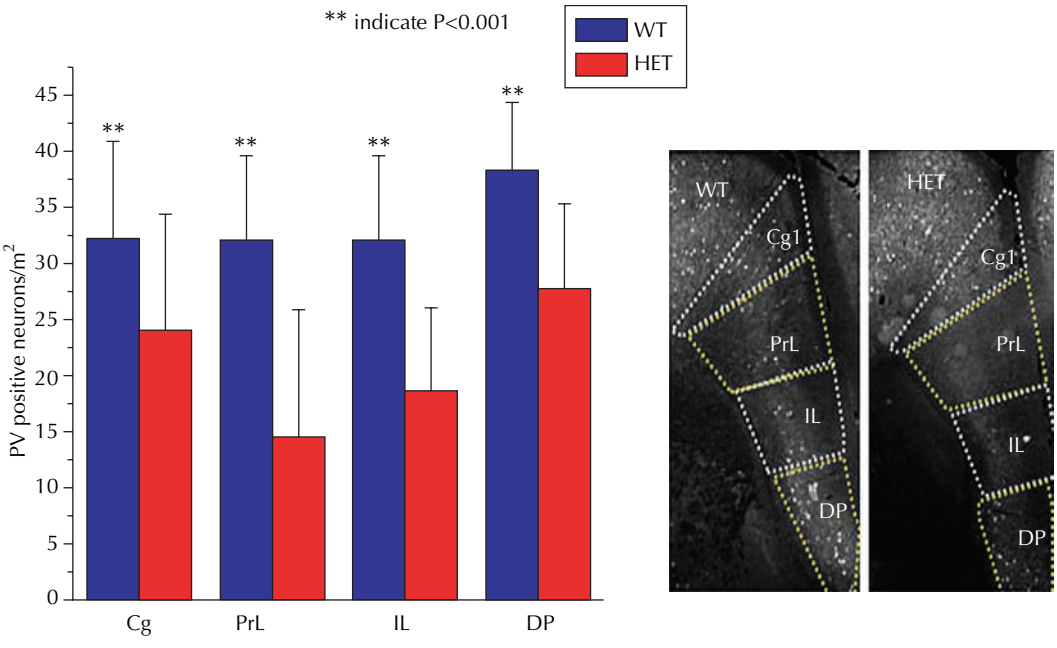

Figure 66–9. Differences in the density of parvalbumin-stained neurons in *Brd2*$^{+/-}$ versus wild-type mice in four brain regions: Cg1, cingulated cortex, area 1; Prl, prelimbic cortex; IL, infralimbic cortex; DP, dorsal peduncular cortex. Densities represent counts from over 100 slices from two *Brd2*$^{+/-}$ and two wild-type mice.

BRD2 JME-related alleles will enable us to do that.

Numerous genes have been identified in densely affected families showing Mendelian inheritance or, like the gene *SCN1A's* role in severe myoclonic epilepsy of infancy, have been shown to play a role in severe epilepsy. However, none of these genes has yet been linked to, associated with, or, by mutation analysis, been shown to play a role in common, genetically complex forms of epilepsy. The results of the RE and JME work on *ELP4* and *BRD2* suggest that common epilepsies are not disorders of a single gene but require several genes for their expression. More important is the suggestion that these disorders result from changes in gene expression that result in subtle changes during development. Investigators studying epilepsy will need to adopt more subtle observational approaches in order to understand these changes.

DISCLOSURE STATEMENT

The authors have no conflicts of interest relating to this chapter.

REFERENCES

1. Cossette P, Liu L, Brisebois K, et al. Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nat Genet*. 2002;31:184–189.
2. Malacarne M, Gennaro E, Madia F, et al. Benign familial infantile convulsions: mapping of a novel locus on chromosome 2q24 and evidence for genetic heterogeneity. *Am J Hum Genet*. 2001;68:1521–1526.
3. Greenberg DA, Durner M, Delgado-Escueta AV. Evidence for multiple gene loci in the expression of the common generalized epilepsies. *Neurology*. 1992;42:56–62.
4. Clarke T, Strug LJ, Murphy PL, et al. High risk of reading disability and speech sound disorder in rolandic epilepsy families: case-control study. *Epilepsia*. 2007;48:2258–2265.
5. Janz D. The idiopathic generalised epilepsies of adolescence with childhood and juvenile age of onset. *Epilepsia*. 1997;38:4–11.
6. Gelisse P, Genton P, Samuelian JC, et al. [Psychiatric disorders in juvenile myoclonic epilepsy]. *Rev Neurol (Paris)*. 2001;157:297–302.
7. Trinka E, Kienpointner G, Unterberger I, et al. Psychiatric comorbidity in juvenile myoclonic epilepsy. *Epilepsia*. 2006;47:2086–2091.
8. Roebling R, Scheerer N, Uttner I, et al. Evaluation of cognition, structural, and functional MRI in juvenile myoclonic epilepsy. *Epilepsia*. 2009;50:2456–2465.
9. Caplan R, Levitt J, Siddarth P, et al. Frontal and temporal volumes in childhood absence epilepsy. *Epilepsia*. 2009;50:2466–2472.

10. Meencke HJ, Janz D. Neuropathological findings in primary generalized epilepsy: a study of eight cases. *Epilepsia.* 1984;25:8–21.
11. Vieland VJ. The replication requirement. *Nat Genet*. 2001;29:244–245.
12. Greenberg DA, Durner M, Resor S, et al. The genetics of idiopathic generalized epilepsies of adolescent onset: differences between juvenile myoclonic epilepsy and epilepsy with random grand mal and with awakening grand mal. *Neurology*. 1995;45:942–946.
13. Durner M, Zhou G, Fu D, et al. Evidence for linkage of adolescent-onset idiopathic generalized epilepsies to chromosome 8-and genetic heterogeneity. *Am J Hum Genet*. 1999;64:1411–1419.
14. Weissbecker KA, Durner M, Janz D, et al. Confirmation of linkage between juvenile myoclonic epilepsy locus and the HLA region of chromosome 6. *Am J Med Genet*. 1991;38:32–36.
15. Durner M, Sander T, Greenberg DA, et al. Localization of idiopathic generalized epilepsy on chromosome 6p in families of juvenile myoclonic epilepsy patients. *Neurology*. 1991;41:1651–1655.
16. Sander T, Bockenkamp B, Hildmann T, et al. Refined mapping of the epilepsy susceptibility locus EJM1 on chromosome 6. *Neurology*. 1997;49:842–847.
17. Greenberg DA, Durner M, Keddache M, et al. Reproducibility and complications in gene searches: linkage on chromosome 6, heterogeneity, association and maternal inheritance in juvenile myoclonic epilepsy. *Am J Hum Genet*. 2000;66:508–516.
18. Liu AW, Delgado-Escueta AV, Serratosa JM, et al. Juvenile myoclonic epilepsy locus in chromosome 6p21.2-p11: linkage to convulsions and electroencephalography trait. *Am J Hum Genet*. 1995;57:368–381.
19. Puranam RS, Jain S, Kleindienst AM, et al. A locus for generalized tonic-clonic seizure susceptibility maps to chromosome 10q25-q26. *Ann Neurol*. 2005;58: 449–458.
20. Pal DK, Durner M, Klotz I, et al. Complex inheritance and parent-of-origin effect in juvenile myoclonic epilepsy. *Brain Dev*. 2006;28:92–98.
21. Durner M, Keddache MA, Tomasini L, et al. Genome scan of idiopathic generalised epilepsy: evidence for major susceptibility gene and modifying genes influencing the seizure type. *Ann Neurol*. 2001;49: 328–335.
22. Greenberg DA, Cayanis E, Strug L, et al. Malic enzyme 2 may underlie susceptibility to adolescent-onset idiopathic generalized epilepsy. *Am J Hum Genet*. 2005;76:139–146.
23. Pal DK, Evgrafov OV, Tabares P, et al. BRD2 (RING3) is a probable major susceptibility gene for common juvenile myoclonic epilepsy. *Am J Hum Genet.* 2003;73:261–270.
24. Greenberg DA, Delgado-Escueta AV, Widelitz H, et al. Juvenile myoclonic epilepsy may be linked to the BF and HLA loci on human chromosome 6. *Am J Med Genet.* 1988;31:185–92.
25. Lorenz S, Taylor KP, Gehrmann A, et al. Association of BRD2 polymorphisms with photoparoxysmal response. *Neurosci Lett*. 2006;400:135–139.
26. Tauer U, Lorenz S, Lenzen KP, et al. Genetic dissection of photosensitivity and its relation to idiopathic generalized epilepsy. *Ann Neurol*. 2005;57:866–873.
27. Strug LJ, Clarke T, Chiang T, et al. Centrotemporal sharp wave EEG trait in rolandic epilepsy maps to elongator protein complex 4 (ELP4). *Eur J Hum Genet.* 2009;17:1171–1181.
28. Pal DK, Strug LJ, Bali B, et al. Pleiotropic effects of the 11p13 locus on developmental verbal dyspraxia and EEG centrotemporal sharp waves. *Genes Brain Behav*. 2010;9:1004–12.
29. Pal DK, Strug LJ, Clarke T, et al. Major genetic loci for reading disability in rolandic epilepsy families. *Epilepsia*. 2007;48:376.
30. Neubauer BA, Fiedler B, Himmelein B, et al. Centrotemporal spikes in families with rolandic epilepsy: linkage to chromosome 15q14. *Neurology.* 1998;51:1608–1612.
31. Guerrini R, Bonanni P, Nardocci N, et al. Autosomal recessive Rolandic epilepsy with paroxysmal exercise-induced dystonia and writer's cramp: delineation of the syndrome and gene mapping to chromosome 16p12–11.2. *Ann Neurol*. 1999;45:344–352.
32. Roll P, Rudolf G, Pereira S, et al. SRPX2 mutations in disorders of language cortex and cognition. *Hum Mol Genet*. 2006;15:1195–1207.
33. Kugler SL, Bali B, Lieberman P, et al. An autosomal dominant genetically heterogeneous variant of rolandic epilepsy and speech disorder. *Epilepsia*. 2008;49: 1086–1090.
34. Strug LJ, Hodge SE, Chiang T, et al. A pure likelihood approach to the analysis of genetic association data: an alternative to Bayesian and Frequentist analysis. *Eur J Hum Genet*. 2010;18(8):933–41.
35. Nguyen L, Humbert S, Saudou F, et al. Elongator—an emerging role in neurological disorders. *Trends Mol Med*. 2010;16:1–6.
36. Wittschieben BO, Otero G, de Bizemont T, et al. A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. *Mol Cell*. 1999;4:123–128.
37. Close P, Hawkes N, Cornez I, et al. Transcription impairment and cell migration defects in elongator-depleted cells: implication for familial dysautonomia. *Mol Cell*. 2006;22:521–531.
38. Kouskouti A, Talianidis I. Histone modifications defining active genes persist after transcriptional and mitotic inactivation. *EMBO J*. 2005;24:347–357.
39. Chen YT, Hims MM, Shetty RS, et al. Loss of mouse Ikbkap, a subunit of elongator, leads to transcriptional deficits and embryonic lethality that can be rescued by human IKBKAP. *Mol Cell Biol*. 2009;29:736–744.
40. Johansen LD, Naumanen T, Knudsen A, et al. IKAP localizes to membrane ruffles with filamin A and regulates actin cytoskeleton organization and cell migration. *J Cell Sci*. 2008;121:854–864.
41. Slaugenhaupt SA, Blumenfeld A, Gill SP, et al. Tissue-specific expression of a splicing mutation in the IKBKAP gene causes familial dysautonomia. *Am J Hum Genet*. 2001;68:598–605.
42. Axelrod FB, Hilz MJ, Berlin D, et al. Neuroimaging supports central pathology in familial dysautonomia. *J Neurol.* 2010;257:198–206.
43. Niedermeyer E, McKusick VA, Brunt P, et al. The EEG in familial dysautonomia (Riley-Day syndrome). *Electroencephalogr Clin Neurophysiol*. 1967;22: 473–475.
44. Creppe C, Malinouskaya L, Volvert ML, et al. Elongator controls the migration and differentiation of cortical neurons through acetylation of alpha-tubulin. *Cell.* 2009;136:551–564.

45. Simpson CL, Lemmens R, Miskiewicz K, et al. Variants of the elongator protein 3 (ELP3) gene are associated with motor neuron degeneration. *Hum Mol Genet*. 2009;18:472–481.
46. Tsuboi T, Christian W. On the genetics of primary generalized epilepsy with sporadic myoclonias of impulsive petit mal. A clinical and electroencephalographic study of 399 probands. *Humangenetik*. 1973;19:155–182.
47. Greenberg DA, Delgado-Escueta AV, Widelitz H, et al. Juvenile myoclonic epilepsy (JME) may be linked to the BF and HLA loci on human chromosome 6. *Am J Med Genet*. 1988;31:185–192.
48. Strug LJ, Clarke T, Chiang T, et al. Centrotemporal sharp wave EEG trait in rolandic epilepsy maps to elongator protein complex 4 (ELP4). *Eur J Hum Genet*. 2009;17:1171–1181.
49. Greenberg DA, Delgado-Escueta AV, Maldonado HM, et al. Segregation analysis of juvenile myoclonic epilepsy. *Genet Epidemiol*. 1988;5:81–94.
50. Shang E, Wang X, Wen D, et al. Double bromodomain-containing gene Brd2 is essential for embryonic development in mouse. *Dev Dyn*. 2009;238: 908–917.
51. Greenberg DA, Shang E, Luo J, et al. Knockout mouse data support BRD2 as a gene for juvenile myoclonic epilepsy. *Epilepsia*. 2007;48, 247.
52. Velíšek L, Shang E, Velíšková J, Chachua T, Macchiarulo S, Maglakelidze G, Wolgemuth DJ, Greenberg DA. GABAergic neuron deficit as an idiopathic generalized epilepsy mechanism: the role of BRD2 haploinsufficiency in juvenile myoclonic epilepsy. *PLoS One*. 2011;6(8):e23656. Epub August 24, 2011.

Chapter 67

Myoclonin1/EFHC1 in Cell Division, Neuroblast Migration, and Synapse/ Dendrite Formation in Juvenile Myoclonic Epilepsy

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Juvenile myoclonic epilepsy (JME) is the most frequent form of idiopathic/genetic generalized epilepsy. It accounts for 2%–12% of all epilepsies. The JME symptoms of myoclonias and tonic-clonic convulsions appear in adolescence in an otherwise normal person with normal neurological and cognitive functions.[1] Five percent of these patients, 5% of their affected family members, and 8% of a 252-patient cohort followed for over 20 years have seizures

only when triggered by external factors such as alcohol use, fatigue, menstruation, and sleep deprivation.

To understand the disease mechanisms underlying the stages of susceptibility and epileptogenesis in JME, many have tried to define its complex heritability and identify the genes corresponding to the 15 chromosomal loci so far linked to the disease.[2]

In 2004, Suzuki et al. identified several heterozygous missense mutations in a gene called *EFHC1*[3] in different unrelated families with JME probands. Since then, heterozygous nonsense, deletion frameshift, and novel missense mutations have been identified in various populations from Italy, Austria, and Chile.[4–7] Nine percent of sporadic JME cases detected in families consecutively seen in epilepsy clinics in Mexico and Honduras and 3% of clinic patients from Japan carry mutations in *EFHC1*. This represents the highest number and percentage of mutations found for a JME-causing gene of any population group.[7] The gene encodes a 75 kDa protein with three DM10 domains of unknown function and a single EF-hand motif, a Ca^{2+}-binding domain. The transcript is observed in many cell types of human tissue, including brain (in particular, in ependymal or periventricular cells), but is also best expressed in dividing cells, with the highest levels in lung and testis.[3] It was first proposed that EFHC1 was pro-apoptotic when overexpression in hippocampal neurons in vitro induced apoptotic cell death. This effect was significantly reduced by any of the five mutations associated with JME. Patch-clamp analysis of BHK (baby hamster kidney) cells transfected with $Ca_v2.3$ VDCC (voltage-dependent calcium channel) and EFHC1 showed significantly increased R-type Ca^{2+} currents. So, the pro-apoptotic effect of EFHC1 was assigned to this enhancing effect on Ca^{2+} through $Ca_v2.3$ VDCC.[3]

In 2005, another research group pointed out that EFHC1 is orthologous to Rib72, an axonemal protein of *Chlamydomonas reinhardtii.* They demonstrated that EFHC1 is abundantly expressed in mouse tissues that have motile cilia or flagella, including the brain, and suggested that it plays a role in the intrinsic properties of these organelles.[8]

One of our laboratories previously reported that the subcellular distribution of EFHC1 in different cell lines varied during the cell cycle. In interphase cells, the protein is present in the cytoplasm and nucleus, except for the nucleoli, and is particularly concentrated at the centrosome. During mitosis, EFHC1 is localized at spindle poles of the mitotic spindle and also at the midbody during cytokinesis.[9] These results suggest that EFHC1 could play an important role during cell division, and in particular during brain development, since mRNA expression is higher at embryonic stages than in the adult.[10] More recently, we demonstrated that EFHC1 is a microtubule-associated protein (MAP) playing a key role in neuronal migration.[11] In this chapter, we review these putative roles of Myoclonin 1/EFHC1 during brain development and during adulthood. We posit the hypothesis that JME is a developmental disease involving neuronal migration and synaptic bouton and dendritic morphogenesis.

EFHC1/MYOCLONIN1, A PROTEIN OF UNKNOWN FUNCTION

The *Myoclonin 1/EFHC1* gene is located on chromosome 6 (6p11–12) between markers D6S1960 and D6S11024, spans 72 kb, and contains 11 exons. This gene encodes a protein of 640 amino acids. A domain search identified three tandemly repeated so-called DM10 domains, a motif with unknown function. This protein also contains a single EF-hand, a well-known Ca^{2+}-binding motif, from which it was named EFHC1 for EF-hand Containing 1 (Fig. 67–1). This motif is located at the C terminus between amino acids 578 and 606 and is encoded by a nucleotide sequence present in exon 10. The transcript undergoes alternative splicing in exon 4, resulting in a C-terminally truncated protein, eliminating the EF-hand domain and two DM10 sequences. This last short form therefore only contains 278 amino acids, the first 240 being common with the entire molecule.

The EF-Hand Motif

Krestinger and Nockolds, when exploring the crystal structure of paravalbumin, first described the EF-hand domain.[12] It corresponds to a helix-loop-helix motif, the loop constituted by 12 amino acids, capable of binding Ca^{2+} and

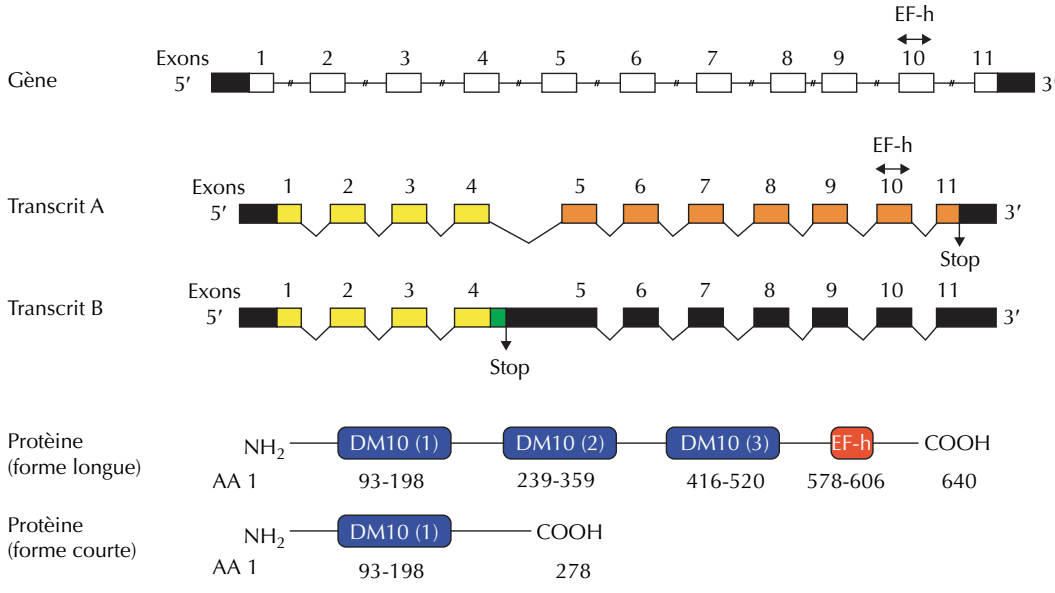

Figure 67–1. Schematic representation of the *EFHC1/Myoclonin1* gene, the long and short forms of the EFHC1 protein, and the different mutations found cosegregated with the JME phenotype.

linking two perpendicular α-helices named E and F (Fig. 67–2). The name *EF-hand* comes from the fact that this motif can be represented with a symbolic right-hand in which the E helix corresponds to the index finger and the F helix to the thumb.[13] The EF-hand proteins belong to the family of Ca^{2+}-binding proteins, which include more than 1000 members classified into 66 different subfamilies. Two groups are distinguished according to their cellular properties: on the one hand, the buffer proteins implicated in the transport and regulation of intracellular calcium concentration (calbindin and calretinin, for instance) and, on the other hand, the calcium sensors that transfer information from calcium influx to specific intracellular molecular pathways (calmodulin, troponin C).[14] Most of the EF-hand proteins, however, contain single or multiple pairs of such helix-loop-helix motifs. They are typically present in globular forms and create a cooperative calcium link between the two motifs, resulting in an increased affinity of the protein for the calcium ion.[15]

At the loop level, the residues in the 1, 3, 5, 7, 9, and 12 positions, also named +X, +Y, +Z, –Y, –X, and –Z, are responsible for binding calcium, forming a geometrical pentagonal bipyramidal structure (Fig. 67–3). The residues +X, +Y, and +Z provide a coordination link, while the residue –Z must provide two of them via the carboxyl groups of its lateral chain. The residue –Y regulates calcium via an oxygen of its principal chain, and the residue –X links calcium indirectly by a molecule of water. The most conserved amino acids of the loop are the aspartate in the +X position and the aspartate or the glutamate in the –Z situation. It is important to note that the EF-hand motif exists in non-canonical positions in which the length or the composition of the calcium binding loop varies, implicating a calcium-binding mechanism with a different system of coordination.[16] The EF-hand domain of EFHC1 is probably one of them, since +Y and –Z residues do not correspond to the consensus sequence of a canonical domain. Moreover, the EFHC1 protein contains only one single EF-hand motif. Therefore, these observations show that EFHC1 is a non-conventional EF-hand protein.

The DM10 Domains

The DM10 domains contain appreciatively 105 residues and are so far of unknown function. The DM10 domains' proteins are found in classes as varied as mammals (human, rat, mouse, dog, cow), fishes (*Tetraodon*, *Danio rerio*), insects (*Anopheles*, *Drosophila*),

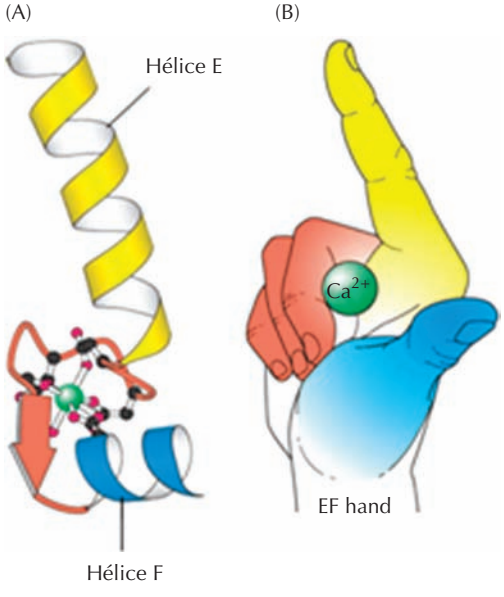

Figure 67–2. The EF-hand domain. **A.** Schematic representation of a EF-hand motif consisting of two helixes, E and F, perpendicularly placed and linked by a Ca^{2+}-binding 12 amino acid sequence. **B.** Symbolic representation of the same motif as a right hand in which the E helix corresponds to the index finger and the F helix corresponds to the thumb.

amphibians (*Xenopus tropicalis*), protists (*Trypanosomia*, *Giardia lamblia*, *Schistosoma japonicum*, *Chlamydomonas reinhardtii*), and nematodes (*C. elegans*). In *Chlamydomonas* and in mammals, these motifs are found in only two types of proteins.[17] The first are type 7 diphosphate nucleoside kinases (NDK7) exhibiting an N-terminal DM10 domain and two catalytic NDK motifs. The NDK catalyze the transfer of the adenosine triphosphate (ATP) terminal gamma-phosphate on the guanosine diphosphate (GDP) to form guanosine triphosphate (GTP) and adenosine diphosphate (ADP). These proteins play crucial roles in cellular proliferation, differentiation, development, and progression of tumors.[18] The second family consists of proteins with three DM10 domains and one C-terminal EF-hand motif, among which one finds EFHC1, its paralog EFHC2, its ortholog Rib72 in *Chlamydomonas* and EFHC1, and EFHC2 in *Drosophila*. In contrast with the NDKs, these proteins do not exhibit any enzymatic activities. Their properties remain to be elucidated; recent findings concerning this family will be reviewed below.

The phylogenetic analysis of these different proteins shows that each of the three DM10 domains is more strongly conserved between the different proteins than among them for the same protein. For instance, the first DM10 domain of EFHC1 is more similar to the first DM10 of Rib72 and EFHC2 compared with the second or third domains of EFHC1.[17] In *Chlamydomonas*, the proteins containing DM10 domains are strongly linked to the flagellar axonema.[19,20] This suggests that these domains could act as flagellar NDK regulators or as specific subunits implicated in axonemal

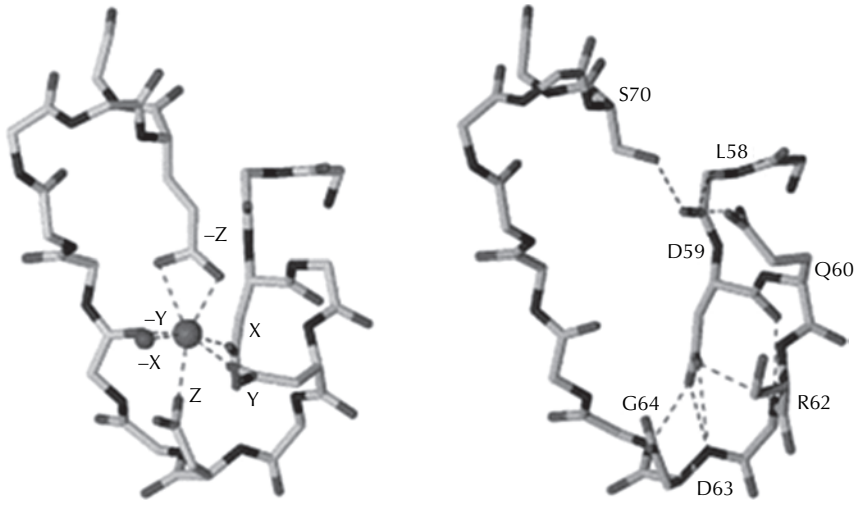

Figure 67–3. Representation of the calcium coordination binding sites by +X, +Y, +Z, -Y, -X, and -Z residues of the EF-hand motif.

assembly or in the localization of these proteins in this structure.[17]

EFHC1/MYOCLONIN1, A WIDELY DISTRIBUTED PROTEIN

So far, the transcript of the Myoclonin 1 has been detected in several structures in various adult and developing tissues or cell models in culture, using either polymerase chain reaction (PCR), Northern blotting, or in situ hybridization.

This last approach, however, produced unconvincing results in determining the presence of the transcript in several tissue, including brain, although PCR on brain tissue samples[21] and cells in culture[9] and Northern blotting from various tissues[3] unequivocally demonstrated its expression. This could indicate a particularly low level of transcription or a fast turnover of the mRNA encoding the Myoclonin1 protein.

Accordingly, using an anti-myoclonin1 polyclonal antibody, the protein EFHC1 has been, in a first approximation, detected by immunohistochemical analyses in several structures of adult mouse brain including hippocampus, cerebellum, cerebral cortex, thalamus, hypothalamus, amygdale, and upper brainstem. In addition, signals were observed in soma and dendrites of both in situ and hippocampal primary culture neurons.[3]

Four years later, however, the same authors[22] created the Efhc1 knockout mouse and, using this null-mutant mouse as a negative control, they found that the signals obtained by the above-mentioned polyclonal antibody remained in the null mutant and that the signals were largely nonspecific. Revisiting the distribution of the protein using a newly generated anti-myoclonin1 monoclonal antibody (yielding more specific myoclonin1 signals in Western blot and immunohistochemical analyses), they showed that myoclonin1 is expressed mainly at cilia on ependymal cells surrounding cerebral ventricles in the postnatal mouse brain. No significant signals were observed in other regions and cell types, including neurons and astrocytes. These observations were, therefore, inconsistent with their previous report. When the authors used a new monoclonal antibody (6A3-mAb) to mouse myoclonin1 and verified its specificity in Western blot and immunohistochemical analyses, using Efhc1-null mice as negative controls, this 6A3-mAb specifically recognized myoclonin1 protein at embryonic choroid plexus and postnatal ependymal cells. However, it should be kept in mind that the sensitivity of these analyses may not be high enough to detect moderate or low levels of myoclonin1 expression in other tissues and cells. In addition, other isoforms of myoclonin1, which the 6A3-mAb cannot recognize, may possibly be expressed in distinct tissues and cells; therefore, further studies are warranted.

We then revisited the distribution and cellular localization of EFHC1 in adult mouse brain[21] studied by immunohistochemistry using a previously well-validated anti-EFHC1 antibody.[8,9] In the adult mouse neocortex, the labeling was present in cells of all cortical layers, particularly in cells resembling pyramidal neurons in cortical layer V and in the piriform cortex. Within the hippocampus, neuronal cells in the CA and the dentate gyrus (DG) regions were stained. Expression was not restricted to the pyramidal cell layer but was also observed in cells located in the striatum oriens, radiatum, and lacunosum-moleculare. At higher magnification, the protein appeared evenly distributed in the cytoplasm. Labeling was also clearly observed in Purkinje cells of the cerebellum and, in particular, in the first-order apical dendrites. In all of these experimental conditions, the immunostaining disappeared or was considerably attenuated after preabsorption of the antibody with purified immunizing peptide.

EFHC1 immunofluorescence, in particular, was clearly observed in cerebellar Purkinje cells with labeling of the soma, the nucleus, and the dendritic arborizations extending throughout the molecular layer. A faint signal was also present in the molecular and granule cell layers. The last one was identified by colabeling using anti-NeuN antibody.

Double immunofluorescence was performed in order to specify the nature of EFHC1-expressing cells in the adult mouse cortex. A count of the number of double- labeled cells indicated that the majority (78%) of EFHC1-positive cells express NeuN and therefore correspond to neurons. In addition, 20% of EFHC1-positive cells express glial fibrillary acidic protein (GFAP) and thus correspond to astrocytes. The labeling was also present in the proximal portion of astrocytic

processes. Another way to illustrate the eventual expression of this gene by neural structures is the use of nerve cell lines in culture. We used this approach extensively and showed clear synthesis of EFHC1 by mouse neuroblastoma cells (N2A cells), as illustrated in the past with HEK-293, HeLa, and COS cell lines.[9] Furthermore, in order to establish how early in culture the *EFHC1* gene was expressed, we investigated its presence in neural stem cells (NSCs). These NSCs were prepared from embryonic mice striata, as previously described.[23] They were allowed to form neurospheres. First, the expression of the *EFHC1* transcript was confirmed by reverse transcriptase-PCR (RT-PCR). Then double immunofluorescence labeling was performed using anti-nestin, a neural stem cell marker, and anti-EFHC1 antibodies. EFHC1 labeling was present in all cells composing neurospheres. Together, these results clearly demonstrate the ability of neuronal cells both in vivo and in vitro to synthesize EFHC1 from the earliest stages of development.

EFHC1/MYOCLONIN1 CALCIUM SIGNALING AND APOPTOSIS

To investigate the functional significance of EFHC1 and its mutants in neurons, Suzuki et al.[22] transfected mouse hippocampal primary culture neurons with enhanced green fluorescent protein (EGFP)-EFHC1 expression constructs. EFHC1-positive neurons had shorter neurites and fewer branches 16 h after transfection and showed signs of neurodegeneration and cell death, including shrinkage of the cell body and fragmentation of processes 48 h after transfection, whereas control cultures seemed to be healthy. Cells transfected with EFHC1 were terminal deoxynucleotidyl deoxyuridine triphosphate nick and labeling (TUNEL)-positive, indicative of apoptosis. Next, Suzuki et al. investigated the effects of EFHC1 mutations on cell survival by counting green fluorescent protein (GFP)-positive surviving cells attached to the dishes at various time points, irrespective of cellular morphologies. The cell-death effect of EFHC1 was substantially reduced by any of the five mutations associated with JME and by the double mutation 229C → A and 662G → A. In contrast, the three coding polymorphisms that were also present in the control population did not affect cell death significantly. Although the number of surviving cells transfected with mutations associated with JME seemed close to that of vector-transfected cells, the cells transfected with mutations associated with JME had unhealthy morphology 48 h after transfection, implying that the mutations did not disrupt EFHC1 function completely. Suzuki et al. also analyzed the effects of EFHC1 isoforms on cell survival. Transfection with a construct-expressing transcript B resulted in moderate cell death, and coexpression of the wild-type transcript and transcript B had intermediate effects. The cellular functions of the protein encoded by transcript B are not known, but the fact that this isoform excludes the mutation 757G → T from its open reading frame suggests that this isoform may not have a large role in the pathogenesis of JME.

As already mentioned, the EF-hand superfamily regulates many aspects of cell function, such as Ca^{2+} buffering in the cytosol, cell proliferation, and signal transduction. In the central nervous system, calmodulin and the related EF-hand containing neuronal Ca^{2+}-sensor proteins have many important roles in neuronal signaling. The basic functional unit consists of a pair of EF-hand motifs, but in the case of human EFHC1, it is not clear if it is present in pairs or not.

Murai et al.[24] reported the first structural and thermodynamic analyses of human EFHC1C-terminus containing the last DM10 domain and the EF-hand motif. The target protein was expressed mainly in soluble form and the purification protocol, including tag removal, was successfully established. The final purified protein demonstrated high stability. The secondary structure was measured by circular dichroism (CD) spectroscopy, showing 34% of α-helices and 17% of β-strands. It was demonstrated that the protein can form dimers in the absence of dithiothreitol (DTT). Tandem mass spectrometry (MS/MS) analysis with the help of the SearchXLinks program suggests that Cys575 participates in intermolecular S–S bond formation. In addition, a dithionitrobenzoic acid (DTNB)assay showed that reduced EFHC1C has only one accessible free thiol per molecule. Isothermal titration calorimetry (ITC) data showed that reduced EFHC1C binds to just one divalent ion (Ca^{2+} or Mg^{2+}), whereas in an oxidized (dimeric) state, EFHC1C has its ion binding site blocked.[24]

To date, it is not clear if EFHC1 is a Ca^{2+}-sensor or a Ca^{2+}-buffer protein. However, full EFHC1 presents 19% identity and 44% similarity with the Ca^{2+} regulator calmodulin, the classical example of an EF-hand protein that suffers conformational changes after ion binding. Additional experiments should be performed in order to better determine the influence of Ca^{2+} or Mg^{2+} binding in the EFHC1 dimerization process. Whether dimerization is a physiological conformation remains to be elucidated.

Because EFHC1 contains a Ca^{2+}-sensing EF-hand motif and because abnormalities of voltage-dependent Ca^{2+} channels (VDCCs) have been described in human and mouse epilepsies, Suzuki et al.[22] investigated whether the observed cell death is due to modulations of VDCCs. Reverse transcriptase-PCR showed that most of the VDCC subtypes were expressed in mouse primary culture neurons, albeit at varied levels. Treatment of EFHC1-transfected primary culture neurons with several antagonists of VDCC subtypes indicated that SNX-482, the antagonist for $Ca_v2.3$, specifically increased the survival rate of EFHC1-positive neurons.

Moreover, patch-clamp analyses of BHK cells stably expressing $Ca_v2.3$ and transiently transfected with EFHC1 showed that EFHC1 substantially increased the R-type Ca^{2+} current generated by $Ca_v2.3$.[3] Cotransfection with constructs expressing P/Q-type VDCC ($Ca_v2.1$) and EFHC1 did not increase Ca^{2+} currents. The effects of EFHC1 on $Ca_v2.3$ were extensive and unique, even when compared with the effects of the auxiliary subunits of the Ca^{2+} channels. These results suggest that EFHC1 enhances Ca^{2+} influx through $Ca_v2.3$ and stimulates programmed cell death.

Mutations associated with JME partly reversed the increase in R-type Ca^{2+} currents by EFHC1. Incomplete reversal of EFHC1-induced Ca^{2+} influx through $Ca_v2.3$ may be responsible for the precarious state of calcium homoeostasis sensitive to the triggering effects of sleep deprivation, fatigue, and alcohol in individuals with JME. The three coding polymorphisms had weaker or no reversal effects, implying that they could be functionally benign or less malignant. Transfection with the transcript B isoform moderately increased the Ca^{2+} current. These results were consistent with those of the cell-death analyses.[3]

EFHC1/MYOCLONIN1 IN MITOSIS AND CELL DIVISION

EFHC1 Colocalizes with Mitotic Spindles

We have shown[9] that the subcellular localization of EFHC1 varied during the cell cycle. The most prominent finding was the association with the mitotic spindle and the midbody. This was observed in different cell lines (HEK293, COS-7, HeLa, NIH3T3, SKNBE) for the overexpressed EGFP-tagged and the endogenous protein. Those results suggest the involvement of EFHC1 in cell division and, in particular, in the organization of the mitotic spindle and the midbody. However, during mitosis, a fraction of EFHC1 remains localized in the cytoplasm, suggesting that only a portion of the protein is recruited by the mitotic apparatus, as is the case for other mitotic apparatus associated proteins.

Colocalization of EFHC1 with α-tubulin was observed at the mitotic spindle, suggesting a possible interaction with microtubules. Such an association was suggested for the *Chlamydomonas* protein Rib72 in flagellar axoneme.[19,20] As the mitotic spindle, axoneme, and cilia are microtubule-based structures, this further supports the hypothesis of a possible interaction of EFHC1 with microtubules.

In nondividing cells, we consistently observed a strong accumulation of EGFP-tagged and endogenous protein in one (occasionally two) perinuclear spot(s). Using anti-γ-tubulin antibody, we identified those spots as centrosomes, suggesting that EFHC1 could be a centrosomal component. This is further supported by the proteomic analysis showing the presence of the *Chlamydomonas* Rib72 in centrioles, a centrosome component.[25] Moreover, the concentration of EFHC1 at spindle poles during mitosis, where centrioles are located, suggests involvement in the functioning and/or organization of the mitotic spindle. Molecular components common to the centrosome and the mitotic spindle have been described.[26–29]

Deletion analyses of EFHC1 demonstrated that its N-terminal portion (construct N92) is required and sufficient for its association with the mitotic spindle and the midbody. In light of our observations made with truncated EFHC1

constructs in mitotic cells, the role of DM10 domains remains unclear.

The association of EFHC1 with the mitotic apparatus and the centrosome constituted at this time a clear, new, and original finding. Correlation between our observations and previous findings, demonstrating the association of the *Chlamydomonas* Rib72 with flagellar axoneme and mRib72-1/Efhc1 with motile cilia and flagella, further supported the concept of EFHC1 interaction with microtubule-based structures. Other axonemal proteins have also been found to be associated with the mitotic spindle and/or the centrosome and vice versa.[25,30–33]

In other respects, our hypothesis of a potential involvement of EFHC1 in cell division is supported by several arguments. It was shown that EFHC1 transcript expression in the mouse brain is higher during embryogenesis, when cell division is prominent, than in the adult brain.[3,10,34] The EFHC1 promoter was also demonstrated to be a putative target of the E2F4 transcription factor,[35] a member of the E2F transcription factor family that plays a crucial role in cell cycle progression and is therefore important in cellular proliferation. Moreover, EFHC1 transcript expression is modulated during the HeLa cell cycle.[36]

EFHC1 Is a Microtubule-Associated Protein (MAP)

To determine more precisely which region of EFHC1 mediates this association, we expressed various EGFP-tagged truncated EFHC1 proteins in HEK293 cells and carried out immunocytochemistry with an antibody to α-tubulin.[11] We found that EGFP-hN45, but not EGFP-hN30, colocalized with the mitotic spindle, suggesting that the first 45-amino-acid region contains a motif that is required for the localization of EFHC1 with the mitotic spindle.

This colocalization prompted us to investigate whether EFHC1 interacts with α- and/or γ-tubulin. Using immunoprecipitation procedures on HEK293 cell lysates, we found that EFHC1 and α-tubulin coprecipitated mutually and that EFHC1 did not interact with γ-tubulin. To determine whether EFHC1 interacts directly with α-tubulin, we used in vitro cosedimentation assays using pure prepolymerized microtubules and different purified glutathione S-transferase (GST)-tagged truncated EFHC1 proteins. We found that GST-hEFHC1 and GST-hN45, but not GST-hN30 and GST, cosedimented with microtubules, indicating that there was a direct interaction between EFHC1 and α-tubulin through the first 45-amino-acid region. This association was not affected by the presence of Ca^{2+}. We therefore estimated the affinity of EFHC1 for microtubules and found an equilibrium dissociation constant of about 1.5 μM, indicating that EFHC1 binds to microtubules with relatively high affinity. Finally, we carried out in vitro tubulin polymerization assays with GST-hEFHC1. We did not detect a substantial change in either the polymerization rate or the nucleation phase in the presence of GST-hEFHC1. This indicates that EFHC1 is not required for the polymerization of microtubules in vitro. It is noteworthy that the region involved in the binding to α-tubulin (i.e., the first 45 amino acids) showed no obvious homology with the microtubule-binding domains (MTBDs) of other known MAPs. Thus, EFHC1 would appear to be a unique MAP, unrelated to conventional previously described MAPs.[11]

EFHC1 Is Required for Mitotic Spindle Organization

In addition to its association with α-tubulin, we found that overexpression of EGFP-hN92, EGFP-hN60, or EGFP-hN45 in HEK293 cells resulted in severe mitotic spindle defects, including monopolar spindle and chromosome alignment failure during metaphase. We quantified their occurrence in cultures overexpressing different EGFP-tagged EFHC1 proteins and found that the percentage of mitotic defects was significantly higher for EGFP-hN92, EGFP-hN60, and EGFP-hN45 compared with EGFP-hEFHC1 or EGFP.

Notably, truncated forms that did not associate with the mitotic spindle did not promote spindle defects, suggesting that binding to microtubules is necessary for this phenotype. EGFP-hDM101, which contains both the N-terminal region and the first DM10 domain, failed to induce mitotic spindle defects. This suggests that at least the first DM10 domain is required for proper EFHC1 function at the

mitotic spindle. Moreover, we observed that monopolar spindles showed an abnormal spherical distribution of chromosomes around a pair of closely and centrally located centrosomes, indicating that the centrosome was duplicated but that there was an insufficient separation to form a bipolar spindle. In the next experiments, we only used the EGFP-hN45 protein, as it produced, in our opinion, the most important phenotype. Using an antibody to the C terminus of EFHC1, we observed that EGFP-hN45 saturated the microtubule's EFHC1 binding sites and, as such, acted as a dominant-negative protein.

Moreover, we transfected HEK293 cells with validated shRNAs and observed a significant increase in the number of disorganized mitotic spindles with the presence of monopolar spindles and misaligned metaphase compared with controls. Expression of rEFHC1, an shRNA-resistant form, rescued the mitotic spindle defects, demonstrating the specificity of the shRNA effect.

Finally, we found that the mitotic index was significantly higher in cultures expressing EGFP-hN45 and hEFHC1 shRNA than in control cultures. In contrast with control cells that were distributed across all mitotic phases, EGFP-hN45- and hEFHC1 shRNA-expressing cells were mostly arrested at prometaphase, with a concomitant decrease in the number of cells in anaphase/telophase and cytokinesis, resulting in subsequent M-phase delay or arrest. Collectively, these results obtained by independent approaches (RNAi and dominant-negative protein) strongly suggest that EFHC1 is involved in mitotic microtubule organization and M-phase progression.[11]

EFHC1 Impairment Induces Microtubule Bundling and Apoptosis

During interphase, EGFP-hN45 and hEFHC1 shRNA expression markedly induced microtubule bundling around the cell periphery. These bundled microtubules were extremely stable and did not depolymerize in the presence of nocodazole. Microtubule bundling was significantly higher in cultures expressing EGFP-hN45 and hEFHC1 shRNA than in those expressing EGFP-hEFHC1, EGFP, or control shRNA. Moreover, using in vitro microtubule bundling assays, we found that GST-hN45, but not GST-hEFHC1 or GST, induced microtubule bundling, indicating that the MTBD of EFHC1 is capable of promoting microtubule bundling formation in vivo and in vitro when expressed alone.

Moreover, in cultures with impaired EFHC1 function, we observed frequent occurrences of nuclear abnormalities such as fragmented and picnotic nuclei, as seen in apoptotic cells. Therefore, we carried out TUNEL assays and found a significant increase of apoptosis in cells overexpressing EGFP-hEFHC1, EGFP-hN45, and hEFHC1 shRNA compared with the EGFP control. Finally, to determine the cell cycle phase distribution of apoptotic cells expressing EGFP or EGFP-hN45, we sorted apoptotic EGFP-positive cells by flow cytometry and carried out cell cycle analysis. We observed a significant enhancement of EGFP-hN45 cells in S and G2/M phases, with a concomitant decrease of cells in the G0/G1 phases compared with EGFP controls, indicating that mitotic spindle defects induced apoptosis.[11]

EFHC1/MYOCLONIN1 AND EARLY NEUROBLAST MIGRATION

EFHC1 Contributes to Radial Migration and Radial Glia Integrity in the Developing Neocortex

On the basis of previous studies reporting higher expression of EFHC1 during brain development,[3,10,34] and on the basis of its key role in cell division, we further investigated the role of EFHC1 in cerebral cortex development by modulating the expression of rEFHC1 in ventricular zone (VZ) cells of the rat developing neocortex using both in utero and ex vivo electroporation (Fig. 67–4). In order to inactivate rEFHC1 functions through RNAi-mediated depletion, we validated the effectiveness of the rEFHC1 shRNA vector by Western blot on extracts from the microdissected ventricular/subventricular zone (VZ/SVZ) after ex vivo electroporation.

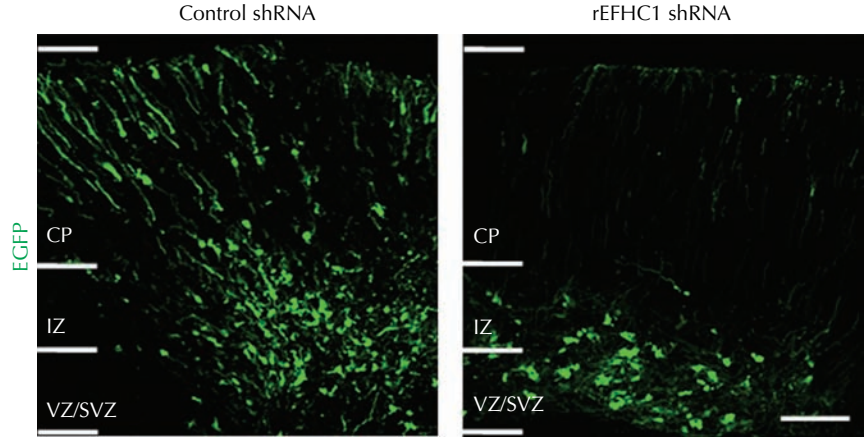

Figure 67–4. Role of EFHC1 in radial neuronal migration. Distribution of EGFP-positive cells (in green) in different cortical regions (VZ/SVZ, IZ, and CP) 4 days after ex vivo electroporation of rat brains at E17 with control shRNA (left) and rEFHC1 shRNA (right) showing significant disruption of neuroblast migration toward the CP when EFHC1 synthesis is invalidated in the cells. Scale bars represent 200 μm (see ref. 11 for more details).

To determine whether EFHC1 influences the radial migration of neocortical neurons in rat neocortex, we examined the position of EGFP$^+$ cells in the VZ/SVZ, intermediate zone (IZ), or cortical plate (CP) 1 and 4 d after ex vivo electroporation at E17 of plasmid encoding Con shRNA, rEFHC1 shRNA, EGFP-hN45, rEFHC1 shRNA, and EGFP-hEFHC1 or EGFP-hEFHC1 alone. On day 1, cells were mostly located within the VZ/SVZ, with no obvious difference in the position of cells in the five different transfected conditions. By day 4, the expression of EGFP-hN45 and rEFHC1 shRNA induced a dramatic disruption of radial migration compared to Con shRNA (Fig. 67–4). In the presence of rEFHC1 shRNA, most of the cells were localized in the VZ/SVZ, whereas few cells reached the CP. RNAi-mediated alteration of radial migration was rescued by the expression of EGFP-hEFHC1, although expression of EGFP-hEFHC1 alone had no significant effect. When electroporated with EGFP-hN45, cells migrated out of the VZ/SVZ but failed to enter the CP and thus accumulated in the IZ. It is interesting to note that similar results have been observed after in utero electroporation of rEFHC1 shRNA (Fig. 67–4). Thus, the impairment of EFHC1 appeared to have a direct effect on radial redistribution to the CP.

Furthermore, immunohistochemistry with anti-brain lipid-binding protein (BLBP) antibody revealed a disruption of extension of radial glial processes with concomitant irregular accumulation of cells strongly stained for BLBP in the VZ/SVZ.

Together, these results indicate a crucial role for EFHC1 in maintaining the integrity of the radial fiber network.

Finally, expression of rEFHC1 shRNA or EGFP-hN45 induced structural disorganization of VZ/SVZ cells; many cells presented an enlarged multipolar morphology and lacked or had only a very short leading process, sometimes not radially oriented. Moreover, we observed the presence of many round-shaped cells with condensed chromosomes, suggesting apoptotic cells. This conclusion was supported by the decreased number of EGFP$^+$ cells in the VZ/SVZ of cortices expressing rEFHC1 shRNA and EGFP-hN45. To test this hypothesis, we performed TUNEL assays and found a significantly increased number of apoptotic cells in brain slices with impaired EFHC1 function.[11]

EFHC1 Is Essential for Mitosis and Cell Cycle Exit of Cortical Progenitor Cells

Since cortical progenitor cell division occurs prior to and is tightly coupled to neuronal migration, we decided to study the influence of EFHC1 on mitosis, proliferation, and cell

cycle exit of cortical progenitors by ex vivo electroporation on E17 rat brains. We analyzed the effect of EGFP-hEFHC1, EGFP-hN45, and rEFHC1 shRNA expression on the spindle mitotic organization of VZ/SVZ cortical progenitors. We observed that both EGFP-hEFHC1 and EGFP-hN45 associated with the mitotic spindle, although EGFP-hN45 and rEFHC1 shRNA induced mitotic spindle defects similar to those observed in vitro (monopolar spindles and misaligned chromosomes at metaphase). To assess whether these abnormalities influence mitotic progression, we performed immunohistochemistry with anti-phospho-Histone H3 (pH3) antibody, a mitotic marker. The mitotic index (the percentage of transfected cells in mitosis) was significantly increased by impairment of EFHC1 function compared to Con shRNA. This accumulation of mitotic cells strongly suggests an M-phase arrest/delay of progenitor cells, as we observed in vitro. Moreover, pH3 immunoreactivity showed ectopic localization of mitotic chromosomes associated with an EFHC1 defect. Whereas most Con shRNA mitotic chromosomes were normally localized along the ventricular lumen, many EFHC1-deficient mitotic chromosomes were found in the region above the ventricular surface.

Furthermore, we explored whether EFHC1 controls the rate of cell cycle exit of cortical progenitors. For this purpose, brains slices were exposed to a short pulse of bromodeoxyuridine (BrdU; 1 h) 24 h after ex vivo electroporation and fixed 24 h later. Triple immunolabeling with antibodies directed against GFP, BrdU, and Ki67 allowed us to establish the cell cycle exit index (the percentage of transfected cells that were exiting the cell cycle within 24 h). We found that the cell cycle exit index was significantly decreased in brains slices expressing rEFHC1 shRNA.

Finally, to quantify the effect of the EFHC1 deficiency on the cortical progenitor population, we determine the percentage of transfected cells expressing Sox2, a progenitor marker, using immunohistochemistry. The fraction of VZ/SVZ cells expressing Sox2 was significantly enhanced by EFHC1 inhibition.

Together, these data suggest that the impairment of EFHC1 function in the developing neocortex interferes with both the cell division and cell cycle exit of cortical progenitors, leading to their accumulation.[11]

EFHC1 Is Required for Locomotion of Postmitotic Neurons

Cortical development is a complex process involving a strictly regulated sequence of neuronal proliferation, differentiation, and migration to specific cell layers.[37] Our results identified an influence of EFHC1 on proliferation and differentiation of cortical progenitors. To investigate the involvement of EFHC1 in radial migration of postmitotic neurons, we performed focal electroporation of plasmid-encoding Con shRNA or rEFHC1 shRNA in the IZ of brain slices at E19 after ex vivo electroporation of red fluorescent protein (RFP) plasmid to label VZ/SVZ progenitors cells. This method allowed us to differentiate progenitors ($EGFP^+/RFP^+$) from postmitotic neurons ($EGFP^+/RFP^-$). We analyzed the cortical scattering of $EGFP^+/RFP^-$ cells 2 days after electroporation using an arbitrary scale divided into 10 bins. Compared to Con shRNA, expression of rEFHC1 shRNA impaired cortical redistribution of postmitotic neurones in the CP, as the majority of cells were located in bins corresponding to the upper IZ, with a concomitant decrease in the number of cells in the CP. In order to confirm these results, we performed ex vivo electroporation at E17 of plasmid-encoding EGFP or EGFP-hN45 under the control of the NeuroD promoter, allowing the expression of protein only in postmitotic neurons. Cortical scattering of $EGFP^+$ cells was analyzed 4 days after electroporation. Compared to EGFP, only a subset of cells had reached the lower CP in brains expressing EGFP-hN45. These results suggest that EFHC1 is also implicated in the locomotion of postmitotic neurons.[11]

EFHC1/MYOCLONIN1, A KEY CILIAR COMPONENT PLAYING A ROLE IN THE FUNCTION OF EPENDYMA

Myoclonin1 is a homolog of P72/Rib72, a flagellar protein of *Chlamydomonas*,[19,20] P72/Rib72 is thought to have important roles in assembly and function of the axoneme. Indeed, it has been shown that Rib72 is an essential component of the flagellar axonemal ribbon (a stable structure formed by protofilaments

of tubulin)[20] that is implicated in calcium-dependent flagellar motility.[19] On the other hand, Rib72 homologs have been observed in organisms containing motile cilia or flagellas, such as humans, mice, sea urchins, and *Drosophila.* Moreover, the mice ortholog of EFHC1 is expressed in many tissues with motile cilia and flagellas, such as lung, testis, and ependymal cells.[8,21,22] Interestingly, in brain, the ependymal cells lining the border of the ventricles are highly ciliated and support, in the adult, a rostral-directed cerebrospinal fluid (CSF) flow pulling along newly forming neuroblasts.[38] One can postulate that a default of EFHC1 highly expressed in these cilia could lead to abnormal migration of newly formed neuroblasts in the adult, modifying the cytoarchitectony of the gabaergic (gamma-aminobutyric acid) cortical interneurons as an epileptogenic mechanism.[17]

Suzuki et al.[22] raise intriguing possibilities that "(choroid) plexusopathy" or "ciliopathy" is the pathological basis of epilepsies caused by the EFHC1 mutations. They found myoclonin1 expression on the hindbrain at the E10 stage and on the choroid plexus at the E14 stage. The surface of choroid plexus is comprised of the choroid epithelium (derived from the same layer of cells that forms the ependymal lining of the ventricles).[39] Since these epithelium cells do not have cilia on the cell surface at the embryonic stages, myoclonin1 might have roles (e.g., CSF secretion) in addition to promoting postnatal ciliary function. Cerebrospinal fluid maintains homeostasis in the extracellular environment of neurons and glial cells.[39]

In 2009, the same authors generated viable *Efhc1*-deficient mice.[40] Most of the mice were normal in outward appearance, and both sexes were found to be fertile. However, the ventricles of the brains were significantly enlarged in the null mutants but not in the heterozygotes. Although the ciliary structure was found to be intact, the ciliary beating frequency was significantly reduced in the null mutants. In adult stages, both the heterozygotes and null mutants developed frequent spontaneous myoclonus. Furthermore, the threshold of seizures induced by pentylenetetrazol was significantly reduced in both heterozygotes and null mutants. These observations seem to further suggest that a decrease or loss of function of myoclonin1 may be the molecular basis for epilepsies caused by *EFHC1* mutations.

THE MAP MYOCLONIN1 IS ESSENTIAL FOR THE NORMAL DEVELOPMENT AND FUNCTION OF THE NEUROMUSCULAR JUNCTION SYNAPSE IN *DROSOPHILA*

Very recently, one of the two *Drosophila* homologs of EFHC1, CG8959, named Defhc1, was functionally characterized.[40a] In agreement with studies in mammalian systems, the investigators found that Defhc1 is capable of binding to microtubules. Transfected Defhc1 distributed both to the cytoplasm and to the nucleus of resting cells, while in dividing cells the protein localized to the mitotic spindle. Defhc1 knockout *Drosophila* displayed normal appearance and behavior. However, the morphology of the neuromuscular junction synapse appeared aberrant, with an increase in the number of satellite boutons, structures that have been regarded as potential ramifications. Defhc1 is capable of binding to microtubules and overlaps in vivo with axonal microtubules. In the neuromuscular junction synapse, disruption of Defhc1 function leads to a decrease in the number of microtubule loops, whose presence correlates with halted bouton division, suggesting that Defhc1 is a negative regulator of this process. The investigators also showed that the increase in satellite boutons requires an intact microtubule cytoskeleton and is accompanied by an increase in spontaneous neurotransmitter release. This important work further shows that loss of Defhc1 induces overgrowth of the dendritic arbor, and in vivo overexpression of Defhc1 in dendritic neurons substantially reduces dendrite elaboration. These results suggest that, at least in this model, Defhc1 functions as an inhibitor of neurite growth by finely tuning microtubule cytoskeleton dynamics. As a corollary, these findings also suggest that the susceptibility stage of EFHC1-dependent JME may result from augmented spontaneous neurotransmitter release due to overgrowth of neuronal processes.

EFHC1 OR MYOCLONIN1 DEFECT CAUSES JME AS A DEVELOPMENTAL DISEASE AFFECTING THE PROPERTIES OF THE MICROTUBULES IN THE NEURONO-GLIAL PROCESSES AND CORTICAL SYNAPTIC FUNCTIONS (MICROTUBULOPATHY)

The works reviewed above all converge to a clear demonstration: *EFHC1* or *Myoclonin 1,* a gene significantly mutated in JME families, is a new MAP. Microtubules affect multiple facets of neuronal development and function, with prominent roles in the growth and maintenance of axons and dendrites[41] but also in the fine tuning of synaptogenesis. Here we will cite some examples from our previously mentioned data supporting the above concept.

As discussed, the mitotic index (the percentage of transfected cells in mitosis) was significantly increased by impairment of EFHC1 function compared to Con shRNA. This accumulation of mitotic cells in VZ/SVZ strongly suggests an M-phase arrest/delay of progenitor cells, as we observed in vitro. The nuclei of the radial glia progenitors (RGPs) perform interkinetic migration along the apico-basal axis in relation to the different phases of the cell cycle. The mitotic cells are located at the border of the ventricular lumen, and the nuclei of the cells in S phase are located in the half basal part of the VZ. The microtubules, the centrosome, and certain MAPs contribute to this important process. It has been shown that the proteins that interact with dynein, lissencephaly 1 (LIS1),and dynactin,[42,43] as well as the centrosomal proteins Cep 120 and TACC,[44] are necessary for the movement of the nuclei from their basal position toward the apical centrosome during the G2 phase. Their dysregulation leads to the occurrence of ectopic cellular divisions. Since EFHC1 appears to be an essential centrosomal MAP, it could play a key role in this mechanism. The nuclei of the EFHC1-depleted cells would then be unable to achieve their G2 phase baso-apical migration, being forced to divide in the basal position and to acquire the basal progenitor phenotype,[45]

Another example concerns the radial glia. These cells play a crucial role during corticogenesis in controlling two important processes of radial migration: somatic translocation and cellular locomotion,[46,47] Defaults in these processes lead to migration defects and disturbances in laminar cortical organization.[48,49] Dynamic regulation of the microtubules by certain MAPS, like MAP1a, MAP4, MAP7, or the tektins, plays a clear role in the extension of the radial glia processes.[50] In addition, the deletion of one of these proteins, doublecortin-like kinase (DCLK), disturbs the cytoarchitectony of the radial glia scaffold.[51] Again, we have shown that invalidation of EFHC1 disrupts the morphology of radial glia organization: the bipolar structure disappears, the cellular extensions are significantly reduced, and sometimes even the radial organization is disrupted. This, in part, can be responsible for the default of migration observed in the postmitotic neuronal progenitors.

The locomotion of these neurons within the VZ is also reduced in cases where EFHC1 is deleted. Migration by locomotion consists of the extension of the anterior process (nucleokinesis) and the retraction of the posterior one. Again, recent studies have shown a key role of lissencephaly 1 (LIS1), dynein, nuclear distribution gene E homolog (A. nidulans)-l (NDEL1), and doublecortin (DCX) in the coupling of the nucleus and centrosome during this phenomenon.[52,53] As a MAP associated with the centrosome, EFHC1 could be implicated in this mechanism. This hypothesis is reinforced by the fact that movement of the nucleus toward the centrosome during interkinetic nuclear migration, nucleokinesis, and locomotion needs the support of the microtubules and the same regulatory proteins.

In summary, all the phenotypes observed with the functional defaults of EFHC1 in the neocortical structures during early brain development mimic those observed with other MAPs, including LIS1, NDEL1, and DCLK. Whether or not these molecules are privileged partners of EFHC1 has still to be determined.

Finally, the *Drosophila* neuromuscular junction synapse has been a useful genetic system in which to assay the role of the microtubule cytoskeleton and model its role in inherited neurological disease. For instance, mutations in the microtubule-interacting protein Futsch (MAP1b) alter synaptic morphology, and VAP-33A regulates synaptic bouton sprouting through interactions with the microtubule cytoskeleton.[54–56] Atypical protein kinase C has been shown to

control bouton formation by regulating the synaptic cytoskeleton,[57] and the hereditary spastic paraplegia genes *spastin* and *spichthyin* regulate synaptic structure and growth through the control of axonal microtubules.[58–61] Furthermore, the *Drosophila fragile X-related* gene has been shown to regulate Futsch/MAP1b, thereby modulating both synaptic architecture and neurotransmission strength.[62]

Microtubules are essential structural components of dendrites as well, and several genes have been reported to influence dendrite growth and arbor expansion through their action on microtubules. For example, *stathmin* regulates dendrite arborization by controlling the dendritic microtubule dynamics,[63] and knockdown of doublecortin, a microtubule-associated protein, in hippocampal neurons results in reduced branching, length, and complexity of the dendrites.[64] Furthermore, Very-(KIND), a KIND domain-containing RasGEF, controls dendrite growth through regulation of microtubule-associated protein 2.[65] Support for an important role of microtubules in regulating dendritic arbor shape has also come from studies in flies demonstrating that in dendritic arborization neurons the Knot transcription factor mediates control of microtubule-based arbor outgrowth by inducing expression of the microtubule-severing protein spastin.[66]

Together, these studies indicate that microtubules play key functions in the control of neurite structure and function, and that mutations perturbing microtubule dynamics are causatively linked with inherited neurological disorders that could therefore be called *microtubulopathies.*

CONCLUSIONS

For the first time, a gene responsible for an idiopathic generalized epilepsy (IGE) has been found to encode a MAP utilizing an unusual MTBD and not an ionic channel. Thus, beside the classical theory of channelopathies causing IGE, our work opens new perspectives in the identification of the precise molecular and cellular mechanisms of IGE, including subtle pathologies of early neuronal migration (microdysgenesis) and/or synaptogenesis. We hypothesize that mutations of *EFHC1* induce subtle neuronal migration or synaptic formation defects that lead to abnormal epileptogenic circuitry during cortical maturation. Eventually, the elucidation of such mechanisms will provide new targets for better pharmacological treatment of epilepsy.

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REFERENCES

1. Delgado-Escueta AV, Enrile-Bacsal F. Juvenile myoclonic epilepsy of Janz. *Neurology.* 1984;34:285–294.
2. Delgado-Escueta AV. Advances in genetics of juvenile myoclonic epilepsies. *Epilepsy Curr.* 2007;7:61–67.
3. Suzuki T, Delgado-Escueta AV, Aguan K, et al. Mutations in EFHC1 cause juvenile myoclonic epilepsy. *Nat Genet.* 2004;36:842–849.
4. Ma S, Blair MA, Abou-Khalil B, et al. Mutations in the GABRA1 and EFHC1 genes are rare in familial juvenile myoclonic epilepsy. *Epilepsy Res.* 2006;71:129–134.
5. Stogmann E, Lichtner P, Baumgartner C, et al. Idiopathic generalized epilepsy phenotypes associated with different EFHC1 mutations. *Neurology.* 2006;67: 2029–2031.
6. Annesi F, Gambardella A, Michelucci R, et al. Mutational analysis of EFHC1 gene in Italian families with juvenile myoclonic epilepsy. *Epilepsia.* 2007;48: 1686–1690.
7. Medina MT, Suzuki T, Alonso ME, et al. Novel mutations in Myoclonin1/EFHC1 in sporadic and familial juvenile myoclonic epilepsy. *Neurology.* 2008;70: 2137–2144.
8. Ikeda T, Ikeda K, Enomoto M, et al. The mouse ortholog of EFHC1 implicated in juvenile myoclonic epilepsy is an axonemal protein widely conserved among organisms with motile cilia and flagella. *FEBS Lett.* 2005;579:819–822.
9. de Nijs L, Lakaye B, Coumans B, et al. EFHC1, a protein mutated in juvenile myoclonic epilepsy, associates with the mitotic spindle through its N-terminus. *Exp Cell Res.* 2006;312:2872–2879.
10. Grisar T, de Nijs L, Chanas G, et al. Some genetic and biochemical aspects of myoclonus. *Neurophysiol Clin.* 2006;36:271–279.

11. de Nijs L, Leon C, Nguyen L, et al. EFHC1 interacts with microtubules to regulate cell division and cortical development. *Nat Neurosci.* 2009;12:1266–1274.
12. Kretsinger RH, Nockolds CE. Carp muscle calcium-binding protein. II. Structure determination and general description. *J Biol Chem.* 1973;248:3313–3326.
13. Lewit-Bentley A, Rety S. EF-hand calcium-binding proteins. *Curr Opin Struct Biol.* 2000;10:637–643.
14. Ikura M. Calcium binding and conformational response in EF-hand proteins. *Trends Biochem Sci.* 1996;21: 14–17.
15. Cates MS, Teodoro ML, Phillips GN Jr. Molecular mechanisms of calcium and magnesium binding to parvalbumin. *Biophys J.* 2002;82:1133–1146.
16. Gifford JL, Walsh MP, Vogel HJ. Structures and metal-ion-binding properties of the Ca^{2+}-binding helix-loop-helix EF-hand motifs. *Biochem J.* 2007;405:199–221.
17. King SM. Axonemal protofilament ribbons, DM10 domains, and the link to juvenile myoclonic epilepsy. *Cell Motil Cytoskeleton.* 2006;63:245–253.
18. Roymans D, Willems R, Vissenberg K, et al. Nucleoside diphosphate kinase beta (Nm23-R1/NDPKbeta) is associated with intermediate filaments and becomes upregulated upon cAMP-induced differentiation of rat C6 glioma. *Exp Cell Res.* 2000;261:127–138.
19. Patel-King RS, Benashski SE, King SM. A bipartite Ca^{2+}-regulated nucleoside-diphosphate kinase system within the Chlamydomonas flagellum. The regulatory subunit p72. *J Biol Chem.* 2002;277:34271–34279.
20. Ikeda K, Brown JA, Yagi T, et al. Rib72, a conserved protein associated with the ribbon compartment of flagellar A-microtubules and potentially involved in the linkage between outer doublet microtubules. *J Biol Chem.* 2003;278:7725–7734.
21. Léon C, de Nijs L, Chanas G, et al. Distribution of EFHC1 or Myoclonin 1 in mouse neural structures. *Epilepsy Res.* 2010;88:196–207.
22. Suzuki T, Inoue I, Yamagata T, et al. Sequential expression of Efhc1/myoclonin1 in choroid plexus and ependymal cell cilia. *Biochem Biophys Res Commun.* 2008;367:226–233.
23. Bez A, Corsini E, Curti D, et al. Neurosphere and neurosphere-forming cells: morphological and ultrastructural characterization. *Brain Res.* 2003;993:18–29.
24. Murai MJ, Sassonia RC, Zamboni AH, et al. Characterization of the C-terminal half of human juvenile myoclonic epilepsy protein EFHC1: dimer formation blocks Ca^{2+} and Mg^{2+} binding to its functional EF-hand. *Arch Biochem Biophys.* 2008;477:131–138.
25. Keller LC, Romijn EP, Zamora I, et al. Proteomic analysis of isolated chlamydomonas centrioles reveals orthologs of ciliary-disease genes. *Curr Biol.* 2005;15:1090–1098.
26. Gartner W, Rossbacher J, Zierhut B, et al. The ATP-dependent helicase RUVBL1/TIP49a associates with tubulin during mitosis. *Cell Motil Cytoskeleton.* 2003;56:79–93.
27. Stenoien DL, Sen S, Mancini MA, et al. Dynamic association of a tumor amplified kinase, Aurora-A, with the centrosome and mitotic spindle. *Cell Motil Cytoskeleton.* 2003;55:134–146.
28. Cassimeris L, Morabito J. TOGp, the human homolog of XMAP215/Dis1, is required for centrosome integrity, spindle pole organization, and bipolar spindle assembly. *Mol Biol Cell.* 2004;15:1580–1590.
29. Patzke S, Hauge H, Sioud M, et al. Identification of a novel centrosome/microtubule-associated coiled-coil protein involved in cell-cycle progression and spindle organization. *Oncogene.* 2005;24:1159–1173.
30. Steffen W, Fajer EA, Linck RW. Centrosomal components immunologically related to tektins from ciliary and flagellar microtubules. *J Cell Sci.* 1994;107 (pt 8): 2095–2105.
31. Li K, Xu EY, Cecil JK, et al. Drosophila centrosomin protein is required for male meiosis and assembly of the flagellar axoneme. *J Cell Biol.* 1998;141:455–467.
32. Larsson M, Norrander J, Graslund S, et al. The spatial and temporal expression of Tekt1, a mouse tektin C homologue, during spermatogenesis suggest that it is involved in the development of the sperm tail basal body and axoneme. *Eur J Cell Biol.* 2000;79:718–725.
33. McKean PG, Baines A, Vaughan S, et al. Gamma-tubulin functions in the nucleation of a discrete subset of microtubules in the eukaryotic flagellum. *Curr Biol.* 2003;13:598–602.
34. Conte FF, Ribeiro PA, Marchesini RB, et al. Expression profile and distribution of Efhc1 gene transcript during rodent brain development. *J Mol Neurosci.* 2009;39: 69–77.
35. Weinmann AS, Yan PS, Oberley MJ, et al. Isolating human transcription factor targets by coupling chromatin immunoprecipitation and CpG island microarray analysis. *Genes Dev.* 2002;16:235–244.
36. Whitfield ML, Sherlock G, Saldanha AJ, et al. Identification of genes periodically expressed in the human cell cycle and their expression in tumors. *Mol Biol Cell.* 2002;13:1977–2000.
37. Gupta A, Tsai LH, Wynshaw-Boris A. Life is a journey: a genetic look at neocortical development. *Nat Rev Genet.* 2002;3:342–355.
38. Sawamoto K, Wichterle H, Gonzalez-Perez O, et al. New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science.* 2006;311:629–632.
39. Chan M, Amin-Hanjani S. Cerebrospinal fluid and its abnormalities. In: *Encyclopedia of Life Sciences (ELS).* John Wiley & Sons, Ltd: Chichester. 2010. DOI: 10.1002/9780470015902.a0002191.pub2.
40. Suzuki T, Miyamoto H, Nakahari T, et al. Efhc1 deficiency causes spontaneous myoclonus and increased seizure susceptibility. *Hum Mol Genet.* 2009;18: 1099–1109.

40a. Rossetto MG, Zanarella E, Orso G, Scorzeto M, Megighian A, Kumar V, Delgado-Escueta AV, Daga A. Deflic1.1, a homologue of the juvenile myoclonic gene EFHC1, modulates architecture and basal activity of the neuromuscular junction in Drosophila. *Hum Mol Genet*. 2011;20:4248–4257.

41. Conde C, Caceres A. Microtubule assembly, organization and dynamics in axons and dendrites. *Nat Rev Neurosci.* 2009;10:319–332.
42. Gambello MJ, Darling DL, Yingling J, et al. Multiple dose-dependent effects of Lis1 on cerebral cortical development. *J Neurosci.* 2003;23:1719–1729.
43. Tsai JW, Chen Y, Kriegstein AR, et al. LIS1 RNA interference blocks neural stem cell division, morphogenesis, and motility at multiple stages. *J Cell Biol.* 2005;170:935–945.
44. Xie Z, Moy LY, Sanada K, et al. Cep120 and TACCs control interkinetic nuclear migration and the neural progenitor pool. *Neuron.* 2007;56:79–93.

45. Cappello S, Attardo A, Wu X, et al. The Rho-GTPase cdc42 regulates neural progenitor fate at the apical surface. *Nat Neurosci.* 2006;9:1099–1107.
46. Nadarajah B, Brunstrom JE, Grutzendler J, et al. Two modes of radial migration in early development of the cerebral cortex. *Nat Neurosci.* 2001;4:143–150.
47. Rakic P. Mode of cell migration to the superficial layers of fetal monkey neocortex. *J Comp Neurol.* 1972;145: 61–83.
48. Halfter W, Dong S, Yip YP, et al. A critical function of the pial basement membrane in cortical histogenesis. *J Neurosci.* 2002;22:6029–6040.
49. Hartfuss E, Forster E, Bock HH, et al. Reelin signaling directly affects radial glia morphology and biochemical maturation. *Development.* 2003;130:4597–4609.
50. Li H, Berlin Y, Hart RP, et al. Microtubules are critical for radial glial morphology: possible regulation by MAPs and MARKs. *Glia.* 2003;44:37–46.
51. Vreugdenhil E, Kolk SM, Boekhoorn K, et al. Doublecortin-like, a microtubule-associated protein expressed in radial glia, is crucial for neuronal precursor division and radial process stability. *Eur J Neurosci.* 2007;25:635–648.
52. Tanaka T, Serneo FF, Higgins C, et al. Lis1 and doublecortin function with dynein to mediate coupling of the nucleus to the centrosome in neuronal migration. *J Cell Biol.* 2004;165:709–721.
53. Tsai LH, Gleeson JG. Nucleokinesis in neuronal migration. *Neuron.* 2005;46:383–388.
54. Hummel T, Krukkert K, Roos J, et al. Drosophila Futsch/22C10 is a MAP1B-like protein required for dendritic and axonal development. *Neuron.* 2000;26: 357–370.
55. Pennetta G, Hiesinger PR, Fabian-Fine R, et al. Drosophila VAP-33A directs bouton formation at neuromuscular junctions in a dosage-dependent manner. *Neuron.* 2002;35:291–306.
56. Roos J, Hummel T, Ng N, et al. Drosophila Futsch regulates synaptic microtubule organization and is necessary for synaptic growth. *Neuron.* 2000;26: 371–382.
57. Ruiz-Canada C, Ashley J, Moeckel-Cole S, et al. New synaptic bouton formation is disrupted by misregulation of microtubule stability in aPKC mutants. *Neuron.* 2004;42:567–580.
58. Orso G, Martinuzzi A, Rossetto MG, et al. Disease-related phenotypes in a Drosophila model of hereditary spastic paraplegia are ameliorated by treatment with vinblastine. *J Clin Invest.* 2005;115: 3026–3034.
59. Sherwood NT, Sun Q, Xue M, et al. Drosophila spastin regulates synaptic microtubule networks and is required for normal motor function. *PLoS Biol.* 2004;2:e429.
60. Trotta N, Orso G, Rossetto MG, et al. The hereditary spastic paraplegia gene, spastin, regulates microtubule stability to modulate synaptic structure and function. *Curr Biol.* 2004;14:1135–1147.
61. Wang X, Shaw WR, Tsang HT, et al. Drosophila spichthyin inhibits BMP signaling and regulates synaptic growth and axonal microtubules. *Nat Neurosci.* 2007;10:177–185.
62. Zhang YQ, Bailey AM, Matthies HJ, et al. Drosophila fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. *Cell.* 2001;107:591–603.
63. Ohkawa N, Fujitani K, Tokunaga E, et al. The microtubule destabilizer stathmin mediates the development of dendritic arbors in neuronal cells. *J Cell Sci.* 2007;120:1447–1456.
64. Cohen D, Segal M, Reiner O. Doublecortin supports the development of dendritic arbors in primary hippocampal neurons. *Dev Neurosci.* 2008;30:187–199.
65. Huang J, Furuya A, Furuichi T. Very-KIND, a KIND domain containing RasGEF, controls dendrite growth by linking Ras small GTPases and MAP2. *J Cell Biol.* 2007;179:539–552.
66. Jinushi-Nakao S, Arvind R, Amikura R, et al. Knot/Collier and cut control different aspects of dendrite cytoskeleton and synergize to define final arbor shape. *Neuron.* 2007;56:963–978.

Chapter 68

Progressive Myoclonus Epilepsy of Lafora

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Lafora disease is an autosomal recessive form of progressive myoclonus epilepsy characterized by a severe course that leads to death in 5–10 years in most patients. Patients present with myoclonic, absence, and generalized tonic-clonic seizures at onset, typically at around age 14–15 years. As the disease progresses, difficulties in speech generation and gait as well as cognitive decline appear. Seizures soon become intractable and, due to a rapidly progressing dementia, patients become severely incapacitated and die. Lafora bodies are the characteristic hallmark and consist of an abnormal, poorly branched, intracellular glucose polymer accumulating in many tissues, including heart, skeletal muscle, liver, and brain. They can be observed on optic microscopy as periodic acid–Schiff-positive (PAS+) cytoplasmic inclusions. Lafora bodies thus resemble glycogen with reduced branching, suggesting an alteration in glycogen metabolism as the cause of their accumulation. Since the localization of the first gene for Lafora disease in 1995, major advances have led to a better understanding of the basic mechanisms involved in this adolescent-onset and deadly disease.

MOLECULAR GENETICS AND GENOTYPE–PHENOTYPE CORRELATIONS

Linkage analysis and homozygosity mapping initially localized a major gene for Lafora disease to chromosome 6q24.[1] Subsequently, the first gene, known as *EPM2A,* encoding laforin was identified.[2,3] Mutation analysis has revealed a marked allelic heterogeneity in *EPM2A*. In spite of this remarkable allelic heterogeneity, one mutation, R241stop, was found in approximately 40% of Lafora disease patients in one study of such patients in Spain.[4] This study also suggested that both a founder effect and recurrence contributed to the relatively high prevalence of the R241stop mutation in Spain.

Chan et al.[5] identified a second gene associated with Lafora disease, *NHLRC1* or *EPM2B*,

which encodes malin, a putative E3 ubiquitin ligase with a RING finger domain and six NHL motifs. Malin was found to colocalize with laforin, and it was suggested that the malin-laforin interplay protected against polyglucosan body formation and epilepsy.

Defects in *EPM2A* and *EPM2B* account for more than 95% of cases, and more than 100 mutations have been reported. A study of 77 families with Lafora disease found that 54 (70.1%) had mutations in *EPM2A*, 21 (27.3%) had mutations in *EPM2B*, and 2 (2.6%) had no mutations in either gene. The course of the disease was longer in patients with *EPM2B* mutations compared to patients with *EPM2A* mutations, suggesting that patients with *EPM2B*-associated Lafora disease tend to have a slightly milder clinical course and a slower progression.[6] This finding was also reported in an Italian family with a *EPM2B* mutation and a remarkably slow, mild course and in a series of Italian patients.[7,8] Singh et al. reported similar findings.[9]

In a meta-analysis performed in 2009,[10] nearly 100 distinct mutations were identified in the two genes in over 200 independent Lafora disease families. Nearly half of them were missense mutations, and deletions accounted for one-quarter. The proportion of patients with *EPM2A* and *EPM2B* mutations varies among countries, and whereas in Spain *EPM2A* mutations are more common, in Italy and France *EPM2B* mutations predominate. In India and in the Arab countries, the mutations are distributed evenly.

FUNCTIONAL STUDIES: MECHANISMS OF LAFORA BODY FORMATION AND NEURODEGENERATION

Lafora bodies consist of an abnormal glycogen known *as polyglucosan*, which has a small number of branches and very long chains of glucose. Lafora bodies resemble starch and have low solubility, with a tendency to accumulate. Glycogen is synthesized through the action of glycogen synthase, which is responsible for chain elongation, and glycogen branching enzyme, which is responsible for chain branching. Glycogen is eliminated through digestion by glycogen phosphorylase and glycogen debranching enzyme. Reversible phosphorylation modulates nearly every step of glycogenesis and glycogenolysis. PTG (protein targeting to glycogen) is an indirect activator of glycogen synthase and an indirect inhibitor of both glycogen phosphorylase and glycogen phosphorylase kinase, the enzyme that activates glycogen phosphorylase. PTG performs this reciprocal activation of synthesis and inhibition of breakdown by binding the protein phosphatase-1 (PP1) through its C terminus, binding glycogen, and through a common region in its N terminus binding glycogen synthase, glycogen phosphorylase, or glycogen phosphorylase kinase, thus targeting PP1 to each of these enzymes. PP1 dephosphorylates each of the three enzymes, activating glycogen synthase and inhibiting glycogen phosphorylase and glycogen phosphorylase kinase.[11]

Malin and laforin colocalize in endoplasmic reticulum (ER) and form centrosomal aggregates when treated with proteasomal inhibitors in both neuronal and nonneuronal cells. Laforin/malin aggregates colocalize with γ-tubulin and cause redistribution of α-tubulin. The centrosomal accumulation of malin, possibly with the help of laforin, may enhance the ubiquitination of its substrates and facilitate their efficient degradation by proteasome. Defects in malin or laforin may thus lead to increased levels of misfolded and/or target proteins, which may eventually affect the physiological processes of the neuron. Thus, defects in protein degradation and clearance are likely to be the primary trigger in the physiopathology of Lafora disease.[12]

The first evidence of the possible role of a dysfunction in glycogen synthesis in the accumulation of polyglucosans came from a study of transgenic mice overexpressing glycogen synthase. The authors proposed an imbalance between glycogen synthase and branching enzyme as the mechanism involved in the production of polyglucosan bodies.[13]

Laforin is a protein of 331 amino acids with two domains, a dual-specificity phosphatase domain and a carbohydrate binding domain. Laforin forms part of a multiprotein complex associated with intracellular glycogen particles and is involved in the regulation of glycogen metabolism. Laforin interacts with itself and with PTG, the glycogen targeting regulatory subunit R5 of PP1. R5 is the human homolog of the murine PTG, a protein that acts as

a molecular scaffold assembling PP1 with its substrate, glycogen synthase, at the intracellular glycogen particles. The majority of *EPM2A* missense mutations found in Lafora disease patients result in lack of phosphatase activity, absence of binding to glycogen, and lack of interaction with PTG.[14]

Polyglucosan formation is catalyzed by glycogen synthase, which is activated through dephosphorylation by glycogen-associated PP1. PTG, one of the proteins that target PP1 to glycogen, was removed from laforin knockout mice. This resulted in near-complete disappearance of polyglucosans and in the resolution of neurodegeneration and myoclonic epilepsy. Blocking of PTG could in this way be a form of treatment for Lafora disease.[11]

Malin is a single-subunit E3 ubiquitin (Ub) ligase. Its RING domain is necessary and sufficient to mediate ubiquitination. Additionally, malin interacts with and polyubiquitinates laforin, leading to its degradation. Missense mutations in malin that are present in Lafora disease patients abolish its ability to polyubiquitinate and signal the degradation of laforin. Laforin is thus a physiological substrate of malin.[15]

The laforin-malin complex suppresses glycogen synthesis in neurons, and its malfunction would explain the accumulation of a poorly branched glycogen.[16] Either the abnormal glucose polymer, a malfunction of other pathways where laforin and malin are involved, or both would result in progressive neurodegeneration and epileptic seizures. The laforin-malin complex also downregulates PTG-induced glycogen synthesis through a mechanism involving ubiquitination and degradation of PTG. The interaction between laforin and malin is a regulated process that is modulated by the adenosine monophosphate (AMP)-activated protein kinase (AMPK).[17] Another theory proposes that excessive phosphorylation of glycogen leads to aberrant branching and Lafora body formation.[18]

MOUSE MODELS BEARING ENGINEERED MUTATIONS IN *Epm2A* AND *Epm2B*

To study the pathology of Lafora disease and the functions of laforin, Ganesh et al. disrupted the *Epm2a* gene in mice.[19] At 2 months of age, homozygous null mutants developed widespread degeneration of neurons, most of which occurred in the absence of Lafora bodies. Dying neurons characteristically exhibited swelling in the ER, Golgi network, and mitochondria in the absence of apoptotic bodies or fragmentation of DNA. The Lafora bodies, present both in neuronal and nonneural tissues, were found to be positive for ubiquitin and advanced glycation end products only in neurons, suggesting different pathological consequences for Lafora inclusions in neuronal tissues. The authors concluded that Lafora disease is a primary neurodegenerative disorder that may utilize a nonapoptotic mechanism of cell death.

Disruption of the *Epm2b* gene in mice resulted in viable animals that, by 3 months of age, had accumulated Lafora bodies in the brain and to a lesser extent in heart and skeletal muscle. Analysis of muscle and brain of the *Epm2b*$^{-/-}$ mice by Western blotting indicated no effect on the levels of glycogen synthase, PTG (type 1 phosphatase-targeting subunit), or debranching enzyme, making it unlikely that these proteins are targeted for destruction by malin, as has been proposed. Total laforin protein was increased in the brains of *Epm2b*$^{-/-}$ mice and, most notably, was redistributed from the soluble, low-speed supernatant to the insoluble, low-speed pellet, which now contained 90% of the total laforin. This result correlated with the elevated insolubility of glycogen and glycogen synthase. Because upregulation of laforin cannot explain Lafora body formation, the authors concluded that malin functions to maintain laforin associated with soluble glycogen and that its absence causes sequestration of laforin to an insoluble polysaccharide fraction, where it is functionally inert.[20]

These mouse models permit a clearer understanding of the pathogenic mechanisms involved in Lafora disease in humans and provide tools to unravel the complex mechanisms involved in the formation of Lafora bodies.

In conclusion, major advances have been achieved in the knowledge of the basic mechanisms involved in Lafora disease since the initial molecular genetic studies of 15 years ago. Studies have focused mainly on the mechanisms of Lafora body formation and reveal some clues concerning how neurodegeneration is produced. However, we do not know how the molecular defects found in Lafora disease result in the production of epileptic

seizures: the role of Lafora bodies is still in question, and it may be that the seizures are the consequence of other dysfunctions. The next steps in research on Lafora disease should lead to the development of experimental test systems to define better therapies.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Serratosa JM, Delgado-Escueta AV, Posada I, Shih S, Drury I, Berciano J, Zabala JA, Antúnez MC, Sparkes RS. The gene for progressive myoclonus epilepsy of the Lafora type maps to chromosome 6q. *Hum Mol Genet*. 1995;49:1657–1663.
2. Minassian BA, Lee JR, Herbrick JA, et al. Mutations in a gene coding a novel protein tyrosine phosphatase cause a progressive myoclonus epilepsy. *Nat Genet.* 1998;20:171–174.
3. Serratosa JM, Gómez-Garre P, Gallardo ME, et al. A novel protein tyrosine phosphatase gene is mutated in progressive myoclonus epilepsy of the Lafora type (EPM2). *Hum Mol Genet.* 1999;8:345–352.
4. Gómez-Garre P, Sanz Y, Rodríguez De Córdoba SR, Serratosa JM. Mutational spectrum of the EPM2A gene in progressive myoclonus epilepsy of Lafora: high degree of allelic heterogeneity and prevalence of deletions. *Eur J Hum Genet*. 2000;8:946–954.
5. Chan EM, Young EJ, Ianzano L, Munteanu I, Zhao X, Christopoulos CC, Avanzini G, Elia M, Ackerley CA, Jovic NJ, Bohlega S, Andermann E, Rouleau GA, Delgado-Escueta AV, Minassian BA, Scherer SW. Mutations in NHLRC1 cause progressive myoclonus epilepsy. *Nat Genet*. 2003;35:125–127.
6. Gómez-Abad C, Gómez-Garre P, Gutiérrez-Delicado E, Saygi. Lafora disease due to *EPM2B* mutations: a clinical and genetic study. *Neurology.* 2005;22;64: 982–986.
7. Baykan B, Striano P, Gianotti S, Bebek N, Gennaro E, Gurses C, Zara F. Late-onset and slow-progressing Lafora disease in four siblings with EPM2B mutation. *Epilepsia.* 2005;46:1695–1697.
8. Franceschetti S, Gambardella A, Canafoglia L, Striano P, Lohi H, Gennaro E, Ianzano L, Veggiotti P, Sofia V, Biondi R, Striano S, Gellera C, Annesi G, Madia F, Civitelli D, Rocca FE, Quattrone A, Avanzini G, Minassian B, Zara F. Clinical and genetic findings in 26 Italian patients with Lafora disease. *Epilepsia.* 2006;47:640–643.
9. Singh S, Sethi I, Francheschetti S, Riggio C, Avanzini G, Yamakawa K, Delgado-Escueta AV, Ganesh S. Novel NHLRC1 mutations and genotype-phenotype correlations in patients with Lafora's progressive myoclonic epilepsy. *J Med Genet*. 2006;43:e48.
10. Singh S, Ganesh S. Lafora progressive myoclonus epilepsy: a meta-analysis of reported mutations in the first decade following the discovery of the EPM2A and NHLRC1 genes. *Hum Mutat*. 2009;30:715–723.
11. Turnbull J, Depaoli-Roach AA, Zhao X, Cortez MA, Pencea N, Tiberia E, Piliguian M, Roach PJ, Wang P, Ackerley CA, Minassian BA. PTG depletion removes Lafora bodies and rescues the fatal epilepsy of Lafora disease. *PLoS Genet*. 2011;7:e1002037.
12. Mittal S, Dubey D, Yamakawa K, Ganesh S. Lafora disease proteins malin and laforin are recruited to aggresomes in response to proteasomal impairment. *Hum Mol Genet*. 2007;16:753–762.
13. Raben N, Danon M, Lu N, Lee E, Shliselfeld L, Skurat AV, Roach PJ, Lawrence JC Jr, Musumeci O, Shanske S, DiMauro S, Plotz P. Surprises of genetic engineering: a possible model of polyglucosan body disease. *Neurology.* 2001;26;56:1739–1745.
14. Fernández-Sánchez ME, Criado-García O, Heath KE, García-Fojeda B, Medraño-Fernández I, Gomez-Garre P, Sanz P, Serratosa JM, Rodríguez de Córdoba S. Laforin, the dual phosphatase responsible for Lafora disease, interacts with R5(PTG), a regulatory subunit of protein phosphatase-1 that enhances glycogen accumulation. *Hum Mol Genet*. 2003;12: 3161–3171.
15. Gentry MS, Worby CA, Dixon JE. Insights into Lafora disease: malin is an E3 ubiquitin ligase that ubiquitinates and promotes the degradation of laforin. *Proc Natl Acad Sci USA*. 2005;102:8501–8506.
16. Vilchez D, Ros S, Cifuentes D, Pujadas L, Vallès J, García-Fojeda B, Criado-García O, Fernández-Sánchez E, Medraño I, Domínguez J, García-Rocha M, Soriano E, Rodríguez de Córdoba S, Guinovart JJ. Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. *Nat Neurosci.* 2007;10:1407–1413.
17. Solaz-Fuster MC, Gimeno-Alcañiz JV, Ros S, Fernandez-Sanchez ME, Garcia-Fojeda B, Criado Garcia O, Vilchez D, Dominguez J, Garcia-Rocha M, Sanchez-Piris M, AguadoC, Knecht E, Serratosa J, Guinovart JJ, Sanz P, Rodriguez de Córdoba S. Regulation of glycogen synthesis by the laforin-malin complex is modulated by the AMP-activated protein kinase pathway. *Hum Mol Genet*. 2008;17:667–678.
18. Tagliabracci VS, Turnbull J, Wang W, Girard JM, Zhao X, Skurat AV,Delgado-Escueta AV, Minassian BA, Depaoli-Roach AA, Roach PJ. Laforin is a glycogen phosphatase, deficiency of which leads to elevated phosphorylation of glycogen in vivo. *Proc Natl Acad Sci USA*. 2007;4;104:19262–19266.
19. Ganesh S, Delgado-Escueta AV, Sakamoto T, Avila MR, Machado-Salas J, Hoshii Y, Akagi T, Gomi H, Suzuki T, Amano K, Agarwala KL, Hasegawa Y, Bai DS, Ishihara T, Hashikawa T, Itohara S, Cornford EM, Niki H, Yamakawa K. Targeted disruption of the Epm2a gene causes formation of Lafora inclusión bodies, neurodegeneration, ataxia, myoclonus epilepsy and impaired behavioral response in mice. *Hum Mol Genet*. 2002;11:1251–1262.
20. DePaoli-Roach AA, Tagliabracci VS, Segvich DM, Meyer CM, Irimia JM, Roach PJ. Genetic depletion of the malin E3 ubiquitin ligase in mice leads to lafora bodies and the accumulation of insoluble laforin. *J Biol Chem.* 2010;285:25372–25381.

Chapter 69

Progressive Myoclonus Epilepsy

Unverricht-Lundborg Disease and Neuronal Ceroid Lipofuscinoses

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UNVERRICHT-LUNDBORG DISEASE

Progressive myoclonus epilepsy of Unverricht-Lundborg type (Unverricht-Lundborg disease; ULD; EPM1) is an autosomal recessive neurodegenerative disorder and the most common single cause of progressive myoclonus epilepsy worldwide.[1] It is enriched in the Finnish population but is also relatively frequent in the western Mediterranean region. EPM1 was previously called *Baltic myoclonus*, *Baltic myoclonic epilepsy*, and *Mediterranean myoclonus*. Following identification of the underlying gene, *CSTB*,[2] and advances in genetic testing, these disorders are now collectively classified as EPM1.

Clinical Features

EPM1 is characterized by onset at 6–16 years, progressive stimulus-sensitive, action-activated myoclonus, and tonic-clonic epileptic seizures.[3] As EPM1 progresses, patients develop ataxia, dysarthria, intentional tremor, and decreased coordination, which reflect widespread neuronal degeneration in the brain. Patients with EPM1show mild intellectual decline over time, but overall their cognitive functions are less impaired than their motor functions. The electroencephalogram (EEG) in EPM1 patients is abnormal, with spontaneous spike-wave discharges, photosensitivity, polyspike discharges during rapid eye movement(REM) sleep, and background slowing.[3,4] The EEG abnormalities

are more pronounced at initial diagnosis, but in general, they diminish as the disease stabilizes. At the time of diagnosis the magnetic resonance imaging (MRI) scan is usually normal. At later stages, atrophy in cortical motor areas, and in pons, medulla, and cerebellar hemispheres, has been reported.[5,6] The limited histopathological findings consist of widespread degenerative changes with no evidence of storage material.

Symptomatic pharmacological and rehabilitative management, including psychosocial support, is the mainstay of EPM1 patients' care.[3] Valproic acid is the first drug of choice, and diminishes both myoclonus and the frequency of tonic-clonic seizures. Clonazepam and piracetam are effective as add-on therapy for myoclonus. Anecdotal evidence exists of the efficacy of levetiracetam, topiramate, and zonisamide, but sodium channel blockers and GABAergic (gamma-aminobutyricacid) drugs should be avoided. With modern antiepileptic medication, the life expectancy of EPM1 patients has gradually increased and is now probably normal. The relative intensity of symptoms and the speed of disease progression vary from one case to another, even within a single family.

The *Cystatin B* Gene and Protein

The *CSTB* gene underlying EPM1 was identified by positional cloning.[2] *CSTB* is alternatively spliced with at least five isoforms of unknown physiological significance, some of which show tissue specificity. The gene has two potential transcription start sites that are located 67 and 78 nucleotides downstream of the dodecamer repeat element in the promoter.

CSTB encodes Cystatin B, a ubiquitously expressed 98 amino acid protein with a molecular weight of approximately 11 kDa. CSTB is a protease inhibitor that inhibits in vitro several lysosomal cysteine proteases, cathepsins, by tight, reversible binding.[7] The main function of cathepsins is nonselective degradation of intracellular proteins, but they also participate in antigen processing and apoptosis.

Cytoplasmic, lysosomal, and nuclear localization has been reported for wild-type CSTB protein in proliferating cells and mainly cytoplasmic localization in differentiated cells,[8] with somewhat conflicting results among different studies, possibly due to the different cellular models and antibodies used. Data suggest that CSTB is attached to the outer side of the lysosomal membrane rather than within the lysosome.[8]

EPM1-Associated *Cystatin B* Gene Mutations

Eleven mutations have been reported to underlie EPM1. The most common mutation is an unstable expansion of a 12-nucleotide dodecamer repeat (5′-CCCCGCCCCGCG-3′) in the promoter region of *CSTB*.[9,10] This is normally polymorphic, with two or three copies present. EPM1-associated alleles have been reported to contain at least 30 repeat copies. The expansion mutation is found in approximately 90% of the disease alleles worldwide and in homozygous form in the majority of patients, especially in populations with a founder effect. No significant correlation between the length of the expanded repeat and the age of onset or disease severity has been reported.

The other EPM1-associated *CSTB* mutations change single amino acids, affect splice sites, or predict truncated proteins. The three reported missense mutations—p.Gly4Arg, p.Gly50Glu, and p.Gln71Pro—are likely to affect the interaction of the CSTB protein with its target cysteine proteases.[8,11] With the exception of the p.Gly4Arg substitution mutation, these mutations have been reported to occur in compound heterozygous form with the repeat expansion.

The expansion mutation causes significant downregulation of *CSTB* mRNA expression, with less than 10% of expression from that in controls.[11] Consequently, CSTB protein expression and its inhibitory activity are also significantly reduced in cells of EPM1 patients. Decreased inhibitory activity of CSTB correlates with enhanced activity of cathepsins B, L, and S in EPM1 patients' cells, providing in vivo evidence for cathepsin regulation by CSTB.[12] Data suggest that reduced CSTB expression is the primary pathological consequence in the majority of EPM1 mutations, with possible exclusion of the amino acid substitution mutations.

When transiently overexpressed in cells, these missense mutant proteins fail to associate

with lysosomes,[8,11] implying an essential role of lysosomal localization for the physiological function of CSTB.

CystatinB-Deficient Mouse Model for EPM1

A mouse model for EPM1 has been generated by targeted disruption of the mouse *Cstb* gene.[13] *Cstb*$^{-/-}$ mice develop myoclonic seizures by 1 month of age and progressive ataxia by 6 months of age and thus recapitulate key clinical features of EPM1. The myoclonic seizures occur during sleep and progress from twitching of isolated muscles to spasms affecting the entire body. Electrocorticogram recordings reveal bilaterally synchronous 4–6Hz repetitive spiking commencing with the myoclonus. No tonic-clonic seizures, photosensitivity, or spike-wave complexes on EEG have been reported. The genetic background has an impact on the clinical outcome, implying that genetic factors influence the phenotype.

The neuropathological hallmark of *Cstb*$^{-/-}$ mice is progressive apoptotic loss of cerebellar granule neurons.[13] There is less marked neuronal apoptosis in the hippocampal formation and entorhinal cortex in young animals and widespread cortical and white matter gliosis in older mice.[13,14] In addition, the superficial neurons of the prosubiculum in the cerebral cortex display prominent cellular atrophy, suggesting that neuronal dysfunction may also contribute to the phenotype. Mutant mice with both seizure-prone and seizure-resistant genetic backgrounds display similar neuropathological changes indicating that neuronal degeneration and loss are consequences of CSTB deficiency independent of seizure events. These data suggest that CSTB has an endogenous neuroprotective role and that EPM1 should be classified as a primary neurodegenerative disorder, with specific neuronal populations affected and with both neuronal death and dysfunction contributing to the phenotype.

Studies of hippocampal slice preparations have revealed hyperexcitability in *Cstb*$^{-/-}$ mice as they responded to afferent stimuli in the CA1 region with multiple population spikes and as kainate perfusion provoked the appearance of epileptic-like activity earlier than in wild-type mice.[15] The density of GABA-positive cells in hippocampus is reduced in *Cstb*$^{-/-}$ mice, and the hyperexcitability may thus be due to loss of inhibition. Moreover, *Cstb*$^{-/-}$ mice have increased susceptibility to kainate-induced seizures, with a shorter latency to seizure onset and more severe seizures compared to wild-type control mice. They also display increased sensitivity to seizure-induced cell death. It has been hypothesized that CSTB acts as a physiological safeguard and that loss of its activity not only triggers hyperexcitability and neurodegeneration, but also makes neurons more susceptible to the prolonged seizure-induced cell death contributing to disease progression in EPM1.[15]

Oxidative Stress in EPM1

A novel function for CSTB in defending cerebellar granule neurons from oxidative stress has been identified.[16] It implies impaired redox homeostasis as a key mechanism by which CSTB deficiency triggers neurodegeneration. Oxidative stress induces CSTB expression in cerebellar granule neurons, and both *Cstb* knockdown in rats and knockout in mice sensitize granule neurons to oxidative stress-induced cell death. The predisposition to oxidative stress in neurons, induced by CSTB deficiency, is mediated by the lysosomal protease Cathepsin B. CSTB deficiency triggers oxidative injury specifically in the cerebellum, leading to diminished antioxidant capacity and elevated lipid peroxidation.[16] Consistent with these findings, cerebellar granule neuron degeneration is reduced in mice deficient in both CSTB and Cathepsin B compared to mice deficient in CSTB only.[17] The double-knockout mice retain myoclonic seizures and ataxia, suggesting that oxidative stress and CSTB may regulate neuronal excitability in EPM1 independently of deregulated Cathepsin B activity.

PRICKLE1 and *SCARB2* Mutations in EPM1-like Patients

Mutations in two genes have been reported in *CSTB* mutation-negative patients presenting with symptoms closely resembling EPM1. A p.Arg104Gln mutation in the *PRICKLE1* gene encoding a protein in the noncanonical (Wnt)

signaling pathway has been described in three possibly related families of Middle Eastern descent.[18] Onset of symptoms is between 5 and 10 years of age, that is, at a slightly younger age than onset in EPM1 patients. The presenting symptom is usually ataxia, with action myoclonus and seizures developing later. Intellect is generally preserved. This disorder was originally named *EPM1B* because of its similarity to EPM1, but it is now referred to as *progressive myoclonus epilepsy with ataxia*.

The second gene harboring mutations in EPM1-like patients is *SCARB2*, which encodes a lysosomal membrane protein. Mutations in *SCARB2* were originally reported in action myoclonus–renal failure syndrome (AMRF).[19] These patients present typically at 15–25 years of age, either with neurological symptoms including tremor, action myoclonus, seizures, and ataxia or with proteinuria that progresses to renal failure. In a subset of patients harboring mutations in *SCARB2* no evidence of renal failure during up to 15 years of follow-up has been observed, implying that mutations in *SCARB2* are an important cause of progressive myoclonus epilepsy cases resembling EPM1 at onset.[20] A missense mutation and five mutations predicting either aberrant splicing or a truncated protein due to a frameshift alteration have been identified in patients with EPM1-like disease. The nature or the location of the mutation in *SCARB2* does not explain the absence of renal features in these patients.

NEURONAL CEROID LIPOFUSCINOSES

The neuronal ceroid lipofuscinoses (NCLs) are a group of inherited neurodegenerative disorders characterized by the accumulation of autofluorescent storage material in neurons and many other cell types. Most are inherited in an autosomal recessive manner and are characterized clinically by epileptic seizures, progressive psychomotor decline, visual failure, and early death.

The NCLs were initially divided into four subtypes according to the age of onset, the clinical phenotype, and the ultrastructure of the storage material. These subtypes, with their eponyms, are infantile NCL (INCL, Haltia-Santavuori), late-infantile NCL (LINCL, Jansky-Bielschowsky), juvenile NCL (Batten, Spielmeyer-Vogt), and adult NCL (Kufs). More recently, several variants of these subtypes, especially of the late-infantile onset, have been characterized, and it has become clear that there is marked locus heterogeneity (mutations in different genes may result in a similar clinical disease phenotype) and allelic heterogeneity (different mutations within the same gene may result in very different clinical disease phenotypes). Estimates of incidence range from 1 in 12,500 to 1 in 100,000.

Eight genes underlying human NCLs have now been identified (*PPT1, TPP1, CLN3, CLN5, CLN6, MFSD8, CLN8*, and *CTSD*), and two are predicted to exist but are not yet isolated (*CLN4, CLN9*).[21] A database of mutations is available (www.ucl.ac.uk/ncl/mutation). In addition, mutations in members of the CLC family of chloride channels and transporters may have a role in NCL, especially *CLCN7, CLCN3*, and *CLCN6*.

Despite much research, including the development of several model organisms for NCLs, the exact biological function of the proteins encoded by NCL genes remains elusive.[22]

They can be usefully divided into the soluble proteins—PPT1, TPP1, CSTD, and CLN5—and the transmembrane proteins coded by *CLN3, CLN6, MFSD8*, and *CLN8*. Although the former have been extensively characterized, their in vivo substrates remain unknown.

Advances in research have enabled diagnosis at the molecular genetic and biochemical levels, and have resulted in prenatal diagnosis and carrier testing availability for many affected families.

Enzyme analysis (for TPP1 and PPT1) and mutation detection for the most common mutations (such as the 1kb deletion in *CLN3*) have become routine and have now replaced more invasive investigations.

There is no specific treatment for the NCLs; the mainstay of treatment is supportive and palliative care. Care is focused on minimizing symptoms, including seizures, behavioral problems, and depression. Antiepilepsy drugs are a mainstay of therapy for the majority of NCL patients. Many promising new therapeutic strategies are presently being investigated, such as chaperone therapy, enzyme replacement therapy, gene therapy, and stem cell therapy. Early diagnosis of

NCL is a key problem, as significant damage occurs between onset of initial symptoms and diagnosis.

CLN1 Disease: *PPT1*

Infantile NCL is caused by mutations in *PPT1*, the gene encoding the enzyme palmitoyl protein thioesterase 1 (PPT1).[23] PPT1 removes fatty acids in covalent linkage to cysteine residues in proteins, ensuring the eventual metabolic disposal of S-acylated cysteine residues during lysosomal degradation. In nonneuronal cells, PPT1 is a soluble lysosomal protein that is targeted to lysosomes through the mannose-6-phosphate pathway. In neuronal cells, it is localized to synaptosomes and synaptic vesicles. Mutations in *PPT1* are associated with granular osmiophilic storage deposits (GRODs) in the cells, where the main components of accumulated protein are the sphingolipid activator proteins (SAPs) A and D.

Over 40 disease-causing mutations distributed throughout the gene have been described in *PPT1*. The most common mutation (c.364A>T) is associated with a founder effect in the Finnish population. This causes disease onset in infancy, as do the majority of the mutations identified.

Early development is normal until 6–18 months, when decreased head growth and developmental retardation appear. Epileptic seizures, hypotonia, ataxia, and visual failure occur, associated with severe brain atrophy and death at 8–15 years. Less severe mutations (missense in peripheral sites) in *PPT1* also cause NCL forms with later onset and protracted progression.

Neurophysiological findings are nonspecific. There is decreased reactivity in EEG to passive eye opening and closing at around the age of 1 year. Sleep spindles are lost by the age of 2 years, and by 3 years the EEG becomes isoelectric. Thalami show markedly decreased signal intensity to the basal ganglia on T2-weighted MR images and increased signal intensity on T1-weighted images even before the clinical disease is apparent. Generalized cerebral and cerebellar atrophy is found by the age of 13 months. Enzyme assay of PPT1 in white cells is the mainstay of diagnosis and has been used for prenatal diagnosis.

CLN2 Disease: *TPP1*

Classical late infantile NCL is caused by mutations in the *TPP1* gene encoding the lysosomal enzyme tripeptidyl peptidase 1 (TPP1).[24] This gene was identified using a proteomics approach to identify the mannose-phosphorylated protein that was missing in brain samples from patients with the disease. TPP1 is a pepstatin-insensitive protease that cleaves tripeptides from the amino termini of polypeptides undergoing degradation. Subunit c of mitochondrial adenosine triphosphate (ATP) synthase, the major protein component of the storage material, particularly in this subtype, is likely to be a substrate. Ultrastructural changes are pure curvilinear membrane-bound lysosmal aggregates.

Over 60 different disease-causing mutations have now been identified in the *TPP1*gene. Two are most common: c.509–1G>C, affecting the splicing of the transcript, and c.622C>T, creating a stop codon (p.Arg208X).

Most mutations in *TPP1* cause classical LINCL. The clinical features usually begin at around the third year of life, with delay of psychomotor development or sudden onset of seizures, which may be generalized tonic-clonic, partial, or often of a severe myoclonic type. Blindness eventually develops due to retinal atrophy. Death usually occurs in mid-childhood. A few of the mutations cause a more protracted or juvenile-onset form of NCL.

The EEG shows characteristic occipital spike responses to slow flash (1–2 Hz) stimulation before the onset of seizures. The electroretinogram is diminished or absent early in the disease even before noticeable visual loss. Severe cerebellar atrophy on MRI is seen at the time of diagnosis. Enzyme assay of TPP1 in white cells is the mainstay of diagnosis.

CLN3 Disease: *CLN3*

Juvenile NCL, the most frequent subtype of the NCLs, is caused by mutations in *CLN3*, which was localized to chromosome 16 by demonstration of linkage to the haptoglobin locus and subsequently identified by a positional cloning strategy.[25] *CLN3* encodes a 438 amino acid transmembrane protein that has been located to various cellular organelles

such as lysosomes, the Golgi complex, and mitochondria.

The function of CLN3 is still unknown, but it is evolutionarily conserved down to yeast, which suggests an important function in eukaryotic cells. Possible roles include maintenance of lysosomal pH homeostasis, inward lysosomal arginine transport, a role in membrane tracking, and an antiapoptotic function. The typical ultrastructural feature is fingerprint profiles.

At least 40 mutations are currently known in *CLN3*. The most common of these is a 1kb deletion (c.462–677del) causing a frameshift after the cysteine residue at position 153 and premature termination. It occurs worldwide but especially in European and North American populations. This mutation is so predominant that most patients are homozygous or at least heterozygous for it. A wide variety of other mutations exist, some of which are associated with a later onset or a more protracted disease course.

Juvenile NCL has a usual age of onset of 4–7 years, with progressive visual failure leading to blindness always occurring first. The ocular pathology is initially a pigmentary retinopathy that may be mistaken for retinitis pigmentosa or cone dystrophy.

Seizures (at a mean age of 10 years) and psychomotor deterioration with behavioral problems follow. The main types of seizures are generalized tonic-clonic seizures, but partial seizures and myoclonic jerks also occur. Seizures tend to increase in frequency and severity, but there is considerable individual variability. In the EEG, progressive background abnormality and an increase in paroxysmal activity are seen.

Extrapyramidal symptoms and signs are noted in about 50% of patients between the ages of 12 and 15 years. Signs include impaired balance, rigidity, hypokinesia, stooped posture, and shuffling gate; tremor is usually mild and inconstant. Death occurs in the second or third decade.

The MRI scan demonstrates variable cerebral and cerebellar atrophy after the age of about 14 years.

Vacuolated lymphocytes visible in the peripheral blood are unique to this form of NCL and are a valuable diagnostic aid. Screening for the 1kb deletion is now widely available as a diagnostic service. The majority (>80%) of children of white Caucasian origin will be homozygous for the common 1kb deletion, and almost all of the rest will be compound heterozygous for the 1kb deletion and another disease-causing mutation that may be unique to the family.

Variant Late Infantile NCLs: *CLN5, CLN6, MFSD8, CLN8*

CLN5 DISEASE

The NCL subtype with mutations in the *CLN5* gene was originally described in the Finnish population and is often referred to as *Finnish variant LI NCL*. The *CLN5* gene was localized to chromosome 13q22 by a genome-wide scan and later identified by a positional cloning strategy.[26] The gene is conserved in vertebrates and encodes a lysosomal glycoprotein with no homology to other known proteins. The function of the CLN5 protein is currently unknown.

Over 25 different disease-causing mutations in *CLN5* have been described. The most common of these, c.1175delAT (p.Tyr392X), presents only in the Finnish population, blocking lysosomal targeting of the mutant protein.

The first disease manifestations include attention deficit and motor clumsiness and usually occur between the ages of 5 and 7 years, later than in the classical form. Thereafter, mental decline and visual failure, myoclonic and tonic-clonic seizures, and ataxia are features of the disease. Specific EEG features include posterior spikes to low-frequency photic stimulation from 7–8 years and giant somatosensory evoked potentials (SEPs). Age of death is between 13 and 21 years.

The MRI scan shows cerebellar atrophy between the ages of 4 and 7 years.

CLN6 DISEASE

A variant late infantile form caused by mutations in the *CLN6* gene is mainly found in the Czech Republic, Croatia, Portugal, Central and South America, and, more rarely, in Central and Northern Europe, Turkey, and the Indian subcontinent. The *CLN6* gene was localized to chromosome 15q21–23 by homozygosity mapping and identified by positional cloning.[27,28] The CLN6 protein has seven transmembrane domains and localizes to the endoplasmic reticulum (ER). It is highly conserved across

vertebrates and has no homology with known proteins; its function is currently unknown.

Over 40 disease-causing mutations have been identified in the *CLN6* gene. One of the two most common of these is a nonsense mutation c.214G>T (p.Glu72X) present in patients of Costa Rican origin.

Ultrastructural deposits include fingerprint, curvilinear, and rectilinear patterns.

Most mutations in *CLN6* cause a clinical phenotype similar to that of classical LINCL with an age of onset between 3 and 5 years, but the range is from 18 months to 8years. Early features may include gait and speech disturbance and epileptic seizures.

CLN7 DISEASE

After a genome-wide scan and homozygosity mapping in nine Turkish families and one Indian family, not linked to any of the known NCL loci, a novel variant LINCL locus was mapped to chromosome 4q28.1-q28.2. Six different mutations were identified in the *MFSD8* gene, which encodes a 518 amino acid membrane protein that belongs to the major facilitator superfamily of transporter proteins.[29] Like the majority of the previously identified NCL proteins, MFSD8 localizes mainly to the lysosomal compartment.

A total of 22 mutations have now been identified in *MFSD8* in patients of various ethnic origins including Italy. A significant group of Roma patients originating from the former Czechoslovakia was shown to bear the c.881C>A (p.Thr294Lys) mutation in *MFSD8*, possibly due to a founder effect. With one exception, these patients presented a phenotype indistinguishable from those of the other variant LINCL forms. In one patient with an in-frame amino acid substitution mutation in *MFSD8*, the disease onset was later and the disease course slower.

CLN8 DISEASE

CLN8 was first identified as a causative gene for Northern epilepsy (also known as *progressive epilepsy with mental retardation*, EPMR) in Finnish patients. Later, recognition of intraneuronal storage material led to this disease being categorized as an NCL. The *CLN8* gene was localized to the short arm of chromosome 8 by linkage analysis and identified by positional cloning.[30] The function of the CLN8 protein is unknown, but it is predicted to be a transmembrane protein with several membrane-spanning domains. In nonneuronal cells, the CLN8 protein is localized to the ER and partially to the ER–Golgi intermediate compartment (ERGIC). In neurons, CLN8 is localized to the ER, but an additional location outside the ER has been suggested in polarized cells. CLN8 has recently been connected to the TRAM-Lag1p-CLN8 (TLC) superfamily of proteins suggested to have a role in biosynthesis, metabolism, and sensing of lipids.

A total of 15 mutations have now been identified, and it is clear that there is allelic heterogeneity with two distinct phenotypes. The most common mutation is c.70C>G (p.Arg24Gly) underlying the EPMR phenotype. The first symptoms in EPMR are epileptic seizures, observed at 5–10 years of age. Two to 5 years after disease onset, patients show progressive mental deterioration and motor and behavioral problems. Additional mutations associated with a variant LINCL phenotype were subsequently identified in Turkish, Israeli, Italian, German, and Pakistani patients, including a novel large deletion in a Turkish family.

CLN10 Disease: *CTSD*

As mutations in the *CSTD* gene had been observed in two naturally occurring animal models for NCL (Swedish Landrace sheep and American bulldogs), *CTSD* was a candidate gene for human NCL. Recently, it was recognized as the causative gene for two separate human NCL phenotypes.

A homozygous duplication (c.764dupA) that creates a premature stop codon at position 255 (p.Tyr255X) results in congenital NCL with absence of CTSD immunostaining in brain.[31] Congenital NCL is the earliest-onset and most aggressive subtype. It presents with extreme brain atrophy, microcephaly, and epilepsy at birth, with death occurring within hours to weeks. In contrast, compound heterozygosity for two missense mutations, with resulting CTSD deficiency, underlies a neurodegenerative phenotype with blindness and psychomotor disability manifesting at an earlyschool age, but no seizures. Ultrastructural features include massive accumulation of GRODs.

The human *CTSD* gene is located on chromosome 11p15.5, contains nine exons, and encodes Cathepsin D, a lysosomal aspartic protease of 412 amino acids that belongs to the pepsin family and is conserved at least down to *Drosophila melanogaster* and *Caenorhabditis elegans*.

CLN4 Disease

The adult-onset form of NCL, Kufs disease, has been assigned the still unidentified gene locus *CLN4*. It is the mildest form of NCL and includes at least two subtypes with an age of onset of about 30 years (range, 10–50 years). The main clinical symptom is dementia. The other clinical features depend on the subtype and include progressive myoclonus epilepsy, ataxia, late pyramidal and extrapyramidal features, behavioral changes, and motor disturbances. There are no ophthalmological abnormalities. The EEG shows generalized fast spike-and-wave discharges with photosensitivity. Diagnosis requires demonstration of ultrastructural changes in skeletal or vascular smooth muscle cells, which may be fingerprint profiles, curvilinear profiles, or GRODs. Both autosomal recessive (most commonly) and dominant inheritance has been reported. Adult-onset NCL can be caused by mutations in the *CLN1* gene, and this may account for some cases.

CLN9 Disease

The most recently reported subtype of NCL was described in two Serbian sisters and two German brothers and is referred to as *CLN9-deficient*, as enzyme screening and sequencing of the coding regions of other NCL genes were negative.[32] The clinical history of these patients was characteristic of juvenile NCL, and curvilinear inclusions, fingerprint profiles, and GRODs were found in neurons, lymphocytes, and conjunctival cells. CLN9-deficient fibroblasts have a distinctive phenotype with rapid growth, increased apoptosis, and diminished levels of ceramide, dihydroceramide, and sphingomyelin. Transfection with *CLN8* but not other NCL genes corrected growth and apoptosis in CLN9-deficient cells. CLN8 is one of the TRAM-Lag1-CLN8 proteins containing a Lag1 motif. The latter imparts dihydroceramide synthase activity to yeast cells, suggesting that the CLN9 protein may be a regulator of dihydroceramide synthase.

SUMMARY

Loss-of-function mutations in *CSTB* are the primary defect in EPM1. In *CSTB* mutation-negative patients, *PRICKLE1* and *SCARB2* should be considered for testing. Lost lysosomal association of CSTB is an important contributing factor to EPM1. CSTB has an endogenous neuroprotective role, with different neuronal populations having different sensitivity to CSTB deficiency. The function of CSTB and the molecular mechanisms of EPM1 remain to be elucidated. Eight genes underlying human NCLs have now been identified: *PPT1*, *TPP1*, *CLN3*, *CLN5*, *CLN6*, *MFSD8*, *CLN8*, and *CSTD*. However, the biological function of the proteins encoded by NCL genes remains elusive, and it is still uncertain whether a common pathway at the molecular level underlies the accumulation of ceroid-lipofuscin. Diagnosis by enzymatic testing or DNA analysis is now available for several subtypes, and new treatment approaches are being developed.

DISCLOSURE STATEMENT

The authors have no conflicts of interest.

REFERENCES

1. Marseille Consensus Group. Classification of progressive myoclonus epilepsies and related disorders. *Ann Neurol*. 1990;28:113–116.
2. Pennacchio LA, Lehesjoki AE, Stone NE, et al. Mutations in the gene encoding cystatin B in progressive myoclonus epilepsy (EPM1). *Science*.1996;271: 1731–1734.
3. Kälviäinen R, Khyuppenen J, Koskenkorva P, et al. Clinical picture of EPM1-Unverricht-Lundborg disease. *Epilepsia*. 2008;49:549–556.
4. Ferlazzo E, Magaudda A, Striano P, et al. Long-term evolution of EEG in Unverricht-Lundborg disease. *Epilepsy Res*. 2007;73:219–227.
5. Koskenkorva P, Khyuppenen J, Niskanen E, et al. Bilateral atrophy of the motor cortex and thalami in Unverricht-Lundborg disease: a voxel-based morphometric study. *Neurology*. 2009;73:606–611.

6. Mascalchi M, Michelucci R, Cosottini M, et al. Brainstem involvement in Unverricht-Lundborg disease (EPM1): an MRI and (1)H MRS study. *Neurology*. 2002;58:1686–1689.
7. Turk V, Bode W. The cystatins: protein inhibitors of cysteine proteinases. *FEBS Lett*.1991;285:213–219.
8. Alakurtti K, Weber E, Rinne R, et al. Loss of lysosomal association of cystatin B proteins representing progressive myoclonus epilepsy, EPM1, mutations. *Eur J Hum Genet*. 2005;13:208–215.
9. Lalioti MD, Scott HS, Buresi C, et al. Dodecamerrepeat expansion in cystatin B gene in progressive myoclonus epilepsy. *Nature.* 1997;386:847–851.
10. Virtaneva K, D'Amato E, Miao J, et al. Unstable minisatellite expansion causing recessively inherited myoclonus epilepsy, EPM1. *Nat Genet*. 1997;15:393–396.
11. Joensuu T, Kuronen M, Alakurtti K, et al. Cystatin B: mutation detection, alternative splicing and expression in progressive myoclonus epilepsy of Unverricht-Lundborg type (EPM1) patients. *Eur J Hum Genet*. 2007;15:185–193.
12. Rinne R, Saukko P, Jarvinen M, et al. Reduced cystatin B activity correlates with enhanced cathepsin activity in progressive myoclonus epilepsy. *Ann Med.* 2002;34:380–385.
13. Pennacchio LA, Bouley DM, Higgins KM, et al. Progressive ataxia, myoclonic epilepsy and cerebellar apoptosis in cystatin B-deficient mice. *Nat Genet*. 1998;20:251–258.
14. Shannon P, Pennacchio LA, Houseweart MK, et al. Neuropathological changes in a mouse model of progressive myoclonus epilepsy: cystatin B deficiency and Unverricht-Lundborg disease. *J Neuropathol Exp Neurol*. 2002;61:1085–1091.
15. Franceschetti S, Sancini G, Buzzi A, et al. A pathogenetic hypothesis of Unverricht-Lundborg disease onset and progression. *Neurobiol Dis*.2007;25: 675–685.
16. Lehtinen MK,Tegelberg S,Schipper H, et al. Cystatin B deficiency sensitizes neurons to oxidative stress in progressive myoclonus epilepsy, EPM1. *J Neurosci*. 2009;29:5910–5915.
17. Houseweart MK, Pennacchio LA, Vilaythong A, et al. Cathepsin B but not cathepsins L or S contributes to the pathogenesis of Unverricht-Lundborg progressive myoclonus epilepsy (EPM1). *J Neurobiol.* 2003;56: 315–327.
18. Bassuk AG, Wallace RH, Buhr A, et al. A homozygous mutation in human PRICKLE1 causes an autosomal-recessive progressive myoclonus epilepsy-ataxia syndrome. *Am J Hum Genet*.2008;83:572–581.
19. Berkovic SF, Dibbens LM, Oshlack A, et al. Array-based gene discovery with three unrelated subjects shows SCARB2/LIMP-2 deficiency causes myoclonus epilepsy and glomerulosclerosis. *Am J Hum Genet*. 2008;82:673–684.
20. Dibbens LM, Michelucci R, Gambardella A, et al. SCARB2 mutations in progressive myoclonus epilepsy without renal failure. *Ann Neurol*. 2009;66:532–536.
21. Siintola E, Lehesjoki AE, Mole SE. Molecular genetics of the NCLs: status and perspectives. *Biochim Biophys Acta.* 2006;1762:857–864.
22. Jalanko A, Braulke T. Neuronal ceroidlipofuscinoses. *Biochim Biophys Acta*. 2009;1793:697–709.
23. Vesa J, Hellsten E, Verkruyse LA, et al. Mutations in the palmitoyl protein thioesterase gene causing infantile neuronal ceroidlipofuscinosis. *Nature.* 1995; 376:584–587.
24. Sleat DE, Donnelly RJ, Lackland H, et al. Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroidlipofuscinosis. *Science*. 1997;277:1802–1805.
25. The International Batten Disease Consortium. Isolation of a novel gene underlying Batten disease. *Cell*. 1995;82:949–957.
26. Savukoski M, Klockars T, Holmberg V, et al. CLN5, a novel gene encoding a putative transmembrane protein mutated in Finnish variant late infantile neuronal ceroidlipofuscinosis. *Nat Genet*.1998;19:286–288.
27. Wheeler RB, Sharp JD, Schultz RA, et al. The gene mutated in variant late-infantile neuronal ceroidlipofuscinosis (CLN6) and in nclf mutant mice encodes a novel predicted transmembrane protein. *Am J Hum Genet*. 2002;70:537–542.
28. Gao H, Boustany RM, Espinola JA, et al. Mutations in a novel CLN6-encoded transmembrane protein cause variant neuronal ceroidlipofuscinosis in man and mouse. *Am J Hum Genet*. 2002;70:324–335.
29. Siintola E, Topcu M, Aula N, et al. The novel neuronal ceroidlipofuscinosis gene MFSD8 encodes a putative lysosomal transporter. *Am J Hum Genet*. 2007;81:136–146.
30. Ranta S, Zhang Y, Ross B, et al. The neuronal ceroid-lipofuscinoses in human EPMR and *mnd* mutant mice are associated with mutations in CLN8. *Nat Genet*. 1999;23:233–236.
31. Siintola E, Partanen S, Strömme P, et al. Cathepsin D deficiency underlies congenital human neuronal ceroid-lipofuscinosis. *Brain*.2006;129:1438–1445.
32. Schulz A, Dhar S, Rylova S, et al. Impaired cell adhesion and apoptosis in a novel CLN9 Batten disease variant. *Ann Neurol*. 2004;56:342–350.

Chapter 70

GABRB3, Epilepsy, and Neurodevelopment

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INTRODUCTION

Three neurodevelopmental disorders—Angelman syndrome (AS), Rett syndrome (RS), and autism spectrum disorders (ASD)—share several clinical features, most notably neurodevelopmental delay and epilepsy. Here, we ask: what common mechanisms do these three neurodevelopmental disorders share that lead to a decline in cognitive development and epilepsy? Based on our observations concerning the genetic regulation of *GABRB3* in childhood absence epilepsy (CAE), we posit that the genetic mutations in these three neurodevelopmental disorders converge on a common disease mechanism involving genetic and epigenetic regulation of *GABRB3*. We first explain the data on human CAE supporting this hypothesis and show that three different point mutations in an alternative signal sequence (exon 1A) and N terminus (exon 2) in *GABRB3* result in hyperglycosylation and decreased gamma-aminobutyric acid (GABA) currents, all of which segregate with CAE. One of the variants of exon 1A, P11S, which is maternally transmitted, links *GABRB3* dysfunction with ASD and provides a possible cause of the seizures in this syndrome. In AS, *GABRB3* deletion contributes to the severe seizure phenotype. Rett syndrome is caused by mutations in MeCp2. Because MeCp2 epigenetically regulates *GABRB3*, reduced expression of *GABRB3* and epilepsy are considered consequences of MeCp2 mutations. We then review the significance of *GABRB3* in embryonic and adult neurogenesis and neuronal development in mammalian brain. We point to a master regulator of neurogenesis, RE1 silencing transcription factor (REST), which binds to repressor element 1 (RE1) in

intron 3 and the 5′ region of *GABRB3*. REST epigenetically regulates tissue and developmental expression of *GABRB3*. Finally, we chart the future challenges and experiments that could prove or disprove our hypothesis that REST and epigenetic regulation are involved in neurogenesis and epileptogenesis of absence seizures.

BACKGROUND

Genomics of *GABRB3*

GABRB3 generates two alternative signal peptide sequences, which are derived from alternative mRNA transcripts (exon 1A and exon 1), along with eight additional exons (exons 2–9). Two different mature polypeptides (NCBI accession number: NM_021912, NM_000814) are produced, each exhibiting a distinct signal peptide sequence with slight variations in residues at the N terminus due to differing cleavage sites.[1]

Exon 1 and exon 1A each can elicit tissue- and temporal-specific expression with the unique signal peptide and N-terminus sequences influencing functions and/or subcellular localization. Both core promoters contain GC-rich regions and are characterized by TATA-less SP1 binding motifs.[1–3] The abundant expression of exon 1A in fetal brain but not adult brain (Fig. 70–1) suggests a role in embryonic neurogenesis, with exon 1 likely contributing to adult brain function. The Human Genome February 2009 (GRCh37/hg19) assembly at the University of California Santa Cruz Genome Browser suggests that additional *GABRB3* sequences are expressed. One isoform found in brain has the first four exons (exons 1A, 1, 2, and 3) truncated, with transcription beginning in intron 3 and extending through exon 9; the other isoform, found in retina, consists of a long mRNA that is transcribed from intron 8 of *GABRA5* and spliced to the short region at the interval between *GABRB3* and *GABRA5* (42 kb upstream from exon 1A) and extending to exon 2 of *GABRB3*. *GABRB3* and *GABRA5* are located in a ~94 kb interval and are transcribed in a head-to-head orientation.[4] The recent reference sequence (NCBI) described four mRNAs of *GABRB3* (variants NM_021912.4, NM_000814.5, NM_001191320.1, NM_001191321.1). Two are truncated mRNAs of *GABRB3* without the first four exons (exons 1A, 1, 2, 3), which have two different lengths of intron 3 added to original variants 1 and 2. However, at present, only two protein sequences of 473 amino acids derived from *GABRB3* have consensus agreement (NP_068712, NP_000805.1).

Thus, *GABRB3* exhibits differing transcription start sites, chromatin accessibility, and histone modification patterns, and/or various splice patterns with stop codons, to produce these two variants. Although some evidence suggests that *GABRB3* is not imprinted, the paternal *GABRB3* gene region has been demonstrated to replicate earlier than maternal replication in

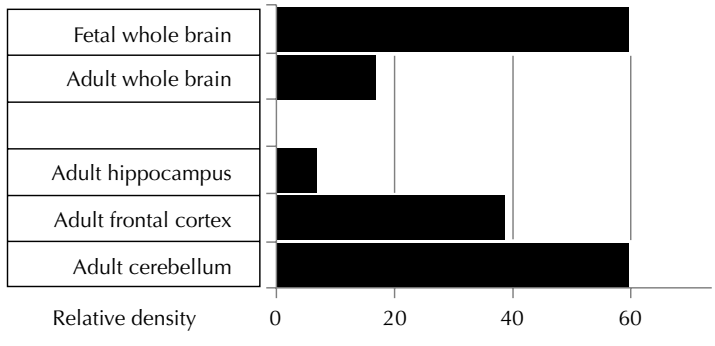

Figure 70–1. Alternative transcripts (*GABRB3* variant 2) of the human $GABA_A$ receptor beta3 subunit detected by RNase protection. The relative RNA content of different sample preparations was assessed by including an antisense riboprobe that was protected by a 152-base segment of the spliced transcript derived from exon 1A and exon 2. The transcript of exon 1A was less abundant than that of exon 1; however, the relative level of alternative transcripts was found to vary among the different brain samples. The transcript of exon 1A was enriched in fetal brain but markedly depleted in adult hippocampus. In frontal cortex and cerebellum, the relative level was similar to that found in adult whole brain. From ref. 3.

T-cell lymphocytes,[5] corresponding with distinct chromatin structure differences between paternal and maternal alleles.[6] This observation suggests paternal expression bias or tissue-specific partial imprinting. In addition, there is evidence that *Gabrb3* expression in brain is not always equivalent between male and female heterozygous mice.[7,8] In a mouse model of AS,[9] maternal-derived heterozygotes for a deletion of the chromosome encompassing *Ube3a* and *Gabrb3* show greater phenotypic correspondence with AS than paternal-derived heterozygotes, consistent with the known imprinting of the disorder; these mice show imprinting in some brain regions, including cerebellum, for *Ube3a* but biallelic expression of *Gabrb3* in the brain regions and ages examined.

GABRB3 in Mammalian Brain Embryogenesis and Adult Neurogenesis

Gamma-aminobutyric acid and $GABA_A$ receptors ($GABA_A$Rs) are the earliest neurotransmitter systems to emerge during development, even prior to glutamatergic synapses. The $GABA_A$Rs mediate excitatory signaling during development and play a significant role in neuronal growth and differentiation.[10,11] The $GABA_A$R β3 subunit mRNA/protein (encoded by *GABRB3*) emerges at embryonic days 14–17 in rat whole brain, and reaches its strongest expression at the perinatal stage, which is 150% of the level expressed in the adult[12] (Fig. 70–2). After birth, the β3 subunit protein decreases to moderate levels in adult cortex while falling rapidly in most thalamic nuclei, except in the reticular thalamic nucleus (NRT), where it remains one of the main components of the $GABA_A$R.[12–14] *GABRB3* therefore plays a significant role in neurodevelopment as well as in adult brain function. By virtue of its location in NRT and cortex, *GABRB3* plays an important role in thalamocortical circuits. As these circuits are essential to sensory processing, it is not surprising that heterozygous disruption of *Gabrb3* in mice would elicit somatosensory disturbances.[8] With *GABRB3* being highly implicated in autism, it is interesting to note that somatosensory disturbances are common to autism.[15]

In adult brain, GABA activates synaptic and extrasynaptic $GABA_A$Rs, producing phasic and tonic inhibitory chloride currents.[16] Opening of chloride conductance channels stabilizes the mature neuron near the resting potential, serving to inhibit neuronal action potentials whether or not the current is hyperpolarizing. Furthermore, $GABA_A$R-mediated chloride conductance can depolarize neural progenitor cells in immature neurons in the adult brain, similar to what occurs in the embryonic nervous system.[10,17] Phasic and tonic $GABA_A$R activity both play an important role in proper regulation of adult neurogenesis within the subventricular zone of the lateral ventricles as well as in the dentate gyrus of the hippocampus.[17]

Recordings of progenitor cells in fresh hippocampal slices from nestin tagged with green fluorescent protein (GFP) mice indicate that these cells receive direct GABAergic inputs, but not glutamatergic inputs, from the hippocampal circuitry. These GABAergic inputs depolarize neuronal progenitor cells, causing an increase in $[Ca^{2+}]_I$, resulting in the induction of expression of NeuroD, a positive regulator of neuronal differentiation. Thus, GABAergic inputs to hippocampal progenitor cells likely promote activity-dependent neuronal differentiation.[18] Since *GABRB3* is one of the main components of $GABA_A$R found in adult hippocampus, *GABRB3* likely contributes

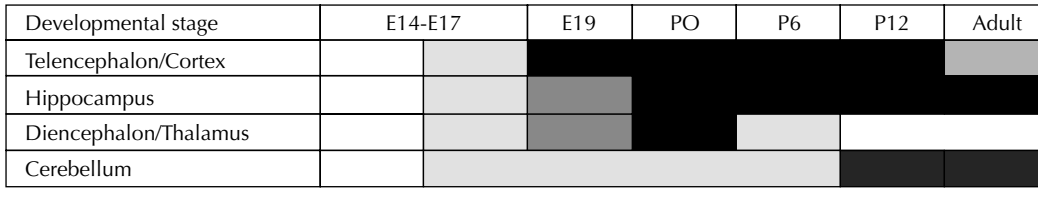

Figure 70–2. Expression level of the $GABA_A$ receptor beta subunit mRNAs in selected regions of embryonic and postnatal rat brain. Black represents a strong signal, dark gray a moderate signal, light gray a weak signal, and white an undetectable signal. From ref. 12.

to these GABAergic inputs. Furthermore, other transcription factors of the basic helix-loop-helix (bHLH) family, to which NeuroD belongs, may likewise be similarly regulated by GABA. *GABRB3* contains NEUROD1 binding motifs within intron 3, as well as a MASH1 binding motif in the 5′ region of exon1A and two NEUROG1 binding motifs in exon 1A (Fig. 70–3). One of the NEUROG1 motifs contains a single nucleotide polymorphism (SNP: rs 25409), which, as described above, we discovered in two CAE families,[1] and is also associated with maternal transmission in autism disorders.[19] This suggests that exon 1A may contain transcription binding sites for essential factors of neurogenesis in addition to its role of encoding a signal peptide. While NEUROD1 regulates neurogenesis,[18,20,21] MASH1 is required for the generation of GABAergic neurons[21,22] and NEUROG1 is required to specify glutamatergic neurons while simultaneously repressing both GABAergic differentiation[23,24] and astrocyte differentiation.[25] Therefore, the SNP rs25409 in the coding sequence may induce malfunction or unbalanced generation of GABAergic and glutamatergic neurons.

The nuclear-localized small modulatory double-stranded RNA (smdsRNA) coding

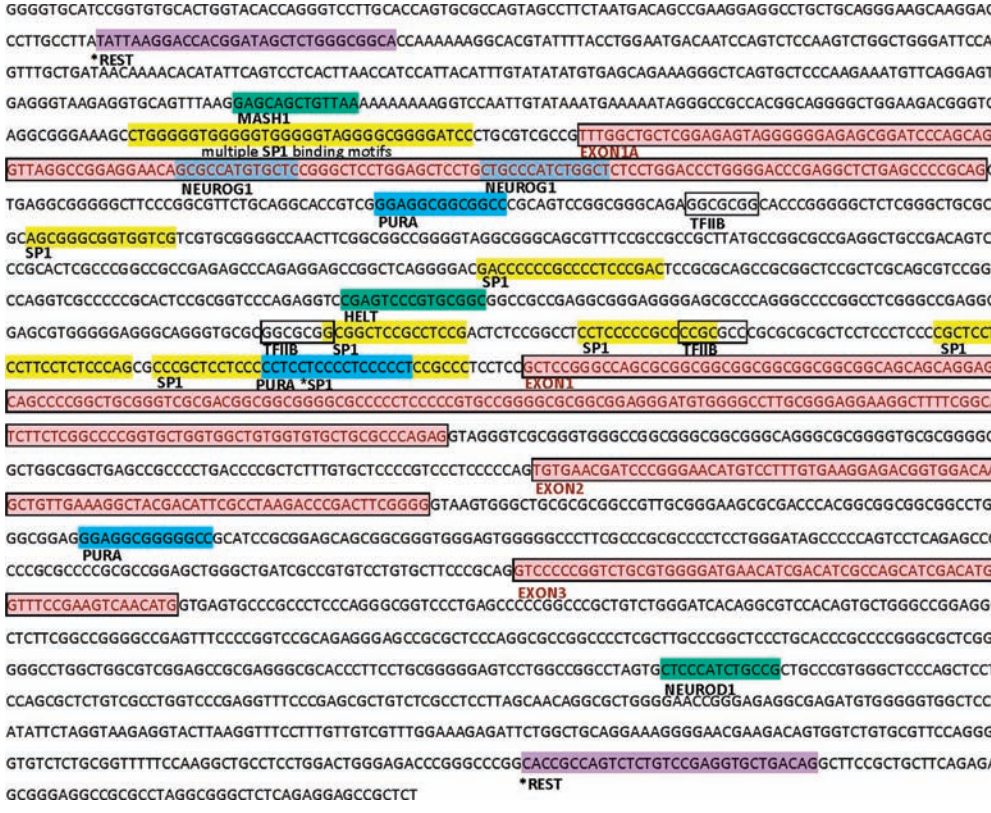

Figure 70–3. Sequence from the 5′ region to intron 3 of human *GABRB3* and predicted transcriptional factor binding motifs. *GABRB3* contains a long intron 3, spanning almost 151 kb. This, along with the first four exons (1A, 1, 2, and 3), represents a GC-rich region of about 1400 bp. Exons are bordered by solid lines. Both signal sequences have multiple SP1 binding sites. The asterisk indicates that the motif is demonstrated by chromatin immunoprecipitation[27] or a gel mobility shift assay with a DNAse I footprinting assay.[3] A Mash binding motif in the 5′ region of exon 1A, a HELT binding motif in intron 1A, two (NEUROG1) binding motifs in the coding region of exon 1A, and a (NEUROD) binding motif in intron 3 are each predicted by the Genomatics MatInspector program 8.0.3 (http://www.genomatics.de). All of these motifs involve GABAergic neuronal determination and differentiation.[20–25] Three predicted PURA motifs in intron 1A, exon 1, and intron 2 are essential for postnatal brain development.[76] The REST binding motif in intron 3 was recognized as two CpG islands by the Methyprimer program (http://www.urogene.org/methprimer/index1.html) and is potentially subject to DNA methylation and MeCP2 binding. This is the same region reported by Hogart et al.[38] as containing MeCP2 binding by chromatin immunoprecipitation.

the RE1-neuron restrictive silencer element (RE1/NRSE) sequence was discovered in hippocampal stem cells and was found to contribute to adult neurogenesis by changing the neuron-restrictive silencer factor (NRSF) from a repressor to an activator for a neuron-specific gene with RE1.[26] Thus, the role of *GABRB3* in adult neurogenesis is promoted by an epigenetic modulator, REST.

Epigenetic Modulation of *GABRB3*

Two NRSF binding motifs are found in *GABRB3*, one about 400 bp upstream from exon 1A and the other within intron 3[27] (Fig. 70–4), both locations corresponding to predicted RE1 sites. The neuron-restrictive silencer factor,, also known as REST, was originally reported as a transcriptional repressor of neuronal differentiation genes in nonneuronal cells and embryonic stem cells.[28] We confirmed the suppression of luciferase promoter activity in a construct containing RE1 in the 5′ region of exon 1A expressed in nonneuronal HEK 293 cells.[2] In contrast, promoter activity was not suppressed in neuron-like NT2 cells.[29]

REST, along with its primary corepressor, CoREST, dynamically recruits cellular cofactors including MeCP2 and other silencing machinery to RE1/NRSE sites to suppress the expression of the target genes, primarily neural genes, by chromatin remodeling.[30] REST not only prevents extraneural expression of target genes but also regulates by repressing the differentiation of neuronal subtypes required for proper tissue differentiation during embryonic development.[28,31,32] REST-mediated regulation of its target genes is not an all-or-none function. It depends on the cellular environment (e.g., intercellular signaling, Ca^{2+} dynamics, cell depolarization) resulting in context-dependent gene repression.[32,33] REST and CoREST can mediate cell type and developmental stage-specific gene repression for protein-coding genes and for several classes of noncoding RNA (ncRNA; e.g., micro RNA and long ncRNAs). The REST and CoREST network is highly integrated with ncRNA and mediates neural gene expression programs including bidirectional feedback.[34,35] REST is an essential epigenetic modulator for neuronal differentiation, homeostasis, and plasticity. Deregulation of REST and ncRNAs are implicated in cancer, neurodegenerative diseases, and neurodevelopmental diseases, including epilepsy.[30,36,37]

Recent studies concluded that REST regulates neurogenesis by reciprocal actions of REST and ncdsRNAs that exhibit RE1 sequence homology; this directly interacts with the REST transcriptional machinery in adult hippocampal neuronal stem cells.[26] The interaction transforms the REST complex into a transcriptional activator, inducing neuronal differentiation. The expression of *GABRB3*, which has two RE1/NRSE sites, is regulated

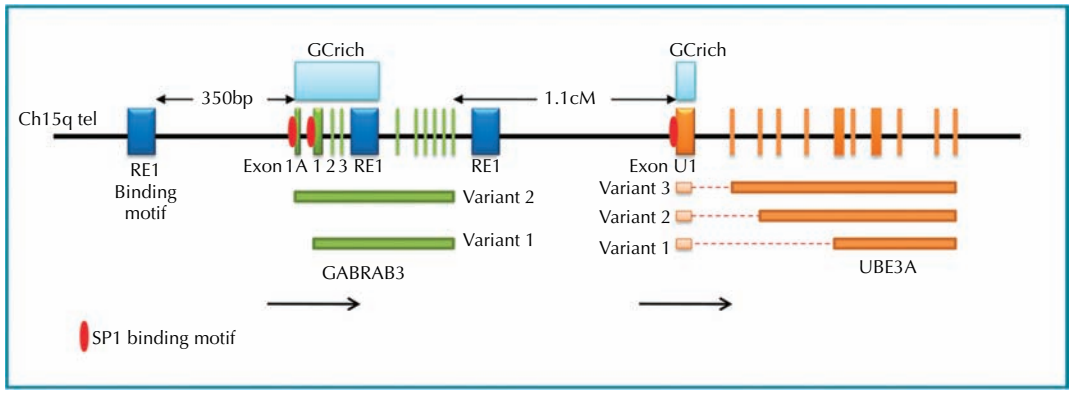

Figure 70–4. Chromosome 15q11.2-q12: *GABRB3* and *UBE3A*. The initial exons of *GABRB3* and *UBE3A* contain SP1 binding motifs in the proximal promoter regions, which are GC-rich regions and are indicated by the light blue painted squares. Green squares indicate exons of *GABRB3* with exon numbers below. Orange squares indicate *UBE3A* exons. Bottom arrows indicate the direction of transcription. Both *GABRB3* and *UBE3A* are transcribed in the same direction. One NRSF binding site lies at 350 bp upstream from exon 1A of *GABRB3*, the second NRSF binding site is in intron 3, and the third NRSF binding site is located 89 kb downstream from *GABRB3* (Quest 2.4 database).

by REST. In addition, Hogart et al.[38] found a MeCP2 binding site in intron 3 of *GABRB3* close to the REST binding site (RE1). MeCP2, a methylated DNA binding protein, is an epigenetic regulator that is required for development and maintenance of neurons.[39] Hogart et al.[38] reported that *GABRB3* expression is biallelic but paternally biased in human prefrontal cortex (Brodmann field 9) via MeCP2 activation of *GABRB3* expression. In other words, REST, which connects with various cofactors including MeCP2, could modulate *GABRB3* expression in age-dependent, tissue-specific development, not only in stem cells, but also in mature neurons through environmental stimulation that could occur throughout life.

NEURODEVELOPMENTAL DISORDERS

GABRB3, Epilepsy, and Autism

Various clinical reports, animal studies, and genetic association and basic molecular studies have provided evidence that *GABRB3* is involved in developmental neurological disorders including epilepsy.[40–46] Three missense mutations (P11S, S15F, G32A) found in an alternative signal peptide and in the N terminus of *GABRB3* segregated with remitting childhood absence epilepsy (rCAE) within four families. Two multiplex and multigenerational families containing eight affected members (Fig. 70–5A) and one proband of a singleton have mutations in an alternative signal peptide. When expressed in vitro, each of the mutations in the signal peptide coding region, exon 1A of *GABRB3,* caused hyperglycosylation of the $GABA_AR$ β3 subunit protein with concurrent reduction in GABA currents.[1] Furthermore, the promoter region of exon 1A of *GABRB3* displayed a significant reduction in promoter activity for a common SNP in the promoter region.[2] We concluded that the functional abnormality resulting from missense mutations, as well as certain combinations of SNPs in multiple regulatory elements in the 5' region and 5′UTR of *GABRB3*, causes reduced expression of *GABRB3* and a concurrent reduction in inhibition, leading to an increase in susceptibility to absence seizures. The CAE disease phenotype exhibits paternal transmission (Fig. 7–5A) of the rare *GABRB3* signal peptide variant (rs 25409: P11S), whereas maternal transmission of the same variant appears to be associated with autism (Fig. 70–5B). The association of phenotype transmission with parental gender suggests that parent-of-origin gene expression is likely occurring in certain brain region(s) within some temporal range (partial imprinting). Further genetic studies are needed to determine conclusively the importance of these rare *GABRB3* variants in CAE and autism.

GABRB3 in Chromosome 15Q11–13, AS, and Prader-Willi Syndrome

GABRB3 is spatially clustered with *GABRA5* and *GABRG3* on chromosome 15q11-q13, with *UBE3A* (ubiquitin protein ligase E3A) lying about 1 cM upstream (centromeric) from *GABRB3*. This region contains a variety of imprinted genes (Fig. 70–6) and exhibits genomic rearrangements at five common breakpoints (BP1–BP5) that often result in deletions and duplications of these genes.[47] Parent-of-origin phenotypes are characteristic of chromosome 15q11-q13 abnormalities. For example, AS,[48] caused by deficiencies of maternal genomic information on chromosome 15q11-q13, exhibits a severe phenotype. In contrast, Prader-Willi syndrome (PWS), resulting from a deficiency of paternal genes from the same region, exhibits a less severe phenotype.[49] The nature of the genomic defect also influences the severity of the phenotype; for instance, a de novo 15q11-q13 deletion on the maternal genome, occurring in 70% of AS cases, is characterized by severe mental retardation, motor dysfunction, and early-onset intractable epilepsy (primarily absences and myoclonic seizures). In contrast, 10% of AS probands exhibit only a mutation in *UBE3A*, also found on chromosome 15q11–13,[49] and are less severely affected than individuals with the full 15q11-q13 deletion. Such patients typically display only mild epilepsy.[50] Recurrence risk and severity of epilepsy also differ according to the genetic type of AS. Minassian et al.[50] suggested that the severe epilepsy in deletion cases of AS appears to be due to the lack of maternal *GABRB3* in addition to the AS gene, *UBE3A*. This is consistent with the epi-

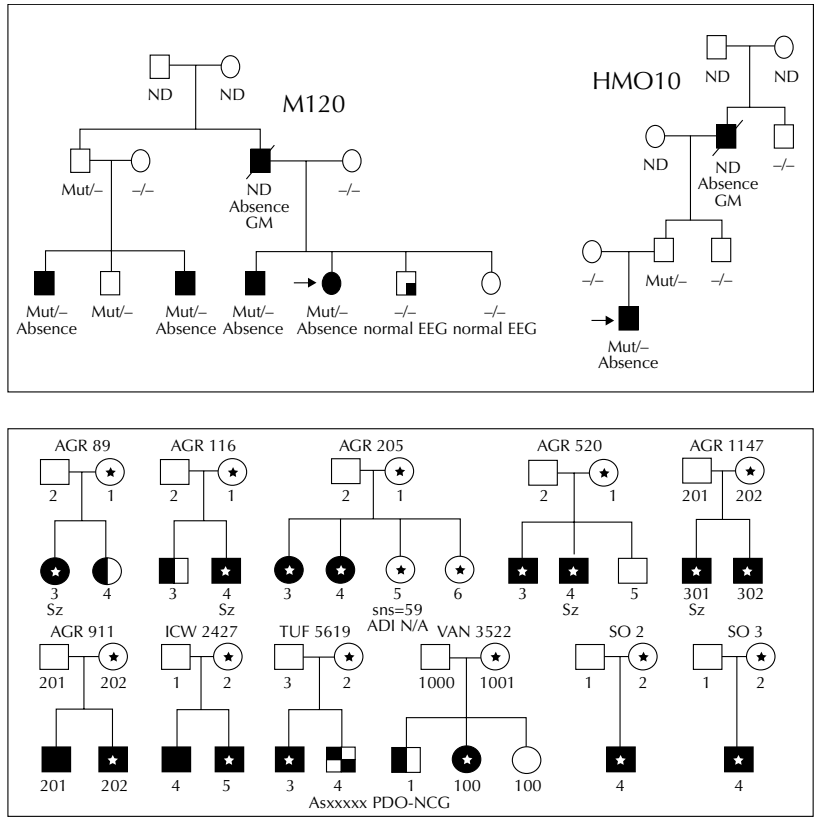

Figure 70–5. Top: Paternally transmitted rs 25409 (P11S) in CAE. The rare variant (dbSNP ID, rs 25409 [P11S]) is segregated in affected persons in two of four families with rCAE probands, as reported.[1] Black circles (female) and squares (males) represent epilepsy-affected probands. Asymptomatic persons who have 3 Hz diffuse bilateral spike-wave complexes or 5 to 6 Hz sharp waves on encephalography are represented by half black circles or squares. From ref. 1. **Bottom:** Maternally transmitted rs 25409 (P11S) in autism. The strictly autism phenotype was shown to be significant in maternal transmission with a genotype relative risk of 6.00 (95% confidence interval 1.62–22.16, $P = 0.007$). Only probands (4 out of 17) with P11S exhibited seizures in this study. One of these probands exhibited paternal transmission, while the other three phenotypes were maternally transmitted. Individuals who meet the criteria for autism on the Autism Diagnostic Interview[19] are represented by completely filled black symbols. Individuals who meet the criteria for ASD1 or ASD2 are represented by half-filled black symbols. ASD1: meets criteria in the Social domain and in either the Communication or Behavior domain. ASD2: meets criteria in the Social and Communication domains or meets criteria in the Social domain and is within 2 points of the Communication domain or meets criteria in the Communication domain and is within 2 points of the Social domain or is within 1 point in both the Social and Communication domains.[77] From ref. 19.

lepsy present in human *GABRB3*[1] and mouse *Gabrb3* mutations.[45] Epilepsy in most AS cases is refractory to medication but shows improvement with age.[51] However, PWS, which results from paternal deletion of the same chromosome 15q11-q13 region, displays a very different phenotype than AS, with mild mental retardation, obesity, and a lower likelihood of epilepsy. Furthermore, Wang et al.[52] reported that 8 of 38 PWS patients (21%) with large deletions of paternal 15q11-q13 genes, including *GABRB3*, had seizures. In contrast, none of the 12 PWS patients without a *GABRB3* deletion had seizures. These differences further support the involvement of *GABRB3* in the epilepsy displayed by these probands.[52] It is also important to note that duplications or micro-deletions of the maternal 15q11-q13 region are one of the more frequently reported observations (~2%) in ASD.[53,54] Interestingly, individuals with paternally derived duplications of 15q11-q13 rarely display autistic symptoms.[55]

The above evidence suggests a significant role for imprinted genes in neurodevelopmental disorders like AS and PWS. Consequently, their control by the imprinting center (IC)

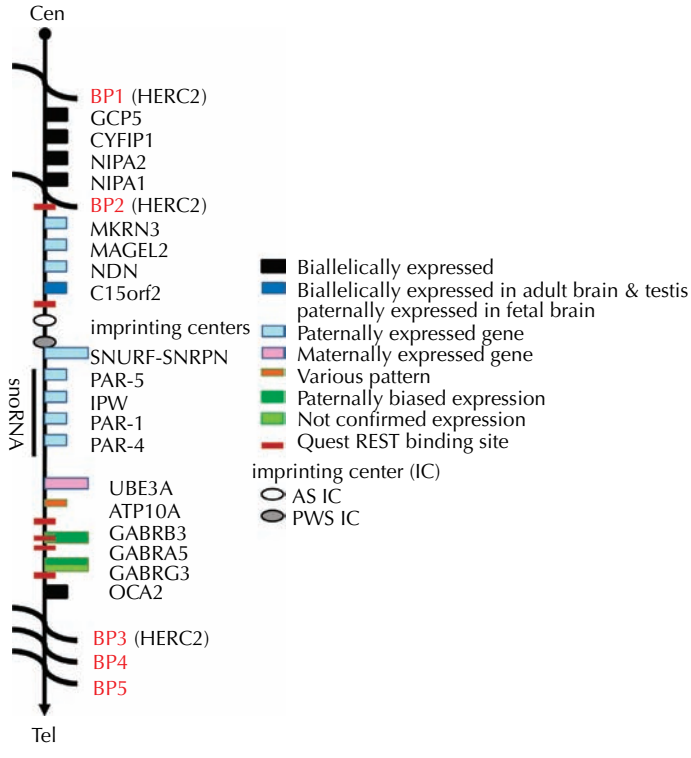

Figure 70–6. Gene order and REST binding sites on chromosome 15q11–13. Chromosome 15 contains segmental duplications and repeated transcribed DNA sequences (i.e., *HERC2* genes) located at the proximal and distal ends of the 15q11-q13 region. The typical deletion of the 15q11-q13 region causes PWS and AS, presumably due to unequal crossing over in meiosis at the breakpoint (BP1–5). Most common deletions extend the region from either of two proximally positioned breakpoints (BP1 or BP2) to a distal breakpoint (BP3). The breakpoint is shown by the curved line. Modified from ref. 78.

on chromosome 15q11-q13 is important. The IC (Fig. 70–6) was originally demonstrated and defined[56,57] by the smallest microdeletion (currently 880 bp) that resulted in a block of resetting the imprint, stabilizing on that chromosome the parental imprint (epigenotype) on which the mutation arose. The IC for AS is located centromeric to the IC for PWS, which includes the first exon of the *SNRPN* gene (Fig. 70–6). None of the paternally imprinted genes (*ZNF127*, *SNRPN*, *PAR-5*, *IPW*, and *PAR-1*), located in the vicinity of the IC, are not expressed when the PWS IC is mutated. By contrast, these same genes are expressed biparentally in mutations of the AS IC. However, these AS probands lack expression of *UBE3A* (the AS gene), located 250–1000 kb distally to the IC. To quote Saitoh et al.,[56] "the paternal chromosome of these PWS (IC mutation) patients carries an ancestral maternal epigenotype, and the maternal chromosome of these AS (IC mutation) patients carries an ancestral paternal epigenotype. The IC therefore functions to reset the maternal and paternal imprints throughout a 2 Mb imprinted domain (in cis) within human chromosome 15q11-q13 during gametogenesis." The two IC sequences presumably also interact in a DNA methylation-regulated manner[57] with proteins and noncoding mRNAs that act in trans with target imprinted genes.[58,59] At this time, the neurological defects in AS are considered to be due primarily to reduced *UBE3A* function needed for inactivation of crucial gene products by ubiquitin-dependent proteasome degradation.[59] Dysfunction of the latter perturbs neuronal growth and development, possibly involving dendritic spine morphology[60] and/or excitatory alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-type glutamatergic receptor trafficking.[61] Localization of $GABA_A$ receptors, including the β3 subunit in postmortem AS brain, would be interesting to examine.

GABRB3 and *UBE3A* in Hippocampus

In the hippocampus, *GABRB3* expression levels remain constant throughout maturation and into adulthood. Exon 1 of *GABRB3* is strongly expressed in many regions of adult human brain, including the hippocampus (Fig. 70–2), and initiates transcription by SP1 as well as other transcriptional factor(s).[3] The AS gene, *UBE3A*, located at 1.1 megabase upstream from *GABRB3* in chromosome 15q11.2-q12, is a maternally imprinted gene. Although it has been accepted that *UBE3A* displays maternally imprinted expression in hippocampus, cerebellum, and several lobes of neocortex,[62,63] recent studies utilizing immunoblotting and immunohistochemistry methods found that maternally predominant Ube3a protein expression is not limited to these regions but instead occurs throughout all brain regions.[64]

Three variants of *UBE3A* are transcribed from the same exon in the 5′ region, but different N termini are produced by various splice patterns, including stop codons.[65] The common 5′ region contains a GC- rich region as well as a SP1 binding motif, as predicted by MatInspector software. This suggests that the common ubiquitous transcriptional factor SP1 could transcribe both the *UBE3A* and *GABRB3* genes on the same chromatin at the same time.

UBE3A and *GABRB3* Deficiency with MeCP2 Dysregulation: RS and AS

Angelman syndrome (AS), RS, and ASD each exhibit mental retardation and epilepsy of varying severity as part of their phenotypes. Reduced *GABRB3* in prefrontal cortex is common to these three disorders.[38,46] Rett syndrome, caused by a *MeCP2* mutation, displays deficiency of *GABRB3* and *UBE3A*,[46] even though the genes for *GABRB3* and *UBE3A* are intact. Therefore, MeCP2 deficiency alone can result in reduced *UBE3A* and *GABRB3*. In contrast, the findings in studies of MeCP2-deficient mice were inconsistent,[46,66,67] suggesting that epigenetic regulation that includes RE1 sites is not necessarily the same between humans and mice. Makedonski et al.[68] found that the PWS- imprinting center (PWS-IC) on the maternal allele is normally methylated. This allows MeCP2 to bind and recruit histone deacetylases 1 and 2, which deacetylate histone H3 and histone methyltransferase to methylate H3(K9). This chain of events leads to heterochromatinization and silencing of all paternally expressed genes. However, in the absence of MeCP2, heterochromatin cannot form, allowing an open chromatin structure that is sufficient to transcribe antisense *UBE3A* RNA, thereby reducing *UBE3A* transcription. MeCP2 deficiency in RS can lead to epigenetic aberrations via the IC.[68] REST is degraded by the ubiquitin-proteasome system through SCFbeta-TRCP.[69] UBE3A is therefore hypothesized to degrade REST. Deficient *UBE3A* could then affect expression of *GABRB3* and other neuronal genes through elevated REST/NRSF.

CONCLUSIONS

GABRB3 plays an important role in embryonic/adult neurogenesis and neuronal development in mammalian brain. *GABRB3* is expressed at the embryonic and perinatal stages as well as in childhood brain as a main component of $GABA_A$Rs in most brain regions. This includes many thalamic nuclei that mediate the electroencephalographic spike-wave complex of absence seizures. In adult brain, *GABRB3* is dominantly expressed in the hippocampus and cerebellum but is very low in most parts of the thalamus, the only exception being the nucleus reticularis, where it remains relatively high. *GABRB3* mutations expressed in embryonic and neonatal but not adult thalamic regions of the brain could explain the reduction of seizures in AS and the remission of CAE with age, since CAE remits during puberty, when expression of *GABRB3* diminishes/ceases in most thalamic nuclei.

We suggest that transcript variant 1 and variant 2 of *GABRB3* need to be examined in thalamus and hippocampus as a function of development, in comparison to *UBE3A* expression, in order to establish the significance of *GABRB3* in neurodevelopment and epilepsy. In mouse, such assays of *GABRB3* expression could be performed on animals engineered to produce

a maternal-derived heterozygous deletion of the chromosomal region encompassing both *UBE3A* and *GABRB3*, an animal model of AS.[9]

Future experiments also should consider the epigenetic modulation of REST and MeCP2 in ASD, AS, RS, and CAE. One study[70] has already broadened the view of REST and CoREST action by genome-wide analysis of the binding status of REST and CoREST to various promoters in embryonic mouse forebrain. Expression of REST and CoREST varies in different brain regions and is specific for neuronal subtypes of GABAergic, glutamatergic, and cholinergic neurons. Differential expression of REST and CoREST in brain regions also affects the generation of factors responsible for neuronal diversity, such as homeostasis, cell cycle dynamics, cell viability, stress responses, and epigenetic regulation. REST is likely to have multiple roles, not only in immature neurons but also in mature ones.[70] In situ hybridization analysis in normal brains has already revealed that REST mRNA is highest in adult hippocampus,[71] and REST protein expression studies utilizing mature hippocampal neurons have replicated the in situ hybridization results.[72] Epileptic insults and ischemic changes have also been observed to elevate REST mRNA.[32,71,73]

UBE3A is hypothesized to regulate REST by degradation through the ubiquitin pathway, with altered *GABRB3* expression resulting from maladjustment of REST. When *UBE3A* is mutated, reduced degradation of REST results in excessive REST. When REST acts as a repressor, excessive REST in a developing brain could hypothetically reduce *GABRB3* expression, resulting in cognitive decline and epilepsy. Experiments that would demonstrate the above hypothesis are warranted. The deletion of *UBE3A*, thereby influencing REST, could also lead to a severe phenotype due to inappropriate regulation of a number of other targets, including *GABRB3*, with which REST interacts. Future experiments should also consider whether epigenetic malregulation of *GABRB3* by REST and/or MeCP2 produces the phenotypes of mental retardation and epilepsy even without a *GABRB3* mutation.[74,75] Understanding these epigenetic mechanisms is likely to lead to novel interventions for neurodevelopmental diseases like ASD, AS, RS, and CAE.

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DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Tanaka M, Olsen RW, Medina MT, Schwartz E, Alonso ME, Duron RM, Castro-Ortega R, Martinez-Juarez IE, Pascual-Castroviejo I, Machado-Salas J, Silva R, Bailey JN, Bai D, Ochoa A, Jara-Prado A, Pineda G, Macdonald RL, Delgado-Escueta AV. Hyperglycosylation and reduced GABA currents of mutated GABRB3 polypeptide in remitting childhood absence epilepsy. *Am J Hum Genet.* 2008;82: 1249–1261.
2. Tanaka M, Bailey JN, Ishikawa-Brush Y, Bai D, Delgado-Escueta AV, Olsen RW. Effects on promoter activity of common SNPs in 5′ region of GABRB3 exon1A. *Epilepsia* 2012, in press..
3. Kirkness EF, Fraser CM. A strong promoter element is located between alternative exons of a gene encoding the human gamma-aminobutyric acid-type A receptor beta 3 subunit (GABRB3). *J Biol Chem*. 1993;268: 4420–4428.
4. Glatt K, Glatt H, Lalande M. Structure and organization of GABRB3 and GABRA5. *Genomics.* 1997;41: 63–69.
5. LaSalle JM, Lalande M. Domain organization of allele-specific replication within the GABRB3 gene cluster requires a biparental 15q11–13 contribution. *Nat Genet*. 1995;9:386–394.
6. ENCODE Project Consortium. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature.* 2007;447:799–816.
7. Liljelund P, Handforth A, Homanics GE, Olsen RW. $GABA_A$ receptor beta3 subunit gene-deficient heterozygous mice show parent-of-origin and gender-related differences in beta3 subunit levels, EEG, and behavior. *Brain Res Dev Brain Res*. 2005;157:150–161.
8. DeLorey TM, Sahbaie P, Hashemi E, Li WW, Salehi A, Clark DJ. Somatosensory and sensorimotor consequences associated with the heterozygous disruption of the autism candidate gene, GABRB3. *Behav Brain Res.* 2011;216:36–45.
9. Jiang Y-H, Pan Y, Zhu L, Landa Yoo,J, Spencer C, Lorenzo I, Brilliant M, Noebels J, Beaudet AL. Altered ultrasonic vocalization and impaired learning and memory in Angelman syndrome mouse model with a large maternal deletion from Ube3a to Gabrb3. *PLOS One.* 2010;5(8):e12278.

10. Ben-Ari Y, Khazipov R, Leinekugel X, Caillard O, Gaiarsa JL. $GABA_A$, NMDA and AMPA receptors: a developmentally regulated "ménage à trois." *Trends Neurosci*. 1997;20:523–529.
11. Herlenius E, Lagercrantz H. Development of neurotransmitter systems during critical periods. *Exp Neurol*. 2004;190(supp1):S8–S21.
12. Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen $GABA_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J Neurosci*. 1992;12:4151–4172.
13. Zhang JH, Sato M, Tohyama M. Different postnatal development profiles of neurons containing distinct $GABA_A$ receptor beta subunit mRNAs (beta 1, beta 2, and beta 3) in the rat thalamus. *Brain Res Dev Brain Res*. 1991;58:289–292
14. Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience*. 2000;101:815–885
15. Greenspan SI, Weider S. Developmental patterns and outcomes in infants and children with disorder in relating and communicating: a chart review of 200 cases of children with autistic spectrum diagnosis. *J Devel Learn Disord*. 1997;1:87–141.
16. Olsen RW, Sieghart W. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid (A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev*. 2008;60:243–260.
17. Ge S, Pradhan DA, Ming GL, Song H. GABA sets the tempo for activity-dependent adult neurogenesis. *Trends Neurosci*. 2007;30:1–8.
18. Tozuka Y, Fukuda S, Namba T, Seki T, Hisatsune T. GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. *Neuron*. 2005;47:803–815.
19. Delahanty RJ, Kang JQ, Brune CW, Kistner EO, Courchesne E, Cox NJ, Cook EH Jr, Macdonald RL, Sutcliffe JS. Maternal transmission of a rare GABRB3 signal peptide variant is associated with autism. *Mol Psychiatry*. 2011;16:86–96.
20. Boutin C, Hardt O, de Chevigny A, Coré N, Goebbels S, Seidenfaden R, Bosio A, Cremer H. NeuroD1 induces terminal neuronal differentiation in olfactory neurogenesis. *Proc Natl Acad Sci USA*. 2010;107: 1201–1206.
21. Roybon L, Mastracci TL, Ribeiro D, Sussel L, Brundin P, Li JY. GABAergic differentiation induced by Mash1 is compromised by the bHLH proteins Neurogenin2, NeuroD1, and NeuroD2. *Cereb Cortex*. 2010;20:1234–1244.
22. Fode C, Ma Q, Casarosa S, Ang SL, Anderson DJ, Guillemot F. A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. *Genes Dev*. 2000;14:67–80.
23. Schuurmans C, Armant O, Nieto M, Stenman JM, Britz O, Klenin N, Brown C, Langevin LM, Seibt J, Tang H, Cunningham JM, Dyck R, Walsh C, Campbell K, Polleux F, Guillemot F. Sequential phases of cortical specification involve Neurogenin-dependent and -independent pathways. *EMBO J*. 2004 23;14:2892–2902.
24. Nakatani T, Minaki Y, Kumai M, Ono Y. Helt determines GABAergic over glutamatergic neuronal fate by repressing Ngn genes in the developing mesencephalon. *Development*. 2007;134:2783–2793.
25. Luo Y, Shan G, Guo W, Smart RD, Johnson EB, Li X, Pfeiffer RL, Szulwach KE, Duan R, Barkho BZ, Li W, Liu C, Jin P, Zhao X. Fragile X mental retardation protein regulates proliferation and differentiation of adult neural stem/progenitor cells. *PLoS Genet*. 2010;6:e1000898.
26. Kuwabara T, Hsieh J, Nakashima K, Taira K, Gage FH. A small modulatory dsRNA specifies the fate of adult neural stem cells. *Cell*. 2004;116:779–793.
27. Valouev A, Johnson DS, Sundquist A, Medina C, Anton E, Batzoglou S, Myers RM, Sidow A. Genomewide analysis of transcription factor binding sites based on ChIP-Seq data. *Nat Methods*. 2008;5:829–834.
28. Chong JA, Tapia-Ramírez J, Kim S, Toledo-Aral JJ, Zheng Y, Boutros MC, Altshuller YM, Frohman MA, Kraner SD, Mandel G. REST: a mammalian silencer protein that restricts sodium channel gene expression to neurons. *Cell*. 1995;80:949–957.
29. Urak L, Feucht M, Fathi N, Hornik K, Fuchs K. A GABRB3 promoter haplotype associated with childhood absence epilepsy impairs transcriptional activity. *Hum Mol Genet*. 2006;15:2533–2541.
30. Majumder S. REST in good times and bad: roles in tumor suppressor and oncogenic activities. *Cell Cycle*. 2006;5:1929–1935.
31. Jones FS, Meech R. Knockout of REST/NRSF shows that the protein is a potent repressor of neuronally expressed genes in non-neural tissues. *Bioessays*. 1999;21:372–376.
32. Ballas N, Grunseich C, Lu DD, Speh JC, Mandel G. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell*. 2005;121:645–657.
33. Ariano P, Zamburlin P, D'Alessandro R, Meldolesi J, Lovisolo D. Differential repression by the transcription factor REST/NRSF of the various Ca^{2+} signaling mechanisms in pheochromocytoma PC12 cells. *Cell Calcium*. 2010;47:360–368.
34. Conaco C, Otto S, Han JJ, Mandel G. Reciprocal actions of REST and a microRNA promote neuronal identity. *Proc Natl Acad Sci USA*. 2006;103:2422–2427.
35. Qureshi IA, Mehler MF. Regulation of non-coding RNA networks in the nervous system—what's the REST of the story? *Neurosci Lett*. 2009;466:73–80.
36. Bassuk AG, Wallace RH, Buhr A, Buller AR, Afawi Z, Shimojo M, Miyata S, Chen S, Gonzalez-Alegre P, Griesbach HL, Wu S, Nashelsky M, Vladar EK, Antic D, Ferguson PJ, Cirak S, Voit T, Scott MP, Axelrod JD, Gurnett C, Daoud AS, Kivity S, Neufeld MY, Mazarib A, Straussberg R, Walid S, Korczyn AD, Slusarski DC, Berkovic SF, El-Shanti HI. A homozygous mutation in human PRICKLE1 causes an autosomal-recessive progressive myoclonus epilepsy-ataxia syndrome. *Am J Hum Genet*. 2008;83:572–581.
37. Garriga-Canut M, Schoenike B, Qazi R, Bergendahl K, Daley TJ, Pfender RM, Morrison J. Ockuly J, Stafstrom C, Sutula T, Roopra A. 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. *Nat Neurosci*. 2006;9;1382–1387.
38. Hogart A, Nagarajan RP, Patzel KA, Yasui DH, Lasalle JM. 15q11–13 $GABA_A$ receptor genes are normally biallelically expressed in brain yet are subject to epigenetic dysregulation in autism-spectrum disorders. *Hum Mol Genet*. 2007;16:691–703.

39. Ghosh RP, Nikitina T, Horowitz-Scherer RA, Gierasch LM, Uversky VN, Hite K, Hansen JC, Woodcock CL. Unique physical properties and interactions of the domains of methylated DNA binding protein 2. *Biochemistry.* 2010;49:4395–4410.
40. Schinzel AA, Brecevic L, Bernasconi F, Binkert F, Berthet F, Wuilloud A, Robinson WP. Intrachromosomal triplication of 15q11-q13. *J Med Genet*. 1994;31:798–803.
41. Battaglia A, Guerrini R. Chromosomal disorders associated with epilepsy. *Epileptic Disord*. 2005;7:181–192.
42. Kim SA, Kim JH, Park M, Cho IH, Yoo HJ. Association of GABRB3 polymorphisms with autism spectrum disorders in Korean trios. *Neuropsychobiology.* 2006;54: 160–165.
43. Lü JJ, Zhang YH, Pan H, Chen YC, Liu XY, Jiang YW, Bao XH, Shen Y, Wu HS, Xu KM, Wu XR. Case-control study and transmission/disequilibrium tests of the genes encoding GABRA5 and GABRB3 in a Chinese population affected by childhood absence epilepsy. *Chin Med J*. 2004;117:1497–1501.
44. Feucht M, Fuchs K, Pichlbauer E, Hornik K, Scharfetter J, Goessler R, Füreder T, Cvetkovic N, Sieghart W, Kasper S, Aschauer H. Possible association between childhood absence epilepsy and the gene encoding GABRB3. *Biol Psychiatry*. 1999;46: 997–1002.
45. DeLorey TM, Handforth A, Anagnostaras SG, Homanics GE, Minassian BA, Asatourian A, Fanselow MS, Delgado-Escueta A, Ellison GD, Olsen RW. Mice lacking the beta3 subunit of the $GABA_A$ receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. *J Neurosci*.1998;18:8505–8514.
46. Samaco RC, Hogart A, LaSalle JM. Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3. *Hum Mol Genet*. 2005;14:483–492.
47. Robinson WP, Dutly F, Nicholls RD, Bernasconi F, Peñaherrera M, Michaelis RC, Abeliovich D, Schinzel AA. The mechanisms involved in formation of deletions and duplications of 15q11-q13. *J Med Genet*. 1998;35:130–136.
48. Lossie AC, Whitney MM, Amidon D, Dong HJ, Chen P, Theriaque D, Hutson A, Nicholls RD, Zori RT, Williams CA, Driscoll DJ. Distinct phenotypes distinguish the molecular classes of Angelman syndrome. *J Med Genet*. 2001;38:12:834–845.
49. Nicholls RD, Saitoh S, Horsthemke B. Imprinting in Prader-Willi and Angelman syndromes. *Trends Genet.* 1998;14:194–200.
50. Minassian BA, DeLorey TM, Olsen RW, Philippart M, Bronstein Y, Zhang Q, Guerrini R, Van Ness P, Livet MO, Delgado-Escueta AV. Angelman syndrome: correlations between epilepsy phenotypes and genotypes. *Ann Neurol*. 1998;43:485–493.
51. Valente KD, Koiffmann CP, Fridman C, Varella M, Kok F, Andrade JQ, Grossmann RM, Marques-Dias MJ. Epilepsy in patients with Angelman syndrome caused by deletion of the chromosome 15q11–13. *Arch Neurol*. 2006;63:122–128.
52. Wang PJ, Hou JW, Sue WC, Lee WT. Electroclinical characteristics of seizures—comparing Prader-Willi syndrome with Angelman syndrome. *Brain Dev.* 2005;27:101–107.
53. Veenstra-VanderWeele J, Cook EH Jr. Molecular genetics of autism spectrum disorder. Mol Psychiatry [review]. 2004;9:819–832.
54. Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L. Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol Psychiatry*. 2006;11:18–28.
55. Cook EH Jr, Lindgren V, Leventhal BL, Courchesne R, Lincoln A, Shulman C, Lord C, Courchesne E. Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. *Am J Hum Genet*. 1997;60:928–934.
56. Saitoh S, Buiting K, Rogan PK, Buxton JL, Driscoll DJ, Arnemann J, König R, Malcolm S, Horsthemke B, Nicholls RD. Minimal definition of the imprinting center and fixation of chromosome 15q11-q13 epigenotype by imprinting mutations. *Proc Natl Acad Sci USA.* 1996;13:7811–7815.
57. Biliya S, Bulla LA Jr. Genomic imprinting: the influence of differential methylation in the two sexes [review]. *Exp Biol Med*. 2010;235:139–147.
58. Horsthemke B, Wagstaff J. Mechanisms of imprinting of the Prader-Willi/Angelman region. *Am J Med Genet A.* 2008;146A:2041–2052.
59. Chamberlain SJ, Lalande M. Angelman syndrome, a genomic imprinting disorder of the brain. *J Neurosci.* 2010;30:9958–9963.
60. Dindot SV, Antalffy BA, Bhattacharjee MB, Beaudet AL. The Angelman syndrome ubiquitin ligase localizes to the synapse and nucleus, and maternal deficiency results in abnormal dendritic spine morphology. *Hum Mol Genet.* 2008;17:111–118.
61. Greer PL, Hanayama R, Bloodgood BL, Mardinly AR, Lipton DM, Flavell SW, Kim TK, Griffith EC, Waldon Z, Maehr R, Ploegh HL, Chowdhury S, Worley PF, Steen J, Greenberg ME. The Angelman Syndrome protein Ube3A regulates synapse development by ubiquitinating arc. *Cell.* 2010;140: 704–716.
62. Jiang YH, Armstrong D, Albrecht U, Atkins CM, Noebels JL, Eichele G, Sweatt JD, Beaudet AL. Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. *Neuron*. 1998;21:799–811.
63. Miura K, Kishino T, Li E, Webber H, Dikkes P, Holmes GL, Wagstaff J. Neurobehavioral and electroencephalographic abnormalities in Ube3a maternal-deficient mice. *Neurobiol Dis*. 2002;9:149–159.
64. Gustin RM, Bichell TJ, Bubser M, Daily J, Filonova I, Mrelashvili D, Deutch AY, Colbran RJ, Weeber EJ, Haas KF. Tissue-specific variation of Ube3a protein expression in rodents and in a mouse model of Angelman syndrome. *Neurobiol Dis.* 2010;39:283–291.
65. Yamamoto Y, Huibregtse JM, Howley PM. The human E6-AP gene (UBE3A) encodes three potential protein isoforms generated by differential splicing. *Genomics*. 1997;41:263–266.
66. Lawson-Yuen A, Liu D, Han L, Jiang ZI, Tsai GE, Basu AC, Picker J, Feng J, Coyle JT. Ube3a mRNA and protein expression are not decreased in Mecp2R168X mutant mice. *Brain Res*. 2007;1180:1–6.
67. Jordan C, Francke U. Ube3a expression is not altered in Mecp2 mutant mice. *Hum Mol Genet*. 2006;15: 2210–2215.

68. Makedonski K, Abuhatzira L, Kaufman Y, Razin A, Shemer R. MeCP2 deficiency in Rett syndrome causes epigenetic aberrations at the PWS/AS imprinting center that affects UBE3A expression. *Hum Mol Genet*. 2005;14:1049–1058.
69. Westbrook TF, Hu G, Ang XL, Mulligan P, Pavlova NN, Liang A, Leng Y, Maehr R, Shi Y, Harper JW, Elledge SJ. SCFbeta-TRCP controls oncogenic transformation and neural differentiation through REST degradation. *Nature*. 2008;452:370–374.
70. Abrajano JJ, Qureshi IA, Gokhan S, Zheng D, Bergman A, Mehler MF. REST and CoREST modulate neuronal subtype specification, maturation and maintenance. *PLoS One*. 2009;4:e7936.
71. Palm K, Belluardo N, Metsis M, Timmusk T. Neuronal expression of zinc finger transcription factor REST/NRSF/XBR gene. *J Neurosci*. 1998;18:1280–1296.
72. Sun YM, Greenway DJ, Johnson R, Street M, Belyaev ND, Deuchars J, Bee T, Wilde S, Buckley NJ. Distinct profiles of REST interactions with its target genes at different stages of neuronal development. *Mol Biol Cell*. 2005;16:12:5630–5638.
73. Tanaka H, Calderone A, Jover T, Grooms SY, Yokota H, Zukin RS, Bennett MV. Ischemic preconditioning acts upstream of GluR2 down-regulation to afford neuroprotection in the hippocampal CA1. *Proc Natl Acad Sci USA*. 2002;99:2362–2367.
74. Kinney DK, Munir KM, Crowley DJ, Miller AM. Prenatal stress and risk for autism. *Neurosci Biobehav Rev*. 2008;32:1519–1532.
75. Qureshi IA, Mehler MF. Epigenetic mechanisms underlying human epileptic disorders and the process of epileptogenesis. *Neurobiol Dis*. 2010;39:1:53–60.
76. Khalili K, Del Valle L, Muralidharan V, Gault WJ, Darbinian N, Otte J, Meier E, Johnson EM, Daniel DC, Kinoshita Y, Amini S, Gordon J. Puralpha is essential for postnatal brain development and developmentally coupled cellular proliferation as revealed by genetic inactivation in the mouse. *Mol Cell Biol*. 2003:23:6857–6875.
77. Risi S, Lord C, Gotham K, Corsello C, Chrysler C, Szatmari P, Cook EH Jr, Leventhal BL, Pickles A. Combining information from multiple sources in the diagnosis of autism spectrum disorders. *J Am Acad Child Adolesc Psychiatry*. 2006;45:1094–1103.
78. Bittel DC, Kibiryeva N, Butler MG. Expression of 4 genes between chromosome 15 breakpoints 1 and 2 and behavioral outcomes in Prader-Willi syndrome. *Pediatrics*. 2006;118:1276–1283.

Chapter 71

Pathophysiology of Epilepsy in Autism Spectrum Disorders

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INTRODUCTION

Why are seizures so common in children with autism? This relatively straightforward question does not, unfortunately, have a straightforward answer. In this chapter, we explore this question from clinical, pathophysiological, and molecular perspectives, using as examples two genetic disorders that share a high prevalence of autism and epilepsy—fragile X syndrome (FXS) and tuberous sclerosis complex (TSC)—with the hope that understanding the pathophysiology of these monogenic conditions will lead to broader understanding of neural hyperexcitability in other autism syndromes. We conclude by discussing cellular and network dysfunctions that might be amenable to targeted treatments in these disorders, with potential wider applicability to idiopathic autism.

Autism spectrum disorders (ASDs) are neurodevelopmental disorders that share abnormalities in three domains: language development, social interaction, and motor behavior with stereotypies and restricted interests. In this chapter, the term *autism spectrum disorder* encompasses classic childhood autism as originally described by Kanner,[1] Asperger syndrome, and pervasive developmental disorder not otherwise specified. The signs and symptoms of ASD can usually be recognized before age 3 years,[2] although recent evidence of an early, but slow, loss of skills in about three-quarters of the infants who develop ASD is observed by 12 months.[3] Later, some individuals with ASD experience regression of language or behavior (autistic regression), a phenomenon that has been hypothesized to be related to epilepsy or epileptic discharges on the electroencephalogram (EEG).[4] The possibility that subclinical epileptiform discharges can contribute to the spectrum of disability in ASD or lead to regression of language or social skills suggests that the brains of individuals with autism are hyperexcitable.

Clinical aspects of seizures and epilepsy within the autism spectrum have been reviewed in detail.[5–9] Up to 30% of individuals with ASD have epilepsy, and ASD is present in about 30% of patients with epilepsy, though these numbers are approximate due to historical differences in the definition of each condition and to different study methodologies.[10] Risk factors for epilepsy in ASD include mental retardation, motor impairment, symptomatic etiology, and seizure onset either early in life (before 5 years of age) or in adolescence.[9] However, the neurobiology of ASD, as well as the mechanisms responsible for cellular hyperexcitability in ASD, are not well understood and likely involve the interplay of genetic, epigenetic, and environmental contributions.[11–14]

There are several possible relationships between brain development, epilepsy, and ASD (Fig. 71–1).[15] First, ASD and epilepsy might be distinct conditions with no causal relationship; however, this possibility is unlikely in view of the high co-occurrence rate (30%) between the two disorders. Second, a common neurobiological antecedent (e.g., structural or developmental lesions, genetic susceptibilities, and/or environmental insults) might lead to abnormal brain development that results in both epilepsy and ASD. Third, epilepsy could lead to autistic behavior or, conversely, abnormal brain

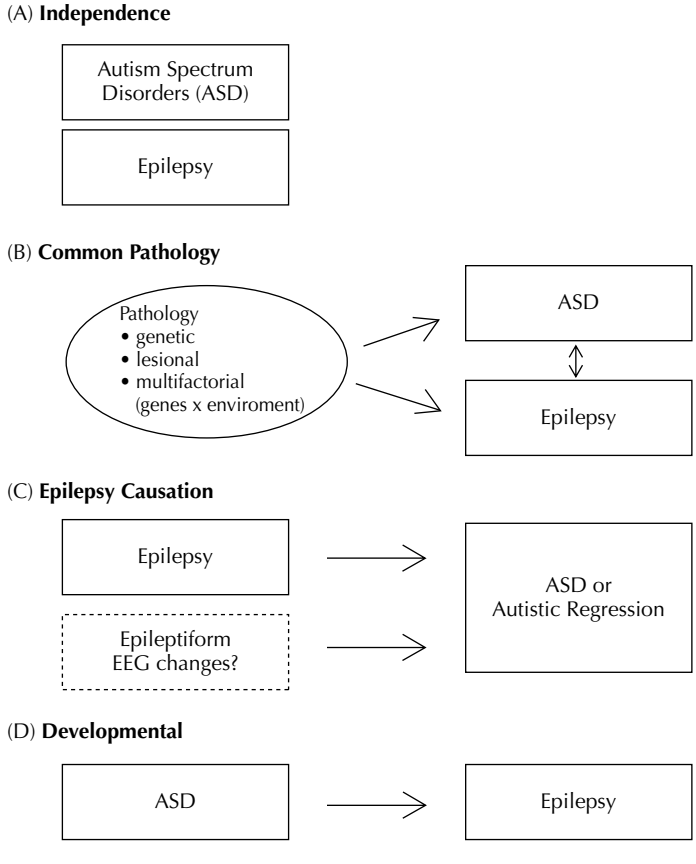

Figure 71–1. Possible relationships between brain development, epilepsy and ASD. **A.** Epilepsy and ASD might be distinct conditions with no causal relationship. This possibility is not likely due to the high comorbidity of these two disorders. **B.** A common neurobiological antecedent (e.g., abnormal brain development, genetic defect) could lead to both epilepsy and ASD. Another possibility is that there is interaction between the pathophysiology of neural circuits underlying established ASD and epilepsy (i.e., at the level of the double-headed arrow). **C.** Epilepsy or epileptogenic EEG changes (the dashed box indicates uncertainty) could lead to ASD. **D.** Conversely, abnormal brain circuitry underlying ASD could predispose the brain to seizures. These relationships are not mutually exclusive or unidirectional, so that mechanisms of epilepsy and ASD are interdependent and targeted therapies for one disorder could benefit the other.

circuitry underlying ASD could predispose the brain to seizures. The second and third possibilities are not mutually exclusive, leading to the hypothesis that mechanisms of epilepsy and ASD are interdependent and that targeted therapies for one condition could ameliorate the impact or severity of the other, as will be discussed in more depth below. The possibility that common developmental mechanisms of epilepsy and ASD exist arises from observations that both disorders, though etiologically heterogeneous, involve abnormal brain plasticity, that is, *dysplasticity* or the ability of neural circuits to function normally with regard to cognitive and social function.[13]

The etiologies of ASD are diverse and can be either idiopathic (nonsyndromic) or secondary to an identifiable underlying medical or genetic disorder (syndromic). The risk of epilepsy is increased in both idiopathic and syndromic forms of ASD, suggesting that there might be common pathophysiological alterations that lower the seizure threshold. Specific medical or genetic/genomic abnormalities have been identified in approximately 20% of ASD cases, though one study reports a 40% diagnostic yield.[16] In the future, the category of "idiopathic" autism might disappear as the molecular and genetic bases of ASD disorder become more fully defined. For now, the existence of known etiologies permits investigation into molecular and physiological aspects of brain function that lead to autistic behaviors.[17] Understanding the pathophysiological mechanisms of increased seizure susceptibility is facilitated by examination of genetic mutations leading to ASD. So far, the results of these studies are weighted toward defects in postsynaptic function and subcellular signaling.[18,19]

While much of idiopathic ASD is likely to be multigenic with complex genetics, a small but increasing proportion of ASD has been identified with specific gene mutations; some single-gene defects are associated with both ASD and seizures.[20] Examples include FXS, caused by mutation of the fragile X mental retardation 1 (*FMR1*) gene, and TSC, due to mutation of the *TSC1* or *TSC2* genes involved in the control of cell growth and differentiation. Other novel mutations with concurrent ASD and epilepsy are rapidly appearing in the literature and are reviewed in detail in elsewhere.[13] Several of these mutations involve genes regulating proteins critical for synapse development (e.g., neuroligins and neurexins)[21] or interneuron function (e.g., aristaless-related homeobox X-linked [*ARX*] gene mutations).[22] Likewise, patients with Rett syndrome, a neurodevelopmental disorder with progressive deterioration of motor skills, language, cognition, and behavior (autism), have a high risk of developing epilepsy.[23] Rett syndrome is due to mutation in the gene encoding methyl-CpG binding protein 2 (*MeCP2*), a transcriptional regulator of numerous genes. Whether genetic mutations associated with syndromic ASD converge on common mechanisms that lead to neuronal hyperexcitability and epilepsy remains to be established.

FRAGILE X SYNDROME

Clinical and Genetic Aspects

Fragile X syndrome is the most common inherited form of cognitive impairment and the leading known monogenic disorder associated with ASD.[24] Using strict diagnostic criteria, 15%–30% of males with FXS have autism.[25] Approximately 20% of children with FXS have seizures, many of which are relatively benign and resolve beyond childhood. Autism in FXS ranges from mild to severe and tends to improve with age.[26,27] Among children with FXS, those with comorbid autism have greater cognitive and verbal impairments than FXS children without autism.[27] Predominant impairments are in the communication domain.[28]

Fragile X syndrome arises when a CGG-repeat tract in the 5′ noncoding region of *FMR1* exceeds 200 repeats (i.e., the "full mutation" range), at which point the gene becomes hypermethylated and transcriptionally silent.[29] The absence of the *FMR1* fragile X mental retardation protein (FMRP) is responsible for the clinical phenotype and physical features, which include prominent ears, long face, high-arched palate, macroorchidism, and hyperextensible finger joints.[30] Approximately 85% of males and 25% of females experience cognitive impairment (IQ <70); nearly all patients have behavioral problems, with males tending to present with attention deficit hyperactivity disorder (ADHD) and aggression, while females are more prone to shyness and social withdrawal.[31] Individuals with CGG expansions in

the premutation range (55–200 CGG repeats) display a range of clinical features, including behavioral and cognitive involvement in children[31–33] and a late-adult-onset neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS).[34] The prevalence of seizures in individuals with the premutation is reported to be in excess of 20%.[35]

The fragile X mental retardation protein is an RNA-binding protein that is believed to have multiple functions, including dendritic transport of various mRNA species[36] and the translational regulation of mRNAs whose protein products are involved in synaptic development, function, and plasticity.[37] Among the known targets of FMRP-coupled translational downregulation are the microtubule-associated protein 1B (MAP1B), which is important for modulating microtubule-coupled growth of dendritic spines and for dendritic arborization,[38] and Arc, which plays a role in the internalization of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor subunits, a subtype of ionotropic glutamate receptors.[39]

Seizures occur in 10%–20% of FXS individuals with full mutations.[40–42] Interictal EEG patterns are similar to those seen in benign childhood epilepsy with centrotemporal spikes (rolandic epilepsy). In one study involving 16 children with FXS and epilepsy, 12 children had partial seizures, with 10 of the 12 having an EEG with centrotemporal spikes.[41] In addition, 23% of the children who did not have seizures displayed abnormal EEG patterns, typically centrotemporal spikes. In most children with FXS, seizures are readily controlled and tend to disappear in adolescence. Therefore, there are similarities between epilepsy in individuals with rolandic epilepsy and FXS, and any mechanism postulated to explain epileptogenesis in FXS must account not only for the relatively benign seizure manifestations, but also for their absence in the majority of FXS cases.[43]

Pathophysiology

THE METABOTROPIC GLUTAMATE RECEPTOR THEORY FOR FXS

Numerous animal models and electrophysiological studies have examined the pathogenesis of FXS and the synaptic dysfunction that underlies the hyperexcitability and epileptiform features associated with the disorder. A key advance in the understanding of the molecular basis of FXS was that mice lacking FMRP displayed enhanced long-term depression (LTD) in hippocampal neurons and that this LTD was dependent on protein synthesis.[44,45] Long-term depression is a form of synaptic plasticity that underlies learning and memory, so dysfunctional LTD in FXS could reflect the cognitive deficiencies seen in patients.[46] In this model, LTD could be inhibited by blocking the metabotropic glutamate receptor 5 (mGluR5) with agents such as 2-methyl-6-(phenylethynyl)-pyridine (MPEP). Further, FMRP normally functions to downregulate the translation of proteins, such as Arc, that are involved with the internalization of AMPA receptors from the postsynaptic surface. Thus, in the absence of postsynaptic FMRP, stimulation of mGluR5, either by receptor agonists or presynaptic glutamate release, results in increased postsynaptic protein translation, leading to excess internalization of AMPA receptors and eventual weakening of synaptic transmission (Fig. 71–2). By contrast, premutation-associated disorders (FXTAS, fragile X-associated primary ovarian insufficiency, neurodevelopmental involvement)[47] are not a consequence of FMRP deficiency per se; rather, mounting evidence from both human and animal studies indicates that these premutation-specific disorders are caused by a direct toxic gain of function of the CGG-repeat *FMR1* mRNA.[48,49] Furthermore, reductions in hippocampal volume, activation, and associated memory deficits, as well as reduced amygdala activation[50] and psychopathology,[51] appear much earlier in adulthood than do the symptoms of FXTAS, suggesting that the processes that ultimately will lead to FXTAS may be operating at a much earlier age. The reports of ADHD and ASD in young boys with the premutation also suggest a neurodevelopmental component to the premutation.[33] Recently, low-density hippocampal neuronal cultures from neonatal mice in the premutation range were shown to recapitulate neurodevelopmental and neurodegenerative aspects described in vivo.[52]

The mGluR model accounts for a number of the physical and behavioral features of FXS and predicts several aspects of the phenotype in various animal models, including enhanced seizure activity in an *Fmr1* knockout mouse model with audiogenic seizures.[53–55] One

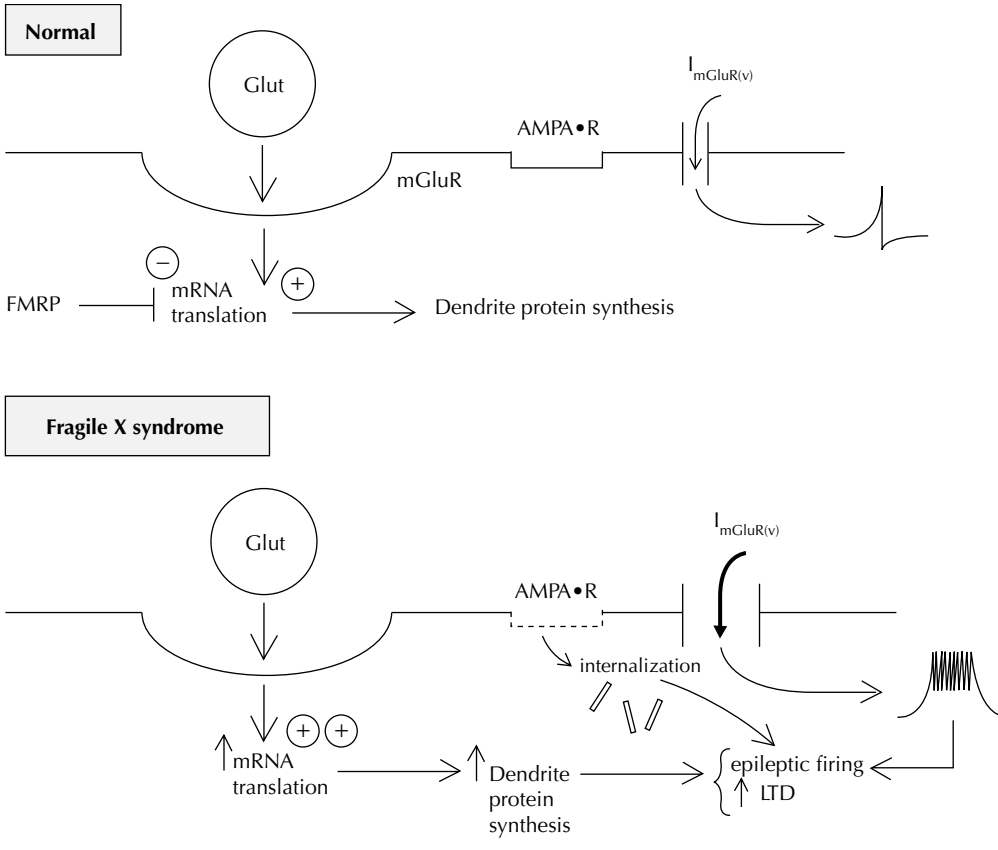

Figure 71–2. Possible mechanisms for seizures in FXS. In the normal case (top), glutamate (Glut) activation of dendritic group I metabotropic receptors (especially mGluR5) enables intracellular gene transcription, leading to translation of dendritic proteins. This process is modulated by FMRP, which keeps protein synthesis in check and prevents synaptic LTD. Activation of group 1 mGluR also activates a phospholipase C β1-dependent, voltage-gated inward current ($I_{mGluR(V)}$), which, under normal conditions, allows hippocampal CA3 neurons to fire in brief bursts (thin curved arrow). In FXS (bottom), with absent FMRP, there is no inhibition of the downstream effects of mGluR activation, leading to excessive protein synthesis, increased LTD, and prolonged $I_{mGluR(V)}$ (thick curved arrow), which can lead to epileptic firing. Internalization of AMPA receptors as a consequence of mGluR activation enhances the tendency toward epileptic firing. The concepts depicted here are derived from the work of several investigators (e.g., refs. 39, 59, 60, 123).

consequence of enhanced protein synthesis in the absence of postsynaptic FMRP in the knockout mouse is increased internalization of AMPA receptors at the postsynaptic surface. The augmented AMPA receptor internalization (a facet of the increased LTD observed in the knockout mouse) no longer requires protein synthesis, suggesting that the elevated protein levels present in the postsynaptic compartment are sufficient to establish the enhanced LTD.[56]

A potential caveat is that agents used in many animal studies, particularly those involving mGluR5 inhibitors (e.g., MPEP) or AMPA-receptor agonists, may have off-target effects that mimic the desired effect. To resolve this uncertainty, *Fmr1* knockout mice were crossed with animals heterozygous for deletion of the *Grm5* gene (50% reduction in mGluR5), thus mimicking drug-induced reductions in mGluR5 activity.[57] The resulting mice, *Fmr1*(–/Y) *Grm5*$^{+/-}$, displayed substantial correction of defects in experience and conditioning (i.e., ocular dominance plasticity and inhibitory avoidance extinction), normalization of dendritic spine density, a return to normal basal protein synthesis, attenuated susceptibility to audiogenic seizures, and rescue from early accelerated growth. These results clearly establish that the enhanced response to stimulation of the mGluR5 receptor plays a critical role in many of the phenotypic characteristics of FXS. Finally, it was shown that kindling promotes

prolonged seizure activity and severe mossy fiber sprouting in the *Fmr1* knockout mouse and that this behavior could be at least partially blocked using either *N*-methyl-d-aspartate (NMDA)-receptor or mGluR5 inhibitors.[58]

EPILEPTOGENIC MECHANISMS IN FXS

While the mGluR theory explains many of the phenotypic features of FXS, altered postsynaptic function arising from the absence of FMRP does not readily explain the central nervous system (CNS) hyperexcitability and seizure susceptibility associated with FXS. However, several recent studies have begun to reveal the connection. A recent investigation provides evidence that a voltage-gated inward current, $I_{mGluR(V)}$, is the cellular basis for the epileptogenic behavior induced by activation of the mGluR5 receptor[59,60] (Fig. 71–2). Specifically, stimulation of mGluR5 by the agonist dihydroxyphenylglycine in mouse hippocampal slices led to prolonged epileptiform discharges that lasted for more than 1 h after washout of the agonist. Moreover, this inward current could be suppressed by inhibitors of downstream signaling pathways that mediate group I mGluR-coupled translation (e.g., tyrosine kinase, extracellular signal-regulated kinase [ERK]1/2).[61,62] Remarkably, glutamate stimulation of glutaminergic synapses did not recapitulate this effect in wild-type mice, whereas $I_{mGluR(V)}$ was activated in hippocampal preparations from *Fmr1* knockout mice. The authors conclude that the induction of $I_{mGluR(V)}$ serves as a form of synaptic plasticity to predispose to epileptogenesis. Thus, activation of mGluR5 at multiple synapses in the absence of FMRP translational control leads to heightened electrical excitability. The carrier of $I_{mGluR(V)}$ is not yet known, though evidence supports one or more of the transient receptor potential canonical (TRPC) channels that mediate Ca^{2+} entry in response to depletion of endoplasmic reticulum Ca^{2+} stores.[63]

Evidence for a connection between the absence of *Fmr1* and epileptogenesis in the knockout mice was extended in a study of neocortical circuits.[64] In agreement with prior observations,[60] the authors documented increased intrinsic excitability in excitatory neurons from *Fmr1* knockout mice. However, there was an imbalance between this excitability and a relatively decreased excitatory drive present at fast-spiking inhibitory neurons. The net result was prolonged neocortical circuit activity (termed the *UP state*) induced by thalamic input. The heightened circuit activity, coupled with less synchronous network inhibition, was proposed as the underlying mechanism that leads to EEG abnormalities and epilepsy in FXS. Thus, the failure to properly modulate the mGluR5 response in the absence of FMRP results in neuronal hyperexcitability, mediated in part by the generation of a voltage-gated inward current, which in turn reduces excitatory input to inhibitory neurons and results in net increased excitability.[65]

ROLE OF GABA RECEPTORS

The increased excitability of hippocampal and neocortical circuits in FXS, due to dysregulation of glutaminergic neurons, can in turn disrupt the normal actions of inhibitory GABAergic neurons. Downregulation of $GABA_A$ receptor (GABR) subunits occurs at both the mRNA and protein levels—a situation that would further increase the excitatory character of limbic and cortical circuits.[66,67] Recently, it was demonstrated that in addition to reductions in $GABA_A$ subunits, there is also lower expression of a number of genes involved in GABA metabolism, including *gad1*, *gat1*, and *gat4,* in the brain of both mouse and *Drosophila* models of FXS.[68]

On a structural level, in the somatosensory cortex of the *Fmr1* knockout mouse, inhibitory circuits were found to be reorganized accompanied by a reduction in the density of GABAergic interneurons.[69] A separate investigation of the function of GABAergic neurons in the subiculum revealed that tonic, but not phasic, $GABA_A$ currents were downregulated in the *Fmr1* knockout mouse relative to wild-type controls.[70] These results were associated with reductions in tonic GABR subunits.

Several classes of pesticides of concern to human environmental health are known to interfere with GABA-mediated neurotransmission because they bind to GABR and block their ability to mediate chloride fluxes. Organochlorine (OC) insecticides that possess polychloroalkane structures are known to bind to GABR in the mammalian brain and potently block their ability to conduct Cl^-, with many having nanomolar affinity for their receptor binding site.[14] Organochlorine insecticides

that are currently being used in the United States include endosulfan, dicofol, and lindane. Because of their chemical stability, global distribution from countries that continue to use these compounds, and their propensity to bioaccumulate, exposure to OC insecticides continues to be a concern for human health. Yet, relatively little is known about their developmental neurotoxicity and the long-term consequences of low-dose exposures.[71] An association between maternal residence near agricultural pesticide applications during key periods of gestation and the development of ASD has been documented.[72] Children of mothers living closest to agricultural fields with the highest endosulfan and dicofol use had a risk factor for autism that was 6.1 times higher than that of mothers not living near agricultural fields. The fact that OC and several newer widely used insecticides impair GABR function suggests that deficiencies in the function of the GABAergic system in the *Fmr1* knockout mouse would further upset the balance between excitatory and inhibitory function in the CNS and may represent a valuable model for studying gene x environment interactions that cause hyperexcitation of the CNS.

Treatment Implications

The growth in knowledge of the pathogenesis of FXS, specifically regarding the linkage between abnormal neural function and epileptogenesis, presents numerous possibilities for targeted interventions.[30,73] Perhaps the most attractive targeted treatment is blockade of the mGluR5 receptor, thus compensating for the absence of downstream control by FMRP. Clinical trials are presently underway with various mGluR5 inhibitors. An open-label single-dose pilot trial in 12 individuals of the mGluR5 agonist, fenobam, demonstrated a reduction in anxiety and hyperactivity, with no significant adverse effects.[74] A second open-label treatment trial of 15 patients assessed the effects of lithium, which reduces mGluR5 activation of downstream processes.[75] There was significant improvement in behavior and verbal memory. Larger clinical trials are needed with both agents.

The GABR subtypes comprise another potential therapeutic target.[76] *Fmr1* mutant *Drosophila* die during development if fed a high-glutamate diet, consistent with the mGluR model of excess activation.[77] The authors exploited this lethal phenotype to screen for small molecules that would rescue the flies and found several, including GABA, that rescued the phenotype, providing additional evidence that GABR agonists might have a beneficial therapeutic effect. Finally, there is substantial evidence that GABR agonists, such as the neurosteroid allopregnanolone and a related analog, ganaxolone, possess significant antiseizure activity.[78]

Other approaches include an open-label trial of minocycline, a metalloproteinase inhibitor that improves dendritic spine morphology in *Fmr1* knockout mice.[79] These and other pharmacological agents will be used singly and in combination to target some of the most troubling behavioral manifestations in FXS.

In summary, epilepsy associated with FXS represents an opportunity to explore mechanisms of hyperexcitability in a disorder for which the molecular pathophysiology is unique and specific.[80] Seizures occurring in conjunction with FXS are generally mild, tend to disappear in childhood, typically respond to anticonvulsant treatment, and are associated with an EEG pattern of centrotemporal spikes. In several respects, the clinical and electrographic aspects of seizures in FXS resemble those of the benign focal epilepsies of childhood. Whether these similarities are coincidental or related mechanistically is an intriguing question for future investigation. The type of (and even need for) antiepileptic therapy for individuals with FXS must be weighed against potential adverse effects, which could be unique in this syndrome. Ideally, pathophysiological insights, as reviewed here, will lead to therapeutic interventions targeted to the specific molecular defects in FXS.

TUBEROUS SCLEROSIS COMPLEX

Clinical and Genetic Aspects

Tuberous sclerosis complex is a multisystem genetic disorder caused by a mutation in the *TSC1* or *TSC2* gene. *TSC1* (on chromosome 9q34) and *TSC2* (on chromosome 16p13) code for proteins (hamartin and tuberin, respectively) that form a dimeric complex that has

guanosine triphosphatase (GTPase) activity and inhibits excessive cell growth and proliferation via the mammalian target of rapamycin (mTOR) signaling pathway.[81] Mutation of either gene causes dysregulation of mTOR, resulting in abnormal cellular proliferation, growth, and differentiation. This leads to the formation of tumors, usually benign, in many organs, including brain, kidney, and heart. Cellular regulation of the mTOR pathway and its dysfunction is discussed briefly below and is elaborated by Wong and Crino (Chapter 65, this volume). Thus, TSC is a malformation of cortical development with a specific genetic basis that leads to a spectrum of neurological disability including multifocal epilepsy, mental retardation, and ASD.[82,83] Here we focus on the relationship between epilepsy and ASD in TSC.

While any organ system can be affected in TSC, the brain is involved extensively. The neuropathological hallmark of TSC is cortical tubers.[84] Tubers are hamartomatous collections of dysplastic cells of both glial and neuronal origin with abnormal morphology, size, orientation, lamination, and cellular connectivity. As such, tubers or nearby extratuberal tissue are extremely epileptogenic, and their extensive and varied cortical localization gives rise to multifocal sites of seizure generation.

In TSC, seizures are very common, affecting 60%–90% of patients.[82,85,86] Seizures in TSC often begin in the first year of life, putting the developing brain at risk for seizure-induced neuroplastic changes.[87] Seizures of any type can be seen, particularly complex partial (related to the multifocal pathology), generalized tonic-clonic, and infantile spasms (IS). Infantile spasms are very common in TSC and are frequently associated with subsequent autism.[88,89] Seizures in TSC are often severe and intractable, with remissions occurring only rarely. In some cases, surgical resection of an offending tuber, especially if performed early in life, can result in seizure reduction and a more favorable developmental profile.[90,91] However, there is a critical need to develop a nonsurgical treatment of TSC that not only reduces seizure occurrence but also prevents epileptogenesis and, by extension, its cognitive and behavioral/autistic sequelae.

About 25%–50% of children with TSC have ASD, with girls and boys affected to a similar degree, in marked contrast to the male predominance in nonsyndromic ASD.[92] The tuber burden (number, extent) and location correlate with the degree of mental retardation, ASD, and seizure predisposition. Tubers located in the temporal lobes are highly correlated with ASD in TSC patients.[89,93] Presumably, disruption of limbic circuitry, perhaps by the hypersynchronous neuronal activity comprising seizures or epileptiform EEG discharges, leads to abnormalities of language development and cognitive processing.[94,95] There is also a high rate of ASD in TSC patients with tubers in the cerebellum.[96] Patients with *TSC2* mutations tend to have earlier onset of ASD and epilepsy, but there is considerable overlap between those with *TSC1* and *TSC2* mutations.[86,97] Although seizures, especially those with onset early in life, are a risk factor for ASD in TSC,[98,99] ASD also occurs in patients with TSC who do not have epilepsy. Therefore, the relationship between seizures and ASD in TSC is complex, with imprecise genotype–phenotype correlation and the potential for modification by epigenetic and environmental factors.[89,100,101] Since epilepsy precedes ASD in many cases of TSC, it is possible that abnormal neural firing alters the development of language and social function.

Pathophysiology

The mTOR protein is a central regulator of cell growth and proliferation.[102] Figure 71–3 depicts some of the molecules involved in this signal transduction pathway. Components of the mTOR pathway are present at synapses and play an important role in synaptic plasticity via regulation of local protein synthesis. Activation of either growth factor receptors or glutamate receptors sets into motion a subcellular signaling cascade that regulates cell growth and differentiation, mediated through the mTOR pathway that is, in turn, regulated by *TSC1* and *TSC2*. A mutation in *TSC1* or *TSC2* prevents mTOR activation, leading to unbridled protein transcription and translation and hence cell growth and proliferation. Upstream regulation of the *TSC1/2* complex and cellular signaling that can bypass *TSC1/2* function (such as the ERK pathway[103]) provides potential pathways for controlling this unchecked cell growth and epileptogenesis. Downstream from mTOR, a variety of kinases and translation factors serve as modulators of cell growth. Nevertheless,

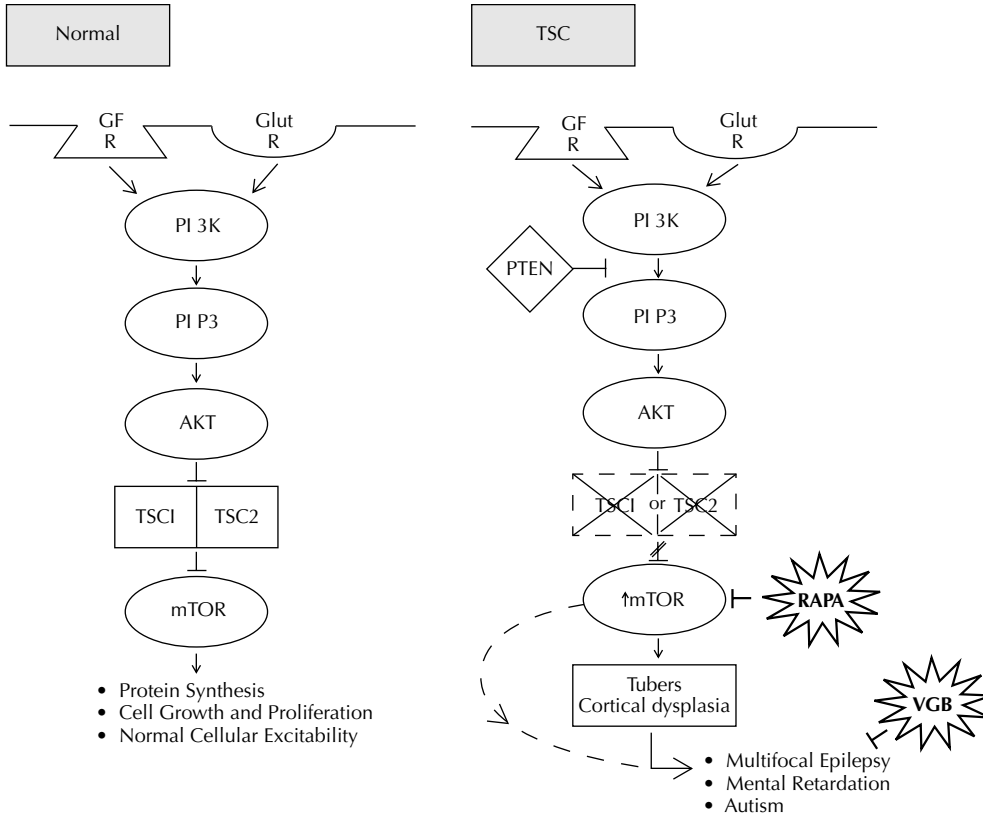

Figure 71–3. Simpified signaling pathway in TSC with possible functional consequences that could lead to increased seizure susceptibility. In the normal situation (left), activation of receptors (R) for growth factors or glutamate leads to an intracellular signaling cascade that limits mTOR activation via the *TSC1* and *TSC2* gene heterodimer. Therefore, protein synthesis is modulated and aberrant cell growth is prevented; likewise, cellular excitability is normal. If *TSC1* or *TSC2* is mutated (right), mTOR is hyperactivated, leading to cellular dysplasia and tuber formation, along with neuronal hyperexcitability, epilepsy, cognitive/behavioral deficits, and autistic symptomatology. It is also possible that epilepsy and ASD can occur independently of dysplasia tubers (dashed arrow). This pathological signaling pathway can be modulated at several points by agents that inhibit mTOR (RAPA) or suppress seizures (VGB). GF, growth factors; Glut, glutamate; R, receptor; PI3K, class I phosphatidylinositol 3-kinase; PIP3, phosphoinositol 3,4,5-triphosphate; AKT, activated tyrosine kinase; TSC, tuberous sclerosis complex; mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homolog on chromosome 10; RAPA, rapamycin; VGB, vigabatrin.

it is presently unclear how mTOR dysregulation leads directly to epilepsy or autism susceptibility.

Animal models of TSC afford the opportunity to study pathophysiological consequences of *TSC1* and *TSC2* mutations. The Eker rat is a spontaneous germline mutation in which one *TSC2* allele is inactivated. Eker rats demonstrate defective long-term potentiation (LTP), a form of synaptic plasticity involved in learning and memory; LTP is even more abnormal after seizures in Eker rats.[104,105] Other models have been created by knocking out *TSC1* or *TSC2* in neurons or glia, allowing investigation of the structural, epileptic, and cognitive effects. *TSC2* knockouts have upregulation of the mTOR pathway and abnormal learning and memory.[106] Conditional *TSC1* knockouts have abnormal hippocampal-dependent learning and memory as well as social behaviors, mimicking key autistic features; they demonstrate abnormal cellular architecture and progressive epilepsy as well.[107] Using such models, a variety of pathophysiological findings have been found, including altered glutamate transporters, potassium channels, and gap junctions, all of which are consistent with enhanced excitability (and seizure activity).[108,109] Other factors that alter the excitation/inhibition balance in the TSC brain have also been documented in

favor of excessive excitation, including specific abnormalities of glutamate receptors.[110]

Treatment Implications

Targets for therapeutic intervention in TSC include several components of the mTOR signaling cascade (Fig. 71–3). There is considerable excitement about rapamycin, an inhibitor of mTOR that is already in clinical use as an immunosuppressant, which ameliorates the epileptic and cognitive consequences of TSC.[111] In *TSC1* knockout mice, rapamycin prevents neuronal hypertrophy.[112] Early treatment of *TSC1* knockout mice with rapamycin prevents epileptogenesis, and seizures return if rapamycin is discontinued.[106,113] A case report of a clinical trial with rapamycin in a 10-year-old girl with TSC claims marked seizure reduction without adverse side effects.[114] Obviously, this finding must be expanded in case series. Rapamycin may have utility in acquired epilepsy as well. In a model of temporal lobe epilepsy in mice induced by kainic acid, rapamycin blocked both acute and chronic phases of seizure-induced mTOR activation. It also prevented seizure-induced cell death and reduced subsequent epileptogenesis.[115]

Another strategy in TSC is to suppress seizures with an anticonvulsant drug. Vigabatrin inhibits GABA reuptake into the presynaptic terminal, thus prolonging the availability of GABA to mediate inhibition at its postsynaptic receptors. This agent is particularly useful for treatment of IS in TSC patients, lessening the subsequent risk for epilepsy and autistic/cognitive deficiencies.[116,117] In fact, vigabatrin appears to be uniquely effective for IS in TSC, suggesting that there is some specificity of the drug for the pathophysiological mechanism.

CONCLUSION: IS THERE A CONVERGENT PATHWAY BETWEEN AUTISM AND EPILEPSY?

Returning to the question that opens this chapter, why is epilepsy so common in children with ASD? The heterogeneous etiologies of ASD and epilepsy make it unlikely that a single common mechanism explains seizure predisposition in both disorders, and recent genetic studies point to numerous diverse gene mutations that have autism and epilepsy as joint sequelae.[20] Yet, hints about the pathogenesis of at least some forms of ASD are emerging. First, the majority of mutations focus on the synapse.[17,19] This association is not surprising, as neuronal excitability is governed by the function and dysfunction of synaptic elements such as receptors and their subtypes; neurotransmitters and their synthesis, metabolism, and vesicular release; developmental regulation of cell adhesion; and the ratio of excitation to inhibition as a result of the above factors. Alterations in synaptic plasticity could underlie autistic and cognitive symptoms, especially if selected circuits are involved.

Second, as discussed here, dysfunction in subcellular signaling pathways of diverse conditions (e.g., FXS, TSC) may in fact have common convergence points contributing to pathophysiology, as exemplified by mTOR dysregulation. In dendrites, mTOR is activated by stimulation of mGluR. The reported involvement of the mTOR pathway in FXS emphasizes a potential commonality between FXS and TSC, with a pathophysiological link to abnormal cellular signaling that could lead to ASD. In *Fmr1* knockout mice, the mTOR pathway is upregulated, providing a functional link between mGluR overactivation and abnormal synaptic plasticity.[118] Additional evidence for the mTOR pathway in autism and epilepsy comes from mutations in the tumor suppressor gene *PTEN* (phosphatase and tensin homolog on chromosome 10), which is involved in upstream regulation of mTOR by inhibiting the interaction of phosphatidylinositol 3-kinase (PI3K) and phosphoinositol 3,4,5-triphosphate (PIP3; Fig. 71–3). Conditional knockout of *Pten* in mice results in an increase in mTOR activation and clinical manifestations that include spontaneous seizures and ASD-like symptoms of anxiety and deficiencies in social interaction.[119] Rapamycin treatment rescues all of these neurological deficits.[120]

Third, thinking beyond the synapse to neuronal function on a network scale, both epilepsy and autism involve abnormal synchrony of widespread systems of the brain.[121] Alterations in functional connectivity between brain structures critical for language and social development are also pivotal in the synchronization that occurs in many epilepsy syndromes.[122] Information about the functional pathology

of such systems is just emerging. Unexpected mechanisms may evolve from genetic studies, involving proteins not expected to participate in the control of cognition or cellular excitability. Such molecules are already being linked to ASD and represent additional sites of intervention that give hope for the future.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to disclose.

REFERENCES

1. Kanner L. Autistic disturbances of affective contact. *Nerv Child*. 1943;2:217–250.
2. Johnson CP, Myers SM. American Academy of Pediatrics Council on Children with Disabilities. Identification and evaluation of children with autism spectrum disorders. *Pediatrics.* 2007;120:1183–1215.
3. Ozonoff S, Iosif A-M, Baguio F, Cook IC, Hill MM, Hutman T, Rogers SJ, Rozga A, Sangha S, Sigman M, Steinfeld MB, Young GS. A prospective study of the early behavioral signs of autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2010;49:258–268.
4. Tuchman R. Autism and epilepsy: what has regression got to do with it? *Epilepsy Curr*. 2006;6:107–111.
5. Tuchman R, Rapin I. Epilepsy in autism. *Lancet Neurol*. 2002;1:352–358.
6. Danielsson S, Gillberg IC, Billstedt E, Gillberg C, Olsson I. Epilepsy in young adults with autism: a prospective population-based follow-up study of 120 individuals diagnosed in childhood. *Epilepsia*. 2005;46: 918–923.
7. Canitano R. Epilepsy in autism spectrum disorders. *Eur Child Adolesc Psychiatry.* 2007;16:61–66.
8. Levisohn PM. The autism-epilepsy connection. *Epilepsia*. 2007;48(suppl 9):33–35.
9. Spence SJ, Schneider MT. The role of epilepsy and epileptiform EEGs in autism spectrum disorders. *Pediatr Res*. 2009;65:599–606.
10. Tuchman R, Cuccaro M, Alessandri M. Autism and epilepsy: historical perspective. *Brain Dev*. 2010;32: 709–718.
11. Moldin SO, Rubenstein JLR, Hyman SE. Can autism speak to neuroscience? *J Neurosci.* 2006;26: 6893–6896.
12. Tuchman R, Moshé SL, Rapin I. Convulsing toward the pathophysiology of autism. *Brain Dev*. 2009;31:95–103.
13. Brooks-Kayal A. Epilepsy and autism spectrum disorders: are there common developmental mechanisms? *Brain Dev*. 2010;32:731–738.
14. Pessah IN, Lein P. Evidence for environmental susceptibility in autism. What we know and what we need to know about gene x environment interactions. In: Zimmerman AW, ed. *Autism: Current Theories and Evidence.* Totowa, NJ: Humana Press; 2008:409–428.
15. Deonna T, Roulet E. Autistic spectrum disorder: evaluating a possible contributing or causal role of epilepsy. *Epilepsia.* 2006;47(suppl 2):79–82.
16. Schaefer GB, Lutz RE. Diagnostic yield in the clinical genetic evaluation of autism spectrum disorders. *Genet Med.* 2006;8:549–556.
17. Sutcliffe JS. Insights into the pathogenesis of autism. *Science*. 2008;321:208–209.
18. Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Almeida J, Bacchelli E, Bader GD, Bailey AJ, Baird G, Battaglia A, Berney T, Bolshakova N, Bölte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Bryson SE, Carson AR, Casallo G, Casey J, Chung BH, Cochrane L, Corsello C, Crawford EL, Crossett A, Cytrynbaum C, Dawson G, de Jonge M, Delorme R, Drmic I, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Freitag CM, Gilbert J, Gillberg C, Glessner JT, Goldberg J, Green A, Green J, Guter SJ, Hakonarson H, Heron EA, Hill M, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Kim C, Klauck SM, Kolevzon A, Korvatska O, Kustanovich V, Lajonchere CM, Lamb JA, Laskawiec M, Leboyer M, Le Couteur A, Leventhal BL, Lionel AC, Liu XQ, Lord C, Lotspeich L, Lund SC, Maestrini E, Mahoney W, Mantoulan C, Marshall CR, McConachie H, McDougle CJ, McGrath J, McMahon WM, Merikangas A, Migita O, Minshew NJ, Mirza GK, Munson J, Nelson SF, Noakes C, Noor A, Nygren G, Oliveira G, Papanikolaou K, Parr JR, Parrini B, Paton T, Pickles A, Pilorge M, Piven J, Ponting CP, Posey DJ, Poustka A, Poustka F, Prasad A, Ragoussis J, Renshaw K, Rickaby J, Roberts W, Roeder K, Roge B, Rutter ML, Bierut LJ, Rice JP, Salt J, Sansom K, Sato D, Segurado R, Sequeira AF, Senman L, Shah N, Sheffield VC, Soorya L, Sousa I, Stein O, Sykes N, Stoppioni V, Strawbridge C, Tancredi R, Tansey K, Thiruvahindrapduram B, Thompson AP, Thomson S, Tryfon A, Tsiantis J, Van Engeland H, Vincent JB, Volkmar F, Wallace S, Wang K, Wang Z, Wassink TH, Webber C, Weksberg R, Wing K, Wittemeyer K, Wood S, Wu J, Yaspan BL, Zurawiecki D, Zwaigenbaum L, Buxbaum JD, Cantor RM, Cook EH, Coon H, Cuccaro ML, Devlin B, Ennis S, Gallagher L, Geschwind DH, Gill M, Haines JL, Hallmayer J, Miller J, Monaco AP, Nurnberger JI Jr, Paterson AD, Pericak-Vance MA, Schellenberg GD, Szatmari P, Vicente AM, Vieland VJ, Wijsman EM, Scherer SW, Sutcliffe JS, Betancur C. Functional impact of global rare copy number variation in autism spectrum disorders. *Nature*. 2010;466:368–372.
19. Bourgeron T. A synaptic trek to autism. Curr Opin Neurobiol 2009;19:231–234.
20. Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. *Nat Rev Genet*. 2008;9:341–355.
21. Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, Südhof TC. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. *Science*. 2007;318:71–76.
22. Marsh E, Fulp C, Gomez E, Nasrallah I, Minarcik J, Sudi J, Christian SL, Mancini G, Labosky P, Dobyns W, Brooks-Kayal A, Golden JA. Targeted loss of Arx results in a developmental epilepsy mouse model and recapitulates the human phenotype in heterozygous females. *Brain*. 2009;132(pt 6):1563–1576.

23. Glaze DG, Percy AK, Skinner S, Motil KJ, Neul JL, Barrish JO, Lane JB, Geerts SP, Annese F, Graham J, McNair L, Lee HS. Epilepsy and the natural history of Rett syndrome. *Neurology*. 2010;74:909–912.
24. Hagerman RJ, Rivera SM, Hagerman PJ. The fragile X family of disorders: a model for autism and targeted treatments. *Curr Pediatr Rev*. 2008;4:40–52.
25. Loesch DZ, Bui QM, Dissanayake C, Clifford S, Gould E, Bulhak-Paterson D, Tassone F, Taylor AK, Hessl D, Hagerman R, Huggins RM. Molecular and cognitive predictors of the continuum of autistic behaviours in fragile X. *Neurosci Biobehav Rev*. 2007;31:315–326.
26. Rogers SJ, Wehner DE, Hagerman RJ. The behavioral phenotype in fragile X: symptoms of autism in very young children with fragile X syndrome, idiopathic autism, and other developmental disorders. *J Dev Behav Pediatr*. 2001;22:409–417.
27. Kaufmann WE, Cortell R, Kau AS, Bukelis I, Tierney E, Gray RM, Cox C, Capone GT, Stanard P. Autism spectrum disorder in fragile X syndrome: communication, social interaction, and specific behaviors. *Am J Med Genet*. 2004;129A:225–234.
28. McDuffie A, Abbeduto L, Lewis P, Kover S, Kim JS, Weber A, Brown WT. Autism spectrum disorder in children and adolescents with fragile X syndrome: within-syndrome differences and age-related changes. *Am J Intell Dev Disabil*. 2010;115:307–326.
29. Penagarikano O, Mulle JG, Warren ST. The pathophysiology of fragile X syndrome. *Annu Rev Genom Human Genet*. 2007;8:109–129.
30. Hagerman RJ, Berry-Kravis E, Kaufmann WE, Ono MY, Tartaglia N, Lachiewicz A, Kronk R, Delahunty C, Hessl D, Visootsak J, Picker J, Gane L, Tranfaglia, M. Advances in the treatment of fragile X syndrome. *Pediatrics*. 2009;123:378–309.
31. Hagerman PJ. Lessons from fragile X regarding neurobiology, autism, and neurodegeneration. *J Dev Behav Pediatr*. 2006;27:63–74.
32. Hessl D, Tassone F, Loesch DZ, Berry-Kravis E, Leehey MA, Game LW, Barbato I, Rice C, Gould E, Hall DA, Grigsby J, Wegelin JA, Harris S, Lewin F, Weinberg D, Hagerman PJ, Hagerman RJ. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. *Am J Med Genet B Neuropsychiatr Genet*. 2005;139B:115–121.
33. Farzin F, Perry H, Hessl D, Loesch D, Cohen J, Bacalman S, Gane L, Tassone F, Hagerman P, Hagerman R. Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. *J Dev Behav Pediatr*. 2006;27: S137–S144.
34. Garcia-Arocena D, Hagerman PJ. Advances in understanding the molecular basis of FXTAS. *Hum Mol Genet*. 2010;19(R1):R83–R89.
35. Coffey SM, Cook K, Tartaglia N, Tassone F, Nguyen DV, Pan R, Bronsky HE, Yuhas J, Borodyanskaya M, Grigsby J, Doerflinger M, Hagerman PJ, Hagerman RJ. Expanded clinical phenotype of women with the FMR1 premutation. *Am J Med Genet A*. 2008;146A: 1009–1016.
36. Dictenberg JB, Swanger SA, Antar LN, Singer RH, Bassell GJ. A direct role for FMRP in activity-dependent dendritic mRNA transport links filopodial-spine morphogenesis to fragile X syndrome. *Dev Cell*. 2008;14:926–939.
37. Bagni C, Greenough WT. From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. *Nat Rev Neurosci*. 2005;6:376–387.
38. Bassell GJ, Warren ST. Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron*. 2008;60:201–214.
39. Nakamoto M, Nalavadi V, Epstein MP, Narayanan U, Bassell GJ, Warren ST. Fragile X mental retardation protein deficiency leads to excessive mGluR5-dependent internalization of AMPA receptors. *Proc Natl Acad Sci USA*. 2007;104:15537–15542.
40. Musumeci SA, Hagerman J, Ferri R, Bosco P, Dalla Bernadina B, Tassinari CA, De Sarro GB, Elia M. Epilepsy and EEG findings in males with fragile X syndrome. *Epilepsia*. 1999;40:1092–1099.
41. Berry-Kravis E. Epilepsy in fragile X syndrome. *Dev Med Child Neurol*. 2002;44:724–728.
42. Incorpora G, Sorge G, Sorge A, Pavone L. Epilepsy in fragile X syndrome. *Brain Dev*. 2002;24:766–769.
43. Hagerman PJ, Stafstrom CE. Origins of epilepsy in fragile X syndrome. *Epilepsy Curr*. 2009;9: 108–112.
44. Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile-X mental retardation. *Proc Natl Acad Sci USA*. 2002;99:7746–7750.
45. Huber KM, Kayser MS, Bear MF. Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science*. 2000;288:1254–1257.
46. Collingridge GL, Peineau S, Howland JG, Wang YT. Long-term depression in the CNS. *Nat Rev Neurosci*. 2010;11:459–473.
47. Chonchaiya W, Utari A, Pereira GM, Tassone F, Hessl D, Hagerman RJ. Broad clinical involvement in a family affected by the fragile X premutation. *J Dev Behav Pediatr*. 2009;30:544–551.
48. Jacquemont S, Farzin F, Hall D, Leehey M, Tassone F, Gane L, Zhang L, Grigsby J, Jardini T, Lewin F, Berry-Kravis E, Hagerman PJ, Hagerman RJ. Aging in individuals with the FMR1 mutation. *Am J Ment Retard*. 2004;109:154–164.
49. Raske C, Hagerman PJ. Molecular pathogenesis of fragile X-associated tremor/ataxia syndrome. *J Investig Med*. 2009;57:825–829.
50. Cornish KM, Li L, Kogan CS, Jacquemont S, Turk J, Dalton A, Hagerman RJ, Hagerman PJ. Age-dependent cognitive changes in carriers of the fragile X syndrome. *Cortex*. 2008;44:628–636.
51. Roberts J, Bailey D, Mankowski J, Ford A, Sideris J, Weisenfeld L, Heath TM, Golden R. Mood and anxiety disorders in females with the FMR1 premutation. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B:130–139.
52. Chen Y, Tassone F, Berman RF, Hagerman PJ, Hagerman RJ, Willemsen R, Pessah IN. Murine hippocampal neurons expressing Fmr1 gene premutations show early developmental deficits and late degeneration. *Hum Mol Genet*. 2010;19:196–208.
53. Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. *Trends Neurosci*. 2004;27:370–377.
54. Chuang SC, Zhao W, Bauchwitz R, Yan Q, Bianchi R, Wong RK. Prolonged epileptiform discharges induced by altered group 1 metabotropic glutamate receptor-mediated synaptic responses in hippocampal slices

of a fragile X mouse model. *J Neurosci*. 2005;25: 8048–8055.
55. Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major fragile X syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. *Neuropharmacology*. 2005;49:1053–1066.
56. Nosyreva ED, Huber KM. Metabotropic receptor-dependent long-term depression persists in the absence of protein synthesis in the mouse model of fragile X syndrome. *J Neurophysiol*. 2006;95:3291–3295.
57. Dolen G, Osterweil E, Shankaranarayana Rao BS, Smith GB, Auerbach BD, Chattarji S, Bear MF. Correction of fragile X syndrome in mice. *Neuron*. 2007;56:955–962.
58. Qiu LF, Lu TJ, Hu XL, Yi YH, Liao WP, Xiong ZQ. Limbic epileptogenesis in a mouse model of fragile X syndrome. *Cereb Cortex*. 2009;19:1504–1514.
59. Chuang SC, Bianchi R, Wong RK. Group I mGluR activation turns on a voltage-gated inward current in hippocampal pyramidal cells. *J Neurophysiol*. 2000;83: 2844–2853.
60. Bianchi R, Chuang SC, Zhao W, Young SR, Wong RKS. Cellular plasticity for group I mGluR-mediated epileptogenesis. *J Neurosci*. 2009;29:3497–3507.
61. Kim SH, Markham JA, Weiler IJ, Greenough WT. Aberrant early-phase ERK inactivation impedes neuronal function in fragile X syndrome. *Proc Natl Acad Sci USA*. 2008;105:4429–4434.
62. Zhao W, Bianchi R, Wang M, Wong RK. Extracellular signal-regulated kinase 1/2 is required for the induction of group I metabotropic glutamate receptor-mediated epileptiform dicharges. *J Neurosci*. 2004;24:76–84.
63. Wang M, Bianchi R, Chuang SC, Zhao W, Wong RK. Group I metabotropic glutamate receptor-dependent TRPC channel trafficking in hippocampal neurons. *J Neurochem*. 2007;101:411–421.
64. Gibson JR, Bartley AF, Hays SA, Huber KM. Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. *J Neurophysiol*. 2008;100:2615–2626.
65. Pfeiffer BE, Huber KM. The state of synapses in fragile X syndrome. *The Neuroscientist*. 2009;15:549–567.
66. El Idrissi A, Ding XH, Scalia J, Trenkner E, Brown WT, Dobkin C. Decreased GABA(A) receptor expression in the seizure-prone fragile X mouse. *Neurosci Lett*. 2005;377:141–146.
67. Gantois I, Vandesompele J, Speleman F, Reyniers E, D'Hooge R, Severijnen LA, Willemsen R, Tassone F, Kooy RF. Expression profiling suggests underexpression of the GABA(A) receptor subunit delta in the fragile X knockout mouse model. *Neurobiol Dis*. 2006;21:346–357.
68. D'Hulst C, Heulens I, Brouwer JR, Willemsen R, De Geest N, Reeve SP, De Deyn PP, Hassan BA, Kooy RF. Expression of the GABAergic system in animal models for fragile X syndrome and fragile X associated tremor/ataxia syndrome (FXTAS). *Brain Res*. 2009;1253:176–183.
69. Selby L, Zhang C, Sun QQ. Major defects in neocortical GABAergic inhibitory circuits in mice lacking the fragile X mental retardation protein. *Neurosci Lett*. 2007;412:227–232.
70. Curia G, Papouin T, Séguéla P, Avoli M. Downregulation of tonic GABAergic inhibition in a mouse model of fragile X syndrome. *Cereb Cortex*. 2009;19:1515–1520.
71. Slotkin TA, MacKillop EA, Ryde IT, Tate CA, Seidler FJ. Screening for developmental neurotoxicity using PC12 cells: comparisons of organophosphates with a carbamate, an organochlorine, and divalent nickel. *Environ Health Perspect*. 2007;115:93–101.
72. Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. *Environ Health Perspect*. 2007;115:1482–1489.
73. Wang LW, Berry-Kravis E, Hagerman RJ. Fragile X: leading the way for targeted treatments in autism. *Neurotherapeutics*. 2010;7:264–274.
74. Berry-Kravis EM, Hessl D, Coffey S, Hervey C, Schneider A, Yuhas J, Hutchison J, Snape M, Tranfaglia M, Nguyen DV, Hagerman R. A pilot open-label single-dose trial of fenobam in adults with fragile X syndrome. *J Med Genet*. 2009;46:266–271.
75. Berry-Kravis EM, Sumis A, Hervey C, Nelson M, Porges SW, Weng N, Weiler IJ, Greenough WT. Open-label treatment trial of lithium to target the underlying defect in fragile X syndrome. *J Dev Behav Pediatr*. 2008;29:293–302.
76. D'Hulst C, Kooy RF. The GABAA receptor: a novel target for treatment of fragile X? *Trends Neurosci*. 2007;30:425–431.
77. Chang S, Bray SM, Li Z, Zarnescu DC, He C, Jin P, Warren ST. Identification of small molecules rescuing fragile X syndrome phenotypes in *Drosophila*. *Nat Chem Biol*. 2008;4:256–263.
78. Reddy DS, Rogawski MA. Ganaxolone suppression of behavioral and electrographic seizures in the mouse amygdala kindling model. *Epilepsy Res*. 2010;89:254–260.
79. Bilousova TV, Dansie L, Ngo M, Aye J, Charles JR, Ethell DW, Ethell IM. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *J Med Genet*. 2009;46:94–102.
80. Stafstrom CE. Mechanisms of epilepsy in mental retardation: insights from Angelman syndrome, Down syndrome and fragile X syndrome. In: Sillanpää M, Gram L, Johannessen SI, Tomson T, eds. *Epilepsy and Mental Retardation*. Petersfield, Hampshire, UK: Wrightson Biomedical Publishing; 1999:7–40.
81. Inoki K, Corradetti MN, Guan KL. Dysregulation of the TSC-mTOR pathway in human disease. *Nat Genet*. 2005;37:19–24.
82. Holmes GL, Stafstrom CE, The Tuberous Sclerosis Study Group. Tuberous sclerosis complex and epilepsy: recent developments and future challenges. *Epilepsia*. 2007;48:617–630.
83. Winterkorn EB, Pulsifer MB, Thiele EA. Cognitive prognosis of patients with tuberous sclerosis complex. *Neurology*. 2007;68:62–64.
84. Mizuguchi M, Takashima S. Neuropathology of tuberous sclerosis. *Brain Dev*. 2001;23:508–515.
85. Curatolo P, D'Argenzio L, Cerminara C, Bombardieri R. Management of epilepsy in tuberous sclerosis omplex. *Expert Rev Neurother*. 2008;8:457–467.
86. Chu-Shore CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia*. 2010;51:1236–1241.
87. Thiele EA. Managing epilepsy in tuberous sclerosis complex. *J Child Neurol*. 2004;19:680–686.
88. Goh S, Kwiatkowski DJ, Dorer DJ, Thiele EA. Infantile spasms and intellectual outcomes in

children with tuberous sclerosis complex. *Neurology*. 2005;65:235–238.
89. Curatolo P, Napolioni V, Moavero R. Autism spectrum disorders in tuberous sclerosis: pathogenetic pathways and implications for treatment. *J Child Neurol.* 2010;25:873–880.
90. Weiner HL, Carlson C, Ridgway EB, Zaroff CM, Miles D, LaJoie J, Devinsky O. Epilepsy surgery in young children with tuberous sclerosis: results of a novel approach. *Pediatrics.* 2006;117:1494–1502.
91. Liang S, Li A, Zhao M, Jiang H, Yu S, Meng X, Sun Y. Epilepsy surgery in tuberous sclerosis complex: emphasis on surgical candidate and neuropsychology. *Epilepsia*. 2010;51:2316–2321.
92. Wiznitzer M. Autism and tuberous sclerosis. *J Child Neurol*. 2004;19:675–679.
93. Bolton PF. Neuroepileptic correlates of autistic symptomatology in tuberous sclerosis. *Ment Retard Dev Disabil Res Rev*. 2004;10:126–131.
94. Asano E, Chugani DC, Muzik O, Behen M, Janisse J, Rothermel R, Mangner TJ, Chakraborty PK, Chugani HT. Autism in tuberous sclerosis complex is related to both cortical and subcortical dysfunction. *Neurology.* 2001;57:1269–1277.
95. Bolton PF, Park RJ, Higgins JN, Griffiths PD, Pickles A. Neuro-epileptic determinants of autism spectrum disorders in tuberous sclerosis complex. *Brain*. 2002;125:1247–1255.
96. Eluvathingal TJ, Behen ME, Chugani HT, Janisse J, Bernardi B, Chakraborty P, Juhasz C, Muzik O, Chugani DC. Cerebellar lesions in tuberous sclerosis complex: neurobehavioral and neuroimaging correlates. *J Child Neurol*. 2006;21:846–851.
97. Jansen FE, Braams O, Vincken KL, Algra A, Anbeek P, Jennekens-Schinkel A, Halley D, Zonnenberg BA, van den Ouweland A, van Huffelen AC, van Nieuwenhuizen O, Nellist M. Overlapping neurologic and cognitive phenotypes in patients with TSC1 or TSC2 mutations. *Neurology.* 2008;70:908–915.
98. Saemundsen E, Ludvigsson P, Hilmarsdottir I, Rafnsson V. Autism spectrum disorders in children with seizures in the first year of life—a population-based study. *Epilepsia*. 2007;48:1724–1730.
99. Jeste SS, Sahin M, Bolton P, Ploubidis GB, Humphrey A. Characterization of autism in young children with tuberous sclerosis complex. *J Child Neurol.* 2008;23:520–525.
100. Wong V. Study of the relationship between tuberous sclerosis complex and autistic disorder. *J Child Neurol.* 2006;21:199–204.
101. Jansen FE, Vincken KL, Algra A, Anbeek P, Braams O, Nellist M, Zonnenberg BA, Jennekens-Schinkel A, van den Ouweland A, Halley D, van Huffelen AC, van Nieuwenhuizen O. Cognitive impairment in tuberous sclerosis complex is a multifactorial condition. *Neurology*. 2008;70:916–923.
102. Foster KG, Fingar DC. Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony. *J Biol Chem*. 2010;285:14071–14077.
103. Mi R, Ma J, Zhang D, Li L, Zhang H. Efficacy of combined inhibition of mTOR and ERK/MAPK pathways in treating a tuberous sclerosis complex cell model. *J Genet Genomics*. 2009;36:355–361.
104. Stafstrom CE. Progress toward understanding epileptogenesis in tuberous sclerosis complex: two hits, no outs, and the Eker rat is up to bat. *Epilepsy Curr*. 2005;5:136–138.
105. von der Brelie C, Waltereit R, Zhang L, Beck H, Kirschstein T. Impaired synaptic plasticity in a rat model of tuberous sclerosis. *Eur J Neurosci.* 2006;6:686–692.
106. Ehninger D, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ, Ramesh V, Silva AJ. Reversal of learning deficits in a $Tsc2^{+/-}$ mouse model of tuberous sclerosis. *Nat Med*. 2008;14:843–848.
107. Meikle L, Talos DM, Onda H, Pollizzi K, Rotenberg A, Sahin M, Jensen FE, Kwiatkowski DJ. A mouse model of tuberous sclerosis: neuronal loss of Tsc1 causes dysplastic and ectopic neurons, reduced myelination, seizure activity, and limited survival. *J Neurosci*. 2007;27:5546–5558.
108. Wong M, Ess KC, Uhlmann EJ, Jansen LA, Li W, Crino PB, Mennerick S, Yamada KA, Gutmann DH. Impaired glial glutamate transport in a mouse tuberous sclerosis epilepsy model. *Ann Neurol*. 2003;54:251–256.
109. Jansen LA, Uhlmann EJ, Crino PB, Gutmann DH, Wong M. Epileptogenesis and reduced inward rectifier potassium current in tuberous sclerosis complex-1-deficient astrocytes. *Epilepsia.* 2005;46:1871–1880.
110. Talos DM, Kwiatkowski DJ, Cordero K, Black PM, Jensen FE. Cell-specific alterations of glutamate receptor expression in tuberous sclerosis complex cortical tubers. *Ann Neurol*. 2008;63:454–465.
111. Franz DN, Leonard J, Tudor C, Chuck G, Care M, Sethuraman G, Dinopoulos A, Thomas G, Crone KR. Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. *Ann Neurol.* 2006;59:490–498.
112. Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, Kwiatkowski DJ. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci*. 2008;28:5422–5432.
113. Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol.* 2008;63:444–453.
114. Muncy J, Butler IJ, Koenig MK. Rapamycin reduces seizure frequency in tuberous sclerosis complex. *J Child Neurol*. 2009;24:477.
115. Zeng LH, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J Neurosci*. 2009;29:6964–6972.
116. Parisi P, Bombardieri R, Curatolo P. Current role of vigabatrin in infantile spasms. *Eur J Paediatr Neurol*. 2007;11:331–336.
117. Willmore LJ, Abelson MB, Ben-Menachem E, Pellock JM, Shields WD. Vigabatrin: 2008 update. *Epilepsia.* 2009;50:163–173.
118. Sharma A, Hoeffer CA, Takayasu Y, Miyawaki T, McBride SM, Klann E, Zukin RS. Dysregulation of mTOR signaling in fragile X syndrome. *J Neurosci.* 2010;30:694–702.
119. Ogawa S, Kwon CH, Zhou J, Koovakkattu D, Parada LF, Sinton CM. A seizure-prone phenotype is associated with altered free-running rhythm in Pten mutant mice. *Brain Res.* 2007;1168:112–123.
120. Ljungberg MC, Sunnen CN, Lugo JN, Anderson AE, D'Arcangelo G. Rapamycin suppresses seizures and neuronal hypertrophy in a mouse model of cortical dysplasia. *Dis Model Mech*. 2009;2:389–398.

121. Uhlhaas PJ, Singer W. Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. *Neuron.* 2006;52:155–168.
122. Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ. Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. *Cereb Cortex.* 2007;17: 951–961.
123. Dolen G, Bear MF. Role for metabotropic glutamate receptor 5 (mGluR5) in the pathogenesis of fragile X syndrome. *J Physiol*. 2008;586.6:1503–1508.

Chapter 72

Cognitive and Behavioral Comorbidities of Epilepsy

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Among the comorbidities associated with epilepsy, cognitive and behavioral abnormalities are the most common and severe.[1,2] Mental retardation, learning disabilities, memory impairment, attention deficit hyperactivity disorder, autism, anxiety, and conduct disorders are greatly overrepresented in individuals with epilepsy,[2,3] and the consequences of such comorbidities greatly diminish the patients' quality of life. Indeed, many people with epilepsy, and their families, consider the cognitive and behavioral consequences of seizures to be at least as troubling as the seizures themselves. Cognitive disorders can be found in a wide range of seizure disorders including temporal and frontal lobe epilepsies, primarily generalized idiopathic epilepsies, and epileptic encephalopathies. While our understanding of the mechanisms responsible for epilepsy-related cognitive and behavioral problems lags far behind our knowledge of the mechanism of epilepsy, increased attention has recently been directed to investigating these comorbidities.

When considering the pathophysiological mechanisms of the cognitive and behavioral consequences of epilepsy, it is helpful to distinguish between impairments that are permanent and those that are dynamic, that is, progressive or transient. Permanent deficits originate from a large number of etiologies in epilepsy: trauma, hypoxic-ischemic insults, genetic disorders, mesial temporal sclerosis as a result of status epilepticus, and malformations of cortical development. In addition to causing seizures, these disorders may result in cognitive and behavioral disturbances, with the severity of such disturbances related to the severity of the etiology. While the cognitive and behavioral

issues associated with these brain insults may evolve over time due to maturational and aging changes in the brain, they are relatively fixed and remain attributable to the underlying brain disorder. Only if the underlying cause of epilepsy is remedied do the cognitive and behavioral deficits improve. This aspect of cognitive and behavioral impairments in the context of epilepsy is particularly underinvestigated.

The second type of impairment is dynamic in the sense that the deficits are either happening in stages or transiently affecting the patients. These cognitive and behavioral deficits can occur as a result of the seizures themselves, interictal epileptiform abnormalities, or antiepileptic drug therapy. Although the dynamic impairments can be observed in the absence of the permanent ones, they often contribute together in affecting the patient's quality of life. Understanding how these chronic and dynamic changes influence behavior and cognitive abilities is instrumental to developing therapeutic interventions.

In this chapter, the mechanisms of both permanent and dynamic impairments in cognition and behavior associated with epilepsy will be reviewed. As will be discussed, there is now considerable evidence that the final common pathway for the cognitive and behavioral disturbances is likely through epilepsy-induced altered neuronal signaling resulting in malfunctioning network activity.

MECHANISMS OF PERMANENT DEFICITS IN COGNITION AND BEHAVIOR

It is commonly accepted that the major factor determining the cognitive and behavioral outcomes in epilepsy is the underlying etiology. The cause of the epilepsy determines, to a great degree, the nature and severity of the behavioral and cognitive outcomes. Because these outcomes affect the balance between excitability and inhibition, it can be argued that they also affect the neural function necessary for cognitive processes.

Permanent Cognitive Deficits

Permanent deficits in cognition can be secondary to innate and acquired neurological disorders. Innate causes of epilepsies can be genetic, congenital, or developmental. The epileptic syndromes that they cause often result in severe cognitive impairments. For instance, the channelopathies are a group of genetic disorders caused by disturbed function of ion channel subunits or the proteins that regulate them. The end result of the channelopathies is altered excitability. These disorders include diseases such as Dravet syndrome (Na^{2+} channels), benign familial neonatal convulsions (K^{+} channels), and autosomal dominant nocturnal frontal lobe epilepsy (nicotinic acetylcholine channels). Genetic and congenital diseases induce malformations of brain tissue such as focal cortical dysplasia, tuberous sclerosis, and arteriovenous malformations. Finally, autoimmune factors, such as those involved in Rasmussen's encephalitis, can cause seizures. The cognitive decline witnessed in the first several years following the diagnosis of some of these conditions likely relates to the progressive alterations in neural activity during the developmental period.

Acquired epilepsy etiologies also result in chronic cognitive and behavioral deficits. An example of an acquired insult is status epilepticus (SE), which has been well studied through the use of animal models, producing a chronic condition that reproduces many aspects of the clinical syndrome of temporal lobe epilepsy. Other commonly acquired conditions resulting in epilepsy include head trauma, inflammatory disorders, cerebrovascular insults, and hypoxia-ischemia. In this section, we will use SE as the model for elucidating mechanisms of impaired cognition and behavior.

When studied weeks or months following SE, rats are found to be impaired in spatial memory tasks such as the Morris water maze[4,5] and the radial arm maze.[6,7] The spatial deficits become apparent shortly after SE develops, before spontaneous seizures occur.[8] Deficits in spatial cognition following SE appear to be age dependent. Following SE, very young rats (<2 weeks of age) usually do not show impairments in the water maze task,[9] whereas pubescent and older rats show patterns in this task similar to those of adult rats subjected to SE.

Paralleling this development of cognitive impairment, a number of morphological and physiological changes occur in brain networks as a result of SE. In the adult, neuronal loss becomes apparent in hippocampal fields CA1, CA3, and the dentate hilus,[10,11] with the pattern

of cell loss dependent upon the agent used to induce the initial SE.[12,13] In addition to cell death, prolonged seizures in the adult brain lead to synaptic reorganization, with aberrant growth (sprouting) of granule cell axons (the so-called *mossy fibers*) in the supragranular zone of the fascia and infrapyramidale region of CA3.[14,15] Sprouting and new synapse formation occur in other brain regions as well—notably in the CA1 pyramidal neurons, where it has been shown that newly formed synapses produce more frequent glutamatergic spontaneous synaptic currents.[16] In rat pups, SE produces no cell loss or sprouting in the hippocampus. However, neonatal SE does result in long-standing changes in long-term potentiation (LTP) and depression (LTD),[17] alterations in the subunit configuration of glutamate[17] and gamma-aminobutyric acid (GABA) receptors,[18] and increases in the primary subsynaptic scaffold, PSD-95.[17]

Morphological and physiological alterations following SE are not limited to the hippocampus. The entorhinal cortex, another area deeply involved in cognitive processing, shows a layer-specific loss of neurons and development of aberrant recurrent circuits. Like the hippocampus, the entorhinal cortex shows altered activity after SE as well.[19,20]

In addition to contributing to sporadic generation of seizures, these and other lasting changes in neural circuitry are likely to directly influence the ability of the affected structure to process information normally. For instance, the loss of interneurons during SE[21,22] leads to a loss of inhibition that is not only critical for seizure prevention, but also fundamental for synaptic integration, oscillatory activity, and information processing in general. The relationship between SE-induced network reorganization and cognitive dysfunction is probably best illustrated by single-unit recordings in freely moving rats.

A subset of neurons in the hippocampus called *place cells* elicit action potentials in frequencies that correspond to the animal's location within its environment. Specifically, these hippocampal pyramidal neurons selectively discharge when the animal enters certain locations of the environment, called the cells' *firing fields* (Fig. 72–1). Field location, size, and shape are specific to each cell and each environment, and fields tend to cover the surface of the environment homogeneously when a large number of neurons are being recorded simultaneously. For a given environment, in normal rats, place cell firing patterns remain unchanged, even between exposures separated by months.[23–27]. Since there is a relationship between place cell activity and the ongoing spatial behavior of rats,[28,29] it is believed that such signals provide the animal with a spatial representation in order to navigate efficiently within the environment. These cells provide a very useful surrogate for spatial memory. Adult rats that have experienced SE and have impaired learning in the water maze task have defective place cells.[4,5] Place cells from SE rats have less precise firing fields and less stable firing fields from session to session[4] (Fig. 72–1).

One of the potential mechanisms by which post-SE changes may affectthe cognition and behavior is through alterations of brain oscillations. Seizure-induced changes at the molecular and structural levels, notably through loss of interneurons, are extremely likely to affect the fine tuning of rhythmic activity of large groups of cooperating neurons, as measured by local field potentials. Oscillations in brain structures provide temporal windows that allow local computations, binding cooperating neuronal assemblies for the representation, processing, storage, and retrieval of information. The theta rhythm (4–12 Hz) is critically involved in mnemonic function of the hippocampus.[30,31] Information arriving in the hippocampus during theta oscillations is processed, whereas information arriving in the absence of normal theta activity is believed not to be encoded, or not encoded with the same degree of precision as when theta activity is present.[31–33] Additionally, the phase of theta activity is critical in learning and memory. Tetanic stimulation in CA1 produces LTP when administered at the peak of theta activity and LTD when delivered at the trough.[34] Similarly, gamma oscillations (30–100 Hz) are critical in the processing or perceiving of sensory information,[35,36] consciousness,[37,38] storage of immediate memories,[39–41] and memory recall.[42] Recent work has provided evidence that epileptic rats have alterations in hippocampal theta rhythm magnitude,[8] providing insights into the mechanisms of spatial cognitive defects following SE.

In the hippocampus, pyramidal place cells are characterized not only by their location-specific firing, but also by their precise temporal firing relationship with hippocampal theta oscillations.[5,41,43–45] When the firing field is entered by the rat, place cells will fire

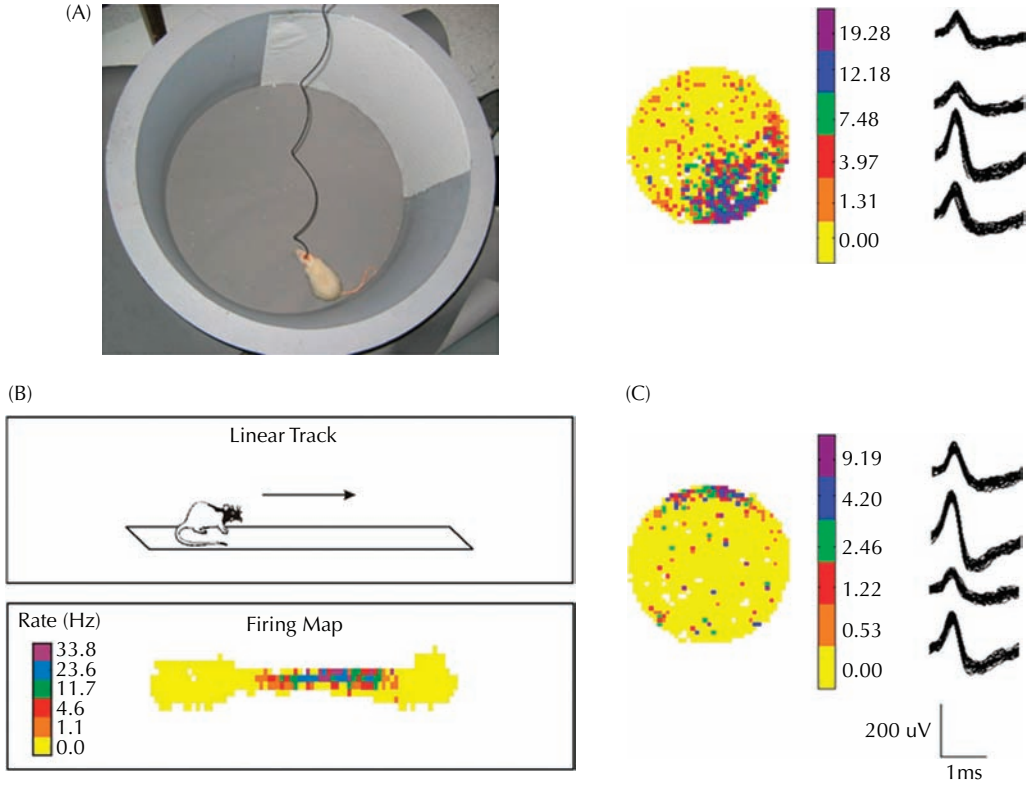

Figure 72–1. Place cell recordings. **A.** Rat in a recording cylinder. The white cue card is placed on the wall of the cylinder to provide orientation for the rat. The environment remains stable from trial to trial. The rat runs about the cylinder chasing food pellets. **B.** Rat on a linear track. The rat runs from one end to the other to obtain a food award. The firing field of a place cell is shown below the track. **C.** Place cells from a rat running in a cylinder. The firing field for the place cell in the upper figure is at around 0500–0600, while the firing field in the lower figure is at 1200. The firing rate was coded in the sequence: yellow, orange, red, green, blue, and purple, from lowest firing rate to highest. The firing rate was zero for yellow pixels. To the right of the firing fields are the complex action potentials recorded using tetrodes. Note the different amplitude of the same action potential in each electrode.

preferentially on the negative phase of the CA1 recorded theta cycle (Fig. 72–2). As the rat crosses the field, the cells fire earlier on successive theta peaks, a phenomenon called *phase precession*.[46,47] Because of this characteristic, two cells with partially overlapping fields will fire at a specific but different phase of the ongoing theta cycle. Their relative firing interval will be constant and directly related to the distance separating their fields. As a result, the sequence of events experienced by the animal, as well as its timing (the rat crossed field A x milliseconds before field B), is encoded: the time difference between A and B is observed on a large time scale (the time it takes to get from field A to field B) and also on the order of tens of milliseconds. The firing sequences of cell assemblies observed in the running time are compressed in a time window short enough to induce LTP-like synaptic changes.[48,49] Using these measurements, a time compression index can be defined, for all possible pairs of cells, as the ratio of two spike-timing measures: (1) the time necessary for the animal to go from one field to the other and (2) the time lag between the spikes of the two corresponding place cells within one theta cycle.[50] Rats subjected to SE have aberrant phase precession and impaired time compression of firing among pairs of neurons,[5] indicating that SE results in impaired temporal coding of information (Fig. 72–3).

Small errors in the timing of neuronal and oscillatory activity can amplify across complex networks and perhaps can even be magnified when synthesized with corresponding cerebral cortical activity.[51] It is likely that such errors are

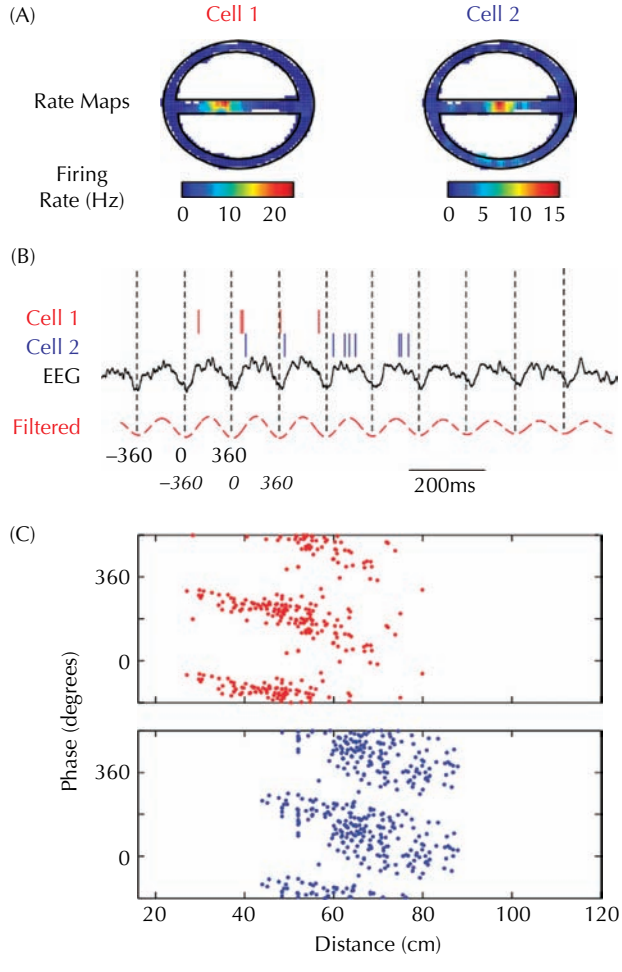

Figure 72–2. Place cell firing and its relationship with the EEG. **A.** Firing rate maps of two cells recorded simultaneously in a rat engaged in a spatial task in a figure 8 maze. Firing activity of the same cells (each vertical bar corresponds to an action potential [AP]) and a simultaneously recorded EEG (black: wideband; red: filtered in the theta band) during a single crossing of the central arm (from left to right). For each cell, as the rat crosses the field, the timing of APs within subsequent theta cycles shifts forward (phase precession). Because the rat enters the field of cell 1 before the field of cell 2, both cells will fire at a different phase on each theta cycle. Within each theta cycle, there is a linear relationship between the time difference of the action potentials of each cell and the distance separating the two fields. Therefore, the cell activation sequence is compressed in a single theta cycle. **B.** Phase/distance plots for the cells represented above. **C.** Each dot corresponds to an action potential. To allow a representation of each cell in relationship to theta activity, each action potential is displayed twice over 360 degrees.

both prominent and perpetual in the chronic epilepsies, and perhaps are even more detrimental in developing animals, in which oscillations drive circuit formation and stabilization.

Permanent Behavioral Deficits

In addition to cognitive abnormalities, chronic epilepsy can produce perpetual behavior problems in both children and adults. Following SE, adult animals become irritable and aggressive on the handling test,[9,52] hyperactive,[9] and anxious.[53] In addition, SE results in impaired socialization; while the animals are more aggressive, irritable, and difficult to handle by experimenters, they display increased passivity toward *an intruder animal* in the home cage intruder test.[7,54] The mechanisms responsible for behavioral changes following SE have not

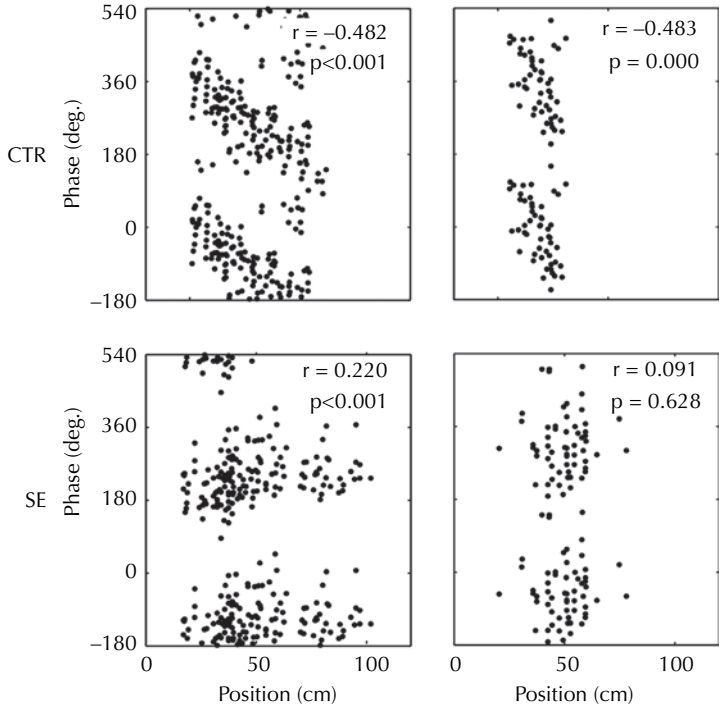

Figure 72–3. Aberrant phase precession in a rat model of epilepsy. Compared to control (CTR) animals, rats with a past history of SE have aberrant phase precession patterns.

been established. However, it is known that SE results in cell loss and synaptic reorganization throughout the behavior-related areas of the brain including the prefrontal cortex, hippocampus, amygdala, and thalamus. While not yet characterized, aberrant signaling changes in these networks likely contribute to these behavioral abnormalities.

MECHANISMS OF DYANAMIC DEFICTS IN COGNITION AND BEHAVIOR

As opposed to permanent deficits in cognition and behavior that are usually attributed to the etiology of the epilepsy, dynamic deficits can also occur. These transient impairments are believed to be caused by the temporary disruption of neural activity patterns. In general, these dynamic cognitive impairments are associated with seizures, epileptiform abnormalities, and the medication used to treat the seizures. While only seizures and interictal epileptiform activity will be discussed here, antiepileptic drug-associated neurobehavioral adverse effects also occur and have been previously reviewed.[55]

Dynamic Deficits Secondary to Seizures

Spontaneous recurrent seizures, a key feature of epilepsy, seriously affect the patient's cognitive ability. In addition to the obvious inabilities during seizures, the postictal state usually corresponds to a period of drastically decreased cognitive ability. After the behavioral symptoms of lethargy and inattention subside, lingering cognitive deficits may persist for minutes to days, depending on the type and severity of the seizure.[56–58]

As an illustration, Lin et al.[59] trained adult rats extensively in the spatial accuracy task, a dry-land analog of the Morris water maze. The authors found a cumulative degradation in spatial performance over 11 days of flurothyl-produced seizures (one per day). However,

the deficits reversed after the seizures were stopped, such that performance returned to baseline. Intriguingly, the rate of learning to an asymptote, the rate of performance decline during one-per-day seizures, and the rate of relearning during the recovery period were all similar. These findings suggest that deficits following a small number of seizures are reversible after a period of time, likely paralleling the return to neurological homeostasis. Similarly, Boukhezra et al.[60] found that generalized seizures following asymptote levels of learning in the Morris water maze task resulted in impaired performance, with the duration of the cognitive deficits exceeding the length of the seizures. Interestingly, the animal's neurological status was a factor in the duration of cognitive impairment following seizures; animals with a prior history of SE had a longer period of impairment following a seizure than animals without such a history.

Zhou et al.[61] assessed the effects of 10 flurothyl-induced seizures in adult rats on LTP and place cell function. Recurrent flurothyl seizures were associated with marked impairment in LTP and a reduction in the frequency of the peak theta power. Compared to baseline recordings, place cell firing patterns following recurrent seizures were significantly less precise, had lower firing rates, and were less stable. Impaired place cell firing was seen as early as after two seizures. Paralleling place cell firing patterns, water maze performance was impaired in animals that underwent a series of seizures. These results demonstrated that significant and long-standing alterations in hippocampal homeostasis occur with relatively brief excitatory events, although the duration of these postictal effects was not measured.

The most commonly provided explanation for the postictal phenomenon is that the prolonged, synchronous neural activity during seizures depletes neurotransmitters and available glucose, which understandably could prevent normal information processing.[62–64]. However, spontaneous seizures are followed by a drastic alteration of place cell firing.[65] After seizures, a marked decrease in the firing rate of action potentials from place cells occurs, whereas interneuron firing is unchanged. In addition, when place cell firing fields persisted or returned, they were aberrant, with reduced coherence and information content. In addition to postictal suppression of firing patterns, seizures lead to the emergence of firing fields in previously silent cells, demonstrating a postictal remapping of the hippocampus. These findings demonstrate that postictal alterations in behavior are not due solely to reduced neuronal firing. Rather, the postictal period is characterized by robust and dynamic changes in cell-firing patterns resulting in the remapping of the hippocampus.

Dynamic Deficits Secondary to Interictal Spikes

In addition to seizures, there is increasing evidence that interictal abnormalities can result in cognitive impairment, though this is much more short-lived than the impairment of the postictal period. Epileptiform abnormalities, including interictal spikes (IIS) or spike-and-wave discharges, represent an aberrant discharge of a large number of neurons near the recording site. These ephemeral events can produce brief disturbances in neural processing, resulting in a phenomenon called *transitory cognitive impairment*.[66] However, they rarely produce overt cognitive or behavioral disturbances.

In a seminal study, Aarts et al.[67] noted that IIS can briefly disrupt neural processes within the brain region where they occur. The authors analyzed the effect of IIS on verbal or nonverbal short-term memory in patients with various epileptic conditions who had no overt clinical manifestations during these discharges, thus targeting the hidden (subclinical) manifestations of IIS. They found that left-hemisphere IIS resulted mainly in verbal task errors, whereas right-hemisphere IIS were associated with errors on the nonverbal task. Electroencephalographic (EEG) discharges interfered mainly when they occurred simultaneously with the presentation of the stimulus, corresponding with the encoding phase of the task. Shewmon and Erwin[68–71] further localized the effect, noting that occipital IIS could disrupt visual perception. A number of ensuing clinical studies demonstrated that IIS in the cortex can result in transitory cognitive impairment.[67–73] One study examined IIS using intracranial electrodes in patients with temporal lobe epilepsy and found transient declines in working memory due to IIS.[74]

To investigate the transient effects of IIS on cognition, Kleen et al.[75] used a within-subject analysis to systematically analyze how IIS might independently affect multiple processes in the hippocampus, a structure critically important for learning and memory and highly prone to IIS in temporal lobe epilepsy. These researchers studied rats that developed chronic IIS following intrahippocampal pilocarpine administration in a hippocampal-dependent operant behavior task, the delayed-match-to-sample. Hippocampal IIS that occurred during memory retrieval strongly impaired performance (Fig. 72–4). However, IIS that occurred during memory encoding or memory maintenance did not affect performance in those trials. Hippocampal IIS also affected response latency, adding approximately 0.48 s to the time taken to respond. Interictal spikes were most harmful if they occurred when hippocampal function was critical, similar to the findings in human studies, showing that cortical spikes are most

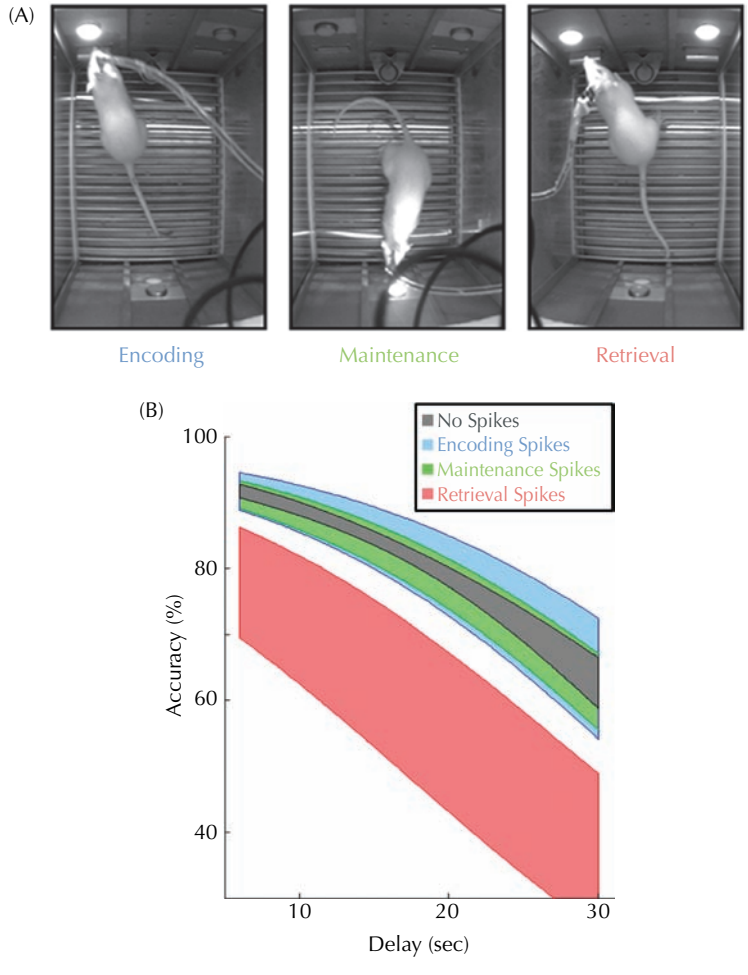

Figure 72–4. Influence of IIS on different memory processes. **A.** Montage of the delayed-match-to-sample task, a useful paradigm for assessing the dynamic influence of IIS on memory. Rats were trained to press a randomly presented lever (e.g., the left lever in this example), then wait for a variable time interval near the opposite wall of the chamber. Subsequently, both levers were presented, and the rat needed to choose the lever it had pressed before. Each trial thereby involved memory encoding, maintenance, and retrieval for accurate performance. **B.** Thousands of trials among five rat trials were separated first by whether an IIS occurred during them and then by the epoch in which the IIS occurred (encoding, maintenance, or retrieval). Accuracy is plotted against the length of the variable interval between lever presses (delay), with longer delays normally producing decreased accuracy. Colored bands of the modeled 95% confidence intervals indicate that IIS during memory retrieval drastically impair memory, while IIS during encoding or maintenance do not.

disruptive during active cortical functioning. It was suggested that the cumulative effects of spikes could therefore impact general cognitive functioning, although this general effect was not seen in this study, supporting the notion that dynamic disruptions in cognition may not be captured by general cognitive testing.

A few studies to date have shed light on the probable cause of IIS-induced transient cognitive disruption. There is a significant and sustained reduction of action potentials in the hippocampus for up to 2 s following a local IIS. Furthermore, when occurring in flurries, IIS can reduce action potential firing for up to 6 s.[76] The response to IIS is cell-dependent; IIS result in decreases in action potential firing after the IIS among interneurons but not among pyramidal (place) cells. In addition to affecting action potentials, the widespread inhibitory wave immediately after IIS can dramatically reduce the power of gamma oscillations and other oscillatory signals in the hippocampus.[77] Since oscillations are intimately linked to ongoing learning and memory functions, this disruption in oscillations likely contributes to cognitive deficits.[75,78]

Transient impairments in cognition are difficult to capture with standard cognitive tests because of several conditions. First, in order to disrupt a particular process, the IIS must incorporate the neural circuits involved in that process, stressing the importance of matching the affected neural substrate with a cognitive test that assesses its intrinsic function. Second, the IIS must occur at a particular moment in cognitive processing when the process is vulnerable to disruption. Third, the process must not be supported significantly by other interconnected structures that might buffer the information and reintroduce it to the affected area once the IIS effects have passed.

Despite these limitations, if the proper cognitive test is utilized and IIS are frequent enough, it is possible to show relations between overall IIS frequency and the degree of cognitive impairment. This accumulation of dynamic effects can thus resemble a chronic cognitive deficit. For example, patients with Landau-Kleffner syndrome gradually develop a high frequency of IIS at a young age, and the degree of their EEG abnormalities is closely related to the auditory agnosia and aphasia most patients eventually experience. These patients may or may not have seizures; thus, the effect seems exclusively related to the EEG abnormalities. Furthermore, improvements in cognition tend to be accompanied by improvements in the EEG.[79]

HOW DYNAMIC DEFICITS MAY BECOME PERMANENT

While a single seizure episode or IIS usually has transient effects on neuronal activity, there are conditions in which their impact may be more detrimental for cognition and behavior. This is the case for recurrent generalized seizures and also for seizures or IIS that occur during critical periods such as sleep or development.

Recurrent Seizures

One of the more popular models for studying recurrent seizures is kindling. Kindling is a dynamic process whereby repeated application of seizure-evoking stimulation produces neuronal changes that result in an enduring enhancement of susceptibility to seizure-evoking stimulation. Since kindling is a gradually acquired process, behavioral tests can be done during the kindling or after the animal has fully kindled. If testing is done during the acquisition of kindling, the investigator can assess behavior before or following the kindling stimulation. If testing occurs after kindling, the investigator can manipulate the time of the testing to determine the duration of any postkindling effect.

Investigators have examined the effect of kindling on spatial memory with the animal being studied after or during kindling using both the radial arm maze and the water maze. The timing of the kindling stimulation determines the type of deficit. If the kindling stimulation is given prior to the learning trial, there is impaired performance,[80–82] whereas kindling immediately after the learning trial impairs retention.[83] Whether kindling has long-term effects on learning is not clear, with some authors finding impairment following hippocampal kindling,[84,85] and others finding no long-standing effects.[81] However, the effects of kindling on spatial memory are not confined solely to electrical kindling. With repetitive

pentylenetetrazole-induced seizures given every other day for 28 days, rats made more reference errors in the radial arm water maze.[86] Genetically epilepsy-prone rats subjected to 66 audiogenic stimulations showed impairment in both the water maze and the T-maze compared to littermates that were handled and placed in the sound chamber but were not stimulated.[87]

The immature brain appears to be particularly prone to developing permanent deficits following early life seizures. Seizures during the first weeks of life result in deficits of spatial cognition in the Morris water maze,[88–93] impairment of auditory discrimination,[94] and altered activity level in the open field.[91] Despite the detrimental effects of early life seizures on cognitive function, recurrent seizures during the first 2 weeks of life do not result in cell loss.[88,90,95] However, early life seizures can result in synaptic reorganization[88,89,96,97] and decreased neurogenesis.[98] Recurrent seizures during development also result in a number of physiological changes including a persistent decrease in GABA currents in the hippocampus[99] and neocortex,[100,101] enhanced excitation in the neocortex,[101] impairment in spike frequency adaptation,[102] marked reductions in afterhyperpolarizing potentials following spike trains,[102] LTP,[91] alterations in theta power,[92] and impaired place cell coherence and stability.[92]

Rats with developmental seizures have also been found to have abnormal hippocampal single-cell firing patterns when studied as adults. Following a series of 100 brief flurothyl-induced seizures during the first weeks of life (P15–P37), rats were found to have impairment in spatial cognition, with poor performance in the water maze and radial arm maze and impaired hippocampal LTP.[92] Similar to rats following SE, these rats had substantial deficits in action potential firing with impaired place cell precision and reduced place cell stability. These results show that recurrent seizures during early development are associated with significant impairment in spatial learning and that these deficits are paralleled by deficits in the hippocampal map.

Recurrent Interictal Spikes

In elegant studies of the striate cortex function in rabbits, IIS were elicited by either penicillin[103,104] or bicuculline[105,106] through focal epidural application. The IIS were elicited for 6–12 h following each drug application, which was given daily from P8–9 up to P24–30. None of the rabbits had behavioral seizures. In single-unit recordings from the lateral geniculate nucleus, superior colliculus, and occipital cortex ipsilateral to the hemisphere with IIS, there was an abnormal distribution of receptive field types, whereas recordings from the contralateral hemisphere were normal. This finding was age-dependent: rabbits with similarly induced IIS during adulthood had normal development of cells, highlighting an additional vulnerability of developmental periods to cumulative IIS effects over time.

Interictal spikes have also been elicited in young rats with flurothyl, an inhaled convulsant.[107] Rat pups were given a low dose of flurothyl for 4 h over 10 days during continuous EEG monitoring. They developed IIS without seizures, while age-matched controls under similar testing conditions showed few IIS. When rats were tested as adults, there was impairment in reference memory in the probe test of the Morris water maze, reference memory impairment in the four-trial radial arm water maze, and impaired LTP. Early-life IIS also resulted in impaired new cell formation and decreased cell counts in the hippocampus, indicating a potential mechanism by which IIS during development can produce cumulative lasting effects in addition to any dynamic disruptions. It appears from these data that IIS, like seizures, during brain development have a cumulative effect on cognitive function.

While not yet fully studied, it is likely that IIS during sleep may contribute to both cognitive and behavioral problems. Sleep is important for memory consolidation, and IIS, when occurring frequently in conditions such as continuous spike-wave sleep, will affect learning and memory.[108] To mimic IIS, Shatskikh et al.[109] implanted a stimulating microelectrode in the ventral hippocampal commissure and a recording microelectrode in the CA1 region of the hippocampus of normal male rats. Spike patterns were induced using a series of electrical pulses to provoke discharges in the hippocampus that resembled naturally occurring IIS in epileptic rats. When the IIS were introduced while the rat was sleeping, performance in the Morris water maze was impaired compared to times when no IIS occurred during sleep. In this study, none of the rats had seizures, indicating

that IIS during sleep have adverse effects in a test of hippocampal memory.

Effect on Behavior

Kindling also affects behavior, with amygdala-kindled animals exhibiting heightened anxiety[110] and enhanced emotionality expressed by elevated anxiety and a defensive attitude toward other animals.[111] Rats kindled in the amygdala and hippocampus explored less in the open field, were more resistant to capture from the open field, and engaged in more open-arm activity in the elevated plus maze,[112,113] classic behavioral signs of increased anxiety in rodent models. Perirhinal cortex kindling also increased anxiety-related behavior in both the elevated plus and open-field mazes and disrupted spontaneous object recognition.[114] Pentylenetetrazol-treated rats have also been shown to have high anxiety levels in the open-field exploratory maze test.[86]

Amygdala kindling also alters social attraction between rats in the open-field test, with kindled rats showing a higher likelihood of remaining in close proximity to a partner rat.[111] Partial kindling of the ventral perforant path in cats produced a lasting increase in the defense response of cats to both rats and conspecific threat howls. In addition, there was a suppression of approach-attack behaviors directed toward rats.[115] Pentylenetetrazol-treated rats also displayed decreased offensive behaviors in the home cage intruder test.[116] Genetically epilepsy-prone rats subjected to repetitive seizures were less active in the open-field activity test, less aggressive in the home cage intruder test, and more irritable and aggressive in the handling test.[87] In a test that mimics depression, the forced swim test, animals receiving repetitive pentylenetetrazole injections were immobile significantly longer than control rats.[86]

CONCLUSIONS

In extrapolating the animal data to individuals with epilepsy, it is helpful to keep in mind that there are two major processes, permanent and dynamic, that can influence cognitive and behavioral outcomes. Permanent deficits are due primarily to the etiology of the epilepsy. Regardless of whether the etiology is innate or acquired, the deficits seen are due to the underlying brain pathology causing the epilepsy. These deficits would occur regardless of the frequency of recurrent seizures. Treating the underlying condition, if possible, may improve the cognitive and behavioral outcomes. Dynamic changes are caused by the seizures or interictal discharges. In the case of dynamic causes of cognitive and behavioral disturbances, there is a window of opportunity for the clinician to intervene by reducing the number of seizures or suppressing the interictal discharges. Failure to do so can convert dynamic changes into permanent ones. This is particularly the case when dealing with seizures and interictal discharges in the developing brain. The challenge for neuroscientists will be to develop safe and effective mechanistically driven therapies to reduce or prevent the dynamic effects of seizures and interictal discharges.

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REFERENCES

1. Austin JK. The 2007 Judith Hoyer lecture. Epilepsy comorbidities: Lennox and lessons learned. *Epilepsy Behav*. 2009;14:3–7.
2. Hermann B, Seidenberg M, Jones J. The neurobehavioural comorbidities of epilepsy: can a natural history be developed? *Lancet Neurol*. 2008;7:151–160.
3. Pellock JM. Understanding co-morbidities affecting children with epilepsy. *Neurology*. 2004;62:S17–S23.
4. Liu X, Muller RU, Huang LT, Kubie JL, Rotenberg A, Rivard B, Cilio MR, Holmes GL. Seizure-induced changes in place cell physiology: relationship to spatial memory. *J Neurosci*. 2003;23:11505–11515.
5. Lenck-Santini PP, Holmes GL. Altered phase precession and compression of temporal sequences by place cells in epileptic rats. *J Neurosci*. 2008;28:5053–5062.
6. Sayin U, Sutula TP, Stafstrom CE. Seizures in the developing brain cause adverse long-term effects on spatial learning and anxiety. *Epilepsia*. 2004;45:1539–1548.

7. Letty S, Lerner-Natoli M, Rondouin G. Differential impairments of spatial memory and social behavior in two models of limbic epilepsy. *Epilepsia.* 1995;36:973–982.
8. Chauviere L, Rafrafi N, Thinus-Blanc C, Bartolomei F, Esclapez M, Bernard C. Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy. *J Neurosci*. 2009;29:5402–5410.
9. Stafstrom CE, Chronopoulos A, Thurber S, Thompson JL, Holmes GL. Age-dependent cognitive and behavioral deficits after kainic acid seizures. *Epilepsia.* 1993;34:420–432.
10. Olney JW, Fuller T, De Gubareff T. Acute dendrotoxic changes in the hippocampus of kainate treated rats. *Brain Res*. 1979;176:91–100.
11. Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience.* 1985;14: 375–403.
12. Nadler JV. Kainic acid as a tool for the study of temporal lobe epilepsy. *Life Sci.* 1981;29:2031–2042.
13. Ben-Ari Y. Cell death and synaptic reorganizations produced by seizures. *Epilepsia*. 2001;42(Suppl 3):5–7.
14. Represa A, Tremblay E, Ben-Ari Y. Kainate binding sites in the hippocampal mossy fibers: localization and plasticity. *Neuroscience*. 1987;20:739–748.
15. Sutula T, Xiao-Xian H, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science*. 1988;239:1147–1150.
16. Esclapez M, Hirsch J, Ben-Ari Y, Bernard C. Newly formed excitatory pathways provide a substrate for hyperexcitability in experimental temporal lobe epilepsy. *J Comp Neurol*. 1999;408:449–460.
17. Cornejo BJ, Mesches MH, Coultrap S, Browning MD, Benke TA. A single episode of neonatal seizures permanently alters glutamatergic synapses. *Ann Neurol.* 2007;61:411–426.
18. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Kelly ME, Coulter DA. Gamma-aminobutyric acid(A) receptor subunit expression predicts functional changes in hippocampal dentate granule cells during postnatal development. *J Neurochem*. 2001;77:1266–1278.
19. Wozny C, Gabriel S, Jandova K, Schulze K, Heinemann U, Behr J. Entorhinal cortex entrains epileptiform activity in CA1 in pilocarpine-treated rats. *Neurobiol Dis*. 2005;19:451–460.
20. Bragin DE, Sanderson JL, Peterson S, Connor JA, Muller WS. Development of epileptiform excitability in the deep entorhinal cortex after status epilepticus. *Eur J Neurosci*. 2009;30:611–624.
21. Andre V, Marescaux C, Nehlig A, Fritschy JM. Alterations of hippocampal GABAergic system contribute to development of spontaneous recurrent seizures in the rat lithium-pilocarpine model of temporal lobe epilepsy. *Hippocampus.* 2001;11:452–468.
22. Ratte S, Lacaille JC. Selective degeneration and synaptic reorganization of hippocampal interneurons in a chronic model of temporal lobe epilepsy. *Adv Neurol*. 2006;97:69–76.
23. Muller RU, Kubie JL. The effects of changes in the environment on the spatial firing patterns of hippocampal complex-spike cells. *J Neurosci*. 1987;7:1951–1968.
24. Muller RU, Kubie JL. The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *J Neurosci*. 1987;7:1951–1968.
25. Muller RU, Kubie JL, Ranck JB Jr. Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. *J Neurosci.* 1987;7:1935–1950.
26. Thompson LT, Best PJ. Place cells and silent cells in the hippocampus of freely-behaving rats. *J Neurosci*. 1989;9:2382–2390.
27. Thompson LT, Best PJ. Long-term stability of the place-field activity of single units recorded from the dorsal hippocampus of freely behaving rats. *Brain Res.* 1990;509:299–308.
28. Lenck-Santini PP, Muller RU, Save E, Poucet B. Relationships between place cell firing fields and navigational decisions by rats. *J Neurosci*. 2002;22: 9035–9047.
29. Lenck-Santini PP, Save E, Poucet B. Evidence for a relationship between place-cell spatial firing and spatial memory performance. *Hippocampus*. 2001;11: 377–390.
30. Senior TJ, Huxter JR, Allen K, O'Neill J, Csicsvari J. Gamma oscillatory firing reveals distinct populations of pyramidal cells in the CA1 region of the hippocampus. *J Neurosci*. 2008;28:2274–2286.
31. Vertes RP, Kocsis B. Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. *Neuroscience.* 1997;81:893–926.
32. Buzsaki G. Theta oscillations in the hippocampus. *Neuron*. 2002;33:325–340.
33. Itskov V, Pastalkova E, Mizuseki K, Buzsaki G, Harris KD. Theta-mediated dynamics of spatial information in hippocampus. *J Neurosci.* 2008;28:5959–5964.
34. Hyman JM, Wyble BP, Goyal V, Rossi CA, Hasselmo ME. Stimulation in hippocampal region CA1 in behaving rats yields long-term potentiation when delivered to the peak of theta and long-term depression when delivered to the trough. *J Neurosci*. 2003;23:11725–11731.
35. Gray CM, Viana Di PG. Stimulus-dependent neuronal oscillations and local synchronization in striate cortex of the alert cat. *J Neurosci*. 1997;17:3239–3253.
36. Gray CM. Synchronous oscillations in neuronal systems: mechanisms and functions. *J Comput Neurosci*. 1994;1:11–38.
37. Vanderwolf CH. Are neocortical gamma waves related to consciousness? *Brain Res*. 2000;855:217–224.
38. Llinas R, Ribary U. Coherent 40-Hz oscillation characterizes dream state in humans. *Proc Natl Acad Sci USA*. 1993;90:2078–2081.
39. Chrobak JJ, Buzsaki G. High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. *J Neurosci*. 1996;16:3056–3066.
40. Hasselmo ME, Wyble BP, Wallenstein GV. Encoding and retrieval of episodic memories: role of cholinergic and GABAergic modulation in the hippocampus. *Hippocampus*. 1996;6:693–708.
41. Lisman J. The theta/gamma discrete phase code occurring during the hippocampal phase precession may be a more general brain coding scheme. *Hippocampus.* 2005;15:913–922.
42. Montgomery SM, Buzsaki G. Gamma oscillations dynamically couple hippocampal CA3 and CA1 regions during memory task performance. *Proc Natl Acad Sci USA*. 2007;104:14495–14500.
43. Bose A, Booth V, Recce M. A temporal mechanism for generating the phase precession of hippocampal place cells. *J Comput Neurosci*. 2000;9:5–30.

44. Harris KD, Henze DA, Hirase H, Leinekugel X, Dragoi G, Czurko A, Buzsaki G. Spike train dynamics predicts theta-related phase precession in hippocampal pyramidal cells. *Nature*. 2002;417:738–741.
45. Skaggs WE, McNaughton BL, Wilson MA, Barnes CA. Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. *Hippocampus.* 1996;6:149–172.
46. O'Keefe J, Recce M. Phase relationships between hippocampal place units and the EEG theta rhythm. *Hippocampus.* 1993;3:317–330.
47. Skaggs WE, McNaughton BL, Gothard KM, Markus EJ. An information-theoretic approach to deciphering the hippocampal code. In: Hanson SJ, Cowan JD, Giles CL, eds. *Advances in Neural Information Processing Systems.* Vol. 5. San Francisco: Morgan Kaufmann; 1993:1030–1037.
48. Bliss TV. LTP and spatial learning. *J Physiol* (*Paris*). 1996;90:335.
49. Muller D, Nikonenko I, Jourdain P, Alberi S. LTP, memory and structural plasticity. *Curr Mol Med*. 2002;2:605–611.
50. Geisler C, Robbe D, Zugaro M, Sirota A, Buzsaki G. Hippocampal place cell assemblies are speed-controlled oscillators. *Proc Natl Acad Sci USA*. 2007;104: 8149–8154.
51. Buzsaki G. The structure of consciousness. *Nature*. 2007;446:267.
52. Mikati MA, Holmes GL, Chronopoulos A, Hyde P, Thurber S, Gatt A, Liu Z, Werner S, Stafstrom CE. Phenobarbital modifies seizure-related brain injury in the developing brain. *Ann Neurol*. 1994;36:425–433.
53. Dos SJ Jr, Longo BM, Blanco MM, Menezes de Oliveira MG, Mello LE. Behavioral changes resulting from the administration of cycloheximide in the pilocarpine model of epilepsy. *Brain Res*. 2005;1066: 37–48.
54. Mellanby J, Strawbridge P, Collingridge GI, George G, Rands G, Stroud C, Thompson P. Behavioural correlates of an experimental hippocampal epileptiform syndrome in rats. *J Neurol Neurosurg Psychiatry*. 1981;44:1084–1093.
55. Sankar R, Holmes GL. Mechanisms of action for the commonly used antiepileptic drugs: relevance to antiepileptic drug-associated neurobehavioral adverse effects. *J Child Neurol*. 2004;19(suppl 1):S6–S14.
56. Biton V, Gates JR, de Padua SL. Prolonged postictal encephalopathy. *Neurology.* 1990; 40:963–966.
57. Helmstaedter C, Elger CE, Lendt M. Postictal courses of cognitive deficits in focal epilepsies. *Epilepsia.* 1994;35:1073–1078.
58. Aldenkamp AP. Effect of seizures and epileptiform discharges on cognitive function. *Epilepsia*. 1997; 38(Suppl 1):S52–S55.
59. Lin H, Holmes GL, Kubie JL, Muller RU. Recurrent seizures induce a reversible impairment in a spatial hidden goal task. *Hippocampus.* 2009;19:817–827.
60. Boukhezra O, Riviello P, Fu DD, Lui X, Zhao Q, Akman C, Holmes GL. Effect of the postictal state on visual-spatial memory in immature rats. *Epilepsy Res*. 2003;55:165–175.
61. Zhou JL, Shatskikh TN, Liu X, Holmes GL. Impaired single cell firing and long-term potentiation parallels memory impairment following recurrent seizures. *Eur J Neurosci*. 2007;25:3667–3677.
62. Duncan R. Epilepsy, cerebral blood flow, and cerebral metabolic rate. *Cerebrovasc Brain Metab Rev*. 1992;4:105–121.
63. Duncan R, Patterson J, Roberts R, Hadley DM, Bone I. Ictal/postictal SPECT in the pre-surgical localisation of complex partial seizures. *J Neurol Neurosurg Psychiatry*. 1993;56:141–148.
64. Chugani HT, Shewmon DA, Khanna S, Phelps ME. Interictal and postictal focal hypermetabolism on positron emission tomography. *Pediatr Neurol.* 1993;9: 10–15.
65. Zhou JL, Lenck-Santini PP, Holmes GL. Postictal single-cell firing patterns in the hippocampus. *Epilepsia.* 2007;48:713–719.
66. Binnie CD. Cognitive impairment during epileptiform discharges: is it ever justifiable to treat the EEG? *Lancet Neurol.* 2003;2:725–730.
67. Aarts JH, Binnie CD, Smit AM, Wilkins AJ. Selective cognitive impairment during focal and generalized epileptiform EEG activity. *Brain*. 1984;107(pt 1): 293–308.
68. Shewmon DA, Erwin RJ. Transient impairment of visual perception induced by single interictal occipital spikes. *J Clin Exp Neuropsychol*. 1989;11:675–691.
69. Shewmon DA, Erwin RJ. Focal spike-induced cerebral dysfunction is related to the after-coming slow wave. *Ann Neurol*. 1988;23:131–137.
70. Shewmon DA, Erwin RJ. The effect of focal interictal spikes on perception and reaction time. II. Neuroanatomic specificity. *Electroencephalogr Clin Neurophysiol.* 1988;69:338–352.
71. Shewmon DA, Erwin RJ. The effect of focal interictal spikes on perception and reaction time. I. General considerations. *Electroencephalogr Clin Neurophysiol.* 1988;69:319–337.
72. Binnie CD, Channon S, Marston DL. Behavioral correlates of interictal spikes. *Adv Neurol*. 1991;55: 113–126.
73. Binnie CD, Kasteleijn-Nolst Trenite DG, Smit AM, Wilkins AJ. Interactions of epileptiform EEG discharges and cognition. *Epilepsy Res*. 1987;1:239–245.
74. Krauss GL, Summerfield M, Brandt J, Breiter S, Ruchkin D. Mesial temporal spikes interfere with working memory. *Neurology*. 1997;49:975–980.
75. Kleen JK, Scott RC, Holmes GL, Lenck-Santini PP. Hippocampal interictal spikes disrupt cognition in rats. *Ann Neurol*. 2010;67:250–257.
76. Zhou JL, Lenck-Santini PP, Zhao Q, Holmes GL. Effect of interictal spikes on single-cell firing patterns in the hippocampus. *Epilepsia*. 2007;48:720–731.
77. Urrestarazu E, Jirsch JD, Levan P, Hall J, Avoli M, Dubeau F, Gotman J. High-frequency intracerebral EEG activity (100–500 Hz) following interictal spikes. *Epilepsia*. 2006;47:1465–1476.
78. Halasz P, Kelemen A, Clemens B, Saracz J, Rosdy B, Rasonyi G, Szucs A. The perisylvian epileptic network. A unifying concept. *Ideggyogy Sz*. 2005;58:21–31.
79. Smith MC, Hoeppner TJ. Epileptic encephalopathy of late childhood: Landau-Kleffner syndrome and the syndrome of continuous spikes and waves during slow-wave sleep. *J Clin Neurophysiol*. 2003;20:462–472.
80. Robinson GB, McNeill HA, Reed GD. Comparison of the short- and long-lasting effects of perforant path kindling on radial maze learning. *Behav Neurosci.* 1993;107:988–995.

81. McNamara RK, Kirkby RD, dePace GE, Corcoran ME. Limbic seizures, but not kindling, reversibly impair place learning in the Morris water maze. *Behav Brain Res*. 1992;50:167–175.
82. Gilbert TH, Hannesson DK, Corcoran ME. Hippocampal kindled seizures impair spatial cognition in the Morris water maze. *Epilepsy Res*. 2000;38: 115–125.
83. Gilbert TH, McNamara RK, Corcoran ME. Kindling of hippocampal field CA1 impairs spatial learning and retention in the Morris water maze. *Behav Brain Res*. 1996;82:57–66.
84. Leung LS, Boon KA, Kaibara T, Innis NK. Radial maze performance following hippocampal kindling. *Behav Brain Res*. 1990;40:119–129.
85. Leung LS, Shen B. Hippocampal CA1 evoked response and radial 8-arm maze performance after hippocampal kindling. *Brain Res.* 1991;555:353–357.
86. Mortazavi F, Ericson M, Story D, Hulce VD, Dunbar GL. Spatial learning deficits and emotional impairments in pentylenetetrazole-kindled rats. *Epilepsy Behav*. 2005;7:629–638.
87. Holmes GL, Thompson JL, Marchi TA, Gabriel PS, Hogan MA, Carl FG, Feldman DS. Effects of seizures on learning, memory, and behavior in the genetically epilepsy-prone rat. *Ann Neurol.* 1990;27:24–32.
88. Holmes GL, Gairsa JL, Chevassus-Au-Louis N, Ben-Ari Y. Consequences of neonatal seizures in the rat: morphological and behavioral effects. *Ann Neurol.* 1998;44:845–857.
89. Huang L, Cilio MR, Silveira DC, McCabe BK, Sogawa Y, Stafstrom CE, Holmes GL. Long-term effects of neonatal seizures: a behavioral, electrophysiological, and histological study. *Brain Res Dev Brain Res*. 1999;118:99–107.
90. Liu Z, Yang Y, Silveira DC, Sarkisian MR, Tandon P, Huang LT, Stafstrom CE, Holmes GL. Consequences of recurrent seizures during early brain development. *Neuroscience*. 1999;92:1443–1454.
91. Karnam HB, Zhao Q, Shatskikh T, Holmes GL. Effect of age on cognitive sequelae following early life seizures in rats. *Epilepsy Res*. 2009;85:221–230.
92. Karnam HB, Zhou JL, Huang LT, Zhao Q, Shatskikh T, Holmes GL. Early life seizures cause long-standing impairment of the hippocampal map. *Exp Neurol.* 2009;217:378–387.
93. Dube CM, Zhou JL, Hamamura M, Zhao Q, Ring A, Abrahams J, McIntyre K, Nalcioglu O, Shatskih T, Baram TZ, Holmes GL. Cognitive dysfunction after experimental febrile seizures. *Exp Neurol.* 2008;215: 167–177.
94. Neill JC, Liu Z, Sarkisian M, Tandon P, Yang Y, Stafstrom CE, Holmes GL. Recurrent seizures in immature rats: effect on auditory and visual discrimination. *Brain Res Dev Brain Res*. 1996;95:283–292.
95. Riviello P, de Rogalski Landrot I, Holmes GL. Lack of cell loss following recurrent neonatal seizures. *Brain Res Dev Brain Res*. 2002;135:101–104.
96. Huang LT, Yang SN, Liou CW, Hung PL, Lai MC, Wang CL, Wang TJ. Pentylenetetrazol-induced recurrent seizures in rat pups: time course on spatial learning and long-term effects. *Epilepsia*. 2002;43: 567–573.
97. Sogawa Y, Monokoshi M, Silveira DC, Cha BH, Cilio MR, McCabe BK, Liu X, Hu Y, Holmes GL. Timing of cognitive deficits following neonatal seizures: relationship to histological changes in the hippocampus. *Brain Res Dev Brain Res.* 2001;131: 73–83.
98. McCabe BK, Silveira DC, Cilio MR, Cha BH, Liu X, Sogawa Y, Holmes GL. Reduced neurogenesis after neonatal seizures. *J Neurosci*. 2001;21:2094–2103.
99. Isaeva E, Isaev D, Khazipov R, Holmes GL. Selective impairment of GABAergic synaptic transmission in the flurothyl model of neonatal seizures. *Eur J Neurosci*. 2006;23:1559–1566.
100. Isaeva E, Isaev D, Khazipov R, Holmes GL. Long-term suppression of GABAergic activity by neonatal seizures in rat somatosensory cortex. *Epilepsy Res*. 2009;87:286–289.
101. Isaeva E, Isaev D, Savrasova A, Khazipov R, Holmes GL. Recurrent neonatal seizures result in long-term increases in neuronal network excitability in the rat neocortex. *Eur J Neurosci*. 2010;31:1446–1455.
102. Villeneuve N, Ben-Ari Y, Holmes GL, Gaiarsa JL. Neonatal seizures induced persistent changes in intrinsic properties of CA1 rat hippocampal cells. *Ann Neurol.* 2000;47:729–738.
103. Baumbach HD, Chow KL. Visuocortical epileptiform discharges in rabbits: differential effects on neuronal development in the lateral geniculate nucleus and superior colliculus. *Brain Res.* 1981;209:61–76.
104. Crabtree JW, Chow KL, Ostrach LH, Baumbach HD. Development of receptive field properties in the visual cortex of rabbits subjected to early epileptiform cortical discharges. *Brain Res*. 1981;227:269–281.
105. Ostrach LH, Crabtree JW, Campbell BG, Chow KL. Effects of bicuculline-induced epileptiform activity on development of receptive field properties in striate cortex and lateral geniculate nucleus of the rabbit. *Brain Res*. 1984;317:113–123.
106. Campbell BG, Ostrach LH, Crabtree JW, Chow KL. Characterization of penicillin- and bicuculline-induced epileptiform discharges during development of striate cortex in rabbits. *Brain Res*. 1984;317: 125–128.
107. Khan OI, Zhao Q, Miller F, Holmes GL. Interictal spikes in developing rats cause long-standing cognitive deficits. *Neurobiol Dis*. 2010;39:362–371.
108. Holmes GL, Lenck-Santini PP. Role of interictal epileptiform abnormalities in cognitive impairment. Epilepsy Behav. 2006;8:504–515.
109. Shatskikh TN, Raghavendra M, Zhao Q, Cui Z, Holmes GL. Electrical induction of spikes in the hippocampus impairs recognition capacity and spatial memory in rats. *Epilepsy Behav*. 2006;9:549–556.
110. Adamec RE, McKay D. Amygdala kindling, anxiety, and corticotrophin releasing factor (CRF). *Physiol Behav.* 1993;54:423–431.
111. Haimovici A, Wang Y, Cohen E, Mintz M. Social attraction between rats in open field: long-term consequences of kindled seizures. *Brain Res*. 2001;922: 125–134.
112. Kalynchuk LE, Pinel JP, Treit D. Long-term kindling and interictal emotionality in rats: effect of stimulation site. *Brain Res.* 1998;779:149–157.
113. Kalynchuk LE, Pinel JP, Treit D, Barnes SJ, McEachern JC, Kippin TE. Persistence of the interictal emotionality produced by long-term amygdala kindling in rats. *Neuroscience.* 1998;85:1311–1319.

114. Hannesson DK, Howland JG, Pollock M, Mohapel P, Wallace AE, Corcoran ME. Anterior perirhinal cortex kindling produces long-lasting effects on anxiety and object recognition memory. *Eur J Neurosci*. 2005;21: 1081–1090.
115. Adamec RE. Partial kindling of the ventral hippocampus: identification of changes in limbic physiology which accompany changes in feline aggression and defense. *Physiol Behav*. 1991;49: 443–453.
116. Franke H, Kittner H. Morphological alterations of neurons and astrocytes and changes in emotional behavior in pentylenetetrazol-kindled rats. *Pharmacol Biochem Behav*. 2001;70:291–303.

Chapter 73

Migraine and Epilepsy—Shared Mechanisms within the Family of Episodic Disorders

Michael A. Rogawski

INTRODUCTION

In 1906, the British neurologist Sir William R. Gowers delivered a clinical lecture at the National Hospital for the Paralysed and Epileptic, Queen Square, London, in which he pointed out the resemblance between migraine and epilepsy.[1] He argued that migraine is a borderland disease to epilepsy: "near it but not of it." Gowers recognized that migraine and epilepsy often occur together in the same patient and that the two conditions are similar in their "character and nature." In recent years, the association between migraine and epilepsy as comorbid conditions has been confirmed. Moreover, migraine and epilepsy are now recognized to be key members of a large family of episodic disorders that also includes periodic paralyses, cardiac arrhythmias, and episodic movement disorders. Studies of the pathophysiological mechanisms underlying the generation of migraine aura and focal seizures indicate remarkable similarities. The identification of genes responsible for both conditions is perhaps the strongest evidence for shared underlying mechanisms.

Fred Andermann, in the introduction to the book *Migraine and Epilepsy*, published in 1987, proposed the following possible explanations for the comorbidity between migraine and epilepsy: (1) both are common and therefore will co-occur by chance, (2) they are

causally related, with one leading to the other, and (3) there is a shared pathophysiological or genetic basis.[2] In this chapter, I examine the evidence for and against these alternatives, focusing on recent advances in physiology and genetics that support Andermann's third possibility.

COMORBIDITY OF EPILEPSY AND MIGRAINE

Migraine and epilepsy are both common neurological disorders, although migraine is more frequent. Numerous studies have observed an association between the two disorders. Most studies of comorbidity have examined the incidence of migraine in cohorts of subjects with epilepsy. The prevalence of migraine in populations of individuals with epilepsy is estimated at 8%–24%,[3] so that the risk of migraine is approximately twice that in the normal population.[4,5] Recently, the incidence of epilepsy was examined in a large series of children with headache.[3] In this study, children with migraine had a 3.2-fold increased risk of epilepsy compared with those with tension-type headache. Although prior studies have found an association only with migraine with aura,[6] this more recent study observed an increased incidence of epilepsy in subjects with migraine both with aura and without aura. In the majority of cases, epilepsy preceded migraine with aura. Overall, the prevalence of epilepsy in individuals with migraine has been reported to be in the range of 1%–17%, with a median of 5.9%, which is higher than the population prevalence of about 0.5%–1%. There is a small increase in the risk of migraine in those with partial onset seizures compared with generalized onset seizures (relative risk, 1.3).[7] Migraine is particularly frequent in those with epilepsy caused by head trauma, where the relative risk compared with idiopathic or cryptogenic epilepsy is 1.8. The strong association between posttraumatic epilepsy and migraine is believed to occur because head injury is a risk for both conditions.[8]

Apart from the observation that head trauma may lead to both epilepsy and migraine, there are several possible explanations for the general comorbidity between the two conditions. Migraine attacks could be epileptogenic and over time could lead to the development of epilepsy; alternatively, recurrent seizures could lead to the development of migraine. Epidemiological data indicate that these explanations are unlikely.[7,8] If migraine caused epilepsy, for example by inducing brain injury, the incidence of an epilepsy diagnosis should be increased in individuals with preexisting migraine. The epidemiological results demonstrate that there is an excess risk of epilepsy both before and after the onset of migraine, leading to the rejection of the unidirectional causality hypothesis. A similar argument suggests that epilepsy does not lead to the development of migraine.

Do Migraine Attacks Trigger Seizures?

The association between migraine and epilepsy could arise not because migraine attacks are epileptogenic (i.e., cause a permanent change in the brain to an epileptic state), but because migraine attacks may simply trigger seizures. Indeed, in 1960, William G. Lennox and his daughter Margaret A. Lennox, in their book *Epilepsy and Related Disorders*, recognized a condition they termed *migralepsy*, in which "ophthalmic migraine with perhaps nausea and vomiting [is] followed by symptoms characteristic of epilepsy."[9] In recent years, the International Headache Society has included migralepsy in its classification scheme. However, it has been noted that the differentiation of occipital seizures from migraine aura is difficult, leading to the frequent erroneous diagnosis of migralepsy.[10,11] Indeed, a review of the literature suggested that migralepsy is extremely rare.[12] In contrast, occipital seizures are often associated with postictal headache that is often indistinguishable from migraine.[10,13] Therefore, according to Panayiotopoulos,[11] in the majority of cases, occipital epilepsy is the correct diagnosis. An alternative perspective is that there are pathophysiological similarities between migraine and epilepsy that make the sharp distinction between the two disorders artificial. In some cases of occipital epilepsy, migraine mechanisms may come into play, inasmuch as there is an overlapping brain substrate. A particularly extreme example of the concept that shared pathophysiological

mechanisms may underlie migraine and epilepsy in the same patient is familial hemiplegic migraine, discussed in detail below, in which migraine attacks and seizures can occur together.[14] In some instances in familial hemiplegic migraine, true migralepsy occurs in which migraine does trigger a seizure. Also, it should be noted that seizures may be caused by migrainous cerebral infarction or possibly also in basilar artery migraine, although the latter has been disputed.[15]

While triggering of a seizure by migraine is unusual, headache is often associated with seizures. Preictal and ictal headaches occur rarely, but postictal headache is common and may have migraine-like features.[7] Not only does migraine-like postictal headache occur following occipital seizures, it also occurs following generalized tonic-clonic seizures[16] and can also occur following temporal lobe seizures, however, it is less likely following frontal lobe seizures or simple partial seizures.[13] Case reports indicate that postictal headache can respond to sumatriptan.[17] Therefore, it has been proposed that seizures can in some instances trigger trigeminovascular pain mechanisms, as occurs in migraine.

A Hypothesis for Comorbidity

Acknowledging that seizures often trigger headache, which may have migraine-like features, there remains an increased risk of migraine attacks, unassociated with seizures, in persons with epilepsy and vice versa. Given that there is little evidence to support the unilateral causality concept, it might be concluded that shared genetic risk factors are the underlying explanation for the comorbidity. This simple explanation was not supported in the epidemiological study of Ottman and Lipton.[8] Indeed, their result might have been expected from the observation noted above that head trauma is a risk factor for both epilepsy and migraine. Ottman and Lipton therefore discarded the simple genetic risk hypothesis and instead proposed that a state of brain hyperexcitability, which can either be produced by genetic factors or can be acquired (such as in a head injury), increases the risk of both migraine and epilepsy, thus leading to the comorbid association. As discussed below, this concept is supported by a wide range of physiological evidence.

EPISODIC NEUROLOGICAL DISORDERS

Although epilepsy and migraine have distinct clinical manifestations, an important similarity is that they are both episodic disorders in which patients are affected with symptoms sporadically and the interictal interval between the appearance of symptoms is variable; between attacks, affected individuals may be symptom free. Episodic disorders comprise a large group of clinically important conditions that affect excitable tissues in various organs and have diverse outward manifestations that depend upon the organ affected, such as seizures, headache, cardiac arrhythmias, episodic movements, and periodic paralysis.[5] A hallmark of episodic disorders is that they often are due to defects in ion channels or, more generally, ion-translocating transmembrane proteins including Na^+,K^+-ATPase.[18] Paradigmatic of the episodic channelopathies are conditions affecting skeletal muscle that lead to transitory weakness or paralysis, including the periodic paralyses and myotonias. Other well-recognized episodic disorders are the various forms of the long QT syndrome that can cause ventricular tachyarrhythmias and sudden death. In addition to affecting skeletal and cardiac muscles, episodic channelopathies affect the brain. Ryan and Ptáček[18] have defined three broad categories of such episodic disorders: paroxysmal movement disorders (episodic ataxias and paroxysmal dyskinesias), epilepsies, and headache disorders. As episodic disorders, epilepsy and migraine share certain characteristics and a presumption that the underlying pathohysiology relates to alterations in ion channels or ion transporters.

FEATURES THAT CHARACTERIZE EPISODIC DISORDERS

Episodic channelopathies exhibit similar clinical features. The hallmark of all episodic disorders is their paroxysmal nature. A trigger factor of some kind causes the system to assume an

aberrant state that is expressed as an aberrant phenotype. An individual with the disease has a reduced barrier to entry into the aberrant state. In episodic disorders, alterations in the structure of ion channels (more generally, ion transport proteins) decrease the safety margins so that a normally innocuous stressor overcomes homeostatic mechanisms that prevent entry into a pathological state, such as a seizure or migraine attack.[18] This concept implies that unaffected individuals (i.e., individuals who do not enter the aberrant state in the normal course of their daily lives) may enter the aberrant state with sufficient provocation. This is certainly the case for epilepsy and possibly also for migraine. Seizures can be provoked in nonepileptic individuals by head trauma, chemical convulsant agents, electrical stimulation (as in electroconvulsive therapy), or metabolic derangements. Headache can also be provoked by certain chemical agents. For example, glyceryl trinitrate (nitroglycerin) has been known from the time of its discovery to produce intense headache.[19] In addition to the immediate headache that occurs in all individuals, in migraine sufferers glyceryl trinitrate can trigger a delayed headache of greater intensity and with a greater number of migraine-like features than occurs in nonmigrainurs.[20,21] Usually, this is without an aura even in migraineurs who experience an aura, although migraine with a visual aura can be triggered in some cases.[22] Histamine infusion can produce similar effects.[23] Secondary migraine headache can also be triggered in nonmigraineurs by many factors, such as intense physical exercise, head injury, hypoglycemia, chronic renal failure, dialysis, and sickle cell disease.[24]

In addition to being members of the family of episodic disorders and often existing together in the same individual, epilepsy and migraine have other similarities. Seizures and migraine attacks may evolve in four comparable stages, with prodromal symptoms, an aura, an ictus (seizure or headache), and a postdromal or postictal phase. Occasionally, the attacks fail to stop, resulting in status epilepticus or status migrainosus. Although there may be a greater diversity of trigger factors for migraine than for epilepsy, there are a surprisingly large number of similar triggers, such as stress (or letdown from stress), factors related to sleep, photic stimulation, hormonal changes such as those occurring during menstruation, and alcohol or dietary factors. How these factors bring on a migraine attack or epileptic seizures is not well understood. However, there is evidence for enhanced cortical responsiveness to diverse stimuli in migraine as well as in epilepsy, as discussed later in this chapter.

ANTIEPILEPTIC DRUGS IN MIGRAINE

A further similarity between migraine and epilepsy that strongly supports the notion that there are shared underlying mechanisms is that some antiepileptic drugs (AEDs) are useful in both conditions. There is extensive evidence from randomized controlled clinical trials that divalproex sodium (valproate) and topiramate are effective in preventing migraine attacks, and both drugs are approved by the U.S. Food and Drug Administration for this indication.[25] The gabapentinoids gabapentin and pregabalin may also be effective in migraine therapy.[26,27] Other AEDs that have been reported to be useful in migraine prophylaxis are levetiracetam and zonisamide.[28–30] Interestingly, while the limited clinical trial data available do not indicate that lamotrigine reduces the frequency of migraine attacks overall, there is evidence that it may reduce the frequency, duration, and intensity of the migraine aura; in many patients who responded with a reduction in aura, there was a reduction in headache frequency.[31] An effect on aura was also found for the investigational AED tonabersat, which produced a reduction in the number of aura attacks (either isolated aura or aura followed by headache) but not a reduction in the number of migraine headache days.[32] The AEDs reduce neuronal hyperexcitability by various mechanisms.[33] Consequently, these results are consistent with the concept that hyperexcitability is responsible for triggering the aura and the subsequent headache. However, they raise the question of whether, in migraine without aura, different trigger mechanisms may be at play. Some AEDs, including phenytoin, oxcarbazepine, vigabatrin, and clonazepam, are not effective in migraine prophylaxis. Thus, AEDs that primarily act via use-dependent block of voltage-gated sodium channels or that act via GABAergic mechanisms appear to influence

hyperexcitability mechanisms that are not relevant to migraine (perhaps related to the spread of hypersynchronous activity). In any case, it is noteworthy that in both epilepsy and migraine, a proportion of patients are pharmacoresistant (around 30% in both conditions).

CORTICAL SPREADING DEPRESSION

When speaking about migraine in his 1906 lecture on the borderland of epilepsy, Gowers noted, "a peculiar spreading disturbance of the nerve structures is evident."[1] He remarked on the similarity with the Jacksonian march in epilepsy but recognized the different time course in that "The epileptic aura occupies a few seconds," whereas "the premonition of migraine is almost always many minutes, often twenty, in its deliberate course." Gowers' description of the slow spread of the migraine aura is remarkable in that it foreshadows the current view that cortical spreading depression (CSD), a propagating wave of cellular depolarization, is the basis for the aura in migraine and the trigger for the subsequent headache pain.

The phenomenon of CSD was first described by Aristides Leão, who was attempting to develop a model of "experimental epilepsy" by electrically stimulating the cortical surface of the rabbit.[34] Instead, he found that weak electrical (and later mechanical) stimulation elicited a decrease in the spontaneous activity (depression of the electrocorticogram signal) at the stimulated region that slowly spread in all directions at a rate of 3 to 5 mm/min.[34,35] Recovery of the initial pattern of spontaneous activity occurred over 5 to 10 min. Leão found that epileptiform discharges elicited by electrical stimulation, strychnine, or acetylcholine were suppressed by spreading depression. In addition, he noted that various types of discharges occurred in conjunction with the wave of depression, including electrographic events that resembled tonic-clonic seizure discharges. The spreading depression and the tonic-clonic seizure activity involved the same cortical elements. Moreover, Leão ended his classic 1944 paper with the statement "The depression and 'tonic-clonic' activity of experimental cortical epilepsy seem to be closely related phenomena."[35] Although Leão considered the cortical discharges and the suppression of neuronal activity to be independent, Grafstein's subsequent work demonstrated that the depression is actually preceded by neural activation.[36,37] Recording from small, isolated slabs of cortex in *cerveau isolé* (midbrain-transected) cats, Grafstein was able to confirm Leão's observation that spreading depression is associated with a slow negative direct current (DC) shift and depressed neural activity. However, she observed that there is a brief (2–3 s) burst of action potential activity at the initiation of the DC negativity. More recent studies have confirmed the existence of fast, rhythmic discharges in the front of a CSD wave; the discharges are synchronized over considerable distances in the tissue and occur for several seconds before the negative DC shift.[38] Initially, there are subthreshold field oscillations that give rise to a high-frequency (60–70 Hz) burst of populations spikes.

The mechanisms that underlie the initiation and propagation of CSD are still not fully defined. Neurons become silent during the passage of CSD, and recordings from neuronal somata have revealed that the negative DC shift of CSD is associated with collapse of the membrane potential to zero and a severe loss of membrane input resistance.[39] Although neuronal somata become electrically unresponsive during CSD, recent studies indicate that parts of the dendritic tree maintain electrical excitability.[40] The membrane conductances that participate in CSD are not fully defined.[41] Potassium currents, including transient A-type currents, M-type (Kv7/KCNQ) current, and also, to a lesser extent, delayed rectifier current, certainly become activated by the depolarization, leading to large transitory increases in extracellular potassium to 30–60 mM that closely follow the DC shift.[42,43] Ionotropic glutamate receptor channels have also been proposed to play a role. AMPA receptors rapidly desensitize and therefore do not contribute substantially. NMDA receptors are ordinarily blocked by magnesium at resting potential, but the depolarization of CSD is expected to relieve the block. Based on modeling, Makarova et al.[41] have argued that while NMDA receptors do contribute, their contribution is relatively small, particularly since NMDA receptors are inhibited by elevated extracellular potassium concentrations. These authors have proposed that a large, but as yet unidentified, dendritic

conductance is a major determinant of the cellular depolarization in CSD.

The concept that spreading depression is responsible for migraine aura is based on a comparison between the rates of progression of the two phenomena. Most commonly, migraine aura arises in the primary visual cortex and is associated with visual symptoms. The disturbance usually starts at the center of the visual field and propagates to peripheral zones within 10 to 15 min. Function returns to normal within another 10 to 15 min.[44] The rate of development of the visual symptoms suggests that there is a front of hyperactivation in the visual cortex that moves at a speed of approximately 3 mm/min. Milner[45] noted that the speed of propagation of the visual symptoms was the same as that of the wave of spreading depression, leading to the hypothesis that CSD is the physiological basis for the aura. In subjects experiencing somatosensory symptoms, the spread of symptoms along the sensory homunculus occurs at a similar rate.

Numerous neuroimaging studies in humans support the concept that spreading depression-like phenomena in neocortex occur with migraine aura.[46–48] In particular, using functional magnetic resonance imaging, it has been possible to demonstrate slowly propagating neurovascular changes in visual cortex that occur together with visual symptoms in patients experiencing visual aura.[47] Although CSD has been assumed to be relevant only to migraine with aura, there is an increasing body of evidence that CSD-like changes in cerebral blood flow also occur in migraine without aura, providing a unified theory of migraine pathogenesis.[48]

HYPEREXCITABILITY IN CSD AND FOCAL SEIZURES

Given that the propagating wave of CSD is led by oscillatory field activity and the discharge of synchronous field responses,[36,38] it is reasonable to compare the hyperexcitability of CSD with that occurring in a focal seizure discharge. In both cases there is hypersynchronous activity in cortical neural aggregates, although in the case of migraine the current flows are insufficiently broadly distributed spatially to be detected by scalp EEG recordings. Although the way in which CSD generates migraine aura symptoms is poorly understood, the positive symptoms presumably derive from active zones with hypersynchronous activity, whereas negative symptoms (such as scotomas) result from depressed zones; the headache is also generated by depressed cortex. In contrast, in epilepsy, the hypersynchronous activity itself generates the aura and the ictus. The spread of CSD occurs at a stereotypical rate of 3–5 mm/min (50–83 μm/s). In contrast, the spread of epileptiform activity occurs over a wide range of rates that spans three orders of magnitude.[49] Ordinarily, the rate of epileptiform propagation is assumed to be faster than that of CSD. However, in in vitro brain slice models, the speed of propagation depends on the specific model. When synaptic inhibition is reduced (e.g., with $GABA_A$ receptor-blocking agents), neocortical slices exhibit discharges that propagate at a rate of 2–10 mm/s. In contrast, in the low-magnesium model, in which seizures arise as a result of the enhanced activity of NMDA receptors, seizures propagate at rates of approximately 100–250 μm/s, which is in the same range as the rate of propagation of CSD. Similarly, measurements from EEG recordings indicate that human cortical seizures can spread at low or high rates (<200 μm/s to >10,000 μm/s).[49,50] Thus, the rate of propagation of epileptiform discharges that are elicited by unblocking NMDA receptors is similar to that of CSD. Potassium channel blockade with 4-aminopyridine (4-AP) can also induce slowly propagating seizure discharges.[49] These seizures, which are largely due to excessive glutamate-mediated excitatory neurotransmission, are not sensitive to NMDA receptor antagonists but are blocked by AMPA receptor antagonists.[51] Therefore, in general, it appears that epileptiform activity associated with enhanced excitatory neurotransmission propagates at rates comparable to those of CSD, whereas epileptiform activity caused by reduced inhibition propagates much faster.

ROLE OF GLUTAMATE

The evidence presented so far indicates that there are similarities in the physiology of the early stages of the evolution of a migraine attack and a focal epileptic seizure. Both begin with

hypersynchronous activity, and both spread as a wave from the region of detonation. For some types of seizure discharges—specifically, those generated by excessive activation of ionotropic glutamate receptors and where GABAergic inhibition is intact—the rate of spread is similar to that occurring in CSD. Ionotropic glutamate receptors appear to play a special role as a trigger in both instances. Indeed, it has been known since the work of van Harreveld and Fifková in the 1970s that glutamate can trigger CSD.[52] Moreover, in their earliest study, glutamate-induced CSD was found to be inhibited in the presence of high magnesium ion concentrations (10–15 mM). We now know that these concentrations of magnesium are sufficient to block NMDA receptors. Indeed, diverse NMDA receptor antagonists, including MK-801, ketamine, memantine, and 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), have been shown to inhibit CSD.[53–56] Therefore, NMDA receptors are a critical trigger for CSD. In contrast, AMPA receptor antagonists are generally ineffective.[53] The relative importance of NMDA and AMPA receptors in triggering CSD mimics their relative roles in generating the large DC shift of CSD. The opposite situation applies for most in vitro seizure models, where AMPA receptor antagonists effectively inhibit epileptiform activity but NMDA antagonists do not.[57] An exception is the low-magnesium model associated with excessive activation of NMDA receptors, where NMDA receptor antagonists do abolish the epileptiform discharges.[58]

Given the ability of glutamate to trigger CSD, it has been proposed that glutamate release is responsible for the advancing wave front of CSD. This glutamate could come from neurons, but recently glia have been implicated. At least partial support for this idea is provided by studies showing that NMDA receptor antagonists influence the synchronous prodromal oscillations and raise the threshold required to initiate CSD but do not prevent CSD propagation once initiated.[55] It is concluded that NMDA receptors contribute to but are not essential for the spatial spread of CSD; their role is mainly prior to the massive neuronal depolarization, and they contribute little to this depolarization. In any case, it is interesting to draw a parallel between epileptic activity and CSD/migraine, where fast glutamate-mediated excitation is the inciting event in both instances. In CSD, glia may be a major source of this glutamate. In epilepsy, however, while it had been speculated that glia might release glutamate that generates synchronized epileptic discharges,[59,60] more recent evidence raises doubt.[61] A schematic illustration of the related but distinct pathways leading to an ictal event in migraine and epilepsy is presented in Fig. 73–1. An example of a situation in an experimental model where seizure activity and spreading depression occur in the same brain networks following a triggering event (a brief pulse of potassium) is shown in Fig. 73–2. Ordinarily, seizure and migraine mechanisms are not considered as occurring together. However, the clinical evidence presented in the first part of this chapter suggests that there are many instances, such as migralepsy or more commonly seizure-induced headache, where both mechanisms may come into play.

REQUIREMENT FOR SYNAPTIC TRANSMISSION

An important difference between seizure discharges and CSD is the role of synaptic mechanisms. In most but perhaps not all epilepsy models,[62,63] ictal activity is blocked by the voltage-gated sodium channel blocker tetrodotoxin and therefore appears to require action potential-dependent synaptic transmission (see ref. 61). Inhibitory transmission, which is also tetrodotoxin sensitive, provides an obligatory synchronizing influence.[64] In contrast, while tetrodotoxin may inhibit the triggering of CSD in some situations, it has generally not been found to inhibit CSD propagation.[65–67] Therefore, one hypothesis to explain the difference between the physiology of CSD and focal seizure activity is that in CSD nonsynaptic glutamate release from glia is a major factor, whereas in epileptic discharges synaptic glutamate release from neurons is required. In addition, in epilepsy there is a predominant dependence on AMPA receptors, whereas triggering of CSD is mainly dependent on NMDA receptors.[68] Once CSD is initiated, other channel mechanisms come into play.

CORTICAL HYPERRESPONSIVITY IN MIGRAINE

While it appears that hyperexcitability is critical to seizures and CSD, this does not explain

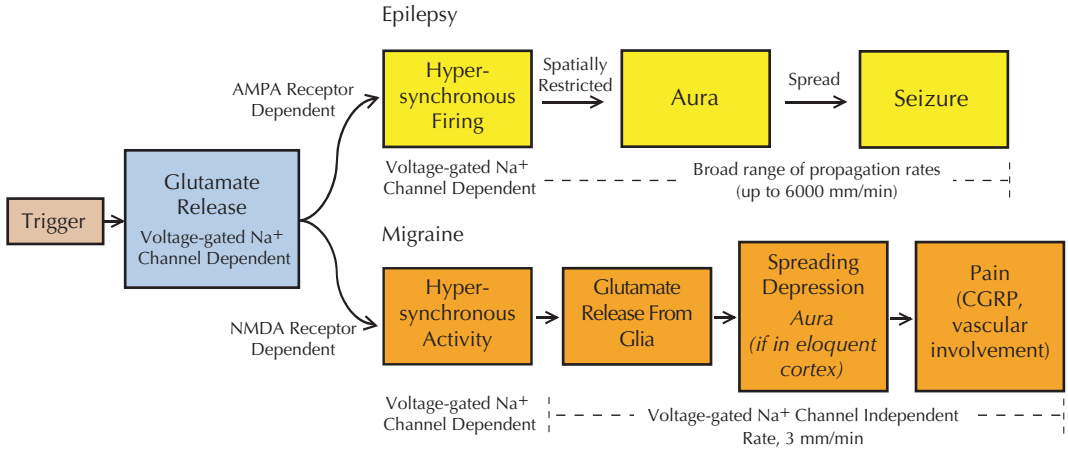

Figure 73–1. chematic illustration of the putative chain of cellular events in the evolution of an epileptic seizure and a migraine attack, highlighting the similarities and differences. In epilepsy, synaptic glutamate release acting through AMPA receptors is a trigger factor and synaptic activity is required (in most instances) for propagation. In migraine, synaptic glutamate acting through NMDA receptors is a trigger factor. Once established, synaptic activity may no longer be necessary and glutamate release from glia is the predominant factor that drives the advancing front of spreading depression. The spreading depression wave triggers the release of mediators that activate the trigeminovascular system, resulting in headache pain. Voltage-gated Na^+ channel dependence (tetrodotoxin sensitivity) implies the involvement of synaptic mechanisms. CGRP, calcitonin gene-related peptide.

the susceptibility of some individuals to clinical attacks of migraine. One possibility is that there is generalized or local cortical hyperresponsivity in susceptible individuals that predisposes to the triggering of attacks. There is a voluminous literature on the sensitivity to sensory stimuli in migraineurs.[69] The techniques that have been used include psychophysical studies; visual, auditory, and somatosensory evoked potentials; magnetoencephalography; and transcranial magnetic stimulation (TMS) of the motor cortex. Although a broad range of results have been obtained, the preponderance of evidence supports the view that there is cortical

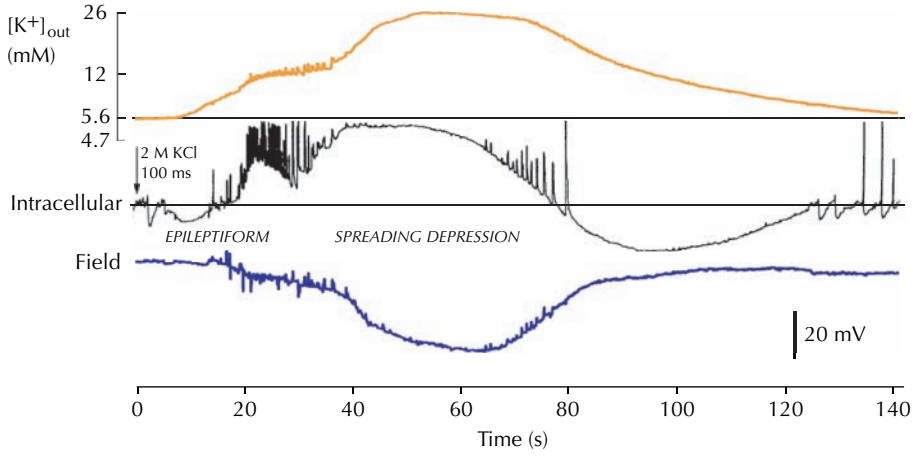

Figure 73–2. Seizure activity and spreading depression can occur together in the same brain substrate. The example shown is from a brain slice recording in the hippocampal CA3 region of a young rabbit. A local micropressure application of 2 M KCl was made at time 0 (arrow). The *top trace* shows the extracellular K^+ level as recorded by an ion-sensitive microelectrode. The *middle trace* is an intracellular recording from a CA3 neuron. The *bottom trace* shows the extracellular field. There is an initial ictal discharge (depolarization and increased action potential generation) followed by full-blown spreading depression with a large negative field potential in conjunction with strong membrane depolarization and a marked increase in extracellular K^+. Adapted from ref. 100.

hypersensitivity to external stimuli in patients with migraine during and between attacks. For example, most but not all studies have shown reduced phosphene thresholds in the visual cortex of migraineurs with TMS.[70] Some studies have found such effects only in subjects who experience migraine with aura, whereas other have found that the visual cortical hyperexcitability extends to subjects who experience migraine without aura.[71,72] Interestingly, the preponderance of TMS data supports the view that untreated patients with generalized epilepsy syndromes also have enhanced cortical excitability.[73]

INSIGHTS FROM GENETICS

Migraine has long been known to have a strong inherited component, but until recently, no accepted linked genetic marker variants have been established for the common forms; a similar lack of information applies to the common forms of epilepsy. Recently, genome-wide association studies have begun to identify genetic variants that confer increased risk for migraine.[74] Some of the variants (such as in the gene for metadherin) have been proposed to influence glutamate signalling although this is still speculative. In contrast, for both migraine and epilepsy, rare Mendelian forms are recognized and single-gene mutations have been identified whose functional implications are better defined. The rare monogenic forms of migraine are of the familial hemiplegic migraine (FHM) subtype. In FHM, attacks typically begin in youth (age range, 5 to 30 years) and include hemiparesis and, in some cases, heminanesthesia, paresthesia, hemianopic visual field disturbances, aphasia, and variable degrees of drowsiness, confusion, or coma. In addition, there is migraine-like unilateral headache that is usually contralateral to the hemparesis. Attacks of nonhemiplegic migraine without aura can occur in FHM patients and family members, which has been interpreted as indicating that FHM is part of the spectrum of migraine.

Calcium Channel

Three different genes cosegregate with FHM. Epilepsy has been reported in all three FHM types. The first to be described was *CACNA1A*, which encodes the pore-forming α_{1A}-subunit ($Ca_V2.1$) of neuronal P/Q-type calcium channels.[75] Mutations in *CACNA1A* account for about one-half of all cases of FHM. In addition to FHM1, mutations in *CACNA1A* are associated with episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6). All of the 21 FHM1 mutations reported to date produce substitutions of conserved amino acids in important functional regions of the $Ca_V2.1$ channel, including the pore lining and the voltage sensors. Studies in heterologous expression systems show that the FHM1 mutations generally are associated with a gain of function of the channel represented as enhanced channel gating. Different studies have revealed a variety of complex effects on channel inactivation. However, Pietrobon[75] notes that a consistent effect seen in studies of the single-channel properties of human $Ca_V2.1$ channels with FHM1 mutations is enhanced channel-opening probability at a wide range of membrane potentials, mainly due to hyperpolarizing shifts in channel activation. These changes in the biophysical properties of single channels result in greater Ca^{2+} flux through the mutant $Ca_V2.1$ channels, which is confirmed by measurements of whole-cell currents in heterologous expression systems and transfected neurons. In addition, while most FHM1 mutations cause a decreased density of functional channels, in some cases the mutations may cause enhanced channel expression, such as with the S218L mutation, which is associated with a particularly severe form of FHM1.[76] Studies of FHM1 knockin mice carrying two different mutations (R192Q and S218L) have confirmed the gain of function observed with recombinant receptors in heterologous expression systems. The gain of function with the S218L mutation was larger than that with the R192Q mutation in accordance with the more severe phenotype of the S218L mutation.[77,78] R192Q mice exhibit a reduced threshold and an increased velocity of CSD[77]; S218L mice are far more sensitive to CSD. These results provide compelling support for the link between CSD and migraine. The functional consequences of the R192Q mutation were studied in neuronal cultures and brain slices from homozygous R192Q knockin mice.[79] There was a gain of function of excitatory neurotransmission between cortical pyramidal cells and between

pyramidal cells and fast-spiking interneurons manifest as increased synaptic strength due to increased action potential-evoked Ca^{2+} influx through mutant P/Q calcium channels. Overall, the results are consistent with the idea that hemiplegic migraine resulting from the R192Q mutation is due to enhanced cortical excitatory neurotransmission. Indeed, inhibitory neurotransmission mediated by fast-spiking interneurons was unaffected. Despite the altered balance between excitation and inhibition, the R192Q mutation is not associated with seizures. However, a genetic marker association study has indicated that *CACNA1A* is associated with idiopathic generalized epilepsy.[80] Moreover, mutations in homologs of the gene can cause absence-like seizures in rodents.[81,82] Interestingly, in mice with these mutations (*tottering* and *leaner*), there is actually a marked resistance to CSD.[82] Among the FHM types, mutations in *CACNA1A* are least likely to be associated with epilepsy. However, seizures (including seizures occurring during headache) have been reported with some mutations, including S218L[14,76,83] as well as I1710T,[84] where a 14-year-old girl had recurrent FHM episodes with status epilepticus.[85] Childhood absence epilepsy (with generalized tonic-clonic seizures) has been associated with a non-FHM-causing point mutation in *CACNA1A* (C5733T).[86] Therefore, diverse mutations in *CACNA1A* can cause migraine without epilepsy, epilepsy without migraine, or both. The underlying pathophysiological mechanisms of absence epilepsy are distinct from those of partial epilepsy. The evidence presented here for a commonality between epilepsy and migraine does not extend to absence epilepsy. Indeed, absence epilepsy mutations in *CACNA1A* are not associated with FHM, and absence epilepsy mutations in *Cacna1a*, the mouse homolog of *CACNA1A*, not only do not promote CSD but actually markedly inhibit it.[82]

Sodium-Potassium Transporter

The second FHM gene to be described was *ATP1A2*, which encodes the α2 isoform of the main catalytic subunit of Na^+-K^+-ATPase (Na^+-K^+ transporter).[87] Among the forms of FHM, this type has the most frequent association with epilepsy (approximately 20% of families). There have been associations with partial seizures,[88] benign familial infantile convulsions (BFIC),[89] and febrile seizures.[90] Epilepsy and migraine can co-occur in the same mutation carriers[90]; in some cases, seizures occur during migraine attacks, representing true migralepsy.[91] Na^+-K^+-ATPase is a highly conserved membrane protein that is expressed in virtually all cells. There are four different isoforms of the catalytic subunit (α1–α4). The α2 isoform is predominantly expressed in astrocytes and skeletal muscle, although it may be expressed in neurons in early development. Therefore, altered Na^+-K^+-ATPase activity in astrocytes accounts for FHM2. Na^+-K^+-ATPase plays a fundamental role in the maintenance of the resting potential of all cells, including astrocytes. The pump, which transports three Na^+ out of the cell in exchange for the countertransport of two K^+ into the cell, allows extracellular K^+ to be maintained at a low level. In addition, the Na^+ gradient produced by Na^+-K^+-ATPase is required for clearance of extracellular glutamate by astrocytes.[92] Glutamate uptake in astrocytes is mediated by glutamate transporters, predominantly GLAST (L-glutamate/L-aspartate transporter; EAAT1) and GLT-1 (glutamate transporter 1; EAAT2). These transporters are expressed along with Na^+-K^+-ATPase in astrocytic processes surrounding glutamatergic synapses. There is tight functional coupling between the transporters and the pump proteins: the uptake of a glutamate molecule is driven by the entry of three Na^+ (and also one H^+) down the electochemical gradient in exchange for one K^+. Recent evidence indicates that the transporters and Na^+-K^+-ATPase are physically associated in the same protein complex.[93] Impairment in the pump function of Na^+-K^+-ATPase is expected to reduce the ability of astrocytes to remove K^+ that accumulates extracellularly during high-frequency neuronal firing. This in itself would promote hyperexcitability (and seizure activity) and CSD. In addition, elevated external K^+ causes reversal of glutamate transporters, so that intracellular glutamate is released into the extracellular space, which would further enhance excitability and predispose to CSD. The FHM mutations in *ATP1A2*, of which more than 36 have now been described, lead to complete inactivation of the protein in some cases,[94] whereas in other cases there is altered kinetics, altered cation affinity, reduced

membrane trafficking, or reduced protein stability.[92,95,96] Familial hemiplegic migraine 2 is an autosomal dominant condition, and it is likely that FHM2 mutations lead to haploinsufficiency with reduced but not absent Na^+-K^+-ATPase. Indeed, *ATP1A2* knockout mice die at birth but heterozygous animals grow normally.[97] Overall, impairment of astrocytic K^+ and glutamate handing are likely to account for the CSD and seizures in FHM2. To the extent that $\alpha 2$ Na^+-K^+-ATPase is expressed in neurons, the neuronal resting potential may be depolarized, which would also enhance excitability. It is noteworthy that both seizures[98,99] and CSD[66,100] can be produced by pharmacological inhibition of Na^+-K^+-ATPase with ouabain, a nonselective inhibitor of astrocytic and also neuronal Na^+-K^+-ATPase.

Sodium Channel

The third FHM gene is *SCN1A*, which encodes the pore-forming $\alpha 1$ subunit of the neuronal type I voltage-gated sodium channel $Na_v1.1$.[101–103] More than 600 sequence variants of this gene have been identified that have been associated with generalized epilepsy with febrile seizures plus (GEFS+) and severe myoclonic epilepsy of infancy (SMEI). To date, only three mutations have been associated with FHM. The first two to be described (Q1489K and L1649Q) cause pure FHM without epilepsy. More recently, in a multigenerational Portuguese family, the L263V mutation was found to be associated not only with FHM but also with generalized tonic-clonic and complex partial seizures.[103] Family members with the mutation invariably had FHM, and some also exhibited seizures that occurred separately from the migraine attacks.Studies with recombinant human $Na_v1.1$ channels bearing the mutations have demonstrated that the pure FHM mutations Q1489K and L1649Q caused biophysical changes in the Na^+ current that were interpreted as predominantly of the loss-of-function type.[104] In contrast, L263V was interpreted as causing gain of function because it produced delayed entry into and accelerated recovery from fast inactivation and increased persistent current as well as other changes in channel gating.[104] Whether these results, which were obtained with recombinant channels expressed in nonneuronal cells, are relevant to the in vivo situation remains to be determined. However, it has generally been the case that mice with loss-of-function mutations in $Na_v1.1$ have impaired function of GABAergic inhibitory neurons, which leads to enhanced circuit excitability. In cases where there is gain of function in $Na_v1.1$, pathological effects may be mediated by the excessive excitability of principal (glutamatergic) neurons that do express the channel, albeit at lower relative levels than do GABAergic neurons.[105,106] The observation that the gain-of-function L263V mutation is associated with both epilepsy and migraine is consistent with the notion that both disorders are triggered by hyperexcitability. Why attacks are manifest as seizures in some instances and as migraine in others remains to be determined. Different loss-of-function *SCN1A* mutations cause pure epilepsy and pure FHM. This provides an opportunity to determine the factors that account for the tendency of circuit hyperexcitability to transition to seizures in one instance and CSD/migraine in the other.

Familial hemiplegic migraine is distinguished from typical migraine by its autosomal dominant Mendelian inheritance and by various clinical features that are not present in typical migraine. Nevertheless, there are sufficient similarities in headache characteristics and triggers to suggest that an understanding of the pathophysiological basis of FHM can shed light on the underlying mechanisms of the far more frequently encountered nonhemiplegic migraine syndromes. Moreover, nonhemiplegic migraine attacks can occur in FHM. It is remarkable that mutations in certain FHM genes can cause either migraine or epilepsy, or in some cases both, clearly demonstrating a commonality between FHM and epilepsy and supporting the notion that migraine generally, like epilepsy, is a disorder of neuronal hyperexcitability.

CONCLUSIONS

Epilepsy and migraine are both episodic functional disorders in which susceptible brain regions are hyperexcitable and attacks begin with hypersynchronous neuronal firing. In epilepsy, the hypersynchronous activity continues, whereas in migraine with aura (and possibly also in migraine without aura) there is CSD. If

CSD occurs in eloquent cortex, there are associated aura symptoms. pain. In both epilepsy and migraine, leads to the release of mediators that elicit headache pain. In both epilepsy and migraine, ionotropic glutamate receptor activation triggers the hyperexcitability. In epilepsy, AMPA receptors play a predominant role in mediating the generation and spread of seizure activity, whereas in migraine NMDA receptors are dominant in triggering CSD but the nature of the ionic conductance that leads to the massive but transitory neuronal depolarization in CSD is yet to be defined. In epilepsy and migraine, various factors can provoke attacks, but how these factors lead to neuronal hypersynchronous activity and the initiation of an attack is not understood. Evidence from human neurophysiology and functional studies of recombinant ion channels and animals with human FHM mutations supports the view that in migraine, as in epilepsy, excitability thresholds are reduced so that a normally innocuous stressor overcomes homeostatic mechanisms that prevent entry into the pathological state (migraine attack or seizure). Key unanswered questions are why some individuals are susceptible to migraine and others to epileptic seizures, and, in those susceptible to both migraine and seizures, why attacks manifest as one or the other at different times.

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REFERENCES

1. Gowers WR. Clinical lectures on the borderland of epilepsy. III. Migraine. *Br Med J.* 1906;2(2397): 1617–1622.
2. Anderman F. Clinical features of migraine-epilepsy syndromes. In: Anderman F, Lugaresi E, eds. *Migraine and Epilepsy*. Boston: Butterworths; 1987:3–30.
3. Toldo I, Perissinotto E, Menegazzo F, Boniver C, Sartori S, Salviati L, Clementi M, Montagna P, Battistella PA. Comorbidity between headache and epilepsy in a pediatric headache center. *J Headache Pain*. 2010;11:235–240.
4. Ottman R, Lipton RB. Comorbidity of migraine and epilepsy. *Neurology.* 1994;44:2105–2110.
5. Haut SR, Bigal ME, Lipton RB. Chronic disorders with episodic manifestations: focus on epilepsy and migraine. *Lancet Neurol*. 2006;5:148–157.
6. Ludvigsson P, Hesdorffer D, Olafsson E, Kjartansson O, Hauser WA. Migraine with aura is a risk factor for unprovoked seizures in children. *Ann Neurol.* 2006;59: 210–213.
7. Silberstein SD, Lipton RB, Haut S. Migraine. In: Engel J Jr, Pedley TA, eds. *Epilepsy: A Comprehensive Textbook*. 2nd ed. Philadelphia: Wolters Kluwer Health/ Lippincott Williams & Wilkins; 2008:2733–2743.
8. Ottman R, Lipton RB. Is the comorbidity of epilepsy and migraine due to a shared genetic susceptibility? *Neurology*. 1996;47:918–924.
9. Lennox WG, Lennox MA. *Epilepsy and Related Disorders*. Boston: Little, Brown;1960.
10. Panayiotopoulos CP. Visual phenomena and headache in occipital epilepsy: a review, a systematic study and differentiation from migraine. *Epileptic Disord*. 1999;1:205–216.
11. Panayiotopoulos CP. "Migralepsy" and the significance of differentiating occipital seizures from migraine. *Epilepsia*. 2006;47:806–808.
12. Sances G, Guaschino E, Perucca P, Allena M, Ghiotto N, Manni R. Migralepsy: a call for a revision of the definition. *Epilepsia.* 2009;50:2487–2496.
13. Ito M, Adachi N, Nakamura F, Koyama T, Okamura T, Kato M, Kanemoto K, Nakano T, Matsuura M, Hara S. Characteristics of postictal headache in patients with partial epilepsy. *Cephalalgia*. 2004;24:23–28.
14. Zangaladze A, Asadi-Pooya AA, Ashkenazi A, Sperling MR. Sporadic hemiplegic migraine and epilepsy associated with CACNA1A gene mutation. *Epilepsy Behav*. 2010;17:293–295.
15. Panayiotopoulos CP. *The Epilepsies. Seizures, Syndromes and Management*. Oxfordshire, UK: Bladon Medical Publishing; 2005.
16. Schon F, Blau JN. Post-epileptic headache and migraine. *J Neurol Neurosurg Psychiatry*. 1987;50:1148–1152.
17. Jacob J, Goadsby PJ, Duncan JS. Use of sumatriptan in post-ictal migraine headache. *Neurology*. 1996; 47:1104.
18. Ryan DP, Ptáček LJ. Episodic neurological channelopathies. *Neuron*. 2010;68:282–292.
19. Stevenson T, ed. *Taylor's Principles and Practices of Medical Jurisprudence*. 3rd ed. Philadelphia: Henry C. Lea's Son & Co.; 1883.
20. Iversen HK. Human migraine models. *Cephalalgia.* 2001;21:781–785.
21. Olesen J. Nitric oxide-related drug targets in headache. *Neurotherapeutics.* 2010;7:183–190.
22. Afridi S, Kaube H, Goadsby PJ. Occipital activation in glyceryl trinitrate induced migraine with visual aura. *J Neurol Neurosurg Psychiatry*. 2005;76: 1158–1160.
23. Lassen LH, Thomsen LL, Olesen J. Histamine induces migraine via the H1-receptor. Support for the NO hypothesis of migraine. *Neuroreport.* 1995;6: 1475–1479.
24. Abend NS, Younkin D, Lewis DW. Secondary headaches in children and adolescents. *Semin Pediatr Neurol*. 2010;17:123–133.

25. Rogawski MA. Antiepileptic drugs and migraine. In: Olesen J, Ramadan N, eds. *Innovative Drug Development for Headache Disorders*. Frontiers in Headache Research, Vol. 16. New York: Oxford University Press; 2008:153–178.
26. D'Amico D. Pharmacological prophylaxis of chronic migraine: a review of double-blind placebo-controlled trials. *Neurol Sci*. 2010;31(suppl 1):S23–S28.
27. Calandre EP, Garcia-Leiva JM, Rico-Villademoros F, Vilchez JS, Rodriguez-Lopez CM. Pregabalin in the treatment of chronic migraine: an open-label study. *Clin Neuropharmacol*. 2010;33:35–39.
28. Drake ME, Greathouse NI, Armentbright AD, Renner JB. Levetiracetam for preventative treatment of migraine. *Cephalalgia*. 2001; 373(abst P2-I13).
29. Bermejo PE, Dorado R. Zonisamide for migraine prophylaxis in patients refractory to topiramate. *Clin Neuropharmacol*. 2009;32:103–106.
30. Villani V, Ciuffoli A, Prosperini L, Sette G. Zonisamide for migraine prophylaxis in topiramate-intolerant patients: an observational study. *Headache*. 2011;51: 287–291.
31. Lampl C, Katsarava Z, Diener HC, Limmroth V. Lamotrigine reduces migraine aura and migraine attacks in patients with migraine with aura. *J Neurol Neurosurg Psychiatry*. 2005;76:1730–1732.
32. Hauge AW, Asghar MS, Schytz HW, Christensen K, Olesen J. Effects of tonabersat on migraine with aura: a randomised, double-blind, placebo-controlled crossover study. *Lancet Neurol*. 2009;8:718–723.
33. Macdonald RL, Rogawski MA. Cellular effects of antiepileptic drugs. In: Engel J Jr, Pedley TA, eds. *Epilepsy: A Comprehensive textbook*. 2nd ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; 2008:1433–1445.
34. Somjen GG. Aristides Leão's discovery of cortical spreading depression. *J Neurophysiol*. 2005;94:2–4.
35. Leão AAP. Spreading depression of activity in the cerebral cortex. *J Neurophysiol*. 1944;7:359–390.
36. Grafstein B. Mechanism of spreading cortical depression. *J Neurophysiol*. 1956:19:154–171.
37. Strong AJ. Dr. Bernice Grafstein's paper on the mechanism of spreading depression. *J Neurophysiol*. 2005;94:5–7.
38. Herreras O, Largo C, Ibarz JM, Somjen GG, Martín del Río R. Role of neuronal synchronizing mechanisms in the propagation of spreading depression in the in vivo hippocampus. *J Neurosci*. 1994;14(11 pt 2): 7087–7098.
39. Snow RW, Taylor CP, Dudek FE. Electrophysiological and optical changes in slices of rat hippocampus during spreading depression. *J Neurophysiol*. 1983;50: 561–572.
40. Canals S, Makarova I, López-Aguado L, Largo C, Ibarz JM, Herreras O. Longitudinal depolarization gradients along the somatodendritic axis of CA1 pyramidal cells: a novel feature of spreading depression. *J Neurophysiol*. 2005;94:943–951.
41. Makarova J, Ibarz JM, Canals S, Herreras O. A steady-state model of spreading depression predicts the importance of an unknown conductance in specific dendritic domains. *Biophys J*. 2007;92:4216–4232.
42. Brinley FJ Jr, Kandel ER, Marshall WH. Potassium outflux from rabbit cortex during spreading depression. *J Neurophysiol*. 1960;23:246–256.
43. Chang JC, Shook LL, Biag J, Nguyen EN, Toga AW, Charles AC, Brennan KC. Biphasic direct current shift, haemoglobin desaturation and neurovascular uncoupling in cortical spreading depression. *Brain*. 2010;133(pt 4):996–1012.
44. Lauritzen M. Cortical spreading depression in migraine. *Cephalalgia*. 2001;21:757–760.
45. Milner PM. Note on a possible correspondence between the scotomas of migraine and spreading depression of Leão. *Electroencephalogr Clin Neurophysiol*. 1959;10:705.
46. Olesen J, Friberg L, Skyhøj Olsen T, Iversen HK, Lassen NA, Andersen AR, Karle A. Timing and topography of cerebral blood flow aura, and headache during migraine attacks. *Ann Neurol*. 1990;28:791–798.
47. Hadjikhani N, Sanchez Del Rio M, Wu O, Schwartz D, Bakker D, Fischl B, Kwong KK, Cutrer FM, Rosen BR, Tootell RB, Sorensen AG, Moskowitz MA. Mechanisms of migraine aura revealed by functional MRI in human visual cortex. *Proc Natl Acad Sci USA*. 2001;98:4687–4692.
48. Schwedt TJ, Dodick DW. Advanced neuroimaging of migraine. *Lancet Neurol*. 2009;8:560–568.
49. Trevelyan AJ, Sussillo D, Yuste R. Feedforward inhibition contributes to the control of epileptiform propagation speed. *J Neurosci*. 2007;27:3383–3387.
50. Jasper HH. Mechanisms of propagation: extracellular studies. In: Jasper HH, Ward AA, Pope A, eds. *Basic Mechanisms of the Epilepsies*. Boston: Little, Brown; 1969:421–440.
51. Yamaguchi S, Donevan SD, Rogawski MA. Anticonvulsant activity of AMPA/kainate antagonists: comparison of GYKI 52466 and NBOX in maximal electroshock and chemoconvulsant seizure models. *Epilepsy Res*. 1993;15:179–184.
52. Van Harreveld A, Fifková E. Mechanisms involved in spreading depression. *J Neurobiol*. 1973;4:375–387.
53. Psarropoulou C, Avoli M. CPP, an NMDA-receptor antagonist, blocks 4-aminopyridine-induced spreading depression episodes but not epileptiform activity in immature rat hippocampal slices. *Neurosci Lett*. 1992;135:139–143.
54. Peters O, Schipke CG, Hashimoto Y, Kettenmann H. Different mechanisms promote astrocyte Ca^{2+} waves and spreading depression in the mouse neocortex. *J Neurosci*. 2003;23:9888–9896.
55. Larrosa B, Pastor J, López-Aguado L, Herreras O. A role for glutamate and glia in the fast network oscillations preceding spreading depression. *Neuroscience*. 2006;141:1057–1068.
56. Peeters M, Gunthorpe MJ, Strijbos PJ, Goldsmith P, Upton N, James MF. Effects of pan- and subtype-selective *N*-methyl-D-aspartate receptor antagonists on cortical spreading depression in the rat: therapeutic potential for migraine. *J Pharmacol Exp Ther*. 2007;321:564–572.
57. Rogawski MA. Revisiting AMPA receptors as an antiepileptic drug target. *Epilepsy Curr*. 2011;11:56–63..
58. Köhr G, Heinemann U. Effects of NMDA antagonists on picrotoxin-, low Mg^{2+}- and low Ca^{2+}-induced epileptogenesis and on evoked changes in extracellular Na^{+} and Ca^{2+} concentrations in rat hippocampal slices. *Epilepsy Res*. 1989;4:187–200.
59. Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J,

Nedergaard M. An astrocytic basis of epilepsy. *Nat Med*. 2005;11:973–981.
60. Rogawski MA. Astrocytes get in the act in epilepsy. *Nat Med*. 2005;11:919–920.
61. Fellin T, Gomez-Gonzalo M, Gobbo S, Carmignoto G, Haydon PG. Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices. *J Neurosci*. 2006;26:9312–9322.
62. Schweitzer JS, Patrylo PR, Dudek FE. Prolonged field bursts in the dentate gyrus: dependence on low calcium, high potassium, and nonsynaptic mechanisms. *J Neurophysiol*. 1992;68:2016–2025.
63. Dudek FE, Yasumura T, Rash JE. "Non-synaptic" mechanisms in seizures and epileptogenesis. *Cell Biol Int*. 1998;22(11–12):793–805.
64. Khazipov R, Holmes GL. Synchronization of kainate-induced epileptic activity via GABAergic inhibition in the superfused rat hippocampus in vivo. *J Neurosci*. 2003;23:5337–5341.
65. Sheardown MJ. The triggering of spreading depression in the chicken retina: a pharmacological study. *Brain Res.* 1993;607:189–194.
66. Wu J, Fisher RS. Hyperthermic spreading depressions in the immature rat hippocampal slice. *J Neurophysiol*. 2000;84:1355–1360.
67. Akerman S, Holland PR, Goadsby PJ. Mechanically-induced cortical spreading depression associated regional cerebral blood flow changes are blocked by Na^+ ion channel blockade. *Brain Res.* 2008;1229:27–36.
68. Gorelova NA, Koroleva VI, Amemori T, Pavlík V, Burěs J. Ketamine blockade of cortical spreading depression in rats. *Electroencephalogr Clin Neurophysiol*. 1987;66:440–447.
69. Coppola G, Pierelli F, Schoenen J. Is the cerebral cortex hyperexcitable or hyperresponsive in migraine? *Cephalalgia.* 2007;27:1427–1439.
70. Aurora SK, Ahmad BK, Welch KM, Bhardhwaj P, Ramadan NM. Transcranial magnetic stimulation confirms hyperexcitability of occipital cortex in migraine. *Neurology.* 1998;50:1111–1114.
71. Siniatchkin M, Reich AL, Shepherd AJ, van Baalen A, Siebner HR, Stephani U. Peri-ictal changes of cortical excitability in children suffering from migraine without aura. *Pain.* 2009;147:132–140.
72. Chronicle EP, Pearson AJ, Mulleners WM. Objective assessment of cortical excitability in migraine with and without aura. *Cephalalgia*. 2006;26:801–808.
73. Theodore WH. Transcranial magnetic stimulation in epilepsy. *Epilepsy Curr.* 2003;3:191–197.
74. Schürks M. Genetics of migraine in the age of genome-wide association studies. *J Headache Pain.* 2011; doi 10.1007/s10194-011-0399-0.
75. Pietrobon D. $Ca_V2.1$ channelopathies. *Pflugers Arch*. 2010;460:375–393.
76. Debiais S, Hommet C, Bonnaud I, Barthez MA, Rimbaux S, Riant F, Autret A. The FHM1 mutation S218L: a severe clinical phenotype? A case report and review of the literature. *Cephalalgia*. 2009;29: 1337–1339.
77. van den Maagdenberg AMJM, Pietrobon D, Pizzorusso T, Kaja S, Broos LAM, Cesetti T, van de Ven RCG, Tottene A, van der Kaa J, Plomp JJ, Frants RR, Ferrari MD. A *Cacna1a* knockin migraine mouse model with increased susceptibility to cortical spreading depression. *Neuron*. 2004;41:701–710.
78. van den Maagdenberg AMJM, Pizzorusso T, Kaja S, Terpolilli N, Shapovalova M, Hoebeek FE, Barrett CF, Gherardini L, van de Ven RC, Todorov B, Broos LA, Tottene A, Gao Z, Fodor M, De Zeeuw CI, Frants RR, Plesnila N, Plomp JJ, Pietrobon D, Ferrari MD. High cortical spreading depression susceptibility and migraine-associated symptoms in $Ca_V2.1$ S218L mice. *Ann Neurol*. 2010;67:85–98.
79. Tottene A, Conti R, Fabbro A, Vecchia D, Shapovalova M, Santello M, van den Maagdenberg AMJM, Ferrari MD, Pietrobon D. Enhanced excitatory transmission at cortical synapses as the basis for facilitated spreading depression in $Ca_V2.1$ knockin migraine mice. *Neuron.* 2009;61:762–773.
80. Chioza B, Wilkie H, Nashef L, Blower J, McCormick D, Sham P, Asherson P, Makoff AJ. Association between the α_{1a} calcium channel gene *CACNA1A* and idiopathic generalized epilepsy. *Neurology*. 2001;56: 1245–1246.
81. Tokuda S, Kuramoto T, Tanaka K, Kaneko S, Takeuchi IK, Sasa M, Serikawa T. The ataxic groggy rat has a missense mutation in the P/Q-type voltage-gated Ca^{2+} channel α_{1A} subunit gene and exhibits absence seizures. *Brain Res*. 2007;1133:168–177.
82. Ayata C, Shimizu-Sasamata M, Lo EH, Noebels JL, Moskowitz MA. Impaired neurotransmitter release and elevated threshold for cortical spreading depression in mice with mutations in the α1A subunit of P/Q type calcium channels. *Neuroscience*. 2000;95:639–645.
83. Chan YC, Burgunder JM, Wilder-Smith E, Chew SE, Lam-Mok-Sing KM, Sharma V, Ong BK. Electroencephalographic changes and seizures in familial hemiplegic migraine patients with the *CACNA1A* gene S218L mutation. *J Clin Neurosci.* 2008;15: 891–894.
84. Kors EE, Melberg A, Vanmolkot KR, Kumlien E, Haan J, Raininko R, Flink R, Ginjaar HB, Frants RR, Ferrari MD, van den Maagdenberg AM. Childhood epilepsy, familial hemiplegic migraine, cerebellar ataxia, and a new *CACNA1A* mutation. *Neurology.* 2004;63:1136–1137.
85. Beauvais K, Cavé-Riant F, De Barace C, Tardieu M, Tournier-Lasserve E, Furby A. New CACNA1A gene mutation in a case of familial hemiplegic migraine with status epilepticus. *Eur Neurol*. 2004;52:58–61.
86. Jouvenceau A, Eunson LH, Spauschus A, Ramesh V, Zuberi SM, Kullmann DM, Hanna MG. Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. *Lancet*. 2001;358(9284):801–807.
87. De Fusco M, Marconi R, Silvestri L, Atorino L, Rampoldi L, Morgante L, Ballabio A, Aridon P, Casari G. Haploinsufficiency of ATP1A2 encoding the Na^+/K^+ pump α2 subunit associated with familial hemiplegic migraine type 2. *Nat Genet*. 2003;33:192–196.
88. Deprez L, Weckhuysen S, Peeters K, Deconinck T, Claeys KG, Claes LR, Suls A, Van Dyck T, Palmini A, Matthijs G, Van Paesschen W, De Jonghe P. Epilepsy as part of the phenotype associated with ATP1A2 mutations. *Epilepsia*. 2008;49:500–508.
89. Vanmolkot KR, Kors EE, Hottenga JJ, Terwindt GM, Haan J, Hoefnagels WA, Black DF, Sandkuijl LA, Frants RR, Ferrari MD, van den Maagdenberg AM. Novel mutations in the Na^+, K^+-ATPase pump gene ATP1A2 associated with familial hemiplegic migraine and benign familial infantile convulsions. *Ann Neurol*. 2003;54:360–366.

90. de Vries B, Stam AH, Kirkpatrick M, Vanmolkot KR, Koenderink JB, van den Heuvel JJ, Stunnenberg B, Goudie D, Shetty J, Jain V, van Vark J, Terwindt GM, Frants RR, Haan J, van den Maagdenberg AM, Ferrari MD. Familial hemiplegic migraine is associated with febrile seizures in an FHM2 family with a novel de novo ATP1A2 mutation. *Epilepsia*. 2009;50:2503–2504.
91. Lebas A, Guégan-Massardier E, Guyant-Maréchal L. Epilepsy and familial hemiplegic migraine. Genetic and clinical aspects. In: Parain D, Guerrini R, Hesdorffer D, Ryvlin P, eds. *Epilepsy and Migraine*. Current Problems in Epilepsy, vol. 22. Montrouge, France: John Libby Eurotext; 2009:99–108.
92. Benarroch EE. Na^+, K^+-ATPase: functions in the nervous system and involvement in neurologic disease. *Neurology*. 2011;76:287–293.
93. Rose EM, Koo JC, Antflick JE, Ahmed SM, Angers S, Hampson DR. Glutamate transporter coupling to Na,K-ATPase. *J Neurosci*. 2009;29:8143–8155.
94. Koenderink JB, Zifarelli G, Qiu LY, Schwarz W, De Pont JJ, Bamberg E, Friedrich T. Na,K-ATPase mutations in familial hemiplegic migraine lead to functional inactivation. *Biochim Biophys Acta*. 2005;1669: 61–68.
95. Morth JP, Poulsen H, Toustrup-Jensen MS, Schack VR, Egebjerg J, Andersen JP, Vilsen B, Nissen P. The structure of the Na^+,K^+-ATPase and mapping of isoform differences and disease-related mutations. *Philos Trans R Soc Lond B Biol Sci*. 2009;364:217–227.
96. Tavraz NN, Dürr KL, Koenderink JB, Freilinger T, Bamberg E, Dichgans M, Friedrich T. Impaired plasma membrane targeting or protein stability by certain ATP1A2 mutations identified in sporadic or familial hemiplegic migraine. *Channels* (*Austin*). 2009;3: 82–87.
97. Moseley AE, Lieske SP, Wetzel RK, James PF, He S, Shelly DA, Paul RJ, Boivin GP, Witte DP, Ramirez JM, Sweadner KJ, Lingrel JB. The Na,K-ATPase $\alpha 2$ isoform is expressed in neurons, and its absence disrupts neuronal activity in newborn mice. *J Biol Chem*. 2003;278:5317–5324.
98. Pedley TA, Zuckermann EC, Glaser GH. Epileptogenic effects of localized ventricular perfusion of ouabain on dorsal hippocampus. *Exp Neurol*. 1969;25: 207–219.
99. Stone WE, Javid MJ. Interactions of phenytoin with ouabain and other chemical convulsants. *Arch Int Pharmacodyn Ther*. 1982;260:28–35.
100. Haglund MM, Schwartzkroin PA. Role of Na-K pump potassium regulation and IPSPs in seizures and spreading depression in immature rabbit hippocampal slices. *J Neurophysiol*. 1990;63:225–239.
101. Dichgans M, Freilinger T, Eckstein G, Babini E, Lorenz-Depiereux B, Biskup S, Ferrari MD, Herzog J, van den Maagdenberg AM, Pusch M, Strom TM. Mutation in the neuronal voltage-gated sodium channel *SCN1A* in familial hemiplegic migraine. *Lancet*. 2005;366(9483):371–377.
102. Vanmolkot KRJ, Babini E, de Vries B, Stam AH, Freilinger T, Terwindt GM, Norris L, Haan J, Frants RR, Ramadan NM, Ferrari MD, Pusch M, van den Maagdenberg AMJM, Dichgans M. The novel p.L1649Q mutation in the *SCN1A* epilepsy gene is associated with familial hemiplegic migraine: genetic and functional studies. Mutation in brief #957. *Hum Mutat*. 2007;28:522.
103. Castro M-J, Stam AH, Lemos C, de Vries B, Vanmolkot KRJ, Barros J, Terwindt GM, Frants RR, Sequeiros J, Ferrari MD, Pereira-Monteiro JM, van den Maagdenberg AMJM. First mutation in the voltage-gated Nav1.1 subunit gene *SCN1A* with co-occurring familial hemiplegic migraine and epilepsy. *Cephalalgia*. 2009;29:308–313.
104. Kahlig KM, Lepist I, Leung K, Rajamani S, George AL. Ranolazine selectively blocks persistent current evoked by epilepsy-associated $Na_V1.1$ mutations. *Br J Pharmacol*. 2010;161:1414–1426.
105. Escayg A, Goldin AL. Sodium channel *SCN1A* and epilepsy: mutations and mechanisms. *Epilepsia*. 2010;51:1650–1658.
106. Catterall WA, Kalume F, Oakley JC. $Na_V1.1$ channels and epilepsy. *J Physiol*. 2010;588(pt 11):1849–1859.

Chapter 74

Neurobiology of Depression as a Comorbidity of Epilepsy

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In his review on melancholia, Lewis describes Hippocrates as having had the perception that the condition was reciprocally connected to epilepsy.[1] Recent epidemiological studies have supported the notion that depression is more frequent among patients with epilepsy (preceding the diagnosis of epilepsy) than among case controls[2] and that depression is seven times more likely among adults presenting with a new-onset seizure disorder than among controls.[3] Depression has been identified as the most frequent psychiatric comorbidity in patients with epilepsy.[4] Hermann et al. have summarized estimates of the prevalence of lifetime-to-date major depression among patients with epilepsy as ranging from 8% to 48%, with a mean and a median approximating 30%.[5] Hermann and colleagues also stated that psychiatric comorbidities contributed to a lowered health-related quality of life (HRQOL) in epilepsy.[6] Further, their study revealed an association of symptom checklist scores with the chronicity of temporal lobe epilepsy (TLE). Interictal psychiatric symptoms adversely influenced the HRQOL to a greater extent than the frequency, severity, and chronicity of seizures.[7] Consideration of the psychiatric comorbidities in treatment decisions involving drug selection have been reviewed for adult[8] as well as pediatric[9] patients with epilepsy.

Depression in a patient with epilepsy can reflect a neurobiological connection between

the disorders, but can also result in part from socioeconomic factors impacting on epilepsy or due to iatrogenic causes. The iatrogenic causes based on pharmacotherapeutics are reflected in the well-recognized emergence of depression due to barbiturate therapy or an improvement in mood associated with anticonvulsant medications like carbamazepine or lamotrigine.[10–12] A strong case for the neurobiological basis of depression is the finding that patients with the condition seem to be at higher risk for developing epilepsy than controls.[13–15] Another interesting finding regarding depression in human epilepsy is that the involvement of distinct networks may influence the presentation in specific epilepsy syndromes. Altshuler et al.[16] found that patients with seizures of temporal lobe origin were significantly more likely to have a history of depression than those with seizure foci of extratemporal lobe origin and suggested a role for limbic dysfunction in increasing the risk for depression. Piazzini et al.[17] found that while all patients with epilepsy scored higher on depression and anxiety scales than controls, patients with partial epilepsy scored higher than those with generalized epilepsy. Further, those with TLE were affected significantly more than those with frontal lobe epilepsy (FLE).[17]

This relationship regarding depression was confirmed in a cohort of pediatric patients in a study that found a difference between depression and anxiety; depression was more frequent in those with complex partial seizures, while anxiety was more common in childhood absence epilepsy.[18] Other pediatric studies[19,20] were not adequately powered to discern a relationship with the seizure type, but all of the studies failed to find a relationship between seizure frequency and the extent of mood disturbance.[18–20]

In adults with TLE, Gilliam et al.[21] found that while seizure frequency was not a determinant of the extent of depression, the degree of hippocampal dysfunction, as measured by proton magnetic resonance spectroscopy (1H-MRS), was predictive of the severity of depression. Hippocampal functional integrity was assessed by the creatine/*N*-acetyl aspartate (Cr/NAA) ratio. Multiple studies have suggested that depression and anxiety symptoms improved in patients who became seizure-free after temporal lobectomy.[22–24] Interestingly, recovery of Cr/NAA ratios was shown to occur during longitudinal follow-up only in patients who become seizure free.[25,26] The reversal of neuronal metabolic dysfunction suggested by the recovery of the Cr/NAA has a time course consistent with an exponential function with a half-life of 6 months, with 95% recovery achieved by 25 months after surgery.[27] Together, these data support the notion that while short-term seizure frequency does not correlate with the severity of depression, a surgical cure of the epileptic state results in favorable plasticity reflected by improving Cr/NAA scores and mood.

A study that prospectively assessed various quality-of-life measures including anxiety and depression prior to surgery, as well as 2, 12, and 24 months afterward, showed that most of the improvement in anxiety and depression occurred within the first 3 months after surgery and remained stable for up to 24 months.[28] While this outcome does not support the conclusion stated in the previous paragraph, the authors of this study did see a trend toward divergence in long-term outcome between the group that remained in remission from seizures and the one that experienced a relapse.[28]

DEPRESSION IN ANIMAL MODELS OF EPILEPSIES

Animal models of epilepsy allow the study of the emergence of depression-like behaviors, and the accompanying changes in the neurochemical milieu, without the confounding effects of socioeconomic and iatrogenic factors. All of the studies that follow involve rodent models of epilepsy.

In rodents, the state of despair is commonly examined using the forced swim test (FST), which is based on their innate ability to adopt active strategies in the inescapable stressful situation.[29,30] In the FST, animals exhibit two alternating behaviors—active escaping and/or exploring behavior, and relative immobility, when they move only enough to maintain their heads above water and to avoid drowning. The increased immobility time in the FST has been regarded and validated[31] as an indicator of the state of despair. The brain systems underlying this susceptibility to helplessness and

depression have been reviewed[32] and include a dissociated hypothalamic-pituitary-adrenocortical axis, which is discussed further below, and other systems.

The examination of anhedonia in rodents relies on their innate preference for sweets and involves a saccharin consumption test.[33] In this test, healthy subjects, when given access to both tap water and saccharin solution, strongly prefer the latter, whereas animals with experimental depression consume equal amounts of water and saccharin. Loss of the preference for saccharin has been regarded and validated[34] as an indicator of anhedonia.

DEPRESSION IN AN ANIMAL MODEL OF GENETIC ABSENCE EPILEPSY

Spontaneous generalized spike-wave discharges (SWDs) and accompanying staring spells have been found in a number of rodent strains. Of special interest are inbred strains of Wistar rats that develop staring spells and SWDs in the absence of other overt neurological findings such as ataxia, tremor, and other motor system disturbances. These strains include the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) and the Wistar Albino Glaxo/Rijswijk[35] (WAG/Rij), as well as Long Evans rats. The two former strains have been much more studied as models for human absence epilepsy since the SWDs are seen early in life.

A number of behavioral studies have demonstrated despair and anhedonia in the WAG/Rij rat[36] and have been reviewed in detail by Sarkisova and van Luijtelaar.[37] When WAG/Rij rat pups were treated with ethosuximide, an antiabsence agent, beginning at postnatal day 21 for 5 months to prevent the emergence of spontaneous SWDs, as described by Blumenfeld et al.,[38] they exhibited no symptoms of depression-like behavior, in contrast to untreated WAG/Rij animals.[39] The authors did not find an antidepressant effect attributable to ethosuximide in the control Wistar rats, and concluded that early treatment prevented the emergence of SWDs and depression, that is, absence seizures and comorbid behavioral depression-like symptoms.

DEPRESSION IN ANIMAL MODELS OF LIMBIC EPILEPSY

Epileptogenesis in animals after experimental status epilepticus (SE) is often accompanied by significant brain damage, so it is not clear whether the anatomical lesion itself may contribute to behavioral alterations. Behavioral testing can be affected by recurring seizures. It is important to establish whether the epileptic state (increased seizure susceptibility) per se, rather than epileptic seizures and morphological changes, is accompanied by symptoms of depression.

DEPRESSION AFTER KINDLING EPILEPTOGENESIS

Kindling is a model of limbic epilepsy that is characterized by a sustained increase in seizure susceptibility. Kindling is different from post-SE TLE in two major respects: the absence or minimal extent of neuronal injury and the absence of spontaneous recurrent seizures. At the same time, kindled animals exhibit persistent enhanced seizure susceptibility. Hence, kindling appears to be especially suitable for studying the epilepsy-associated depression that results from neuronal plastic changes. A limitation of traditional kindling protocols is that rapid brain and skull growth in developing animals displace electrodes. Lothman and coworkers[40,41] described rapid kindling, which allows the creation of enhanced susceptibility within several hours. We have adapted this approach to evaluating the age-specific and pharmacological properties of epileptogenesis.[42]

In our experiments involving postnatal day 21 (P21) rats,[43] the afterdischarge threshold and duration were detected by applying electrical stimuli consisting of 10 s train duration, 20 Hz, 1 ms pulse duration, square wave monophasic stimuli, starting at 0.2 mA, at 0.1 mA increments, delivered every 10 min. Stimuli were applied to the ventral hippocampus. The rapid kindling procedure, which started 10 min after detecting the afterdischarge, consisted of 84 trains delivered every 5 min using the parameters described above at a current of 0.1 mA over the afterdischarge threshold; the

total procedure duration was 7 h. Sham animals were implanted with electrodes but did not receive kindling stimuli. Behavioral seizures were scored using the following scale: 1: motor arrest and twitching vibrissae; 2: chewing and head bobbing; 3: forelimb clonus; 4: forelimb clonus and rearing; 5: rearing and falling.

The kindled animals as well as the naive and sham animals (implanted with electrodes but not stimulated) underwent behavioral testing 2 and 4 weeks after rapid kindling. Both naive and sham animals showed similar immobility time during the 2- and 4-week recording sessions (Fig. 74–1A). However, the kindled animals spent significantly more time immobile in the tank both 2 weeks after (Fig. 74–1A) and 4 weeks after kindling compared to controls. We further examined whether the immobility time under conditions of FST correlated with the severity of behavioral seizures in response to test stimulations in individual animals. We found a strong positive correlation between the time spent immobile during FST both 2 and 4 weeks after kindling and seizure score both 24 h and 4 weeks after kindling (Fig. 74–1B,C). As shown in Fig. 74–2, the kindled rats demonstrated a loss of preference for saccharin-sweetened water at both 2 and 4 weeks after rapid kindling. Surprisingly, in the sucrose consumption test 4 weeks after kindling, the animals' behavior was reversed—from loss of preference to enhanced preference—even when compared to naive subjects. In contrast to saccharin, sucrose has caloric value. It is

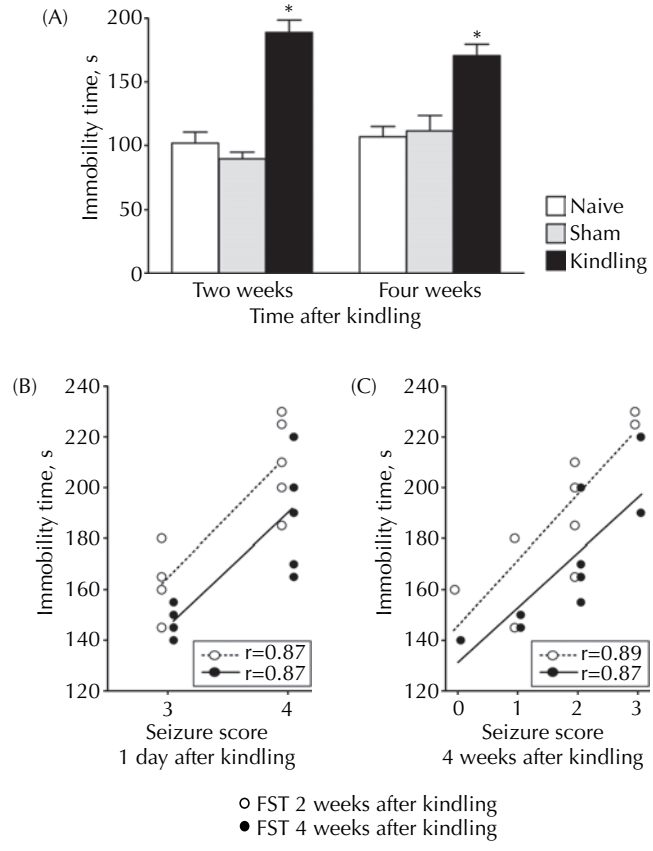

Figure 74–1. The FST before and after rapid kindling. **A.** Mean ± SEM values 2 and 4 weeks after kindling. *$p < 0.05$ versus both naive and sham (one-way ANOVA + Bonferroni test). There were no statistical differences ($p > 0.1$) between the findings at 2 and 4 weeks for any group (paired *t*-test). Seizure scores of individual animals in response to threshold stimulation 1 day (**B**) and 4 weeks (**C**) after kindling are plotted against immobility time in the FST 2 weeks (open circles) and 4 weeks (black circles) after kindling. The coefficient of correlation (*r*), calculated using the Spearman test, is indicated for each dataset; a positive correlation was statistically significant in all cases ($p < 0.05$).

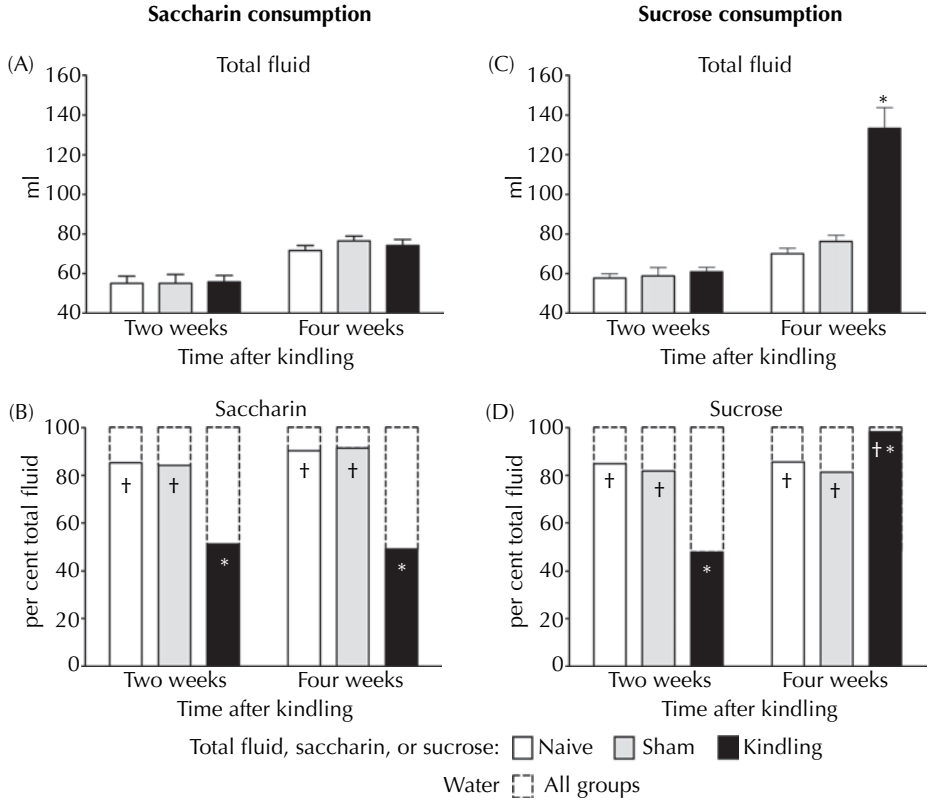

Figure 74–2. Twenty-four-hour saccharin (**A,B**) and sucrose (**C,D**) consumption 2 and 4 weeks after rapid kindling. **A.** No differences were observed at any age in total fluid (tap water + saccharin) intake between control and experimental groups. **B.** Although the naive and sham animals exhibited a strong preference for saccharin versus tap water, the kindled animals exhibited loss of the taste preference for saccharin both 2 and 4 weeks after kindling. **C.** No differences were observed in total fluid (tap water + sucrose) intake between naive and sham animals. However, the total volume of fluid consumed was significantly larger in kindled animals. **D.** Both naive and sham animals preferred sucrose to tap water. Kindled animals did not exhibit a sucrose preference 2 weeks after kindling. However, at 4 weeks, kindled animals consumed significantly more sucrose solution compared with both sham and naive rats. Data in **A** and **C** are presented as means + SEM and those in **B** and **D** as means. $^{*}p < 0.05$ versus both naive and sham rats (one-way ANOVA + Bonferroni test). †$p < 0.05$, percentage of either saccharin or sucrose (bottom bars in the stacks) versus percentage of water (top bars in the stacks, *t*-test).

known that depression can be accompanied by both the loss and the increase of appetite.[44,45]

These data provided evidence that rapid kindling in young animals leads to sustained changes in forced swimming and taste preference tests that can be interpreted as behavioral correlates of depression. In contrast to post-SE epilepsy, kindling is not accompanied by hippocampal sclerosis and spontaneous seizures. Eliminating spontaneous seizures as a variable permits this model to accommodate an important clinical feature of epilepsy-associated depression observed by Attarian et al.,[46] Quigg et al.,[47] and Gilliam et al.[23] Those authors found that the prevalence depression was independent of the degree of seizure control.

Thus, the important findings of our experiments were as follows: (1) the evolution of depression accompanying epileptogenesis can be modeled in young animals and (2) epilepsy-associated depression can be demonstrated in this model without either overt brain injury or ongoing spontaneous seizures. Having established that features of depression can evolve in the absence of brain injury, we decided to extend our observations to a better-established model of rodent epilepsy, one that follows SE induced by lithium-pilocarpine treatment. The latency to the development of spontaneous seizures in this model precludes narrowly defined early developmental aspects of the process.

DEPRESSION ACCOMPANIES EPILEPTOGENESIS FOLLOWING SE

We subjected P35 rats to lithium-pilocarpine-induced SE, a well-studied model of TLE. Four weeks after SE, the animals underwent 1 week of seizure monitoring with continuous electroencephalography (EEG). Subsequently, they underwent physiological interrogations for afterdischarge threshold and duration and were divided into two groups. One group received saline daily for the following 10 days, while the experimental group received daily injections of fluoxetine. After a repetition of the physiological tests, the animals were subjected to the behavioral tests for despair (i.e., FST) and anhedonia (i.e., taste preference for saccharin-sweetened water).

The results confirmed that the post-SE rats had indeed developed increased immobility time in the FST, and they also demonstrated a loss of taste preference for saccharin consumption, as had younger animals subjected to rapid kindling. In our studies, post-SE animals spent a significantly longer time immobile (50% of a total 5 min test duration) compared both with naive rats and with themselves prior to SE (25% of a total test duration), thus suggesting the state of despair. These depression-associated behaviors could not be correlated with the number of seizures and appeared resistant to treatment with the selective serotonin reuptake inhibitor, fluoxetine, despite the adequacy of the fluoxetine treatment regimen in reducing the number of seizures.[48]

EPILEPTOGENESIS IS ACCOMPANIED BY CHANGES IN SEROTONIN TURNOVER AND EVOKED SEROTONIN RELEASE

In our study of depression in post-SE rats, we determined the serotonin (5-hydroxytryptamine, 5-HT) concentration and turnover in the hippocampal tissue using a high-performance liquid chromatography method described previously.[49] In living animals, the evoked release of serotonin upon high-frequency stimulation of the raphe nucleus was determined using fast cyclic voltammetry (FCV).[48] This was accomplished by placing carbon fiber FCV electrodes in the CA1 and CA3 of the hippocampus to provide a ramp current 1 s after raphe stimulation to facilitate the oxidation of the phenolic hydroxyl group in 5-HT released to the quinonoid form; the measured Faradaic peak response provides for quantitative determination of the evoked 5-HT release.

THE EPILEPTIC STATE AFFECTS THE HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL (HPA) AXIS TO PRODUCE EFFECTS ON SEROTONIN RELEASE

We studied the functional state of the HPA axis in our post-SE model of TLE in rats. Young adult animals (45–55 days old) were subjected to lithium-pilocarpine SE and 10-week-long video monitoring beginning 4 weeks after SE. We obtained a basal concentration of plasma corticosterone (CORT) in our rats 3–7 days before and 6–8 weeks after SE. In addition, rats were subjected to challenges with dexamethasone (DEX) and corticotropin releasing hormone (CRH). We were able to conclude that HPA axis dysregulation was present in the post-SE rats by demonstrating that DEX failed to decrease the level of plasma CORT and that exogenously administered CRH led to an increase in the level of CORT.[50] The CRH challenge was added to the conventional DEX test based on the finding of Watson et al.[51] that the DEX/CRH test had higher sensitivity and specificity in a patient population. Care was taken to draw blood samples only from animals that had not experienced a seizure within 6 h prior to specimen collection. In separate experiments, this time period produced CORT levels similar to those at time points separated from the preceding spontaneous seizure by periods of up to 4 days.

In these studies,[50] out of the 16 animals that had undergone SE, spontaneous seizures were observed in 10; however, all of the post-SE animals showed a statistically similar increase in hippocampal excitability that was evident as a lower afterdischarge threshold (ADT) and a longer afterdischarge duration (ADD) compared with controls (data not presented in this chapter); furthermore, unlike the control animals, all post-SE rats developed behavioral seizures in response to the threshold stimulation.

The data shown in Fig. 74–3 demonstrate that the HPA axis dysregulation was similar in rats in which spontaneous seizures were commonly seen and in those with rare or no documented recurrent seizures. This was also the case with regard to the observed immobility time in the FST, reminiscent of our previous observations[48] and those of Gilliam et al.[21] Gilliam and coworkers[21] had shown that in a study of 31 patients with TLE in whom the extent of depression was represented by the Profile of Mood States (POMS) scores and who underwent magnetic resonance spectroscopic imaging (1H-MRSI) to assess the functional state of the hippocampus (Cr]/NAA metabolite ratio maps were derived), hippocampal 1H-MRSI correlated with severity of depression symptoms in TLE rather than with seizure frequency. Likewise, we have found that the HPA axis dysfunction as well as the immobility time in the FST correlated with measures of hippocampal physiology (ADT and ADD) rather than with the frequency of spontaneous seizures.

How does the exaggerated CORT response in post-SE animals influence the serotonergic system? The hippocampus receives serotonergic input from neurons located in median and dorsal raphe nuclei. The release of 5-HT from raphe serotonergic neurons is regulated by a number of intrinsic and external mechanisms.

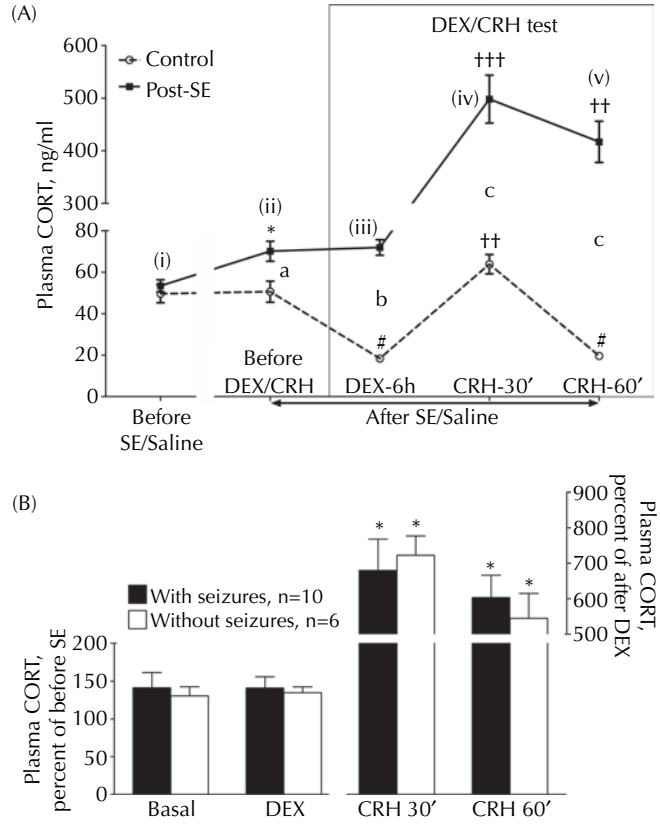

Figure 74–3. Plasma CORT levels in control and post-SE rats under basal conditions and in response to the combined DEX/CRH test. **A.** (i) Basal CORT levels before SE (saline in control rats); (ii) basal CORT levels 6–8 weeks after SE; (iii) CORT levels 6 h after DEX injection; (iv) and (v) CORT levels 30 and 60 min after CRH injection (and 6.5 and 7 h, respectively after DEX injection). Post-SE animals showed an increase in basal CORT level, loss of a response to DEX, and an exacerbated and longer-lasting response to CRH. Data are presented as mean ± SEM. $^{*}p < 0.05$ vs. "Before SE/Saline"; $\#p < 0.01$ vs. "Before DEX/CRH"; $\dagger\dagger p < 0.01$ and $\dagger\dagger\dagger p < 0.001$ vs. "DEX-6 h" (repeated measures ANOVA + Tukey test). $ap < 0.05$; $bp < 0.01$; $cp < 0.001$ for SE vs. Control (Student's *t*-test). **B**. No statistical differences were observed between the subsets of post-SE animals with and without documented spontaneous seizures for both basal CORT concentrations and in response to the combined DEX/CRH test. ($p > 0.05$, Student's *t*-test). $^{*}p < 0.05$ vs. both "Basal" and "DEX" (repeated measures ANOVA + Tukey test). Data are presented as mean ± SEM.

The most efficient mechanism consists of the short feedback autoinhibitory loop that involves somatodendritic 5-HT1A receptors (autoreceptors). The activation of raphe 5-HT1A autoreceptors by locally released serotonin inhibits firing of serotonergic neurons and further neurotransmitter release.[52] Hence, the upregulation of raphe 5-HT1A receptors would be expected to result in compromised raphe-hippocampal serotonergic transmission. In TLE patients with concurrent depression, binding affinity of raphe 5-HT1A receptors was increased, and the increase correlated positively with the severity of depression.[53] Evidence supporting the positive modulation of 5-HT1A receptor function by the glucocorticoid corticosterone exists.[54,55] We found that chronic treatment of post-SE rats with chronic raphe injections of the glucocorticoid receptor antagonist mifepristone or the 5-HT1A antagonist (WAY-100635) could restore evoked 5-HT release, as measured by FCV, as well as reduce immobility time in the FST (unpublished data).

INFLAMMATION CONTRIBUTES TO HPA AXIS DYSREGULATION AND IMPAIRED SEROTONERGIC FUNCTION AND PROLONGS IMMOBILITY TIME IN THE FST

We explored the possible role of hippocampal neuroinflammation and, in particular, enhanced interleukin-1β (IL-1β) signaling. Indeed, activation of hippocampal IL-1β and its receptor has been an established hallmark of TLE in both clinical and experimental settings.[56] Furthermore, IL-1β may lead to depression, conceivably by inducing perturbation in the HPA axis, as suggested by clinical observations and confirmed by experimental studies.[57] In order to evaluate this hypothesis, we subjected animals with post-SE depression to 2 weeks of sustained treatment with a recombinant human interleukin receptor antagonist (IL-1Ra) using osmotic mini-pumps for continuous delivery of the peptide into the hippocampus bilaterally.[58] Experimental animals received IL-1ra, while control animals received either saline or heat-inactivated IL-1Ra (iIL-1Ra). While this treatment had no effect on the behavior of rats that had not undergone SE, in post-SE animals treatment with IL-1Ra restored taste preference in the saccharin consumption test and significant shortening of the immobility time in the FST (Fig. 74–4). Treatment with IL-1Ra

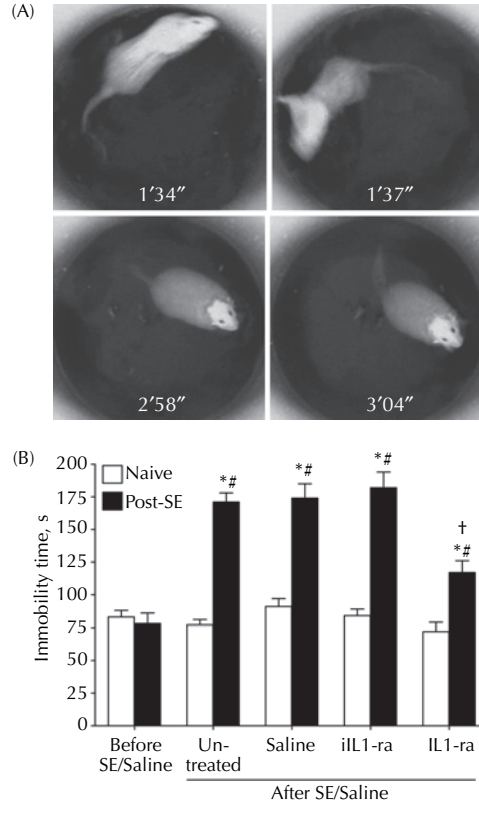

Figure 74–4. Effects of IL-1ra treatment on the forced swimming behavior. **A.** Sample snapshots taken from a prerecorded video during the FST. The time after the start of the test is indicated on each image. Examples of active swimming that reflects active escape strategies are presented at 1 min 34 s and 1 min 37 s. Note the change in the rat's position in the tank, which occurred during the 3 s period, and the fuzziness of images due to the animal's movement. Examples of immobility when animals moved only enough to avoid drowning are presented at 2 min 58 s and 3 min 04 s. Note that the animal's position in the tank did not change during 6 s of recording and that the body is positioned vertically in the water. **B.** Immobility time in naive and post-SE animals: untreated or treated with saline, heat-inactivated IL-1ra (iIL-1ra), or active IL-1ra. Note the increase in the cumulative immobility time in untreated, saline-treated, and iIL-1ratreated post-SE animals and its partial reversal following IL-1ra administration. Data are presented as mean ± SEM. $^{*}p < 0.05$ after SE vs. before SE (repeated measure ANOVA + Newman–Keuls post hoc test); $\#p < 0.05$ post-SE vs. naive; $\dagger p < 0.05$ post-SE IL-1ra vs. post-SE saline (one-way ANOVA + Newman–Keuls post hoc test).

also produced the expected modifications in CORT levels in the DEX/CRH test and partially restored the compromise to evoked serotonin release, as measured by Faradaic currents in the FCV test. These effects were not a consequence of seizure modification, since the frequency of behavioral spontaneous seizures, as assessed by video monitoring, was not altered by the treatment. While hrIL-1Ra can have an anticonvulsant effect,[56] that effect is not seen when it is administered in the manner (dose and site of infusion) used in our experiments.

In our most recent set of experiments,[59] we delved further into aspects of how raphe-hippocampal transmission was affected in the post-SE animals in terms of the presynaptic and postsynaptic 5-HT1A receptors. Tritiated WAY-10065 binding was studied in the raphe and the hippocampus to evaluate receptor numbers, while the functional capacity of the 5-HT1A receptors to activate G-protein was examined using guanosine 5′-O-gamma-thio]triphosphate ([35S]GTPγS) autoradiography.[60] In the dorsal raphe, [3H]WAY-10065 binding was similar in both moderately and severely affected post-SE animals compared to naive animals. However, [35S]GTPγS binding suggested an enhancement in second messenger function. In the hippocampus, [3H]WAY-10065 binding was unaffected in Ammon's horn, while second messenger function showed a diminution when the receptors were activated maximally. Receptor binding was enhanced in the dentate gyrus, where no change in second messenger function was discernible. Administration of mifepristone into the raphe for 1 week improved evoked 5HT-release and mitigated against the impairments in the FST only in those animals that were severely affected. Intrahippocampal mifepristone reduced immobility time in both moderately and severely affected animals.

Based on the studies performed to date, we suggest that the following sequence of events may lead to depression associated with TLE[61] (Fig. 74–5). Chronic epilepsy leads to dysregulation of the HPA axis via several putative mechanisms, one being the activation of hippocampal IL-1β signaling. The enhanced level of circulating glucocorticoids and the hyperactivity of the HPA axis upregulate raphe 5-HT1A

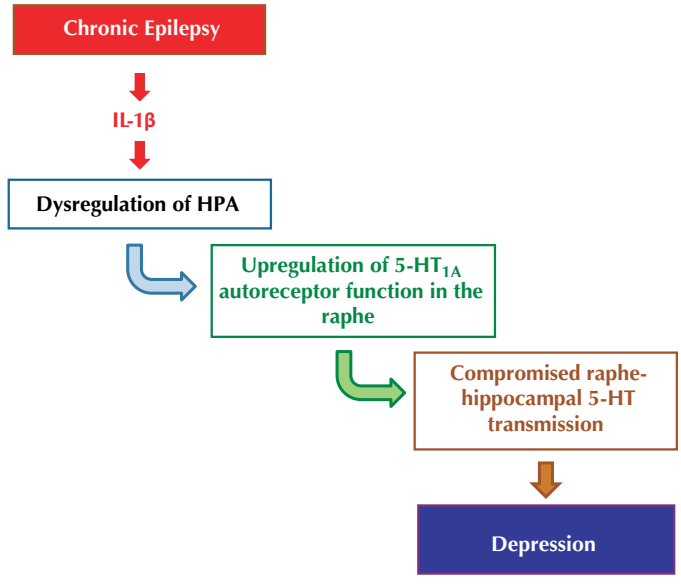

Figure 74–5. Proposed mechanisms of depression as a comorbidity of TLE. Chronic epilepsy leads to dysregulation of the HPA axis[50] via several putative mechanisms, one being activation of IL-1β signaling in the hippocampus.[58] One result of dysregulation of the HPA axis is the upregulation of 5-HT1A somatodendritic receptors in dorsal raphe and subsequent increased autoinhibition of serotonin release in the raphe-hippocampal pathway, as well as changes in the postsynaptic receptors in the hippocampus compromising serotonergic signaling and transuction.[61] Behavioral and biochemical experiments involved the administration of glucocorticoid and 5-HT1A blockers into the raphe of post-SE rats as well as binding studies.[61] The resulting compromised raphe-hippocampal serotonergic transmission[17] leads to behavioral symptoms of depression.[48,50,58]

autoreceptor function and downregulate postsynaptic 5-HT1A receptor function, thus compromising raphe-hippocampal serotonergic transmission. The latter serotonergic deficit leads to the development of clinical symptoms of depression.

CONCLUSIONS

Studies involving an animal model of absence epilepsy suggest that preemptive treatment before the onset of the epileptic syndrome may protect against both the evolution of the epilepsy and the comorbid depressive symptoms. Translation of this concept to humans is difficult at present since we cannot predict with high reliability who will develop the epileptic syndrome and also acquire the comorbidity. Thus, treatment of asymptomatic children with medications that may produce neurobehavioral effects of their own, and may also involve other systemic risks, is not ethically acceptable.

We have demonstrated that symptoms of depression-like behavior can be elicited in rodents upon kindling epileptogenesis, dissociating the emergence of depression from both seizure-related cellular injury and spontaneous seizure frequency. Our studies on limbic epilepsy have focused on the role of raphe-hippocampal serotonergic transmission and how it is influenced by inflammatory signaling by IL-1β and perturbations in the HPA axis.

Clinical depression is a multifactorial and multisymptomatic disorder, and apparently, so is the depression associated with epilepsy. Other lines of experimental evidence involving vagus nerve stimulation suggest that other brain stem nuclei, such as the locus coeruleus and the noradrenergic pathways, may work in partnership with the raphe to ameliorate both epilepsy and depression.[62] Neuropeptide galanin,[63] which is both an anticonvulsant and an antidepressant, also seems to interact with serotonergic and noradrenergic systems at the brainstem.[49,64] Thus, many targets may be relevant for the management of epilepsy and the comorbidity of depression.

Therefore, the validation of the model of comorbidity between epilepsy and depression should continue with examination of other hallmarks of depression and with the investigation of other mechanisms involved. Our studies to date show that the pilocarpine model of epilepsy may be regarded as a model of comorbidity between epilepsy and depression that is useful both for mechanistic studies and as a screening platform for mechanism-driven therapeutic interventions.

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R.S. has served as a consultant to UCB Pharma, GSK, Lundbeck, Sunovion, and Neurotherapeutics Pharma. He has served on the speakers bureau of UCB, GSK, and Lundbeck. He has received clinical research grants from Pfizer. He has also received grant support for a multicenter NINDS, NIH study on Childhood Absence Epilepsy (NS046516). He serves on the Professional Advisory Board of the Epilepsy Foundation and is a member of the Commission on Neurobiology of the International League Against Epilepsy.

A.M. has no relationships to disclose.

REFERENCES

1. Lewis A. Melancholia: a historic review. *J Mental Sci*. 1934;80:1–42.
2. Forsgren L, Nyström L. An incident case-referent study of epileptic seizures in adults. *Epilepsy Res*. 1990;6:66–81.
3. Hesdorffer DC, Hauser WA, Annegers JF, Cascino G. Major depression is a risk factor for seizures in older adults. *Ann Neurol*. 2000;47:246–249.
4. Kanner AM. Depression in epilepsy: a neurobiologic perspective. *Epilepsy Curr*. 2005;5: 21–27.
5. Hermann BP, Seidenberg M, Bell B. Psychiatric comorbidity in chronic epilepsy: identification, consequences, and treatment of major depression. *Epilepsia*. 2000;41(suppl 2):31–41.
6. Hermann BP, Seidenberg M, Bell B, Woodard A, Rutecki P, Sheth R. Comorbid psychiatric symptoms in temporal lobe epilepsy: association with chronicity

of epilepsy and impact on quality of life. *Epilepsy Behav*. 2000;1:184–190.
7. Johnson EK, Jones JE, Seidenberg M, Hermann BP. The relative impact of anxiety, depression, and clinical seizure features on health-related quality of life in epilepsy. *Epilepsia*. 2004;45:544–555.
8. Harden CL, Goldstein MA. Mood disorders in patients with epilepsy: epidemiology and management. *CNS Drugs*. 2002;16:291–302.
9. Sankar R. Initial treatment of epilepsy with antiepileptic drugs: pediatric issues. *Neurology*. 2004;63(suppl 4): S30–S39.
10. Mula M, Sander JW. Negative effects of antiepileptic drugs on mood in patients with epilepsy. *Drug Saf*. 2007;30:555–567.
11. Miller JM, Kustra RP, Vuong A, Hammer AE, Messenheimer JA. Depressive symptoms in epilepsy: prevalence, impact, aetiology, biological correlates and effect of treatment with antiepileptic drugs. *Drugs*. 2008;68:1493–1509.
12. Ketter TA, Post RM, Theodore WH. Positive and negative psychiatric effects of antiepileptic drugs in patients with seizure disorders. *Neurology*. 1999; 53(5 suppl 2):S53–S67.
13. Forsgren L, Nystrom L. An incident case referent study of epileptic seizures in adults. *Epilepsy Res*. 1990;6:66–81.
14. Hesdorffer DC, Hauser WA, Annegers JF, Cascino G. Major depression is a risk factor for seizures in older adults. *Ann Neurol*. 2000;47:246–249.
15. Hesdorffer DC, Hauser WA, Olafsson E, Ludvigsson P. Kjartansson O. Depression and suicidal attempt as risk factor for incidental unprovoked seizures. *Ann Neurol*. 59 2006;59:35–41.
16. Altshuler L, Rausch R, Delrahim S, Kay J, Crandall P. Temporal lobe epilepsy, temporal lobectomy, and major depression. *J Neuropsychiatry Clin Neurosci*. 1999;11:436–443.
17. Piazzini A, Canevini MP, Maggiori G, Canger R. Depression and anxiety in patients with epilepsy. *Epilepsy Behav*. 2001;2:481–489.
18. Caplan R, Siddarth P, Gurbani S, Hanson R, Sankar R, Shields WD. Depression and anxiety disorders in pediatric epilepsy. *Epilepsia*. 2005;46:720–730.
19. Ettinger AB, Weisbrot DM, Nolan EE, Gadow KD, Vitale SA, Andriola MR, Lenn NJ, Novak GP, Hermann BP. Symptoms of depression and anxiety in pediatric epilepsy patients. *Epilepsia*. 1998;39:595–599.
20. Oguz A, Kurul S, Dirik E. Relationship of epilepsy-related factors to anxiety and depression scores in epileptic children. *J Child Neurol*. 2002;17:37–40.
21. Gilliam FG, Maton BM, Martin RC, Sawrie SM, Faught RE, Hugg JW, Viikinsalo M, Kuzniecky RI. Hippocampal 1H-MRSI correlates with severity of depression symptoms in temporal lobe epilepsy. *Neurology*. 2007;68:364–368.
22. Hermann BP, Wyler AR. Depression, locus of control, and the effects of epilepsy surgery. *Epilepsia*. 1989;30:332–338.
23. Blumer D, Wakhlus, Davies K, Hermann B. Psychiatric outcome of temporal lobectomy for epilepsy: incidence and treatment of psychiatric complications. *Epilepsia*. 1998;39:478–486.
24. Devinsky O, Barr WB, Vickrey BG, Berg AT, Bazil CW, Pacia SV, Langfitt JT, Walczak TS, Sperling MR, Shinnar S, Spencer SS. Changes in depression and anxiety after resective surgery for epilepsy. *Neurology*. 2005;65:1744–1749.
25. Hugg JW, Kuzniecky RI, Gilliam FG, Morawetz RB, Faught RE, Hetherington HP. Normalization of contralateral metabolic function following temporal lobectomy demonstrated by H-1 magnetic resonance spectroscopic imaging. *Ann Neurol*. 1996;40:236–239.
26. Cendes F, Andermann F, Dubeau F, Matthews PM, Arnold DL. Normalization of neuronal metabolic dysfunction after surgery for temporal lobe epilepsy. Evidence from proton MR spectroscopic imaging. *Neurology*. 1997;49:1525–1533.
27. Serles W, Li LM, Antel SB, Cendes F, Gotman J, Olivier A, Andermann F, Dubeau F, Arnold DL. Time course of postoperative recovery of *N*-acetyl-aspartate in temporal lobe epilepsy. *Epilepsia*. 2001;42:190–197.
28. Spencer SS, Berg AT, Vickrey BG, Sperling MR, Bazil CW, Shinnar S, Langfitt JT, Walczak TS, Pacia SV, Ebrahimi N, Frobish D; Multicenter Study of Epilepsy Surgery. Initial outcomes in the Multicenter Study of Epilepsy Surgery. *Neurology*. 2003;61:1680–1685.
29. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature*. 1977;266:730–732.
30. Pucilowski O, Overstreet DH. Effect of chronic antidepressant treatment on responses to apomorphine in selectively bred rat strains. *Brain Res Bull*. 1993;32: 471–475.
31. Willner P, Mitchell PJ. The validity of animal models of predisposition to depression. *Behav Pharmacol*. 2002;13:169–188.
32. Shumake J, Gonzalez-Lima F. Brain systems underlying susceptibility to helplessness and depression. *Behav Cognit Neurosci Rev*. 2003;2:198–221.
33. Pucilowski O, Overstreet DH, Rezvani AH, Janowsky DS. Chronic mild stress-induced anhedonia: greater effect in a genetic rat model of depression. *Physiol Behav*. 1993;54:1215–1220.
34. Moreau JL. Validation of an animal model of anhedonia, a major symptom of depression. *Encephale*. 1997;23:280–289.
35. Depaulis A, van Luijtelaar G. Genetic models of absence epilepsy in the rat. In: Pitkänen A, Schwartzkroin P, Moshé S, eds. *Animal Models of Seizures and Epilepsy*. San Diego, CA: Elsevier; 2006:233–248.
36. Sarkisova KYu, Kulikov MA. The WAG/Rij strain of rats: a new genetically-based animal model of depression? In: Kuznetsova GD, Coenen A, Chepurnov SA, van Luijtelaar G, eds. *The WAG/Rij Rat Model of Absence Epilepsy: The Nijmegen-Moscow Research. A Tribute to Five Years of Co-operation*. Nijmegen, Netherlands: Nijmegen University Press; 2000:105–112.
37. Sarkisova K, van Luijtelaar G. The WAG/Rij strain: a genetic animal model of absence epilepsy with comorbidity of depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010;35:854–876.
38. Blumenfeld H, Klein JP, Schridde U, Vestal M, Rice T, Khera DS, Bashyal C, Giblin K, Paul-Laughinghouse C, Wang F, Phadke A, Mission J, Agarwal RK, Englot DJ, Motelow J, Nersesyan H, Waxman SG, Levin AR. Early treatment suppresses the development of spike-wave epilepsy in a rat model. *Epilepsia*. 2008;49:400–409.
39. Sarkisova KY, Kuznetsova GD, Kulikov MA, van Luijtelaar G. Spike-wave discharges are necessary for the expression of behavioral depression-like symptoms. *Epilepsia*. 2010;51:146–160.

40. Lothman EW, Hatlelid JM, Zorumski CF, Conry JA, Moon PF, Perlin JB. Kindling with rapidly recurring hippocampal seizures. *Brain Res.* 1985;360:83–91.
41. Lothman EW, Williamson JM. Closely spaced recurrent hippocampal seizures elicit two types of heightened epileptogenesis: a rapidly developing, transient kindling and a slowly developing, enduring kindling. *Brain Res.* 1994;649:71–84.
42. Sankar R, Auvin S, Kwon YS, Pineda E, Shin D, Mazarati A. Evaluation of development-specific targets for antiepileptogenic therapy using rapid kindling. *Epilepsia*. 2010;51(suppl 3):39–42.
43. Mazarati A, Shin D, Auvin S, Caplan R, Sankar R. Kindling epileptogenesis in immature rats leads to persistent depressive behavior. *Epilepsy Behav*. 2007;10: 377–383.
44. Overstreet DH, Friedman E, Mathe AA, Yadid G. The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. *Neurosci Biobehav Rev.* 2005;29:739–759.
45. American Psychiatric Association. Mood disorders. In: *Diagnostic and Statistical Manual of Mental Disorders DSM-IV-TR*. 4th ed. VA: American Psychiatric Publishing of Arlington; 2000:345–429.
46. Attarian H, Vahle V, Carter J, Hykes E, Gilliam F. Relationship between depression and intractability of seizures. *Epilepsy Behav*. 2003;4:298–301.
47. Quigg M, Broshek DK, Heidal-Schiltz S, Maedgen JW, Bertram EH 3rd. Depression in intractable partial epilepsy varies by laterality of focus and surgery. *Epilepsia.* 2003;44:419–424.
48. Mazarati A, Siddarth P, Baldwin RA, Shin D, Caplan R, Sankar R. Depression after status epilepticus: behavioural and biochemical deficits and effects of fluoxetine. *Brain.* 2008;131(pt 8):2071–2083.
49. Mazarati AM, Baldwin RA, Shinmei S, Sankar R. In vivo interaction between serotonin and galanin receptors types 1 and 2 in the dorsal raphe: implication for limbic seizures. *J Neurochem*. 2005;95:1495–1503.
50. Mazarati AM, Shin D, Kwon YS, Bragin A, Pineda E, Tio D, Taylor AN, Sankar R. Elevated plasma corticosterone level and depressive behavior in experimental temporal lobe epilepsy. *Neurobiol Dis.* 2009;34:457–461.
51. Watson S, Gallagher P, Smith MS, Ferrier IN, Young AH. The DEX/CRH test—is it better than the DST? *Psychoneuroendocrinology.* 2006;31:889–894.
52. Riad M, Garcia S, Watkins KC, Jodoin N, Doucet E, Langlois X, el Mestikawy S, Hamon M, Descarries L. Somatodendritic localization of 5-HT1A and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. *J Comp Neurol*. 2000;417:181–194.
53. Lothe A, Didelot A, Hammers A, Costes N, Saoud M, Gilliam F, Ryvlin P. Comorbidity between temporal lobe epilepsy and depression: a [18F]MPPF PET study. *Brain*. 2008;131:2765–2782.
54. Man MS, Young AH, McAllister-Williams RH. Corticosterone modulation of somatodendritic 5-HT1A receptor function in mice. *J Psychopharmacol*. 2002;16:245–252.
55. Bellido I, Hansson AC, Gomez-Luque AJ, Andbjer B, Agnati LF, Fuxe K. Corticosterone strongly increases the affinity of dorsal raphe 5-HT1A receptors. *Neuroreport.* 2004;15:1457–1459.
56. Vezzani A, Balosso S, Ravizza T. The role of cytokines in the pathophysiology of epilepsy. *Brain Behav Immun*. 2008;22:797–803.
57. Dunn AJ, Swiergiel AH, de Beaurepaire R. Cytokines as mediators of depression: what can we learn from animal studies? *Neurosci Biobehav Rev*. 2005;29: 891–909.
58. Mazarati AM, Pineda E, Shin D, Tio D, Taylor AN, Sankar R. Comorbidity between epilepsy and depression: role of hippocampal interleukin-1beta. *Neurobiol Dis*. 2010;37:461–467.
59. Pineda EA, Hensler JG, Sankar R, Shin D, Burke TF, Mazarati AM. Plasticity of presynaptic and postsynaptic serotonin 1A receptors in an animal model of epilepsy-associated depression. *Neuropsychopharmacology*. 2011;36:1305–1316.
60. Hensler J, Durgam H. Regulation of 5-HT(1A) receptor-stimulated [35S]-GTP gamma S binding as measured by quantitative autoradiography following chronic agonist administration. *Br J Pharmacol*. 2001;132:605–611.
61. Pineda E, Shin D, Sankar R, Mazarati AM. Comorbidity between epilepsy and depression: experimental evidence for the involvement of serotonergic, glucocorticoid, and neuroinflammatory mechanisms. *Epilepsia*. 2010;51(suppl 3):110–114.
62. Manta S, Dong J, Debonnel G, Blier P. Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. *J Psychiatry Neurosci.* 2009;34:272–280.
63. Bartfai T, Lu X, Badie-Mahdavi H, Barr AM, Mazarati A, Hua XY, Yaksh T, Haberhauer G, Ceide SC, Trembleau L, Somogyi L, Kröck L, Rebek J Jr. Galmic, a nonpeptide galanin receptor agonist, affects behaviors in seizure, pain, and forced-swim tests. *Proc Natl Acad Sci USA*. 2004;101:10470–10475.
64. Lu X, Barr AM, Kinney JW, Sanna P, Conti B, Behrens MM, Bartfai T. A role of galanin in antidepressant actions with a focus on the dorsal raphe nucleus. *Proc Natl Acad Sci USA.* 2005;102:874–879.

SECTION 5

Epilepsy Therapeutics

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Chapter 75

Calcium Channel $\alpha_2\delta$ Subunits in Epilepsy and as Targets for Antiepileptic Drugs

Annette C. Dolphin

Voltage-gated calcium (Ca_V) channels are involved in numerous physiological processes, the most important being muscle contraction, hormone secretion, and synaptic transmission. The pore-forming subunit of Ca_V channels is the $\alpha1$ subunit, and this determines the main biophysical and pharmacological properties of the channels (Fig. 75–1). There are three main subgroups of Ca_V channels, Ca_V1–3. The low-voltage-activated calcium channels (Ca_V3 or T-type channels) appear to be able to function well as monomers. However, the high-voltage activated (HVA) Ca_V1 and Ca_V2 subfamilies are normally heteromeric, with the $\alpha1$ subunit being associated with a $Ca_V\beta$ subunit and an $\alpha_2\delta$ subunit (for reviews see refs. 1–3; Fig. 75–1).

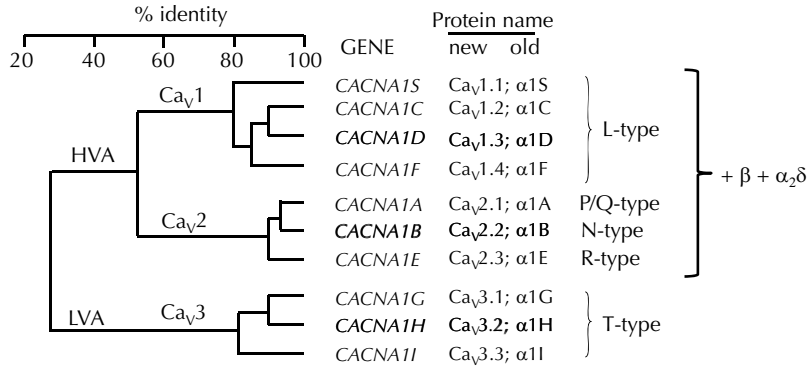

Figure 75–1. Dendrogram showing the similarity of calcium channel α1 subunit genes, with their nomenclature.

Ten mammalian α1 subunits (Fig. 75–1), four β subunits, and four $\alpha_2\delta$ subunits have been cloned. These are described in more detail below.

VOLTAGE-GATED CALCIUM CHANNELS

Discovery of Calcium Channels

A calcium conductance was first identified in crustacean muscle.[4,5] Calcium-dependent action potentials and calcium currents were then investigated in other invertebrate systems[6,7] and subsequently in mammalian systems, first in cardiac Purkinje fibers (e.g., ref. 8), cardiac ventricular cells[9] and subsequently in other excitable cells**.** The identification of 1,4-dihydropyridines (DHPs) and other calcium antagonists as blockers of muscle calcium channels[10] was a major therapeutic advance, but also had the by-product of aiding the isolation of purified Ca_v channels. In skeletal muscle, there is a very high concentration of DHP receptors, representing L-type calcium channels, situated in the T-tubules. Muscle depolarization results in large gating currents but anomalously low calcium flux, which is now explained by the very slow activation of the ionic currents. For this reason, little or no Ca^{2+} passes across the T-tubule membrane during a single action potential. The study of how these skeletal muscle calcium channels are now thought to couple by a direct mechanical process to the release of intracellular Ca^{2+} from the sarcoplasmic reticulum has enhanced our understanding of excitation-contraction coupling in muscle.[11]

Calcium Channel Purification

DHP receptor complexes were first purified from T-tubules because of their relative abundance.[12,13] Three major bands were initially identified: α, β, and γ. Under reducing conditions, the purified skeletal muscle DHP receptor complex was found to contain five components, which were termed α_1 (~175 kDa), α_2 (~150 kDa), β (~54 kDa), δ (17–25 kDa), and γ (~32 kDa), in an approximately stoichiometric ratio. The α1 subunit was shown to bind ^{3}H-DHP ligands, and thus was identified as the pore-forming subunit. In all of the HVA calcium channels in which it has been studied, the $Ca_V\alpha1$ subunit copurifies with a cytoplasmic β subunit ($Ca_V\beta$) and an extracellular $Ca_V\alpha_2$ subunit, which is associated with a membrane-anchored δ subunit.[13–16] In these studies, the association of the $\alpha_2\delta$ subunit with the complex was found to be looser than that of the β subunit.

Calcium Channel Subunit Gene Cloning

Following identification of the α1 subunit as the DHP receptor, by virtue of its ability to bind ^{3}H-azidopine, cloning of its cDNA was accomplished, initially from skeletal muscle,[13] and subsequently from heart, by homology with

the skeletal muscle sequence.[17] Hydropathy analysis indicated that the α_1 subunits have 24 putative transmembrane segments, arranged into four homologous repeated domains, with intracellular linkers and N and C termini. The 10 cloned α_1 subunits all have specialized functions and distributions (Fig. 75–1; for review of nomenclature, see ref. 18). The four members of the Ca_V1 family are all L-type channels, with $Ca_V1.1$ being the skeletal muscle isoform and $Ca_V1.2$ being particularly prevalent in cardiac muscle, whereas the more recently cloned $Ca_V1.3$ and $Ca_V1.4$ are activated at lower voltage thresholds and have a more restricted distribution. All the Ca_V1 family channels are sensitive to the 1,4-DHPs. $Ca_V2.1$ (initially termed α_1A) is the molecular counterpart of P/Q-type calcium channels,[19] and $Ca_V2.2$ or α_1B,[20] is the molecular counterpart of the neuronal N-type calcium channels. $Ca_V2.3$ or α_1E shows more inactivation than the other HVA channels cloned[21] and is thought to contribute to the molecular counterpart of the R-type calcium current. The Ca_V3 group of channels (the molecular counterpart of the T-type channels) are clearly divergent in terms of structure from the HVA channels (for review, see ref. 22).

THE $\alpha_2\delta$ SUBUNITS

$\alpha_2\delta$ Gene Cloning

Four mammalian $\alpha_2\delta$ subunit genes have been cloned; these are skeletal muscle $\alpha_2\delta$-1,[23] $\alpha_2\delta$-2,[24] $\alpha_2\delta$-3,[25] and $\alpha_2\delta$-4.[26] Several other similar genes have been identified[27] but have not yet been shown to function as $\alpha_2\delta$ subunits.

Classical Topology and Posttranslational Processing of $\alpha_2\delta$ Subunits

The topological organization of the $\alpha_2\delta$ protein was first determined for $\alpha_2\delta$-1 and has been thought to be similar in all four $\alpha_2\delta$ subunits that have been cloned (for reviews, see refs. 1 and 2). Biochemical studies on purified skeletal muscle DHP receptors, examined under reducing and nonreducing conditions, showed that the α_2 subunit is disulfide-bonded to the smaller δ subunit.[12,14,28] Following the initial cloning of the gene encoding $\alpha_2\delta$-1 and N-terminal sequencing of the δ peptide, it was realized that α_2 and δ are expressed from the same gene, encoding the $\alpha_2\delta$ preprotein,[29] which is then posttranslationally cleaved into α_2 and δ.[30] by an unknown protease. The α_2 and δ moieties then remain associated by one or more disulfide bonds (Fig. 75–2).

Novel Topology: Evidence That $\alpha_2\delta$ Subunits Can Form GPI-Anchored Proteins

The $\alpha_2\delta$ subunits have an exofacial N terminus, as indicated by the presence of an N-terminal signal sequence, that directs the protein into the lumen of the endoplasmic reticulum, where the signal sequence is cotranslationally cleaved (Fig. 75–2). The $\alpha_2\delta$ subunits were first identified to be type I transmembrane proteins since they also have a C-terminal hydrophobic domain. However, in various proteomic prediction programs, some of these $\alpha_2\delta$ proteins are strongly predicted to be glycosyl-phosphatidylinositol (GPI)-anchored.[31–33] We have now obtained evidence consistent with the hypothesis that both heterologously expressed and endogenous $\alpha_2\delta$ proteins form GPI-anchored rather than transmembrane proteins.[33] All the $\alpha_2\delta$ proteins that we have examined ($\alpha_2\delta$-1, –2, and –3 are substrates for phosphatidyl-inositol phospholipase C enzymes (purified from bacteria or trypanosomes), which act extracellularly, to release both heterologously expressed and endogenous $\alpha_2\delta$ from the plasma membrane. This treatment of cells with these enzymes also inhibits calcium currents in cells in which $\alpha_2\delta$ is expressed.[33]

Structure of $\alpha_2\delta$ Subunits in Calcium Channel Complexes

Very little structural information is available on $\alpha_2\delta$ subunits, although all contain a von Willebrand factor A (VWA) domain[27] and two Cache domains.[34] In general, VWA domains are involved in protein–protein interactions, particularly between extracellular matrix proteins via a metal ion-dependent adhesion site

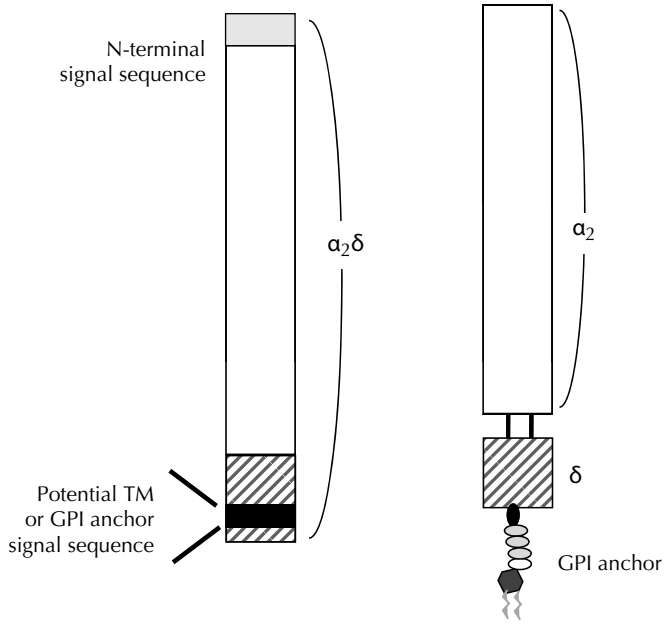

Figure 75–2. Diagram of $\alpha_2\delta$ subunit structure and posttranslational modification.

(MIDAS) motif.[27] We showed that mutation of MIDAS in the VWA domain markedly reduced the functionality of $\alpha_2\delta$ subunits.[35] The VWA domains of $\alpha_2\delta$-2 [35] and $\alpha_2\delta$-1[36] have been subjected to homology modeling, because many template domains are available. The $\alpha_2\delta$ subunit has also been identified in single-particle electron microscopic studies of calcium channel complexes purified from skeletal muscle[37,38] and cardiac muscle.[39]

Function of $\alpha_2\delta$ Subunits in Calcium Channel Complexes

The $\alpha_2\delta$ subunits enhance the forward trafficking of the α1 calcium channel subunits and decrease their turnover at the plasma membrane.[35,40] They also influence the biophysical properties of the channels, increasing the inactivation rate to varying extents. In some studies, hyperpolarization of steady-state inactivation has been observed, as well as an increase in its voltage dependence.[33,35,41] The loss of $\alpha^2\delta$ subunits, as in the naturally occurring $\alpha_2\delta$-2 knockout strains of mice, *ducky* and *ducky*^{2J},[42] results in a reduction in calcium channel currents in Purkinje neurons, where $\alpha_2\delta$-2 is normally strongly expressed. This is accompanied by a marked reduction in both spontaneous and evoked Purkinje cell firing.[43] It was originally mentioned, in several reviews of $\alpha_2\delta$ proteins, that mice in which the more ubiquitously expressed $\alpha_2\delta$-1 is knocked out have a neonatal-lethal phenotype (e.g., refs. 44 and 45). However, a viable $\alpha_2\delta$-1 knockout has recently been published, with a cardiac phenotype, showing reduced cardiac calcium currents and decreased myocardial contractility.[46] The mechanism(s) involved in the function of $\alpha_2\delta$ subunits with respect to calcium channel trafficking and function are still unclear, although we have determined that this process involves their VWA domain.[35] Although the exact site at which the $\alpha_2\delta$ subunits intervene in the calcium channel trafficking process remains to be established, it is assumed that they interact with one or more exofacial domains of the channel α1 subunit. For example, it has been found that the α_2 subunit of $\alpha_2\delta$-1 binds to domain III of $Ca_V1.1$ as one site of interaction.[47,48] Evidence was also obtained previously that the transmembrane segment of δ interacts with α1 subunits.[47,49] However, this needs to be reconsidered in the light of our finding that $\alpha_2\delta$ subunits form GPI-anchored proteins.[33]

Distribution and Function of $\alpha_2\delta$ Proteins in Specific Tissues

SKELETAL MUSCLE

In skeletal muscle T-tubules, $\alpha_2\delta$-1 is found in association with the L-type calcium channel complex including $Ca_V1.1$, β1a, and γ1.[23] In the T-tubule junction with the sarcoplasmic reticulum, the DHP receptors form a characteristic tetrad structure associated with ryanodine receptors on the sarcoplasmic reticulum.[50,51] However, it has been found that although partial loss of $\alpha_2\delta$-1 using small interfering RNA (siRNA) caused a significant increase in the rate of activation of the L-type Ca^{2+} current in myotubes, it had little or no effect on skeletal muscle excitation-contraction coupling.[52] More extensive knockdown, using viral infection of siRNA in myotubes, confirmed the effect on current kinetics but found that the size and spacing of tetradic particles were unaffected by the loss of $\alpha_2\delta$-1, indicating that the visible particles probably represent the α1S ($Ca_V1.1$) subunit.[53] Further, there was a complete loss of excitation-coupled calcium entry during potassium chloride (KCl) depolarization and a more rapid decay of Ca^{2+} transients. However, it was found that $\alpha_2\delta$-1 was not necessary for myotube growth, or for differentiation from myoblasts to form myotubes, or in the expression levels or targeting of $Ca_V1.1$ to the calcium release units apposed to ryanodine receptors.[53] In contrast, another study of developing myocytes showed that $\alpha_2\delta$-1 was concentrated at the ends of the myocytes. In that study, when the expression of $\alpha_2\delta$-1 was reduced with siRNA, migration, attachment and spreading of myoblasts were impaired, while the L-type calcium current remained unaffected.[54]

CARDIAC AND SMOOTH MUSCLE

The $\alpha_2\delta$-1 subunit is strongly expressed in cardiac and smooth muscle and is likely to be the main $\alpha_2\delta$ subunit associated with calcium channels in these tissues.[55,56] It was also found that the $\alpha_2\delta$-4 subunit transcript was expressed in cardiac muscle, although very little $\alpha_2\delta$-4 protein was observed in cardiac tissue.[26]

NERVOUS SYSTEM

The $\alpha_2\delta$-1, $\alpha_2\delta$-2, and $\alpha_2\delta$-3 subunits are widely expressed in brain, with $\alpha_2\delta$-1 being expressed in many neuronal cell types.[57] The $\alpha_2\delta$-1 protein is mainly present in presynaptic terminals, and to a much lower extent in cell bodies under physiological conditions.[58,59] This is illustrated in Fig. 75–3 with respect to the distribution of $\alpha_2\delta$-1 in the hippocampus, where it is clearly absent from the main cell body layers. The expression of $\alpha_2\delta$-1 was also found to be correlated with excitatory rather than inhibitory neurons,[57] and it is strongly expressed in dorsal root ganglion neurons.[59] In contrast, $\alpha_2\delta$-2 expression is more restricted and appears to correlate with GABAergic (gamma-aminobutyric acid) neurons, including cerebellar Purkinje neurons, where it is strongly expressed.[42,57] The $\alpha_2\delta$-3 protein is widely expressed throughout the brain, particularly in the caudate-putamen.[57] In contrast, $\alpha_2\delta$-4 protein is expressed in specific endocrine tissues and at a low level in the brain.[26] It is also expressed in the retina, where its mutation results in a form of night blindness.[60]

Splice Variants of $\alpha_2\delta$ Subunits

It was originally noted that the main $\alpha_2\delta$-1 subunit splice variant expressed in rat brain was different from that in skeletal muscle.[61] Three alternatively spliced regions have been identified from multiple sequence alignments, termed *region A*, *region B*, and *region C*, and five different transcripts consisting of different combinations of these alternatively spliced regions were originally found in mouse brain, skeletal muscle, heart, smooth muscle, and aorta[62] (Fig. 75–4). Other splice variants have been mentioned in abstract form.[63] Differences in the ability of different splice variants to bind ^{3}H-gabapentin have been mentioned in these publications, but it remains unknown whether changes in alternative splicing may correlate with disease states or with the therapeutic efficacy of $\alpha_2\delta$ ligand drugs. A number of splice variants of the other $\alpha_2\delta$ subunits have also been described.[26,64]

Membrane Localization, Trafficking, and Endocytosis of $\alpha_2\delta$ Subunits

Glycosyl-phosphatidylinositol anchoring has major implications for the structure and function

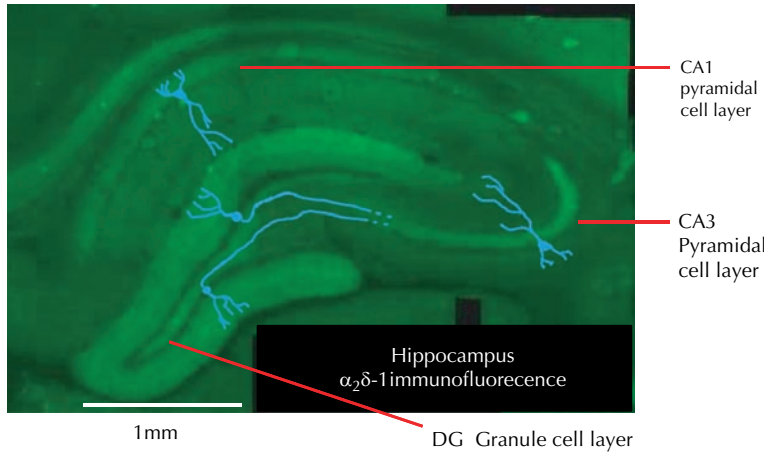

Figure 75–3. Localization of $\alpha_2\delta$-1 in a section of rat hippocampus. The section was 25 μm thick and was prepared and $\alpha_2\delta$-1 was visualized as previously described.[59] Note the lack of $\alpha_2\delta$-1 staining in the hippocampal granule and pyramidal cell body layers. The scale bar is 1 mm.

of $\alpha_2\delta$ proteins, but it also affects their membrane localization and their intracellular trafficking and endocytosis, since these proteins cannot signal across the lipid bilayer to interact directly with sorting proteins.[65,66] Moreover, it explains the marked localization of $\alpha_2\delta$ proteins in a cholesterol-rich detergent-resistant membrane (DRM) fraction, also termed *lipid rafts*, that we have noted previously.[67] Lipid raft localization is shown by many proteins that are GPI-linked to the membrane, for example prion proteins, thy-1, neural cell adhesion molecule (N-CAM), and carcinoembryonic antigen (CEA). Lipid rafts may represent distinct membrane microdomains (for review, see ref. 68), although heterogeneity in the GPI-anchored protein distribution has also been demonstrated,[69] which may be in part a result of variation in the specific chemistry of different GPI anchors.[70] Furthermore, some proteins have both GPI-anchored and transmembrane forms,[71] and our evidence does not yet preclude this possibility for $\alpha_2\delta$ subunits.

Many GPI-anchored proteins are endocytosed by a route involving GPI-enriched early endosomal compartments (GEECs) that is independent of the clathrin pathway.[66,72] The GEECs are cholesterol-rich compartments, and we have recently found that the $\alpha_2\delta$ subunits show constitutive endocytosis.[41,59,73]

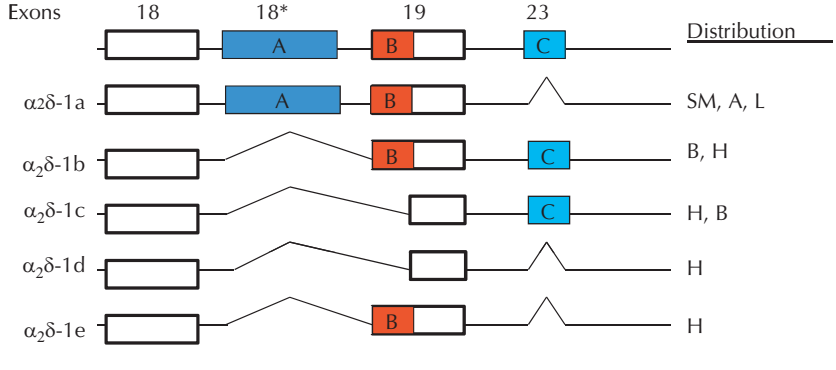

Figure 75–4. Regions of splice variation in $\alpha_2\delta$-1; details are taken from ref. 62. Region A is encoded by a separate exon, here called 18*. Region B is part of exon 19, generated by an alternative splice site. Main tissues showing expression of these splice variants involving regions A, B, and C are given. SM, smooth muscle; A, aorta; L, lung; B, brain; H, heart.

Presynaptic Localization and Transport of $\alpha_2\delta$ Subunits

As described above, $\alpha_2\delta$-1 is mainly associated with terminal fields rather than cell bodies.[58] With regard to $\alpha_2\delta$-3, where similar immunohistochemical studies are lacking, mutations in the *Drosophila* $\alpha_2\delta$-3 subunit have revealed it to be the likely binding partner for the cacophony calcium channel that is involved in presynaptic vesicle release and calcium channel expression at active zones.[74] This suggests that $\alpha_2\delta$-3 has a role in targeting calcium channels to correct presynaptic sites. It has also been found that in *Caenorhabditis. elegans* the $\alpha_2\delta$ subunit (UNC36) is required for the correct presynaptic localization of the Ca_V2 calcium channel (UNC2).[75]

In the peripheral nervous system, $\alpha_2\delta$-1 is strongly expressed in dorsal root ganglion neurons (DRGs), particularly in the small nociceptors.[58,76] We have utilized spinal nerve ligation (SNL) as a rodent model of neuropathic pain to study the distribution of $\alpha_2\delta$-1, following its upregulation in DRGs in vivo as a consequence of SNL.[76] Our results showed that the increase in $\alpha_2\delta$-1 protein occurs in all DRG neuronal subtypes. We also observed an elevation within both myelinated and nonmyelinated DRG axons on the side ipsilateral to the ligation.[76] This increase was rapid, starting on the second day after ligation, in the dorsal roots that terminate in the spinal cord dorsal horn. We also found accumulation of $\alpha_2\delta$-1 proximal to the ligation site—the first direct evidence for $\alpha_2\delta$-1 trafficking toward the periphery.[59] We also observed a large increase in $\alpha_2\delta$-1 immunofluorescence in both the superficial and deeper laminae of the dorsal horn, in accordance with our finding that $\alpha_2\delta$-1 was increased in all DRG neurons.[59] Immunoelectron microscopy showed that the $\alpha_2\delta$-1 protein was predominantly presynaptic, being found on excitatory nerve terminals in the spinal cord. However, some $\alpha_2\delta$-1 immunoparticles were also observed both in endocytic vesicles within dendritic structures and at the dendritic plasma membrane. Our results indicate that $\alpha_2\delta$-1 subunits are trafficked from the site of synthesis to the plasma membrane of the DRG presynaptic terminals. However, the upregulation of $\alpha_2\delta$-1 in damaged sensory neurons in various neuropathic pain models,[59,77] and the fact that the elevated $\alpha_2\delta$-1 is transported not only centrally but also peripherally,[59] suggest that it might affect other processes, such as neuronal regeneration and plasticity, as well as calcium channel trafficking.

Involvement of $\alpha_2\delta$ Subunits in Synaptogenesis and Other Processes

Other roles for $\alpha_2\delta$-1 subunits unrelated to calcium channel function have recently been proposed. One suggestion that $\alpha_2\delta$ subunits may have multiple roles comes from the finding that the genes for both $\alpha_2\delta$-2[24] and $\alpha_2\delta$-3[78] have potentially been implicated in tumor susceptibility and tumor growth. As described above, there is disagreement about whether $\alpha_2\delta$-1 subunits are involved in development and migration of myotubes, separate from their role in the calcium channel complex.[53,54] However, $\alpha_2\delta$ subunits are only loosely associated with calcium channel complexes, and a proportion of $\alpha_2\delta$ subunits can be isolated separately by column chromatography.[79] This supports the possibility that these proteins have other roles.

The $\alpha_2\delta$-1 and $\alpha_2\delta$-3 subunits have also been found to have a role in synaptogenesis, which has been described to be independent of their association with calcium channels.[80,81] The $\alpha_2\delta$-1 protein has been found to be one of the binding partners of the extracellular matrix proteins of the thrombospondin (TSP) family,[80] although TSPs also bind to a number of other proteins and mediate many processes, both in the nervous system and elsewhere.[82] It was also found that $\alpha_2\delta$-1 was required postsynaptically for TSP- and astrocyte-induced synapse formation.[80] This potential postsynaptic function of $\alpha_2\delta$-1 is in contrast to the predominantly presynaptic localization of $\alpha_2\delta$-1 in adult mammalian nervous tissue.[58]

Drosophila $\alpha_2\delta$-3 has also been shown to be required for synaptic stabilization of the *cacophony* calcium channel[74,83] and for synaptogenesis.[81] It is unknown whether it plays a similar role in mammalian brain and whether $\alpha_2\delta$-1 and $\alpha_2\delta$-3 have interchangeable functions.

The $\alpha_2\delta$ Subunits in Disease

EPILEPSIES

We have found that *ducky* and *ducky*^{2J} mice, which show a phenotype of cerebellar ataxia and absence epilepsy,[42,43,84] both have mutations in *cacna2d2.* These mutations both predict the expression of a C-terminally truncated protein, and this protein was detected in *ducky* mice.[84] The ataxic phenotype results primarily from the loss of $\alpha_2\delta$-2 in the cerebellar Purkinje cells, where it is strongly expressed[42] and from which the other $\alpha_2\delta$ subunits are largely absent. Another spontaneous mouse mutation, in *cacna2d2* (*entla*), encodes a mutant form of the $\alpha_2\delta$-2 protein with an intact C terminus. This mutant mouse also shows generalized epilepsy,[85] as does a targeted knockout of *cacna2d2.*[86] These mutations are all recessive, with the heterozygotes showing no significant behavioral effects.[42] To date, no human mutations in $\alpha_2\delta$ subunits have been reported to be associated with epileptic phenotypes.

NEUROPATHIC PAIN

As described above, experimental nerve injury is known to result in an increase in the level of $\alpha_2\delta$-1 mRNA in the damaged sensory neurons (DRGs), shown by in situ hybridization,[87] microarray analysis,[88] and quantitative polymerase chain reaction (PCR).[59] There is a corresponding increase in $\alpha_2\delta$-1 protein in DRGs and spinal cord, as determined by Western blot analysis[77] and immunohistochemistry.[59] Furthermore, $\alpha_2\delta$-1-overexpressing mice show a neuropathic phenotype of hyperalgesia and tactile allodynia in the absence of nerve injury,[89] indicating that $\alpha_2\delta$-1 is instrumental in the excitability of DRG neurons and the expression of neuropathy.

NIGHT BLINDNESS

Mutation in the *CACNA2D4* gene (encoding $\alpha 2\delta$-4 subunits) can lead to dysfunction of photoreceptors, resulting in certain forms of night blindness. A spontaneous mouse mutation and human mutations in this gene have been identified, both showing similar phenotypes of autosomal recessive cone dystrophy and night blindness.[60,90] When the gene encoding $\alpha_2\delta$-4 was cloned, it was suggested that it had limited distribution in certain cell types including pituitary, adrenal gland, colon, and fetal liver.[26] However, Northern blot analysis detected widespread distribution of $\alpha_2\delta$-4 transcripts in mouse tissue.[60] Nevertheless, the phenotype resulting from $\alpha_2\delta$-4 loss or dysfunction appears to be confined to the retina, possibly because of the lack of compensation by other $\alpha_2\delta$ subunits in photoreceptors.

MECHANISM OF ACTION OF THE GABAPENTINOID DRUGS ON $\alpha_2\delta$ SUBUNITS

History of Gabapentinoid Drug Development

Gabapentin (2-[1-(aminomethyl)cyclohexyl] acetic acid) and pregabalin (*S*(+)-3-isobutyl GABA) were synthesized as rigid lipophilic analogs of the inhibitory neurotransmitter GABA, with the intention of mimicking the function of this neurotransmitter and suppressing excitatory neurotransmission.[91] However, despite being shown to be efficacious antiepileptic drugs, both in animal models[92] and in clinical studies (for review, see ref. 45), their mechanism of action remained elusive for many years. No clear binding of these drugs to $GABA_A$ or $GABA_B$ receptors was seen in radioligand binding studies (for review, see ref. 45). Although functional effects of gabapentin attributed to activation of $GABA_B$ receptors were reported,[93] no direct evidence that gabapentin activated $GABA_B$ receptors was obtained.[94,95] There is no evidence from in vivo studies that the antiepileptic or antiallodynic effects of these drugs are produced via $GABA_B$ receptors (for review, see ref. 45). Although pregabalin and gabapentin were found to increase glutamic acid decarboxylase activity in vitro, this only occurred at very high concentrations.[96] Nevertheless, in rats, gabapentin was shown to increase GABA turnover in several brain regions.[97] However, as reviewed recently,[45] neither pregabalin nor gabapentin appears to mimic GABA or enhance the action of GABA pharmacologically, suggesting that direct or indirect effects on GABA receptors do not contribute significantly to their pharmacological actions. Furthermore, pregabalin and

gabapentin did not inhibit GABA transport in vitro, as would be expected of an inhibitor of GABA uptake.[98] However, both gabapentin and pregabalin are zwitterions at neutral pH and utilize the large neutral amino acid transporter *system L* for uptake across cell membranes.[91] This adds a degree of complexity to structure–activity relationships for these compounds.

Effects of Gabapentinoids on Synaptic Transmission and Transmitter Release

Acute inhibitory effects of gabapentinoid drugs on excitatory transmitter release and synaptic transmission have been observed in some but not all *in vitro* systems (reviewed in refs. 2 and 45). Furthermore, there is some indirect evidence that gabapentin increased GABA release.[99] In a study in the trigeminal nucleus, an inhibitory effect of gabapentin was only observed on a component of glutamate release that was enhanced following protein kinase C activation.[100] Of interest, calcium channel insertion into the plasma membrane has been shown to be increased by protein kinase C activation.[101] It is possible that gabapentinoid drugs have differential effects on calcium currents in presynaptic terminals resulting in acute inhibition, or that calcium channel turnover is higher in presynaptic terminals, so that effects on calcium channel trafficking are observed more acutely. Thus, gabapentinoid drugs might act more or less rapidly, depending on the interplay between these different processes. While behavioral therapeutic effects of gabapentinoid drugs may be observed acutely,[102] this is not always the case.[103–105]

Identification of the Gabapentin Receptor in Brain as the $\alpha_2\delta$-1 Subunit

Purification of the ^{3}H-gabapentin binding site from pig brain led to its identification by proteomic methods as the $\alpha_2\delta$-1 subunit of Ca_v channels. N-terminal peptide sequencing of the purified protein gave the sequence EPFPSAVTIK.[79] This was initially very surprising, since these proteins are unrelated to GABA function. However, ^{3}H-gabapentin was shown to bind to purified $\alpha_2\delta$-1 with an affinity of about 13 nM, and expression of recombinant $\alpha_2\delta$-1 revealed it also to bind ^{3}H-gabapentin.[79] This affinity has been shown to vary between 7 and 72 nM, depending on the source and purity of the preparation.[106,107] ^{3}H-gabapentin was also subsequently found to bind to $\alpha_2\delta$-2 with an affinity of 156 nM.[106] The variability in reported affinities may depend on the lipid environment and the state of purification of the $\alpha_2\delta$-1 and $\alpha_2\delta$-2 proteins, as we have found that the affinity of ^{3}H-gabapentin binding to $\alpha_2\delta$ from mouse cerebellum (predominantly $\alpha_2\delta$-2) increases from a K_D of ~385 nM in membranes to ~80 nM in lipid raft preparations.[67] The equivalent values for $\alpha_2\delta$-2 expressed in Cos-7 cells were a K_D of ~470 nM in membranes and ~50 nM in the lipid raft fraction.[67] Others have found that the affinity of ^{3}H-gabapentin binding to $\alpha_2\delta$-1 increases on dialysis of the purified protein, and this was attributed to the removal of a heat-stable factor.[108] This might be a large neutral amino acid, since these were found to compete for binding to $\alpha_2\delta$ subunits,[79] or it might be a more complex molecule. Interestingly, pregabalin was found to be slightly more potent in displacing ^{3}H-gabapentin from $\alpha_2\delta$-2 (70 nM) than from $\alpha_2\delta$-1 (106 nM).[106]

It has recently been shown that the binding of gabapentin and pregabalin to $\alpha_2\delta$-1 subunits is essential for their therapeutic effect in neuropathic pain.[102] In this study, a knockin mouse was generated with a mutation (R217A) that abrogated ^{3}H-gabapentin binding to $\alpha_2\delta$-1. These mice developed neuropathic pain normally in response to chronic constriction injury, but this pain was not sensitive to treatment with the gabapentinoid drugs. Similar studies using these R217A $\alpha_2\delta$-1 mice and mice with the equivalent mutation in $\alpha_2\delta$-2 are urgently needed in the epilepsy field in order to clarify further the mechanism of action of the gabapentinoids as antiepileptic drugs.

Acute and Chronic Effects of Gabapentinoids on Calcium Currents

Some initial studies reported either no effect[109] or small acute inhibitory effects of gabapentin on HVA calcium channel currents in

brain neurons[110] and DRG neurons.[111,112] Furthermore, the inhibitory effect of gabapentin on DRG neurons was observed to be dependent on culture conditions.[111] However, in our studies, we found no reproducible acute effects of gabapentin at 100 μM on Ba^{2+} currents in cerebellar Purkinje neurons, although they contain $\alpha_2\delta$-2 as their main $\alpha_2\delta$ subunit,[67] and no effect of up to 1 mM gabapentin on Ba^{2+} currents in DRG neurons or on transfected cells.[41] It was also reported that there was no effect of gabapentin on calcium channel currents in mouse DRG neurons, although when DRGs from $\alpha_2\delta$-1-overexpressing mice were used, they were sensitive to inhibition by gabapentin.[89]

In a recent study, we showed that chronic application of gabapentin markedly reduced cell surface localization of $\alpha_2\delta$ and α1 subunits, and also calcium channel currents, both in expression systems and in DRG neurons.[41] This effect did not occur when $\alpha_2\delta$ subunits were not expressed or when mutant $\alpha_2\delta$ subunits that do not bind gabapentin were coexpressed,[41] suggesting that gabapentin affects the trafficking function of $\alpha_2\delta$ subunits. Accordingly, we also found that chronic application of pregabalin, at the same time as it alleviated neuropathic pain, markedly reduced the trafficking of $\alpha_2\delta$-1 to presynaptic terminals in vivo, thereby inhibiting the function of presynaptic calcium channels[59]

We have found that gabapentinoid drugs do not affect the constitutive endocytosis of $\alpha_2\delta$-1[59] or $\alpha_2\delta$-2.[113] In contrast, gabapentin inhibits post-Golgi forward trafficking of the $\alpha_2\delta$-2 subunit in a manner that is prevented by dominant-negative Rab11, which disrupts trafficking through the recycling endosome compartment.[113] These findings indicate that gabapentinoid drugs may disrupt the interaction between $\alpha_2\delta$ subunits and sorting proteins in this compartment, and that this may represent a rate-limiting step in calcium channel trafficking.

Furthermore, we have found that the gabapentin analog, pregabalin, reduces the ability of $\alpha_2\delta$-1, upregulated in neuropathic pain, to be trafficked from DRG neurons to presynaptic terminals *in vivo*.[59] Both our *in vivo* and *in vitro* data point to a mechanism of action in which the gabapentinoid drugs would inhibit trafficking of the upregulated $\alpha_2\delta$-1 subunits within DRGs following the development of neuropathic pain, and would therefore inhibit the function of presynaptic calcium channels in the dorsal horn, making this a unique mode of action. The extent of this elevation of $\alpha_2\delta$-1 protein in the dorsal horn but not in the DRGs was significantly reduced by chronic treatment with pregabalin for 8 days following spinal nerve ligation, indicating that pregabalin interferes with the transport of $\alpha_2\delta$-1 to its terminal zones.[59]

Gabapentin and pregabalin are effective treatments for neuropathic pain, including diabetic neuropathy and postherpetic neuralgia, with relatively slow onset of action[45,114] and no effect on acute pain.[115] In several studies, the effects of these drugs were observed, or the response was augmented, only after multiple doses,[59,105,114,115] which might be a result of pharmacokinetics or of a slow mechanism of action. This is compatible with a potential effect of these drugs on calcium channel trafficking.[41,59]

Gabapentin was also reported to disrupt the in vitro interaction between $\alpha_2\delta$-1 and TSPs. In this way it has been found to disrupt synaptogenesis, although it did not affect the stability of preformed synapses.[80] This could have major clinical implications for chronic use of this drug, if confirmed.

CONCLUSIONS AND FUTURE RESEARCH

The $\alpha_2\delta$ proteins are classically known as auxiliary subunits of Ca_v channels, but they may have other functions involving binding to the extracellular matrix. The $\alpha_2\delta$-1 and $\alpha_2\delta$-2 proteins represent the binding sites for the gabapentinoid drugs gabapentin and pregabalin. The $\alpha_2\delta$-1 subunits are strongly upregulated in DRG neurons in experimental models of neuropathic pain, and this protein is trafficked from the cell bodies to the presynaptic terminals in the spinal cord. Several parallels can be drawn between neuropathic pain and epileptic seizures in terms of increased neuronal excitability and the fact that certain drugs are effective in the treatment of both conditions. However, there is, as yet, no evidence that any of the $\alpha_2\delta$ proteins are up- or downregulated in seizure models or following seizure activity. Our evidence suggests that the mechanism of action

of the gabapentinoid drugs in the alleviation of neuropathic pain involves in part an inhibition of $\alpha_2\delta$-1 subunit trafficking and associated calcium channel trafficking. However, these gabapentinoid drugs are also antiepileptic, and evidence suggests that $\alpha_2\delta$-2 may play a role in this disease, at least from rodent studies, since mutations in $\alpha_2\delta$-2, and also its targeted knockout, give rise to epilepsy phenotypes in mice. Nevertheless, there is, as yet, no direct evidence whether either $\alpha_2\delta$-1 or $\alpha_2\delta$-2 is a specific target for the gabapentinoid drugs in the alleviation of epileptic seizures. This will require the examination of whether the antiepileptic efficacy of gabapentinoid drugs is reduced in knockin mice with the point mutations described in $\alpha_2\delta$-1 or $\alpha_2\delta$-2 such that these proteins no longer bind the gabapentinoid drugs.

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REFERENCES

1. Arikkath J, Campbell KP. Auxiliary subunits: essential components of the voltage-gated calcium channel complex. *Curr Opin Neurobiol*. 2003;13:298–307.
2. Davies A, Hendrich J, Van Minh AT, Wratten J, Douglas L, Dolphin AC. Functional biology of the alpha(2)delta subunits of voltage-gated calcium channels. *Trends Pharmacol Sci.* 2007;28:220–228.
3. Bauer CS, Tran-Van-Minh A, Kadurin I, Dolphin AC. A new look at calcium channel alpha2delta subunits. *Curr Opin Neurobiol*. 2010;20:563–571.
4. Fatt P, Katz B. The electrical properties of crustacean muscle fibres. *J Physiol.* 1953;120:171–204.
5. Fatt P, Ginsborg BL. The ionic requirements for the production of action potentials in crustacean muscle fibres. *J Physiol*. 1958;142:516–543.
6. Hagiwara S, Ozawa S, Sand O. Voltage clamp analysis of two inward current mechanisms in the egg cell membrane of a starfish. *J Gen Physiol*. 1975;65:617–644.
7. Hagiwara S, Byerly L. Calcium channel. *Annu Rev Neurosci*. 1981;4:69–125.
8. Reuter H. The dependence of slow inward current in Purkinje fibres on the extracellular calcium-concentration. *J Physiol*. 1967;192:479–492.
9. Reuter H, Beeler GW Jr. Calcium current and activation of contraction in ventricular myocardial fibers. *Science.* 1969;163:399–401.
10. Fleckenstein A. History of calcium antagonists. *Circ Res*. 1983;52:I3–16.
11. Beam KG, Adams BA, Niidome T, Numa S, Tanabe T. Function of a truncated dihydropyridine receptor as both voltage sensor and calcium channel. *Nature*. 1992;360:169–171.
12. Takahashi M, Seager MJ, Jones JF, Reber BFX, Catterall WA. Subunit structure of dihydropyridine-sensitive calcium channels from skeletal muscle. *Proc Natl Acad Sci USA*. 1987;84:5478–5482.
13. Tanabe T, Takeshima H, Mikami A, Flockerzi V, Takahashi H, Kangawa K, Kojima M, Matsuo H, Hirose T, Numa S. Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature.* 1987;328:313–318.
14. Chang FC, Hosey MM. Dihydropyridine and phenylalkylamine receptors associated with cardiac and skeletal muscle calcium channels are structurally different. *J Biol Chem*. 1988;263:18929–18937.
15. Witcher DR, De Waard M, Sakamoto J, Franzini-Armstrong C, Pragnell M, Kahl SD, Campbell KP. Subunit identification and reconstitution of the N-type Ca^{2+} channel complex purified from brain. *Science*. 1993;261:486–489.
16. Liu H, De Waard M, Scott VES, Gurnett CA, Lennon VA, Campbell KP. Identification of three subunits of the high affinity w-conotoxin MVIIC-sensitive Ca^{2+} channel. *J Biol Chem*. 1996;271:13804–13810.
17. Mikami A, Imoto K, Tanabe T, Niidome T, Mori Y, Takeshima H, Narumiya S, Numa S. Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. *Nature.* 1989;340:230–233.
18. Ertel EA, Campbell KP, Harpold MM, Hofmann F, Mori Y, Perez-Reyes E, Schwartz A, Snutch TP, Tanabe T, Birnbaumer L, Tsien RW, Catterall WA. Nomenclature of voltage-gated calcium channels. *Neuron.* 2000;25:533–535.
19. Mori Y, Friedrich T, Kim M-S, Mikami A, Nakai J, Ruth P, Bosse E, Hofmann F, Flockerzi V, Furuichi T, Mikoshiba K, Imoto K, Tanabe T, Numa S. Primary structure and functional expression from complementary DNA of a brain calcium channel. *Nature.* 1991;350:398–402.
20. Dubel SJ, Starr TVB, Hell J, Ahlijanian MK, Enyeart JJ, Catterall WA, Snutch TP. Molecular cloning of the α-1 subunit of an w-conotoxin-sensitive calcium channel. *Proc Natl Acad Sci USA.* 1992;89:5058–5062.
21. Soong TW, Stea A, Hodson CD, Dubel SJ, Vincent SR, Snutch TP. Structure and functional expression of a member of the low voltage-activated calcium channel family. *Science*. 1993;260:1133–1136.
22. Perez-Reyes E. Molecular physiology of low-voltage-activated T-type calcium channels. *Physiol Rev*. 2003;83:117–161.
23. Ellis SB, Williams ME, Ways NR, Brenner R, Sharp AH, Leung AT, Campbell KP, McKenna E, Koch WJ, Hui A, Schwartz A, Harpold MM. Sequence and expression of mRNAs encoding the α_1 and α_2 subunits of a DHP-sensitive calcium channel. *Science.* 1988;241:1661–1664.

24. Gao BN, Sekido Y, Maximov A, Saad M, Forgacs E, Latif F, Wei MH, Lerman M, Lee JH, Perez-Reyes E, Bezprozvanny I, Minna JD. Functional properties of a new voltage-dependent calcium channel $\alpha_2\delta$ auxiliary subunit gene (CACNA2D2). *J Biol Chem.* 2000;275: 12237–12242.
25. Hanke S, Bugert P, Chudek J, Kovacs G. Cloning a calcium channel alpha2delta-3 subunit gene from a putative tumor suppressor gene region at chromosome 3p21.1 in conventional renal cell carcinoma. *Gene.* 2001;264:69–75.
26. Qin N, Yagel S, Momplaisir ML, Codd EE, D'Andrea MR. Molecular cloning and characterization of the human voltage-gated calcium channel $\alpha_2\delta$-4 subunit. *Mol Pharmacol*. 2002;62:485–496.
27. Whittaker CA, Hynes RO. Distribution and evolution of von Willebrand/integrin A domains: widely dispersed domains with roles in cell adhesion and elsewhere. *Mol Biol Cell.* 2002;13:3369–3387.
28. Flockerzi V, Oeken H-J, Hofmann F, Pelzer D, Cavalié A, Trautwein W. Purified dihydropyridine-binding site from skeletal muscle t-tubules is a functional calcium channel. *Nature.* 1986;323:66–68.
29. De Jongh KS, Warner C, Catterall WA. Subunits of purified calcium channels. $\alpha 2$ and δ are encoded by the same gene. *J Biol Chem*. 1990;265:14738–14741.
30. Jay SD, Sharp AH, Kahl SD, Vedvick TS, Harpold MM, Campbell KP. Structural characterization of the dihydropyridine-sensitive calcium channel α_2-subunit and the associated δ peptides. *J Biol Chem*. 1991;266: 3287–3293.
31. Fankhauser N, Maser P. Identification of GPI anchor attachment signals by a Kohonen self-organizing map. *Bioinformatics.* 2005;21:1846–1852.
32. Pierleoni A, Martelli PL, Casadio R. PredGPI: a GPI-anchor predictor. *Bioinformatics.* 2008;9:392.
33. Davies A, Kadurin I, Alvarez-Laviada A, Douglas L, Nieto-Rostro M, Bauer CS, Pratt WS, Dolphin AC. The $\alpha 2\delta$ subunits of voltage-gated calcium channels are GPI-anchored rather than trans-membrane proteins, a post-translational modification necessary for function. *Proc Natl Acad Sci USA.* 2010;107:1654–1659.
34. Anantharaman V, Aravind L. Cache-a signalling domain common to animal Ca channel subunits and a class of prokaryotic chemotaxis receptors. *Trends Biochem Sci*. 2000;25:535–537.
35. Canti C, Nieto-Rostro M, Foucault I, Heblich F, Wratten J, Richards MW, Hendrich J, Douglas L, Page KM, Davies A, Dolphin AC. The metal-ion-dependent adhesion site in the Von Willebrand factor: domain of alpha2delta subunits is key to trafficking voltage-gated Ca^{2+} channels. *Proc Natl Acad Sci USA*. 2005;102:11230–11235.
36. Walsh CP, Davies A, Nieto-Rostro M, Dolphin AC, Kitmitto A. Labelling of the 3D structure of the cardiac L-type voltage-gated calcium channel. *Channels* (*Austin*) 2009;3:387–392.
37. Wang M-C, Berrow NS, Ford RC, Dolphin AC, Kitmitto A. 3D structure of the skeletal muscle dihydropyridine receptor. *J Mol Biol.* 2002;323:85–98.
38. Wolf M, Eberhart A, Glossmann H, Striessnig J, Grigorieff N. Visualization of the domain structure of an L-type Ca^{2+} channel using electron cryo-microscopy. *J Mol Biol*. 2003;332:171–182.
39. Walsh CP, Davies A, Butcher AJ, Dolphin AC, Kitmitto A. 3D structure of CaV3.1—comparison with the cardiac L-type voltage-gated calcium channel monomer architecture. *J Biol Chem*. 2009;284: 22310–22321.
40. Bernstein GM, Jones OT. Kinetics of internalization and degradation of N-type voltage-gated calcium channels: role of the alpha(2)/delta subunit. *Cell Calcium*. 2007;41:27–40.
41. Hendrich J, Tran-Van-Minh A, Heblich F, Nieto-Rostro M, Watschinger K, Striessnig J, Wratten J, Davies A, Dolphin AC. Pharmacological disruption of calcium channel trafficking by the $\alpha 2\delta$ ligand gabapentin. *Proc Natl Acad Sci USA*. 2008;105:3628–3633.
42. Barclay J, Balaguero N, Mione M, Ackerman SL, Letts VA, Brodbeck J, Canti C, Meir A, Page KM, Kusumi K, Perez Reyes E, Lander ES, Frankel WN, Gardiner RM, Dolphin AC, Rees M. Ducky mouse phenotype of epilepsy and ataxia is associated with mutations in the Cacna2d2 gene and decreased calcium channel current in cerebellar Purkinje cells. *J Neurosci.* 2001;21:6095–6104.
43. Donato R, Page KM, Koch D, Nieto-Rostro M, Foucault I, Davies A, Wilkinson T, Rees M, Edwards FA, Dolphin AC. The ducky2J mutation in Cacna2d2 results in reduced spontaneous Purkinje cell activity and altered gene expression. *J Neurosci*. 2006;26: 12576–12586.
44. Joshi I, Taylor CP. Pregabalin action at a model synapse: binding to presynaptic calcium channel alpha2-delta subunit reduces neurotransmission in mice. *Eur J Pharmacol*. 2006;553:82–88.
45. Taylor CP, Angelotti T, Fauman E. Pharmacology and mechanism of action of pregabalin: the calcium channel alpha2-delta (alpha2-delta) subunit as a target for antiepileptic drug discovery. *Epilepsy Res*. 2007;73:137–150.
46. Fuller-Bicer GA, Varadi G, Koch SE, Ishii M, Bodi I, Kadeer N, Muth JN, Mikala G, Petrashevskaya NN, Jordan MA, Zhang SP, Qin N, Flores CM, Isaacsohn I, Varadi M, Mori Y, Jones WK, Schwartz A. Targeted disruption of the voltage-dependent Ca^{2+} channel (alpha)2/(delta)-1 subunit. *Am J Physiol Heart Circ Physiol*. 2009;297:H117–H124.
47. Gurnett CA, De Waard M, Campbell KP. Dual function of the voltage-dependent Ca^{2+} channel $\alpha_2\delta$ subunit in current stimulation and subunit interaction. *Neuron.* 1996;16:431–440.
48. Gurnett CA, Felix R, Campbell KP. Extracellular interaction of the voltage-dependent Ca^{2+} channel $\alpha_2\delta$ and α_1 subunits. *J Biol Chem*. 1997;272: 18508–18512.
49. Felix R, Gurnett CA, De Waard M, Campbell KP. Dissection of functional domains of the voltage-dependent Ca^{2+} channel alpha2delta subunit. *J Neurosci.* 1997;17:6884–6891.
50. Fosset M, Jaimovich E, Delpont E, Lazdunski M. [3H]nitrendipine receptors in skeletal muscle. *J Biol Chem*. 1983;258:6086–6092.
51. Block BA, Imagawa T, Campbell KP, Franzini-Armstrong C. Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle. *J Cell Biol.* 1988;107:2587–2600.
52. Obermair GJ, Kugler G, Baumgartner S, Tuluc P, Grabner M, Flucher BE. The Ca^{2+} channel alpha2delta-1 subunit determines Ca^{2+} current kinetics in skeletal muscle but not targeting of alpha1S

or excitation-contraction coupling. *J Biol Chem*. 2005;280:2229–2237.
53. Gach MP, Cherednichenko G, Haarmann C, Lopez JR, Beam KG, Pessah IN, Franzini-Armstrong C, Allen PD. Alpha2delta1 dihydropyridine receptor subunit is a critical element for excitation-coupled calcium entry but not for formation of tetrads in skeletal myotubes. *Biophys J*. 2008;94:3023–3034.
54. Garcia K, Nabhani T, Garcia J. The calcium channel alpha2/delta1 subunit is involved in extracellular signalling. *J Physiol*. 2008;586:727–738.
55. Chang FC, Hosey MM. Dihydropyridine and phenylalkylamine receptors associated with cardiac and skeletal muscle calcium channels are structurally different. *J Biol Chem*. 1988;263:18929–18937.
56. Marais E, Klugbauer N, Hofmann F. Calcium channel alpha(2)delta subunits— structure and gabapentin binding. *Mol Pharmacol*. 2001;59:1243–1248.
57. Cole RL, Lechner SM, Williams ME, Prodanovich P, Bleicher L, Varney MA, Gu G. Differential distribution of voltage-gated calcium channel alpha-2 delta (alpha2delta) subunit mRNA-containing cells in the rat central nervous system and the dorsal root ganglia. *J Comp Neurol*. 2005;491:246–269.
58. Taylor CP, Garrido R. Immunostaining of rat brain, spinal cord, sensory neurons and skeletal muscle for calcium channel alpha2-delta (alpha2-delta) type 1 protein. *Neuroscience*. 2008;155:510–521.
59. Bauer CS, Nieto-Rostro M, Rahman W, Tran-Van-Minh A, Ferron L, Douglas L, Kadurin I, Sri Ranjan Y, Fernandez-Alacid L, Millar NS, Dickenson AH, Lujan R, Dolphin AC. The increased trafficking of the calcium channel subunit $\alpha2\delta$-1 to presynaptic terminals in neuropathic pain is inhibited by the $\alpha2\delta$ ligand pregabalin. *J Neurosci*. 2009;29:4076–4088.
60. Wycisk KA, Budde B, Feil S, Skosyrski S, Buzzi F, Neidhardt J, Glaus E, Nurnberg P, Ruether K, Berger W. Structural and functional abnormalities of retinal ribbon synapses due to Cacna2d4 mutation. *Invest Ophthalmol Vis Sci*. 2006;47:3523–3530.
61. Kim H-L, Kim H, Lee P, King RG, Chin H. Rat brain expresses an alternatively spliced form of the dihydropyridine-sensitive L-type calcium channel $\alpha2$ subunit. *Proc Natl Acad Sci USA*. 1992;89:3251–3255.
62. Angelotti T, Hofmann F. Tissue-specific expression of splice variants of the mouse voltage-gated calcium channel $\alpha2/\delta$ subunit. *FEBS Lett*. 1996;397:331–337.
63. Su T, Meder WP, Woolf MW, Dooley DJ. Multiple alternatively spliced alpha2delta-1 calcium channel subunit variants: binding of [3H]gabapentin. *Soc Neurosci Abstr*. 2004;965:4.
64. Barclay J, Rees M. Genomic organization of the mouse and human $\alpha2\delta2$ voltage-dependent calcium channel subunit genes. *Mamm Genome*. 2000;11:1142–1144.
65. Schuck S, Simons K. Controversy fuels trafficking of GPI-anchored proteins. *J Cell Biol*. 2006;172: 963–965.
66. Nichols B. Endocytosis of lipid-anchored proteins: excluding GEECs from the crowd. *J Cell Biol*. 2009;186:457–459.
67. Davies A, Douglas L, Hendrich J, Wratten J, Tran-Van-Minh A, Foucault I, Koch D, Pratt WS, Saibil H, Dolphin AC. The calcium channel $\alpha2\delta$-2 subunit partitions with CaV2.1 in lipid rafts in cerebellum: implications for localization and function. *J Neurosci*. 2006;26:8748–8757.
68. Brown DA. Lipid rafts, detergent-resistant membranes, and raft targeting signals. *Physiology* (*Bethesda*). 2006;21:430–439.
69. Nicholson TB, Stanners CP. Specific inhibition of GPI-anchored protein function by homing and self-association of specific GPI anchors. *J Cell Biol*. 2006;175:647–659.
70. Paulick MG, Bertozzi CR. The glycosylphosphatidylinositol anchor: a complex membrane-anchoring structure for proteins. *Biochemistry*. 2008;47: 6991–7000.
71. Dustin ML, Selvaraj P, Mattaliano RJ, Springer TA. Anchoring mechanisms for LFA-3 cell adhesion glycoprotein at membrane surface. *Nature*. 1987;329: 846–848.
72. Sabharanjak S, Sharma P, Parton RG, Mayor S. GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway. *Dev Cell*. 2002;2:411–423.
73. Heblich F, Tran-Van-Minh A, Hendrich J, Watschinger K, Dolphin AC. Time course and specificity of the pharmacological disruption of the trafficking of voltage-gated calcium channels by gabapentin. *Channels*. 2008;2:4–9.
74. Dickman DK, Kurshan PT, Schwarz TL. Mutations in a Drosophila alpha2delta voltage-gated calcium channel subunit reveal a crucial synaptic function. *J Neurosci*. 2008;28:31–38.
75. Saheki Y, Bargmann CI. Presynaptic CaV2 calcium channel traffic requires CALF-1 and the alpha(2)delta subunit UNC-36. *Nat Neurosci*. 2009;12:1257–1265.
76. Bennett GJ, Chung JM, Honore M, Seltzer Z. Models of neuropathic pain in the rat. *Curr Protoc Neurosci*. 2003;chapter 9:unit 9.14.
77. Luo ZD, Chaplan SR, Higuera ES, Sorkin LS, Stauderman KA, Williams ME, Yaksh TL. Upregulation of dorsal root ganglion $\alpha_2\delta$ calcium channel subunit and its correlation with allodynia in spinal nerve-injured rats. *J Neurosci*. 2001;21:1868–1875.
78. Wanajo A, Sasaki A, Nagasaki H, Shimada S, Otsubo T, Owaki S, Shimizu Y, Eishi Y, Kojima K, Nakajima Y, Kawano T, Yuasa Y, Akiyama Y. Methylation of the calcium channel-related gene, CACNA2D3, is frequent and a poor prognostic factor in gastric cancer. *Gastroenterology*. 2008;135:580–590.
79. Gee NS, Brown JP, Dissanayake VUK, Offord J, Thurlow R, Woodruff GN. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the $\alpha_2\delta$ subunit of a calcium channel. *J Biol Chem*. 1996;271: 5768–5776.
80. Eroglu C, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, Chakraborty C, Mulinyawe SB, Annis DS, Huberman AD, Green EM, Lawler J, Dolmetsch R, Garcia KC, Smith SJ, Luo ZD, Rosenthal A, Mosher DF, Barres BA. Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell*. 2009;139:380–392.
81. Kurshan PT, Oztan A, Schwarz TL. Presynaptic alpha(2)delta-3 is required for synaptic morphogenesis independent of its Ca(2+)-channel functions. *Nat Neurosci*. 2009;12:1415–1423.
82. Kazerounian S, Yee KO, Lawler J. Thrombospondins in cancer. *Cell Mol Life Sci*. 2008;65:700–712.
83. Ly CV, Yao CK, Verstreken P, Ohyama T, Bellen HJ. Straightjacket is required for the synaptic stabilization

of cacophony, a voltage-gated calcium channel alpha1 subunit. *J Cell Biol*. 2008;181:157–170.
84. Brodbeck J, Davies A, Courtney J-M, Meir A, Balaguero N, Canti C, Moss FJ, Page KM, Pratt WS, Hunt SP, Barclay J, Rees M, Dolphin AC. The ducky mutation in Cacna2d2 results in altered Purkinje cell morphology and is associated with the expression of a truncated a2d-2 protein with abnormal function. *J Biol Chem*. 2002;277:7684–7693.
85. Brill J, Klocke R, Paul D, Boison D, Gouder N, Klugbauer N, Hofmann F, Becker CM, Becker K. entla, a novel epileptic and ataxic Cacna2d2 mutant of the mouse. *J Biol Chem*. 2004;279:7322–7330.
86. Ivanov SV, Ward JM, Tessarollo L, McAreavey D, Sachdev V, Fananapazir L, Banks MK, Morris N, Djurickovic D, Devor-Henneman DE, Wei MH, Alvord GW, Gao B, Richardson JA, Minna JD, Rogawski MA, Lerman MI. Cerebellar ataxia, seizures, premature death, and cardiac abnormalities in mice with targeted disruption of the Cacna2d2 gene. *Am J Pathol.* 2004;165:1007–1018.
87. Newton RA, Bingham S, Case PC, Sanger GJ, Lawson SN. Dorsal root ganglion neurons show increased expression of the calcium channel alpha2delta-1 subunit following partial sciatic nerve injury. *Brain Res Mol Brain Res*. 2001;95:1–8.
88. Wang H, Sun H, Della PK, Benz RJ, Xu J, Gerhold DL, Holder DJ, Koblan KS. Chronic neuropathic pain is accompanied by global changes in gene expression and shares pathobiology with neurodegenerative diseases. *Neuroscience.* 2002;114:529–546.
89. Li CY, Zhang XL, Matthews EA, Li KW, Kurwa A, Boroujerdi A, Gross J, Gold MS, Dickenson AH, Feng G, Luo ZD. Calcium channel alpha(2)delta(1) subunit mediates spinal hyperexcitability in pain modulation. *Pain*. 2006;125:20–34.
90. Wycisk KA, Zeitz C, Feil S, Wittmer M, Forster U, Neidhardt J, Wissinger B, Zrenner E, Wilke R, Kohl S, Berger W. Mutation in the auxiliary calcium-channel subunit CACNA2D4 causes autosomal recessive cone dystrophy. *Am J Hum Genet*. 2006;79:973–977.
91. Belliotti TR, Capiris T, Ekhato IV, Kinsora JJ, Field MJ, Heffner TG, Meltzer LT, Schwarz JB, Taylor CP, Thorpe AJ, Vartanian MG, Wise LD, Zhi-Su T, Weber ML, Wustrow DJ. Structure-activity relationships of pregabalin and analogues that target the alpha(2)-delta protein. *J Med Chem*. 2005;48:2294–2307.
92. Vartanian MG, Radulovic LL, Kinsora JJ, Serpa KA, Vergnes M, Bertram E, Taylor CP. Activity profile of pregabalin in rodent models of epilepsy and ataxia. *Epilepsy Res*. 2006;68:189–205.
93. Bertrand S, Ng GY, Purisai MG, Wolfe SE, Severidt MW, Nouel D, Robitaille R, Low MJ, O'Neill GP, Metters K, Lacaille JC, Chronwall BM, Morris SJ. The anticonvulsant, antihyperalgesic agent gabapentin is an agonist at brain gamma-aminobutyric acid type B receptors negatively coupled to voltage-dependent calcium channels. *J Pharmacol Exp Ther*. 2001;298: 15–24.
94. Lanneau C, Green A, Hirst WD, Wise A, Brown JT, Donnier E, Charles KJ, Wood M, Davies CH, Pangalos MN. Gabapentin is not a $GABA_B$ receptor agonist. *Neuropharmacology*. 2001;41:965–975.
95. Jensen AA, Mosbacher J, Elg S, Lingenhoehl K, Lohmann T, Johansen TN, Abrahamsen B, Mattsson JP, Lehmann A, Bettler B, Brauner-Osborne H. The anticonvulsant gabapentin (neurontin) does not act through gamma-aminobutyric acid-B receptors. *Mol Pharmacol*. 2002;61:1377–1384.
96. Taylor CP, Vartanian MG, Andruszkiewicz R, Silverman RB. 3-Alkyl GABA and 3-alkylglutamic acid analogues: two new classes of anticonvulsant agents. *Epilepsy Res*. 1992;11:103–110.
97. Loscher W, Honack D, Taylor CP. Gabapentin increases amino-oxyacetic acid-induced GABA accumulation in several regions of rat brain. *Neurosci Lett.* 1991;128:150–154.
98. Su TZ, Feng MR, Weber ML. Mediation of highly concentrative uptake of pregabalin by L-type amino acid transport in Chinese hamster ovary and Caco-2 cells. *J Pharmacol Exp Ther*. 2005;313:1406–1415.
99. Honmou O, Oyelese AA, Kocsis JD. The anticonvulsant gabapentin enhances promoted release of GABA in hippocampus: a field potential analysis. *Brain Res.* 1995;692:273–277.
100. Maneuf YP, McKnight AT. Block by gabapentin of the facilitation of glutamate release from rat trigeminal nucleus following activation of protein kinase C or adenylyl cyclase. *Br J Pharmacol*. 2001;134:237–240.
101. Zhang Y, Helm JS, Senatore A, Spafford JD, Kaczmarek LK, Jonas EA. PKC-induced intracellular trafficking of Ca(V)2 precedes its rapid recruitment to the plasma membrane. *J Neurosci.* 2008;28:2601–2612.
102. Field MJ, Cox PJ, Stott E, Melrose H, Offord J, Su TZ, Bramwell S, Corradini L, England S, Winks J, Kinloch RA, Hendrich J, Dolphin AC, Webb T, Williams D. Identification of the $\alpha 2\delta$-1 subunit of voltage-dependent calcium channels as a novel molecular target for pain mediating the analgesic actions of pregabalin. *Proc Natl Acad Sci USA.* 2006;103:17537–17542.
103. Xiao W, Boroujerdi A, Bennett GJ, Luo ZD. Chemotherapy-evoked painful peripheral neuropathy: analgesic effects of gabapentin and effects on expression of the alpha-2-delta type-1 calcium channel subunit. *Neuroscience.* 2007;144:714–720.
104. Hao JX, Xu XJ, Urban L, Wiesenfeld-Hallin Z. Repeated administration of systemic gabapentin alleviates allodynia-like behaviors in spinally injured rats. *Neurosci Lett.* 2000;280:211–214.
105. Fox A, Gentry C, Patel S, Kesingland A, Bevan S. Comparative activity of the anti-convulsants oxcarbazepine, carbamazepine, lamotrigine and gabapentin in a model of neuropathic pain in the rat and guinea-pig. *Pain*. 2003;105:355–362.
106. Gong HC, Hang J, Kohler W, Li L, Su TZ. Tissue-specific expression and gabapentin-binding properties of calcium channel alpha2delta subunit subtypes. *J Membr Biol*. 2001;184:35–43.
107. Klugbauer N, Marais E, Hofmann F. Calcium channel alpha2delta subunits: differential expression, function, and drug binding. *J Bioenerg Biomembr*. 2003;35:639–647.
108. Dissanayake VUK, Gee NS, Brown JP, Woodruff GN. Spermine modulation of specific [^{3}H]-gabapentin binding to the detergent-solubilized porcine cerebral cortex $\alpha_2\delta$ calcium channel subunit. *Br J Pharmacol*. 1997;120:833–840.
109. Schumacher TB, Beck H, Steinhäuser C, Schramm J, Elger CE. Effects of phenytoin, carbamazepine, and gabapentin on calcium channels in hippocampal

granule cells from patients with temporal lobe epilepsy. *Epilepsia.* 1998;39:355–363.
110. Stefani A, Spadoni F, Bernardi G. Gabapentin inhibits calcium currents in isolated rat brain neurons. *Neuropharmacology.* 1998;37:83–91.
111. Martin DJ, McClelland D, Herd MB, Sutton KG, Hall MD, Lee K, Pinnock RD, Scott RH. Gabapentin-mediated inhibition of voltage-activated Ca^{2+} channel currents in cultured sensory neurones is dependent on culture conditions and channel subunit expression. *Neuropharmacology.* 2002;42:353–366.
112. Sutton KG, Martin DJ, Pinnock RD, Lee K, Scott RH. Gabapentin inhibits high-threshold calcium channel currents in cultured rat dorsal root ganglion neurones. *Br J Pharmacol.* 2002;135:257–265.
113. Tran Van Minh A, Dolphin AC. Gabapentin inhibits the Rab11-dependent recycling of the calcium channel subunit α2δ-2". *J Neurosci.* 2010;30:12856–12867.
114. Stacey BR, Barrett JA, Whalen E, Phillips KF, Rowbotham MC. Pregabalin for postherpetic neuralgia: placebo-controlled trial of fixed and flexible dosing regimens on allodynia and time to onset of pain relief. *J Pain.* 2008;9:1006–1017.
115. Moore RA, Straube S, Wiffen PJ, Derry S, McQuay HJ. Pregabalin for acute and chronic pain in adults. *Cochrane Database Syst Rev.* 2009;CD007076.

Chapter 76

Targeting SV2A for Discovery of Antiepileptic Drugs

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Levetiracetam (LEV) is a novel antiepileptic drug (AED) displaying a unique preclinical profile. Despite initial findings showing LEV to be active against audiogenic seizures in mice,[1] later studies failed to show significant activity in classical seizure screening tests such as maximal electroshock (MES) and pentylenetetrazole (PTZ).[2] Interestingly, the same study reported significant seizure protection against fully amygdala-kindled seizures in rats.[2] This unique profile associated with a wide safety margin has also been confirmed in later studies utilizing a range of epilepsy models, including amygdala kindling.[3] Furthermore, LEV significantly inhibited the development of seizure kindling,[4] which may be consistent with its potential antiepileptogenic effect.[5] This unique profile triggered significant interest in unraveling the molecular mechanism of action of LEV. Numerous studies explored the interaction of LEV with mechanisms known to account for the antiseizure activity of established AEDs including GABAergic (gamma-aminobutric acid) and glutamatergic neurotransmission and inhibition of voltage-gated Na^+ and Ca^{2+} channels.[6–8] None of these studies succeeded in identifying any major effects of LEV. In contrast, pioneering studies at UCB more than 15 years ago resulted in the identification of a brain-specific LEV binding site (LBS) for [^{3}H]-LEV that was not shared with any other AED.[9] This breakthrough finding, combined with the later identification of LBS, provided an important rationale to pursue drug discovery efforts aimed at identifying new high-affinity ligands for this novel binding site with antiepileptic properties potentially superior to those of LEV.[10,11] In that context, approximately 12,000 compounds were screened in vitro for binding affinity to LBS; 900 compounds were examined for seizure protection in audiogenic-susceptible mice, and 30 compounds were

characterized broadly in a variety of animal models of seizures and epilepsy. Out of these efforts, two lead candidates, seletracetam and brivaractam, were discovered and underwent clinical testing.[12] Due to a more potent and complete suppression of seizures than provided by LEV in animal models mimicking both partial and generalized seizures, brivaracetam was finally selected for further clinical studies in epilepsy.[13,14] Analysis of the two initial Phase III add-on studies indicated significant efficacy of brivaracetam; however, different results were obtained, depending on patient subpopulations and doses tested (UCB press release, April 28, 2009). Therefore, a confirmatory Phase III program is currently underway. The data accumulated with LEV and brivaracetam support the promise of LBS as a novel target for AED discovery. This highlights the strong interest in pursuing further studies aimed at unraveling the molecular nature and consolidating the validity of LBS as an AED target.

IDENTIFICATION OF SV2A AS THE BINDING TARGET FOR LEV

Our group has demonstrated that LBS is enriched in purified synaptic vesicle fraction. In fact, [^{3}H]ucb 30889, a derivative of LEV, labeled a synaptic vesicle protein with an approximate molecular mass of 90 kDa, which was consistent with the molecular mass of synaptic vesicle protein 2A (SV2A).[15] Binding studies were then performed in brain membranes obtained from SV2A transgenic mice to determine whether SV2A is indeed necessary for LEV binding. We found that [^{3}H]ucb 30889 binds only to membranes from SV2A$^{+/+}$ mice and not to those obtained from SV2A$^{-/-}$ mice.[15] Additional in vitro binding experiments performed in brain membranes confirmed that the B_{max} value for [^{3}H]ucb 30889 is reduced by 50% in SV2A$^{+/-}$ compared to the B_{max} value obtained with brain membranes of their wild-type littermates.[16] These observations were further corroborated by ex vivo binding experiments. Injection of LEV into both SV2A$^{+/+}$ and SV2A$^{+/-}$ mice revealed that that SV2A$^{+/-}$ mice have half of the protein available for binding compared to their wild-type littermates.[16] It should be noted, however, that both the radioligand dissociation constants (K_d) in brain membranes and the inhibitory concentration of 50% (IC_{50}) values of LEV calculated from the ex vivo binding curves were comparable between SV2A$^{+/+}$ and SVA$^{+/-}$ mice, confirming that SV2A binding properties were not modified in transgenic animals.[16] In fact, the binding characteristics of native SV2A in human and rat brain share very similar properties.[17] Furthermore, the binding affinities of several SV2A ligands are also comparable when measured in human brain, in rat brain, and when the recombinant human SV2A protein is expression in Chinese hamster ovary (CHO) cells.[17]

BIOLOGY AND FUNCTION OF SV2

A series of molecular studies described above has led to the identification of SV2A as the molecular correlate of LBS; however, the exact role of this protein in synaptic vesicle cycle and neurotransmitter release still remains elusive. Synaptic vesicle 2 proteins exist as three separate subtypes (SV2A, SV2B, and SV2C) and are an integral part of secretory vesicle membranes localized in different mammalian secretory organs, with the highest expression being observed in the brain.[18–21] Synaptic vesicle 2 proteins have 12 transmembrane domains and are members of the major facilitator superfamily (MFS) of membrane transporters.[18,22] LacY, the prototypic member of the MFS proteins, has been shown to exist under two major conformations, and the transition between these two conformations would allow the transport of lactose.[23] Similar changes in conformation have been proposed for other members of this family of proteins, which also includes neurotransmitter cotransporters.[24] The existence of two major conformations adopted by SV2A proteins in mouse brain was also recently reported by Lynch et al. using protein tomography.[25] One of the conformations has a funnel structure with the opening toward the cytosol, and the second displays a V shape with the opening toward the luminal space. Both conformational states of the protein were present in controls samples and samples treated with LEV, indicating that binding of LEV does not induce an obvious conformational change of SV2A and does not stabilize a specific conformational state of the protein.[25] This apparent lack of effect of LEV on the conformational states of

the SV2A protein may have been hampered by the limitations of the protein tomography technology and its resolution. In that context, as the functional role of SV2A is still largely unknown today, except for its probable involvement in synaptic vesicle cycling and in exo- or endocytosis processes, the downstream consequences of compounds binding to SV2A have not yet been elucidated. One could imagine that upon binding to SV2A, each compound could induce or stabilize a conformational change that would be more or less favorable in terms of function, meaning that compounds could show various degree of intrinsic efficacy. If proven correct, this hypothesis opens the possibility that newer SV2A ligands bearing higher intrinsic efficacy upon binding could either show clinical efficacy at lower levels of SV2A occupancy or even be efficacious in refractory patients.

Synaptic vesicle 2A is the best studied of the three SV2 proteins. To date, the majority of the information about its role in neuronal excitability has been obtained in experiments involving knockout mice.[21,26–29] It appears that SV2A is not crucial for vesicle biogenesis or synaptic function, but instead modulates the exocytosis of transmitter-containing vesicles.[26,28] Mice lacking SV2A are characterized by a decrease in the calcium-dependent exocytotic burst, which is a measure of the availability of neurotransmitter vesicles ready to release their content.[28] Moreover, lack of SV2A results in decreased action potential-dependent neurotransmission, while action potential-independent neurotransmission remains normal.[26,28] Very recent reports seem to confirm the hypothesis that SV2 proteins play an important role in calcium-dependent neurotransmission.[30–32] Other recent data seem to indicate that SV2A also plays a role in structural changes (volume increase) of synaptic vesicles upon loading with glutamate.[33] Finally, SV2A may influence synaptic vesicle priming regulated by binding of adenine nucleotides.[34]

VALIDATION OF SV2A AS A DRUG TARGET FOR AEDS

Affinity–Potency Correlation Studies

A key observation initially suggesting that SV2A is the target site for the principal mechanism of action of LEV was the fact that the anticonvulsant potency of selective SV2A ligands strongly correlated with their in vitro binding affinity. Such correlations between the in vitro affinity of ligands and their potency in inhibiting audiogenic seizures in mice have been documented using SV2A from rat cerebral cortex[9] and human recombinant SV2A expressed in cell lines.[15] Our more recent studies confirmed and extended these initial findings to other animal models of epilepsy, that is, corneal kindling and absence seizures in Genetic Absence Epilepsy Rats from Strasbourg (GAERS).[35] The anticonvulsant potency of SV2A ligands against audiogenic seizures correlated well with their in vitro binding affinity ($r^2 = 0.77$; $p < 0.001$; Fig. 76–1A). Similar correlation ($r^2 = 0.80$; $p < 0.01$) between anticonvulsant activity and SV2A protein in vitro binding was also observed in corneally kindled mice (Fig. 76–1B). Finally, correlation between in vitro SV2A binding and inhibition of spike-wave discharges in GAERS has also been documented ($r^2 = 0.72$; $p < 0.01$; Fig. 76–1C). It is important to note that despite differences in species and epilepsy models, that is, the audiogenic model, corneal kindling, and GAERS, the slopes and intercepts of regression lines were not statistically different. These experiments demonstrated the existence of a strong correlation between SV2A binding affinity and anticonvulsant potency in three distinct preclinical epilepsy models, which reinforced the significance of this molecular target in the mechanism of action of the tested ligands.[35] It also suggested that SV2A-related mechanisms are equally important in protection against seizures irrespective of the model. This was a surprising finding since different mechanisms and brain regions are likely to be involved in the generation of seizure activity observed in these models. Yet, it appears that SV2A protein is essential for neuronal synchronization fundamentally associated with every type of epileptiform activity. Taken together, these data strongly support the notion that targeting SV2A results in an anticonvulsant activity relevant for both partial and generalized epilepsy, and thereby can provide antiepileptic drug candidates with a potential for broad-spectrum clinical efficacy.[35]

Although these correlations were helpful in validating the main target for the anticonvulsant properties of selective SV2A ligands, in vitro data do not take into account individual pharmacokinetic properties of compounds

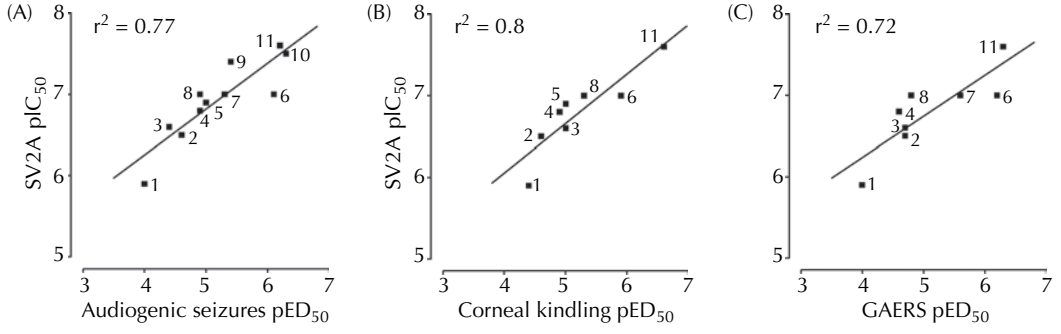

Figure 76–1. Correlation between binding affinity and protective potency of SV2A ligands against convulsive seizures (**A**, audiogenic seizures and **B**, corneal kindling) and (**C**) absence seizures in GAERS rats. Synaptic vesicle protein 2A binding affinities –log IC_{50} (pIC_{50}) were measured in rat brain membranes with the use of [^{3}H]ucb 30889. Antiseizure potencies, based on dose-response studies, are shown as –log ED_{50} (pED_{50}). From ref. 35.

such as protein binding, brain penetration, and metabolism that may affect the concentration of the drug in the compartment of interest and hence binding to its target. The fact that we observed good correlations between in vitro binding and in vivo efficacy indicates that most compounds tested were close structural analogues sharing very similar physicochemical and pharmacokinetic properties. Another major limitation of using in vitro binding data is the inability to predict the level of SV2A occupancy that compounds need to reach in vivo in order to afford seizure protection. To address these issues, we performed ex vivo binding experiments in which binding of compounds with selective affinity for SV2A was measured in vitro in brain homogenates from animals that have been administered the compounds prior to being sacrificed. When corrected for experimental conditions (essentially dilution factors), ex vivo binding data are comparable to in vivo binding data, as shown in Fig. 76–2, where ex vivo and in vivo binding dose-response curves of LEV overlap. The disadvantage of in vivo

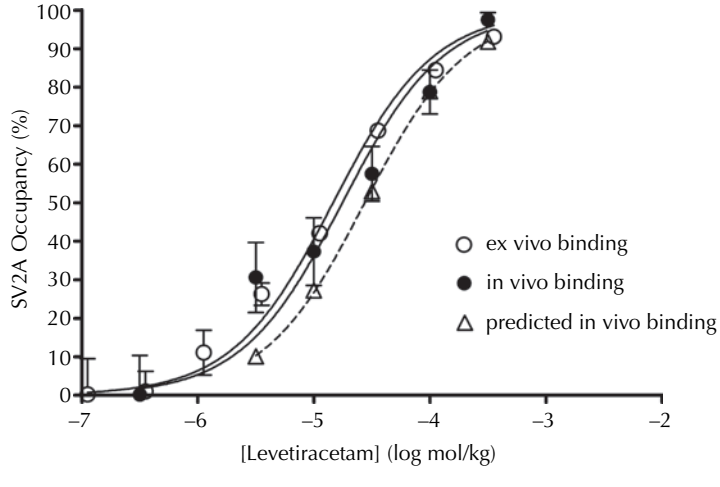

Figure 76–2. Ex vivo and in vivo binding of LEV to SV2A in mouse brain. Increasing doses of LEV were administered intraperitoneally (ip) to mice. The mice were sacrificed 60 min postadministration. For ex vivo binding, the brains were removed and homogenized, and binding using [^{3}H]ucb 30889 was performed as described in Gillard et al.[42] For in vivo binding, [^{3}H] ucb 30889 was injected into the tail vein 5 min prior to sacrifice. The brains were removed, homogenized at 4°C, and quickly filtered to retain the bound radioligand. Synaptic vesicle 2A occupancy of 0% was determined in saline-treated animals, and 100% occupancy was defined by the nonspecific binding measured in the presence of an excess of lLEV (1 mM). Four and six animals per dose were used for ex vivo and in vivo experiments, respectively, and each binding was performed in duplicate or triplicate. Predicted in vivo binding was calculated as explained in the text, taking into account the plasma concentration corrected for protein binding (10%), the brain-to-plasma ratio (0.4), and the affinity of LEV measured at 37°C (8 μM).

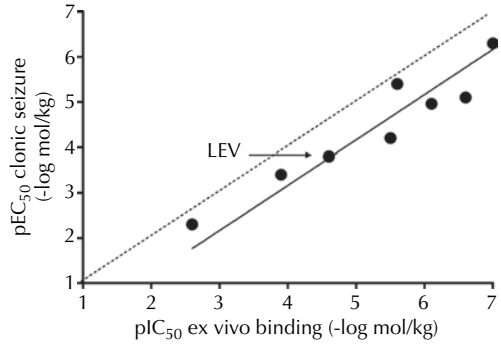

Figure 76–3. Correlation between ex vivo binding affinity and protection against clonic seizures in audiogenic mice. Increasing doses of SV2A ligands were administered ip to mice. The mice were sacrificed 60 min postadministration. The brains were removed and homogenized, and binding using [^{3}H]ucb 30889 was performed as described in Gillard et al.[42] pIC_{50} is the dose of compound leading to 50% of SV2A occupancy ($n = 2$ mice per dose). In parallel experiments, audiogenic seizures were induced by subjecting sound-susceptible mice to a 90 dB, 10 to 20 kHz acoustic stimulus for 30 s. The occurrence of clonic and tonic convulsions was recorded. pED_{50} is the dose leading to 50% protection against clonic seizures ($n = 10$ mice per dose).

compared to ex vivo binding experiments is related to the fact that a substantial amount of radioactive tracer needs to be injected into each animal. Not unexpectedly, we observed a good correlation between SV2A occupancy measured by ex vivo binding and protection against clonic seizures in audiogenic mice, as shown in Fig. 76–3 for a series of compounds. However, it appears quite clear that the doses of compounds needed to occupy 50% of SV2A are generally lower than the doses needed to afford 50% seizure protection ($pIC_{50} > pED_{50}$), that is, high SV2A occupancy is required to provide pharmacological activity (Fig. 76–3).

Anticonvulsant Effects of LEV and Their Relation to SV2A Occupancy

Using the same ex vivo binding approach, we explored the relationship between SV2A occupancy and seizure protection in audiogenic mice as a function of time after single-dose administration. The results obtained with LEV are depicted in Fig. 76–4. We found that the postadministration time needed for LEV to occupy SV2A maximally was well correlated with the time needed for maximal protection against clonic and tonic seizures. Interestingly, protection against the more severe clonic seizures is lost prior to the decrease in protection against tonic seizures. This suggests that lower amounts of SV2A proteins need to be occupied to prevent later-stage less severe (tonic) seizures, while higher occupancy is needed to prevent both forms of seizures (clonic + tonic). We have also accumulated similar data for other SV2A ligands that display a variety of occupancy-protection patterns. Some compounds quickly occupy SV2A and afford maximal protection against seizures faster than LEV, while other compounds display a more rapid decline in occupancy and seizure protection than LEV. This link between SV2A occupancy and efficacy in a time-dependent manner further strengthens the role of SV2A as the relevant target in the mechanism of action of these drugs.

From dose-response and kinetic experiments, as shown in Figs. 76–2 and 76–4, it appears that LEV needs to occupy nearly 90% of SV2A in order to protect against clonic seizures in audiogenic mice. It is also clear that there is a threshold level of occupancy that needs to be reached to afford seizure protection and that minute variations around this threshold are sufficient to prevent or not prevent the occurrence of a seizure. To illustrate this, a group of 10 mice were treated with LEV; 2 h postadministration, 6 mice were protected against sound-elicited clonic seizures and 4 were not. We measured the SV2A occupancy in each mouse and found, quite surprisingly, that the individual SV2A receptor occupancy was nearly identical for all animals. We had expected that in protected mice, the level of SV2A occupancy would be higher than that in nonprotected mice (reflecting interindividual variations due to slight differences in drug administration and/or pharmacokinetics). This might be explained by the fact that a seizure is not a gradual, but rather a binary response, and therefore even small differences in SV2A occupancy might be sufficient to be either above or below the threshold for a seizure to happen. This is in agreement with the rather steep dose-response curves observed for LEV in showing protection against audiogenic[1] or corneally kindled seizures in mice.[36]

To further ascertain whether high occupancy of SV2A is also needed to afford seizure protection in patients, we predicted SV2A occupancy by LEV in the brains of human patients based on affinity and available pharmacokinetic data.

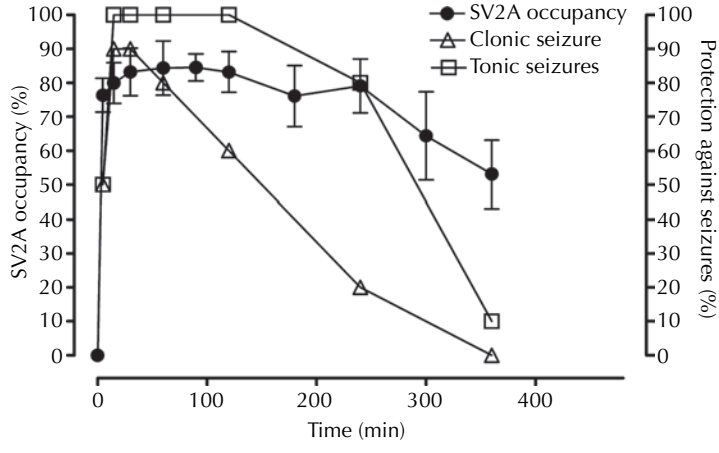

Figure 76–4. Time course of SV2A occupancy and seizure protection in audiogenic mice after a single administration of LEV. A 210 μmol/kg dose of LEV was administered ip. Synaptic vesicle 2A occupancy and protection against tonic and clonic seizures were assessed at several time points after administration. Synaptic vesicle 2A occupancy was measured by ex vivo binding. The mice were sacrificed and the brains were removed and homogenized; binding using [^{3}H]ucb 30889 was performed as described in Gillard et al.[42] Synaptic vesicle 2A occupancy of 0% was determined in saline-treated animals, and 100% occupancy was defined by the nonspecific binding measured in the presence of an excess of LEV (1 mM; $n = 3$ mice per time point). In parallel experiments, audiogenic seizures were induced by subjecting sound-susceptible mice to a 90 dB, 10 to 20 kHz acoustic stimulus for 30 s. The occurrence of clonic and tonic convulsions was recorded ($n = 10$ mice per time point).

We used the following equation describing a single bimolecular interaction between a ligand and a receptor:

[Levetiracetam] refers to the free concentration of LEV in the brain. This was approximated by using total plasma concentrations measured in healthy volunteers and patients (UCB data on file; see ref. 37) corrected for plasma protein binding of 10%.[38] Brain-to-plasma ratios of 0.4 to 1 were considered based on studies in rodents.[39] Ki is the equilibrium dissociation constant (affinity) of LEV for SV2A protein. We took the value of 8 μM that was measured at 37°C in brain tissue. We first validated this approach by predicting, in the same way, the SV2A occupancy in mouse brain 60 min after administration using total plasma concentrations corrected for protein binding, and using a brain-to-plasma ratio of 0.4, as reported for mouse.[39] The predicted SV2A occupancy related to administered dose is shown in Fig. 76–2, and it compares quite satisfactorily with the experimental data obtained from ex vivo and in vivo binding experiments.

The total plasma concentrations of LEV at a clinically active daily dose of 1 g range from 6–8 μg/mL (C_{min}) to 15–20 μg/mL (C_{max}; UCB data on file; see ref. 37). The plasma concentrations have been shown to be linearly proportional to the administered dose[38] and can be adjusted accordingly. Using the above equation and assuming a brain-to-plasma ratio of 1, the predicted SV2A occupancy by LEV in patients varies from 80% to 93% at C_{min} and C_{max} for a 1 g daily dose and from 92% to 98% for a 3 g daily dose. With a less favorable brain-to-plasma ratio of 0.5, the predicted values are 67% (C_{min}) and 87% (C_{max}) for a 1 g daily dose and 86% (C_{min}) and 98% (C_{max}) for a 3 g daily dose. These predicted levels of SV2A occupancy in patients are remarkably close to those measured in animal models of epilepsy at active pharmacological doses and confirm that high SV2A occupancy is needed by current SV2 ligands to afford adequate protection against seizure.

Pharmacology and Phenotyping of SV2A Transgenic Animals in Seizure Models

Direct evidence that the in vivo anticonvulsant activity of LEV is indeed mediated by SV2A has been lacking until recently.[16] Genetically engineered knockout animals, which lack a given drug target, are frequently used to

prove the in vivo selectivity of pharmacological agents and to demonstrate the lack of therapeutic activity in the absence of the molecular target. Similar proof of concept evidence could have been obtained by testing LEV in SV2A$^{-/-}$ homozygous mice. However, these animals suffer from severe seizures starting very early in their development and do not survive beyond 2–3 weeks after birth, which precludes their use in pharmacological in vivo experiments.[26,29] Therefore, we decided to use SV2A$^{+/-}$ mice, which are deficient in the SV2A protein but develop normally after birth. First, we demonstrated by video-electroencephalographic monitoring that SV2A$^{+/-}$ mice do not display any overt epileptic phenotype, but rather show pro-epileptic traits such as decreased seizure thresholds and accelerated kindling development. The pro-epileptic phenotype of SV2A$^{+/-}$ mice was observed in kindling, pilocarpine, kainate, pentylenetetrazol (intravenous) and 6 Hz models, but not in the MES model.[16] Interestingly, the pro-epileptic phenotype of SV2A$^{+/-}$ in a range of different experimental seizure models appeared as a "mirror image" of the unique pharmacological profile of LEV in the same models.[3]

We also demonstrated for the first time a functional involvement of SV2A in mediating the anticonvulsant effect of LEV, which was indeed reduced in SV2A$^{+/-}$ mice.[16] This was illustrated by its failure to produce the same degree of increase in the threshold for induction of 6 Hz seizures in SV2$^{+/-}$ mice as in their wild-type littermates (Fig. 76–5). In contrast, valproate, which has SV2A-unrelated mechanisms of action,[9] produced the same magnitude of threshold increase in both genotypes (Fig. 76–5). We decided to use an unbiased approach for this comparison and ascertain

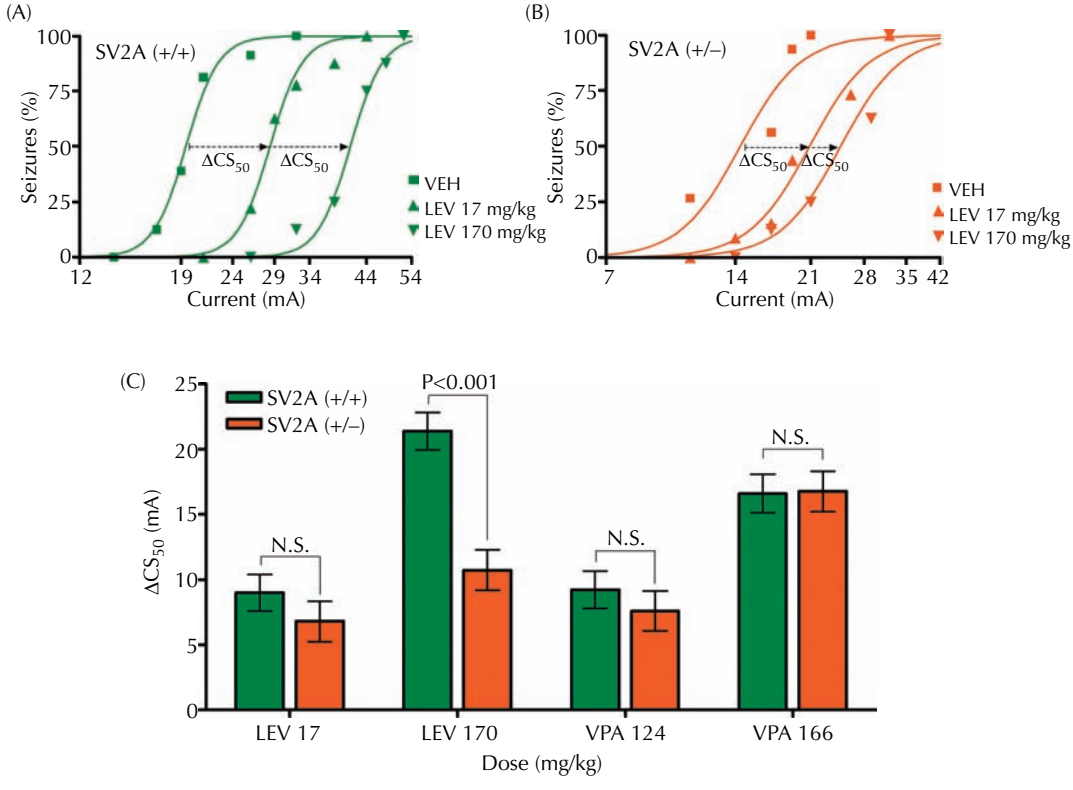

Figure 76–5. Current responses for seizures induced by 6 Hz electrical stimulation after treatment with LEV in SV2A$^{+/+}$ mice (**A**) and SV2A$^{+/-}$ mice (**B**). Points representing the percentage of animals ($n \geq 8$ per group) responding with seizures were used for sigmoidal curve fitting. The CS_{50} value represents the current required to induce convulsions in 50% of animals. **C.** The $\Delta CS_{50s} = [CS_{50\,(DRUG)} — CS_{50\,(SALINE)}] \pm$ SEM value calculated after treatment with two different doses of LEV or valproate (VPA). Values were compared statistically with Student's *t*-test. From ref. 16.

whether the same doses of LEV and valproate would produce the same magnitude of threshold increase for 6 Hz seizures in both $SV2A^{+/+}$ and $SV2A^{+/-}$ mice. Remarkably, this was true only in the case of valproate. A low dose of valproate produced an increase in the threshold for 6 Hz seizures that was comparable between $SV2A^{+/+}$ and $SV2A^{+/-}$ mice (Fig. 76–5). A higher dose of valproate further increased the threshold, which again was almost identical in the two genotypes. Similarly, a low dose of LEV produced comparable increases in the seizure threshold in both genotypes. However, in contrast to valproate, a higher dose of LEV failed to provide an additional threshold increase in the $SV2A^{+/-}$ mice, while the threshold was further increased in $SV2A^{+/+}$ mice. In fact, a higher dose of LEV produced a 50% higher increase in threshold in wild-type mice compared to the increase obtained in $SV2^{+/-}$ mice. It is important to remember that $SV2A^{+/-}$ mice still express 50% of the SV2A protein; thus, occupancy of SV2A by LEV may afford some protection against seizures. Furthermore, $SV2A^{+/-}$ mice have a significantly reduced threshold for 6 Hz seizures, and at lower stimulation currents it might have been somewhat easier to elevate the threshold with the same dose of LEV, which in fact has the same SV2A affinity in both genotypes. The difference in the effects of LEV on the seizure threshold became significant only at the dose that occupied nearly all of the SVA2 binding sites in both genotypes, but since $SV2A^{+/-}$ mice have 50% fewer sites available for LEV binding, the degree of seizure protection was also reduced by approximately 50%.

CHANGES IN SV2A EXPRESSION IN PRECLINICAL MODELS AND HUMAN EPILEPSY

Discovery of SV2A as the binding target for LEV prompted investigations of the potential role of SV2A in the pathophysiology of epilepsy. Van Vliet et al.[40] used immunohistochemistry and Western blot analysis to study SV2A expression patterns during epileptogenesis and chronic epilepsy. Hippocampal samples from autopsy controls, patients who died from status epilepticus, and pharmacoresistant temporal epilepsy (TLE) patients were analyzed. Additionally, SV2A expression was assessed in the hippocampus of rats at different stages of epileptogenesis in a post-status epilepticus model. Remarkable consistency has been observed between human and rat samples. That is, SV2A expression was significantly decreased in the hippocampus of TLE patients with hippocampal sclerosis and also in the mossy fiber terminals during the latent and chronic phases of epileptogenesis in rats.[40] Based on these results, it is not possible to establish a clear cause-effect relationship between reduced expression of SV2A and development of epilepsy, but these data are very consistent with the above-described pro-epileptic phenotype of SV2A-deficnt mice and the accelerated epileptogenesis observed in these animals.[16] Since SV2A is the binding site of LEV and the drug shows reduced efficacy in SV2A-deficient animals,[16] the data of van Vliet et al.[40] may also explain the apparent lack of efficacy of LEV in some patients with TLE. It is conceivable that reduced expression of the target for LEV, namely the SV2A protein, could underlie the cause of nonresponsiveness to the drug in some patients. This hypothesis can be verified by comparison of SV2A expression levels assessed by positron emission tomography (PET) with patients' response or nonresponse to LEV. It is plausible that SV2A expression changes may be responsible for different responses to LEV, because pharmacogenetic studies failed to identify SV2A genetic variants that influence the response to LEV.[41] Future SV2A PET ligands may also allow the study of occupancy–efficacy relationships to allow full translation of the data we have obtained in animal models to human epilepsy.

CONCLUSION

Synaptic vesicle 2A constitutes the unique binding site for LEV and plays an important role in synaptic vesicle function. Affinity–potency correlations in several models of partial and generalized epilepsy indicate that SV2A is a broad-spectrum anticonvulsant target. The anticonvulsant activity of LEV is closely linked with occupancy and availability of SV2A, whereas SV2A deficiency leads to increased seizure vulnerability and accelerated epileptogenesis. Taken together, existing experimental data prove that SV2A plays a crucial role in

mediating the anticonvulsant action of LEV in vivo and indicate that the SV2A protein represents an important and well-validated target for the discovery of novel AEDs. Finally, the finding that SV2A protein is a clinically validated target for epilepsy has triggered further discovery programs exploring the therapeutic potential for SV2A ligands with different binding properties.

DISCLOSURE STATEMENT

All the authors are full-time employees of UCB Pharma, the manufacturer of levetiracetam.

REFERENCES

1. Gower AJ, Noyer M, Verloes R, Gobert J, Wülfert E. ucb L059, a novel anti-convulsant drug: pharmacological profile in animals. *Eur J Pharmacol*. 1992;222: 193–203.
2. Löscher W, Hönack D. Profile of ucb L059, a novel anticonvulsant drug, in models of partial and generalized epilepsy in mice and rats. *Eur J Pharmacol*. 1993;232:147–158.
3. Klitgaard H, Matagne A, Gobert J, Wülfert E. Evidence for a unique profile of levetiracetam in rodent models of seizures and epilepsy. *Eur J Pharmacol.* 1998;353: 191–206.
4. Löscher W, Hönack D, Rundfeldt C. Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. *J Pharmacol Exp Ther*. 1998;284:474–479.
5. Margineanu DG, Matagne A, Kaminski RM, Klitgaard H. Effects of chronic treatment with levetiracetam on hippocampal hyperexcitability developing after pilocarpine-induced status epilepticus in rats. *Brain Res Bull*. 2008;77:282–285.
6. Niespodziany I, Klitgaard H, Margineanu DG. Levetiracetam inhibits the high-voltage-activated Ca^{2+} current in pyramidal neurones of rat hippocampal slices. *Neurosci Lett.* 2001;306:5–8.
7. Lukyanetz EA, Shkryl VM, Kostyuk PG. Selective blockade of N-type calcium channels by levetiracetam. *Epilepsia.* 2002;43:9–18.
8. Rigo J-M, Hans G, Nguyen L, et al. The anti-epileptic drug levetiracetam reverses the inhibition by negative allosteric modulators of neuronal GABA- and glycine-gated currents. *Br J Pharmacol*. 2002; 136:659–672.
9. Noyer M, Gillard M, Matagne A, Henichart JP, Wulfert E. The novel antiepileptic drug levetiracetam (Ucb L059) appears to act via a specific binding site in CNS membranes. *Eur J Pharmacol*. 1995;286:137–146.
10. Klitgaard H. Levetiracetam: the preclinical profile of a new class of antiepileptic drugs? *Epilepsia*. 2001; 42(suppl 4):13–18.
11. Klitgaard H, Verdru P. Levetiracetam: the first SV2A ligand for the treatment of epilepsy. *Expert Opin Drug Disc*. 2007;2:1537–1545.
12. Kenda BM, Matagne AC, Talaga PE, Pasau PM, Differding E, Lallemand BI, Frycia AM, Moureau FG, Klitgaard HV, Gillard MR, Fuks B, Michel P. Discovery of 4-substituted pyrrolidone butanamides as new agents with significant antiepileptic activity. *J Med Chem.* 2004;47:530–549.
13. Matagne A, Margineanu DG, Kenda B, Michel P, Klitgaard H. Anti-convulsive and anti-epileptic properties of brivaracetam (ucb 34714), a high-affinity ligand for the synaptic vesicle protein, SV2A. *Br J Pharmacol*. 2008;154:1662–1671.
14. Matagne A, Margineanu DG, Potschka H, Löscher W, Michel P, Kenda B, Klitgaard H. Profile of the new pyrrolidone derivative seletracetam (ucb 44212) in animal models of epilepsy. *Eur J Pharmacol*. 2009;614: 30–37.
15. Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, Fuks B. The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. *Proc Natl Acad Sci USA*. 2004;101: 9861–9866.
16. Kaminski RM, Gillard M, Leclercq K, Hanon E, Lorent G, Dassesse D, Matagne A, Klitgaard H. Proepileptic phenotype of SV2A-deficient mice is associated with reduced anticonvulsant efficacy of levetiracetam. *Epilepsia.* 2009;50:1729–1740.
17. Gillard M, Chatelain P, Fuks B. Binding characteristics of levetiracetam to synaptic vesicle protein 2A (SV2A) in human brain and in CHO cells expressing the human recombinant protein. *Eur J Pharmacol*. 2006;536:102–108.
18. Bajjalieh SM, Peterson K, Shinghal R, Scheller RH. SV2, a brain synaptic vesicle protein homologous to bacterial transporters. *Science*. 1992;257:1271–1273.
19. Buckley K, Kelly RB. Identification of a transmembrane glycoprotein specific for secretory vesicles of neural and endocrine cells. *J Cell Biol*. 1985;100:1284–1294.
20. Feany MB, Lee S, Edwards RH, Buckley KM. The synaptic vesicle protein SV2 is a novel type of transmembrane transporter. *Cell.* 1992;70:861–867.
21. Janz R, Südhof TC. SV2C is a synaptic vesicle protein with an unusually restricted localization: anatomy of a synaptic vesicle protein family. *Neuroscience.* 1999;94:1279–1290.
22. Saier MH Jr, Beatty JT, Goffeau A, Harley KT, Heijne WH, Huang SC, Jack DL, Jähn PS, Lew K, Liu J, Pao SS, Paulsen IT, Tseng TT, Virk PS. The major facilitator superfamily. *J Mol Microbiol Biotechnol*. 1999;1:257–279.
23. Holyoake J, Sansom MS. Conformational change in an MFS protein: MD simulations of LacY. *Structure.* 2007;15:873–884.
24. DeFelice LJ. Transporter structure and mechanism. *Trends Neurosci*. 2004;27:352–359.
25. Lynch BA, Matagne A, Brannstrom A, von Euler A, Jansson M, Hauzenberger E, Soderhall JA. Visualization of SV2A conformations in situ by the use of protein tomography. *Biochem Biophys Res Commun*. 2008;375:491–495.
26. Crowder KM, Gunther JM, Jones TA, Hale BD, Zhang HZ, Peterson MR, Scheller RH, Chavkin C, Bajjalieh SM. Abnormal neurotransmission in mice

lacking synaptic vesicle protein 2A (SV2A). *Proc Natl Acad Sci USA*. 1999;96,15268–15273.
27. Custer KL, Austin NS, Sullivan JM, Bajjalieh SM. Synaptic vesicle protein 2 enhances release probability at quiescent synapses. *J Neurosci*. 2006;26:1303–1313.
28. Xu T, Bajjalieh SM. SV2 modulates the size of the readily releasable pool of secretory vesicles. *Nat Cell Biol*. 2001;3:691–698.
29. Janz R, Goda Y, Geppert M, Missler M, Südhof TC. SV2A and SV2B function as redundant Ca^{2+} regulators in neurotransmitter release. *Neuron*. 1999;24: 1003–1016.
30. Yao J, Nowack A, Kensel-Hammes P, Gardner RG, Bajjalieh SM. Cotrafficking of SV2 and synaptotagmin at the synapse. *J Neurosci*. 2010;30:5569–5578.
31. Wan Q-F, Zhen-Yu Zhou Z-Y, Thakur P, Vila A, Sherry DM, Janz R, Heidelberger R. SV2 acts via presynaptic calcium to regulate neurotransmitter release. *Neuron*. 2010;66:884–895.
32. Chang WP, Südhof TC. SV2 renders primed synaptic vesicles competent for Ca^{2+}-induced exocytosis. *J Neurosci*. 2009;29:883–897.
33. Budzinski KL, Allen RW, Fujimoto BS, Kensel-Hammes P, Belnap DM, Bajjalieh SM, Chiu DT. Large structural change in isolated synaptic vesicles upon loading with neurotransmitter. *Biophys J*. 2009;97: 2577–2584.
34. Yao J, Bajjalieh SM. Synaptic vesicle protein 2 binds adenine nucleotides. *J Biol Chem*. 2008;283: 20628–20634.
35. Kaminski RM, Matagne A, Leclercq K, Gillard M, Michel P, Kenda B, Talaga P, Klitgaard H. SV2A protein is a broad-spectrum anticonvulsant target: functional correlation between protein binding and seizure protection in models of both partial and generalized epilepsy. *Neuropharmacology*. 2008;54:715–720.
36. Matagne A, Klitgaard H. Validation of corneally kindled mice: a sensitive screening model for partial epilepsy in man. *Epilepsy Res*. 1998;31:59–71.
37. Perruca E, Gidal BE, Baltès E. Effects of antiepileptic co-medication on levetiracetam pharmacokinetics: a pooled analysis of data from randomized adjunctive therapy trials. *Epilepsy Res*. 2003;53:47–56.
38. Patsalos PN. Pharmacokinetic profile of levetiracetam: toward ideal characteristics. *Pharmacol Ther*. 2000;85:77–85.
39. Benedetti MS, Coupez R, Whomsley R, Nicolas JM, Collart P, Baltes E. Comparative pharmacokinetics and metabolism of levetiracetam, a new anti-epileptic agent, in mouse, rat, rabbit and dog. *Xenobiotica*. 2004;34:281–300.
40. van Vliet EA, Aronica E, Redeker S, Boer K, Gorter JA. Decreased expression of synaptic vesicle protein 2A, the binding site for levetiracetam, during epileptogenesis and chronic epilepsy. *Epilepsia*. 2009;50: 422–433.
41. Lynch JM, Tate SK, Kinirons P, Weale ME, Cavalleri GL, Depondt C, Murphy K, O'Rourke D, Doherty CP, Shianna KV, Wood NW, Sander JW, Delanty N, Goldstein DB, Sisodiya SM. No major role of common SV2A variation for predisposition or levetiracetam response in epilepsy. *Epilepsy Res*. 2009;83: 44–51.
42. Gillard M, Fuks B, Michel P, Vertongen P, Massingham R, Chatelain P. Binding characteristics of [3H]ucb 30889 to levetiracetam binding sites in rat brain. *Eur J Pharmacol*. 2003;478:1–9.

Chapter 77

Neurosteroids—Endogenous Regulators of Seizure Susceptibility and Role in the Treatment of Epilepsy

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INTRODUCTION

The term *neurosteroid* was coined in 1981 by the French endocrinologist Étienne-Émile Baulieu to refer to steroids that are synthesized de novo in the nervous system from cholesterol independently of the peripheral steroidogenic endocrine glands.[1] Paul and Purdy then characterized *neuroactive steroids* as "natural or synthetic steroids that rapidly alter the excitability of neurons by binding to membrane-bound receptors such as those for inhibitory and (or) excitatory neurotransmitters."[2] This chapter is concerned with the anticonvulsant and antiepileptogenic properties of neurosteroid-related steroid molecules (i.e., related to endogenously synthesized steroids) that meet the Paul and Purdy definition of neuroactive steroids by virtue of their pharmacological actions as positive allosteric modulators of γ-aminobutyric acid A ($GABA_A$) receptors. The effect of these steroids on $GABA_A$ receptors occurs through a direct action on $GABA_A$ receptors and is not related to interactions with classical steroid hormone receptors that regulate gene transcription. Indeed, $GABA_A$ receptor modulatory

neurosteroids are not themselves active at intracellular steroid receptors. We will make scant mention of other endogenous neurosteroids that exert other types of pharmacological actions, such as inhibition of $GABA_A$ receptors or effects on excitatory amino acid receptors (pregnenolone sulfate is an example of such a steroid), although it is conceivable that neurosteroids with such actions could play a role in regulating seizure susceptibility.

It has been known since the 1940s, from the pioneering work of Hans Selye, that naturally occurring steroids such as the ovarian steroid progesterone and the adrenal steroid deoxycorticosterone (DOC) can exert anesthetic and anticonvulsant actions.[3] Recognizing that some steroids could produce such acute central nervous system effects, researchers at the pharmaceutical company Glaxo identified the synthetic steroid alphaxolone as having anesthetic properties. In the early 1970s, alphaxolone was marketed as a component of the intravenous anesthetic agent Althesin, which also included the less potent anesthetic steroid alphadalone acetate, said to increase the solubility of alphaxolone.[4] Several years later, the mechanism of action of alphaxolone was defined: it was found to enhance synaptic inhibition via an action on $GABA_A$ receptors.[5,6] A major advance occurred when naturally occurring metabolites of progesterone and DOC were also found to enhance and directly activate $GABA_A$ receptors.[7] It was speculated that the anesthetic and hypnotic properties of progesterone and DOC known since the time of Selye were due to their conversion in the body to these metabolites, respectively: allopregnanolone (3α-hydroxy-5α-pregnane-20-one) and allotetrahydrodeoxycorticosterone (3α,21-dihydroxy-5α-pregnan-20-one; THDOC). At the time, it was recognized that the enzymes required for the conversion of the steroid hormone precursors to their active A-ring reduced metabolites are present in brain so that part of the synthesis of the $GABA_A$ receptor active steroids could occur locally. Therefore, the neuroactive steroids allopregnanolone and THDOC came to be referred to as *neurosteroids* even though it was not believed at the time that their synthesis occurred independently of peripherally synthesized precursor steroid hormones. We now know that all of the enzymes required for the synthesis of the $GABA_A$ receptor active steroids from cholesterol are present in the brain.[8] It is well recognized that these steroids readily cross the blood-brain barrier. While it is likely that locally synthesized $GABA_A$ receptor active steroids play a role in modulating circuit excitability, there is little information on the relative importance of de novo local synthesis versus peripheral production of either the active $GABA_A$ receptor modulatory steroids (e.g., allopregnanolone or THDOC) or their precursor hormones (e.g., progesterone or DOC), which are converted locally by brain 5α-reductase and 3α-hydroxysteroidoxidoreductase (3α-HSOR). Nevertheless, it is common to refer to $GABA_A$ receptor modulatory steroids such as allopregnanolone and THDOC as neurosteroids, and we will follow that practice here. This chapter reviews the potential roles of such neurosteroids as endogenous modulators of seizure susceptibility and also the more limited evidence that they can, under certain circumstances, influence epileptogenesis (transformation of the brain to an epileptic state). The discussion of these topics serves as a prelude to the main objective of this chapter, which is to review the evidence supporting the utility of exogenously administered neurosteroid-related agents in the treatment of epilepsy.

DIVERSITY OF NEUROSTEROIDS AND THEIR BIOSYNTHESIS

A variety of $GABA_A$ receptor modulatory neurosteroids are known to be synthesized endogenously (Figs. 77–1 and 77–2). The best recognized of these are the pregnane neurosteroids allopregnanolone and THDOC, which are produced via sequential A-ring reduction of the steroid hormones progesterone and its 21-hydroxylated derivative deoxycorticosterone by 5α-reductase and 3α-HSOR isoenzymes. In the periphery, the steroid precursors are mainly synthesized in the gonads, adrenal gland, and feto-placental unit, but as noted above, synthesis of both of these neurosteroids likely occurs in the brain from cholesterol or from peripherally derived intermediates including the steroid hormone precursors. A-ring reduction can also occur in peripheral tissues such as reproductive endocrine tissues, liver, and skin that are rich in the two reducing activities.[8] Since neurosteroids are highly lipophilic and can readily cross the blood-brain barrier, neurosteroids

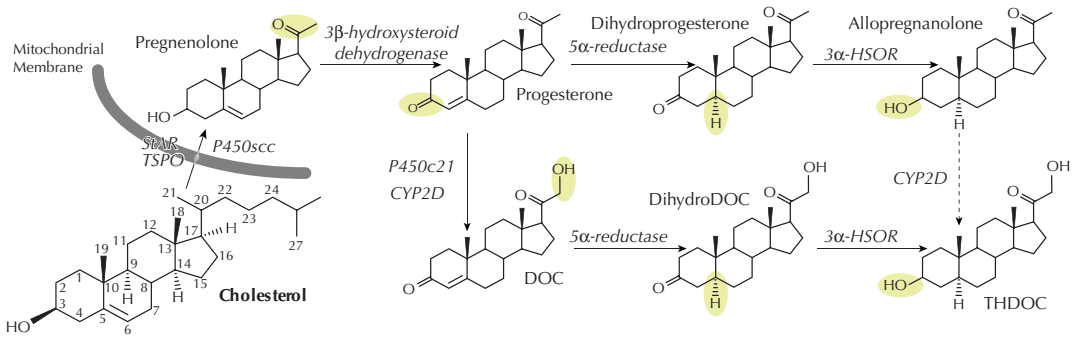

Figure 77–1. Biochemical pathways of neurosteroid biosynthesis. Cholesterol is trafficked by StAR (steroidogenic acute regulatory protein) and TSPO (translocator protein; 18 kDa) to the inner mitochondrial membrane, where it is converted to pregnenolone by P450sc (cytochrome P450 side-chain cleavage). Pregnenolone is the precursor for progesterone that can undergo the two sequential A-ring reduction steps catalyzed by 5α-reductase and 3α-HSOR (3α-hydroxysteroid oxidoreductase) to form the neurosteroid allopregnanolone. Alternatively, in the zona reticulata of the adrenal cortex, P450c21 (cytochrome P450 21-hydroxylase) converts progesterone to DOC (deoxycorticosterone), which is the precursor for the neurosteroid THDOC (allotetrahydrodeoxycorticosterone). In the brain, this reaction appears to be mainly catalyzed by CYP2D isoenzymes, which can also convert allopregnanolone to THDOC.

synthesized in peripheral tissues accumulate in the brain and can influence brain function.

The 5β-isomers of allopregnanolone and THDOC have $GABA_A$ receptor modulatory activity that is only modestly less potent than that of the corresponding 5α-epimers.[9] A steroid 5β-reductase enzyme is distributed widely in vertebrates, including in the gonads, liver, and brain.[10] However, whether progesterone or DOC is a substrate, and the extent to which 5β-reduced epimers of allopregnanolone and THDOC are produced endogenously, is unclear.

The androgenic steroid testosterone differs from progesterone by virtue of a 17-alcohol that replaces the 17-acetyl in progesterone. Testosterone is a substrate for both 5α-reductase and 5β-reductase isoenzymes.

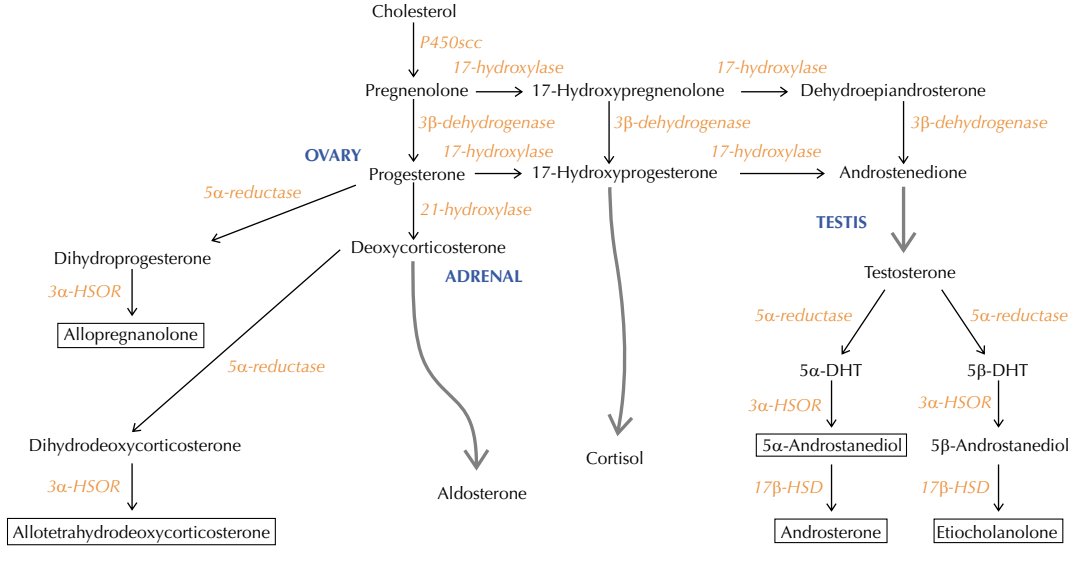

Figure 77–2. General scheme of peripheral steroidogenesis showing side pathways for the production of known neurosteroids. Steroids known to act on $GABA_A$ receptors in a fashion similar to that of the prototypic neurosteroids allopregnanolone and allotetrahydrodeoxycorticosterone are boxed.

The product of 5α-reduction of testosterone, 5α-dihydrotestosterone, is hormonally more active than testosterone itself. However, subsequent 3α-reduction leads to 5α-androstanediol (5α-androstane-3α,17β-diol), which is comparable in potency and efficacy to allopregnanolone as a $GABA_A$ receptor-positive modulator.[11] 5α- and 5β-Androstanediol are further metabolized by 17β-hydroxysteroid dehydrogenase to androsterone and etiocholanolone, respectively, which are considered the major excreted metabolites of testosterone. These compounds and their conjugates are present at high concentrations. Androsterone and etiocholanolone also have $GABA_A$ receptor-positive modulatory activity and represent endogenous neurosteroids.[12] Collectively, the various $GABA_A$ receptor modulatory steroids that lack the pregnane 17β-ethyl moiety, such as 5α-androstanediol, androsterone, and etiocholanolone, can be considered androstane neurosteroids. Finally, substantial amounts of androstenol, the 16-unsaturated form of 5α-androstanediol, are present in mammals, including humans. This compound is considered to be a pheromone that increases sexual receptivity in pigs and possibly other species. Androstenol also is a $GABA_A$ receptor-positive modulator that has similar efficacy but is modestly less potent than allopregnanolone.[13]

PRODUCTION OF NEUROSTEROIDS IN THE BRAIN AND THEIR LOCALIZATION TO PRINCIPAL NEURONS

In addition to peripheral production, it is clear that neurosteroids can be formed from steroid hormone precursors (such as progesterone, DOC, and perhaps testosterone) locally in the brain. 5α-Reductase activity has been identified in both neurons and glial cells in rodent and sheep brain, in regions, such as the neocortex and hippocampus, that are relevant to epilepsy.[14,15] 3α-HSOR is also expressed widely in the brain.[16] In humans, both enzymes have been found in neocortex and hippocampus.[17–19] Thus, it is likely that neurosteroids can be formed from their parent steroid hormone precursors directly in the brain. Steroid precursors readily enter the brain, so pools of peripherally synthesized precursors are available for local neurosteroid biosynthesis. In peripheral tissues 5α-reductase is believed to be the rate-limiting step in the production of neurosteroids because 3α-HSOR is a more ubiquitous enzyme; the same situation likely applies in brain.

It is likely that neurosteroids can be produced locally in brain not only from their steroid hormone precursors but also from more elementary steroid precursors such as cholesterol or pregnenolone. The rate-limiting and initial step in steroidogenesis is the conversion of cholesterol to pregnenolone by the mitochondrial enzyme P450scc (cytochrome P450 cholesterol side-chain cleavage enzyme; CYP11A). Access of cholesterol to P450scc requires StAR (steroidogenic acute regulatory protein), which functions to transfer cholesterol from the outer mitochondrial membrane to the inner membrane where P450scc is located.[20] Translocator protein 18 kD (TSPO), formerly called *peripheral* or *mitochondrial benzodiazepine receptor*, likely functions as a complex with StAR.[21,22] Both proteins are expressed widely in peripheral tissues and in the brain. Activation of TSPO by certain ligands facilitates the intramitochondrial flux of cholesterol and thereby increases the availability of cholesterol to P450scc, enhancing pregnenolone synthesis and ultimately neurosteroid production.[23,24] The observation that TSPO ligands enhance neurosteroid production not only confirms the key role of TSPO in neurosteroidogenesis, but also suggests that such ligands may have therapeutic utility as an alternative to exogenously administered neurosteroids in situations where it is desirable to increase the level of brain neurosteroids.[25]

In addition to P450scc, 3β-hydroxysteroid dehydrogenase, an enzyme required for the conversion of pregnenolone to progesterone, has been demonstrated in the brain.[26] Thus, the enzymes necessary for in situ synthesis of progesterone from cholesterol are present in the brain.

In the adrenal, P450c21 (cytochrome P450 21-hydroxylase) converts progesterone to DOC, which is the precursor for the neurosteroid THDOC. The brain also possesses 21-hydroxylase activity, but it expresses only very small amounts of P450c21 mRNA and protein. It appears that cytochrome P450 2D (CYP2D) isoforms, in particular CYP2D4, present in brain can 21-hydroxylate progesterone to form DOC, which can then be converted to

THDOC.[27] In addition, allopregnanolone itself is a substrate for CYP2D4, so that in brain, allopregnanolone may be converted directly to THDOC. Allopregnanolone and THDOC persist in the brain after adrenalectomy and gonadectomy or after pharmacological suppression of adrenal and gonadal steroid synthesis,[28,29] confirming that these two key neurosteroids can be synthesized independently of peripherally produced steroid hormone precursors. However, the regulatory mechanisms underlying neurosteroid biosynthesis in the brain remain unclear.

In studies with mouse and rat brain, in situ hybridization with mRNA probes to 5α-reductase and 3α-HSOR indicates that the two mRNAs colocalize to glutamatergic principal neurons and not GABAergic inhibitory neurons or glial cells within neocortex, hippocampus, amygdala, and other brain regions.[30] Immunohistochemistry with an antiserum raised against allopregnanolone that also recognizes THDOC confirms that the neurosteroids are concentrated in principal neurons, predominantly in cell bodies and thick dendrites.[31] The highly restricted distribution of neurosteroids to principal neurons suggests that they are mainly derived from local synthesis and not from the circulation, although it is clear that peripheral neurosteroids, as previously noted, readily cross the blood-brain barrier. It is remarkable that brain neurosteroids are localized to the neurons that contain their targets ($GABA_A$ receptors). This observation is consistent with the notion that neurosteroids function in an autocrine fashion in which they reach their targets by lateral membrane diffusion.[32]

NEUROSTEROID MODULATION OF $GABA_A$ RECEPTORS

In electrophysiological studies, allopregnanolone and THDOC at aqueous concentrations in the range 10–1500 nM enhance the activation of $GABA_A$ receptors by GABA.[9,33] At higher concentrations, the steroids directly activate the receptor in the absence of GABA. In addition, like other positive allosteric modulators of $GABA_A$ receptors, neurosteroids exert allosteric effects on these receptors such that there is enhancement of the binding of [^{3}H]flunitrazepam, a benzodiazepine receptor agonist, and [^{3}H]muscimol, a specific GABA-site agonist, as well as inhibition of the binding of [^{35}S]*t*-butylbicycloorthobenzoate (TBPS), a cage convulsant and noncompetitive $GABA_A$ receptor antagonist.[34,35] Neurosteroid enhancement of $GABA_A$ receptors occurs through increases in both channel open frequency and channel open duration.[36–38] Thus, neurosteroids greatly enhance the probability of $GABA_A$ receptor chloride channel opening, thereby enhancing $GABA_A$ receptor-mediated inhibition.

The effects of neurosteroids on $GABA_A$ receptors occur by binding to discrete sites on the receptor-channel complex that are located within the transmembrane domains of the α and β subunits.[38] The binding sites for neurosteroids are distinct from the recognition sites for GABA, benzodiazepines, and barbiturates. Although the exact location of neurosteroid binding has not been mapped, it has been proposed that there are two distinct sites for neurosteroids that act as positive modulators: one for allosteric enhancement of GABA and another for direct activation of the receptor.[37,38] Using site-directed mutagenesis, it has been shown that a highly conserved glutamine at position 241 in the M1 domain (toward the intracellular side) of the α subunit plays a key role in neurosteroid modulation of GABA responses and is believed to contribute to the binding site for modulation.[39] Additional nearby residues in the M4 domain of the same α subunit (tyrosine 410 and asparagines 407, which are located more toward the extracellular side) have also been proposed to contribute to the binding site. Other investigators have found that mutations in serine 240 and tryptophan 245 of the α subunit interfere with neurosteroid potentiation.[40] Studies with structurally diverse steroids have led to the conclusion that the steroid binding pocket on the α subunit is more correctly viewed as a *hydrophobic surface* that can accommodate steroid molecules of different structures.[40] Direct activation of the receptor, in contrast, has been proposed to be due to binding at a site on the interface between β and α subunits formed by a threonine at position 236 in the α subunit and a tyrosine at position 284 in the β subunit.[38] However, more recent models of

the $GABA_A$ receptor have questioned whether these residues reside at the β subunit–α subunit interface.[41] A photo-incorporable analog of the anesthetic etomidate appears to bind at the interface, but binding of this ligand is not competitively inhibited by neurosteroids.[41] In fact, neurosteroids (at concentrations that produce direct receptor activation) enhance binding, presumably due to allosteric effects transmitted upon interaction with a different site on the receptor. The newer topology models do not bring into proximity the residues in the β and α subunits proposed to constitute the site for direct activation. Therefore, at present, the location of this site is uncertain.

A range of steroid structures have activity as positive modulators of $GABA_A$ receptors in line with the hydrophobic surface binding site model. Nevertheless, there are certain strict structural requirements for neurosteroid-positive modulation. A hydrogen bond-donating 3α-hydroxy group on the steroid A-ring and a hydrogen bond-accepting group (typically a keto moiety) on the D-ring at either C20 of the pregnane steroid side chain or C17 of the androstane ring system are critical for positive modulatory activity at $GABA_A$ receptors.[37,42] The orientation of the C5 hydrogen group only modestly influences potency.[9]

Studies with recombinant $GABA_A$ receptor isoforms indicate that neurosteroids act on most subunit combinations.[37,43] This distinguishes neurosteroids from benzodiazepines, which only act on $GABA_A$ receptors that contain γ2 subunits and do not contain α4 or α6 subunits. In general, the specific α subunit type may influence neurosteroid efficacy, whereas the γ subunit type may affect both the efficacy and potency of neurosteroid modulation.[37]

GABA is a relatively low-efficacy agonist of $GABA_A$ receptors in which the δ subunit replaces the more common γ2 subunit, even though it binds with high affinity to such δ-subunit-containing receptors.[44] Neurosteroids therefore have an opportunity to markedly enhance the current generated by δ-subunit-containing $GABA_A$ receptors even in the presence of saturating GABA concentrations. Consequently, $GABA_A$ receptors that contain the δ subunit are highly sensitive to neurosteroid-induced potentiation of GABA responses,[45,46] and mice lacking δ subunits show drastically reduced sensitivity to neurosteroids.[47–49] $GABA_A$ receptors containing δ subunits exhibit low desensitization, and they are located nonsynaptically (perisynaptically/extrasynaptically) since the γ2 subunit is required for synaptic targeting. These properties cause them to be prime candidates for mediating *tonic* $GABA_A$ receptor current that is activated by ambient concentrations of GABA in the extracellular space. Ambient GABA is believed to result from spillover of synaptically released GABA; concentrations would increase with high levels of activity of GABAergic interneurons, as occurs during seizures. Tonic $GABA_A$ receptor current causes a steady inhibition of neurons and reduces their excitability. Neurosteroids could therefore have a general role in setting the level of excitability and might specifically potentiate tonic inhibition during seizures when ambient GABA may rise. Overall, the robust effect of neurosteroids is likely to be due to their action on both synaptic and perisynaptic/extrasynaptic $GABA_A$ receptors.

Although neurosteroids are viewed as high-potency modulators of $GABA_A$ receptors since they are effective at concentrations in the mid-nanomolar to low-micromolar range in aqueous solution, recent studies indicate that neurosteroid binding to the $GABA_A$ receptor is actually of low affinity (K_d, ~1 mM).[32] The high effective potency of neurosteroids results from partitioning of the lipophilic steroids within the plasma membrane, such that the concentrations presented to the receptor are orders of magnitude greater. Neurosteroids access the $GABA_A$ receptor from the lipophilic plasma membrane. The nonspecific accumulation and removal of the neurosteroids from the membrane are the major factors determining the rates of neurosteroid action when applied to cells via aqueous solution; rates of binding and unbinding to the receptor are only secondary factors.[50] It is noteworthy that intracellular delivery through the plasma membrane is compatible with the autocrine mechanism discussed above, in which the neurosteroids act on the $GABA_A$ receptors in the same neurons in which they are produced.

As noted, at high concentrations (>10 μM), neurosteroids can directly activate $GABA_A$ receptor channels in the absence of GABA.[9,51,52] In this respect, neurosteroids resemble barbiturates but not benzodiazepines.[53] Given the high concentrations required, whether direct

actions are relevant to the role of endogenous neurosteroids or to the pharmacological actions of exogenously administered neurosteroid-related agents is not well understood.

ANTICONVULSANT AND ANTIEPILEPTOGENIC EFFECTS OF NEUROSTEROIDS

Exogenously administered neurosteroids, like other agents that act as positive $GABA_A$ receptor modulators, exhibit broad-spectrum anticonvulsant effects in diverse rodent seizure models. They protect against seizures induced by $GABA_A$ receptor antagonists including pentylenetetrazol (PTZ) and bicuculline, and they are effective against pilocarpine-induced limbic seizures and seizures in kindled animals.[9,54–57] However, neurosteroids may exacerbate generalized absence seizures.[58,59] The potencies of neurosteroids in models where they confer seizure protection vary largely in accordance with their activities as positive allosteric modulators of $GABA_A$ receptors. Thus, allopregnanolone has roughly equal potency to THDOC, but androstanediol, androsterone, and etiocholanolone are somewhat less potent.[12,60,61] Like other anticonvulsant agents that act on $GABA_A$ receptors, neurosteroids are inactive or only weakly active against electrically induced tonic extension seizures elicited according to the maximal electroshock (MES) protocol that is widely used for drug screening. However, they are active in the 6 Hz model in mice in which limbic-like seizures are induced by electrical stimulation of lower frequency and longer duration than in the MES test.[62] In general, neurosteroids have comparable potencies in the 6 Hz and PTZ models. Neurosteroids are also highly effective in suppressing seizures due to withdrawal of $GABA_A$ receptor modulator drugs including neurosteroids and benzodiazepines (diazepam), and also due to other types of agents such as ethanol, which may act in part through $GABA_A$ receptors.[63–65] In contrast to benzodiazepines, where utility in the chronic treatment of epilepsy is limited by tolerance, anticonvulsant tolerance is not obtained with neurosteroids.[66,67] Thus, neurosteroids have the potential to be used in the chronic treatment of epilepsy, and this has been borne out in clinical trials (see below). The mechanisms responsible for tolerance to benzodiazepines are not known. However, factors such as uncoupling of the allosteric linkage between the GABA and benzodiazepine sites and changes in receptor subunit turnover with switching of subunits may be contributing mechanisms.[68] Neurosteroids do not act on the benzodiazepine site of $GABA_A$ receptors, and they are able to modulate all isoforms of $GABA_A$ receptors, even those that contain benzodiazepine-insensitive $\alpha 4$ and $\alpha 6$ subunits or do not include the obligatory $\gamma 2$ subunit required for benzodiazepine sensitivity. Thus, it is clear that neurosteroids can act on $GABA_A$ receptors where the proposed benzodiazepine tolerance mechanisms have been invoked. Surprisingly, while chronic neurosteroid exposure does not lead to anticonvulsant tolerance, chronic neurosteroid exposure does lead to tolerance to benzodiazepines.[67] Thus, it appears that the same plastic changes that underlie benzodiazepine tolerance are brought into play by chronic neurosteroid exposure. However, neurosteroids, acting at distinct sites on $GABA_A$ receptors and exhibiting effects on the full range of $GABA_A$ receptor isoforms, do not exhibit anticonvulsant tolerance.

The sulfated neurosteroids pregnenolone sulfate and dehydroepiandosterone sulfate, which act as $GABA_A$ receptor antagonists, are pro-convulsant when administered at high doses into the brain, producing seizures and status epilepticus.[69,70] Compelling evidence that such steroids exist endogenously in the brain is lacking, and in any case it is unlikely that they exist at sufficiently high concentrations to exert pro-convulsant effects, so the physiological relevance is unclear. However, it is known that the seizure-facilitating effects of these steroids can be blocked by coadministration of allopregnanolone or other neurosteroids that positively modulate $GABA_A$ receptors.[71]

In addition to anticonvulsant activity, there is some limited evidence that endogenous neurosteroids play a role in regulating epileptogenesis.[72–74] Following pilocarpine-induced status epilepticus in the rat, the neurosteroidogenic enzyme P450scc is upregulated for several weeks, suggesting that neurosteroidogenesis may be increased. Ordinarily, rats develop spontaneous recurrent seizures following a latent period of similar duration to the period during which P450scc is elevated. Inhibiting neurosteroid synthesis with

finasteride accelerated the onset of spontaneous recurrent seizures, suggesting that endogenous neurosteroids play a role in restraining epileptogenesis or at least that they inhibit the expression of seizures. Exogenous treatment with neurosteroids or with progesterone, which serves as a precursor for neurosteroid synthesis, has also been reported to delay the occurrence of epileptogenesis in some situations.[75] In fact, progesterone may impair epileptogenesis in kindling models, even at doses that do not affect seizure expression.[76,77] If endogenous neurosteroids can be confirmed as endogenous regulators of epileptogenesis, neurosteroids themselves or modulators of neurosteroid disposition could potentially have disease-modifying therapeutic activity.

ROLE OF ENDOGENOUS NEUROSTEROIDS IN THE MODULATION OF SEIZURES

Endogenous neurosteroids may play a role in the physiological regulation of seizure susceptibility in individuals with epilepsy. We will discuss several such situations: catamenial epilepsy, stress, temporal lobe epilepsy, and alcohol withdrawal. However, it is noteworthy that there is no evidence that alterations in neurosteroid levels in the absence of preexisting epilepsy can induce epileptogenesis.

Neurosteroids in Catamenial Epilepsy

Catamenial epilepsy, the cyclical occurrence of seizure exacerbations during particular phases of the menstrual cycle in women with preexisting epilepsy, is a specific form of pharmacoresistant epilepsy. Catamenial seizure exacerbations affect up to 70% of women of childbearing age with epilepsy.[78–80] Although there are several forms of catamenial epilepsy, neurosteroids have been implicated only in the seizure exacerbations that occur in the most common situation, which is when women with normal menstrual cycles experience seizure exacerbations in the perimenstrual period. It is hypothesized that withdrawal of progesterone-derived neurosteroids leads to enhanced brain excitability predisposing to seizures.

During the menstrual cycle, circulating progesterone levels are low in the follicular phase but rise in the midluteal phase for about 10 to 11 days before declining in the late luteal phase. Circulating allopregnanolone levels parallel those of its parent progesterone.[81] Circulating THDOC levels also fluctuate during the menstrual cycle, with higher levels in the luteal phase.[81] Overall, the serum levels of THDOC are lower than those of allopregnanolone, so THDOC is likely to be less relevant to catamenial epilepsy, although it could contribute. An important unanswered question is whether the local brain synthesis of neurosteroids also fluctuates.

In addition to withdrawal of the anticonvulsant effects of neurosteroids in association with the fall in progesterone at the time of menstruation, plasticity in $GABA_A$ receptors, the targets of neurosteroid action, could also play a role in the enhanced brain excitability that is presumed to underlie the increase in seizure susceptibility in perimenstrual catamenial epilepsy. The precise changes in brain $GABA_A$ receptor subunit expression occurring during the human menstrual cycle have not been determined. However, it is now well recognized that prolonged exposure to allopregnanolone in rats causes increased expression of the $\alpha 4$ $GABA_A$ receptor subunit in hippocampus, resulting in decreased benzodiazepine sensitivity of $GABA_A$ receptor currents.[82,83] Although $\alpha 4$ can coassemble with $\gamma 2$ to form synaptic $GABA_A$ receptors, it preferentially coassembles with δ to form nonsynaptic (perisynaptic/extrasynaptic) $GABA_A$ receptors. Treatment of rats with allopregnanolone results in transient increased expression of the δ subunit in hippocampus and increased benzodiazepine-insensitive tonic current.[84,85] Progesterone also increases δ subunit expression, likely as a result of conversion to allopregnanolone. The relevance of the increased δ subunit expression for catamenial epilepsy is unclear, as δ subunit increases may be transitory and followed by *reduced* expression with chronic exposures, as in pregnancy or in the prolonged luteal phase of the human menstrual cycle. Therefore, an important consequence of the incorporation of the normally low-abundance $\alpha 4$ subunit into synaptic $GABA_A$ receptors is that synaptic currents generated by these receptors have accelerated decay kinetics, so that there is less total charge transfer, which results in

reduced inhibition.[86] $GABA_A$ receptor-modulating neurosteroids cause a prolongation of the decay of GABA-mediated synaptic currents. Consequently, in the presence of high levels of allopregnanolone during the luteal phase, the acceleration due to α4 substitution is balanced. However, when neurosteroids are withdrawn at the time of menstruation, synaptic inhibition is diminished from normal, resulting in enhanced excitability, which, among other effects, predisposes to seizures. Indeed, chronic exposure to neurosteroids also is accompanied by downregulation of δ subunit expression and perisynaptic/extrasynaptic $GABA_A$ receptors.[84] This change is believed to be a compensatory mechanism, which would avoid excessive sedation caused by high neurosteroid levels acting on sensitive δ subunit-containing $GABA_A$ receptors. At the time of neurosteroid withdrawal, δ subunit expression rapidly recovers. However, if recovery is not sufficiently fast, there could be an enhancement of excitability due to a reduction in tonic inhibition mediated by perisynaptic/extrasynaptic $GABA_A$ receptors in the relative absence of neurosteroids.

A rodent model has been developed to simulate the hormonal changes that are believed to be relevant to perimenstrual catamenial epilepsy.[87,88] Rodents have a 4 to 5 day estrous cycle, and studies of fluctuations in seizure susceptibility in cycling female rodents have not led to results that are relevant to the human menstrual cycle. In order to provide a model that more closely mimics the human situation, a condition of pseudopregnancy was induced in rats by sequential gonadotropin treatment. This resulted in prolonged high circulating levels of estrogen and progesterone similar to those that occur in the luteal phase of the 28 day human menstrual cycle. Then, to simulate the withdrawal of allopregnanolone that occurs in conjunction with the fall in progesterone levels at the time of menstruation, the animals were treated with finasteride 11 days after the initiation of gonadotropin treatment.

The neurosteroid withdrawal model of catamenial epilepsy was used to investigate therapies for perimenstrual catamenial epilepsy.[63,89] A key result is that conventional antiepileptic drugs, including benzodiazepines and valproate, have reduced potency in protecting against seizures during the period of enhanced seizure susceptibility following neurosteroid withdrawal. This pharmacoresistance seems to mimic the situation in women with catamenial epilepsy in which breakthrough seizures occur despite treatment with antiepileptic drugs. In contrast to the results with conventional antiepileptic drugs, neurosteroids, including allopregnanolone, THDOC, and their 5β-isomers, were found to have enhanced activity in the perimenstrual catamenial epilepsy model.[63] This suggested a "neurosteroid replacement" approach to treat catamenial seizure exacerbations.[88] A neurosteroid could be administered in a "pulse" prior to menstruation and then withdrawn or continuously administered throughout the month. While intermittent administration at the time of increased seizure vulnerability is rational, continuous administration would avoid withdrawal of the therapeutic agent, which itself could predispose to seizures. This factor, as well as the practical difficulty many women experience in predicting the time of their menstrual periods, suggests that continuous administration is preferred. The neurosteroid would be administered at low doses to avoid sedative side effects. Such low doses are expected to contribute little anticonvulsant activity during most of the menstrual cycle. Patients would still require treatment with conventional antiepileptic medications. However, during the period of enhanced seizure susceptibility at the time of menstruation, the increased potency of the neurosteroid would confer protection against perimenstrual seizure exacerbations. It is noteworthy that while the anticonvulsant activity of neurosteroids increases in conjunction with neurosteroid withdrawal, there is no corresponding increase in side effects (mainly sedation), at least as assessed by a measure of motor impairment.[88] Therefore, enhanced side effects, which would negate the potential of the therapeutic approach, would not be expected to occur.

To determine whether the enhanced activity of neurosteroids is due to pharmacokinetic or pharmacodynamic factors, brain and plasma levels of the neurosteroid ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one, discussed below) were determined with a liquid chromatography-mass spectrometric method. Control and neurosteroid withdrawn animals received a single dose of ganaxolone (7 mg/kg, subcutaneously), resulting in an elevation in PTZ threshold that peaked at 30 min and returned to baseline at 120–180 min. Ganaxolone caused a markedly greater (1.8-fold) elevation of the PTZ threshold in the withdrawn animals than in controls,

indicating a greater sensitivity to the anticonvulsant effects of ganaxolone. Surprisingly, plasma and brain ganaxolone levels were reduced in withdrawn animals (69% of control levels). Adjusting for the reduced brain levels, the pharmacodynamic sensitivity to ganaxolone was enhanced 2.3-fold in the withdrawn animals compared with controls. There was a significant increase in clearance (CL) of ganaxolone in the withdrawn animals, which accounts for the reduced plasma and brain levels. Ganaxolone levels reached a peak more slowly in brain ($T_{max\text{-}brain}$, ~30 min) than in plasma ($T_{max\text{-}plasma}$, ~15 min); the $T_{max\text{-}brain}$ value corresponds with the peak elevation in seizure threshold. These studies confirmed the enhanced anticonvulsant activity of ganaxolone in the rat model of catamenial epilepsy. The enhanced activity occurs in the face of decreased plasma and brain ganaxolone levels, indicating a marked increase in pharmacodynamic sensitivity.

Recently, studies have been conducted with the catamenial epilepsy model in female rats that experienced a prolonged bout of status epilepticus induced by lithium-pilocarpine treatment, resulting in a chronic epileptic state with spontaneous recurrent seizures.[90] Epileptic animals in the catamenial epilepsy model exhibited about six seizures per day, each lasting approximately 1 min. When neurosteroids were withdrawn by treatment with finasteride, an enormous (more than 10-fold) increase in seizure frequency was observed. In contrast, finasteride did not induce seizures in normal animals. However, it did induce an increase in seizures in epileptic rats that were not treated with gonadotropins, albeit of smaller magnitude than that in the pseudopregnant animals. The observation that inhibition of the synthesis of endogenous neurosteroids in nonepileptic animals did not lead to seizures indicates that neurosteroid reductions are not epileptogenic. This is consistent with the observation that finasteride does not cause seizures in humans who do not have epilepsy. Finasteride is used clinically for the treatment of benign prostatic hypertrophy and male pattern hair loss. Seizures have not been reported as an adverse effect of the drug. While it is clear that finasteride does not provoke seizures in the general population, there are no prospective studies to determine whether inhibition of 5α-reductase by finasteride influences seizure susceptibility in individuals with epilepsy. There is a single anecdotal report of a woman with epilepsy taking finasteride for male pattern baldness who experienced an increase in seizure frequency and severity in association with finasteride use.[91] The doses of finasteride used clinically are in the range of 1–5 mg/day, which are far less than the doses of 30–100 mg/kg used in rats to inhibit brain neurosteroid synthesis. Furthermore, in humans, finasteride is selective for the type 2 5α-reductase isoform and is less active on the type 1 enzyme that is the isoform predominantly present in the brain. This selectivity is not observed with the rat enzymes. In sum, finasteride, as administered clinically in humans, probably does not block neurosteroidogenesis sufficiently to influence seizure susceptibility under most circumstances. Also, individuals with congenital 5α-reductase deficiency, caused by a mutation in the 5α-reductase type 2 gene (a condition with ambiguous genitalia), do not exhibit epilepsy. While neurosteroid reduction by itself does not lead to epilepsy, it is apparent that endogenous neurosteroids do modulate seizure susceptibility in epileptic animals.

Although it has been assumed that the effect of finasteride on seizure susceptibility is mediated through inhibition of peripheral neurosteroidogenesis, ovariectomized epileptic animals also exhibited large increases in seizure frequency following finasteride treatment, indicating that a major effect of the drug may be to influence neurosteroid synthesis in the brain.[90] Whatever the site of action of finasteride, treatment with exogenous allopregnanolone was found to rapidly terminate the finasteride-induced exacerbation of seizures, providing additional evidence that the increase in seizure frequency is due to a finasteride-induced reduction in neurosteroids and not some other action of the drug. More importantly, it supports the concept that neurosteroid replacement may be useful in the treatment of seizures associated with neurosteroid fluctuations, such as catamenial epilepsy. In catamenial epilepsy, breakthrough seizures occur despite treatment with antiepileptic drugs. Previous studies (reviewed in ref. 88) and the new results from Lawrence et al.[90] support the potential of neurosteroids as a novel treatment approach for these pharmacoresistant seizures.

Although neurosteroids seems to be the most direct approach to the treatment of catamenial

epilepsy, only limited anecdotal data are available to support their use.[92] No neurosteroid is currently approved. In contrast, two open-label trials have shown that adjunctive progesterone therapy produces significant reductions in seizure occurrence.[93] It is recommended that the hormone be administered during the entire second half of the menstrual cycle and tapered gradually, as it is believed that abrupt discontinuation can result in rebound seizure exacerbation. Enthusiasm for the use of progesterone in the treatment of catamenial epilepsy has been tempered by the lack of data from adequately controlled clinical trials. A multicenter prospective controlled trial of 3 months of supplemental progesterone in 462 women, 294 who were randomized 2:1 to progesterone or placebo, has been completed. While the overall response to progesterone was no greater than to placebo in women with or without catamenial epilepsy, as the magnitude of a woman's perimenstrual seizure exacerbation increased there was a correlated increase in the response to progesterone but not to placebo, suggesting that some women may benefit.[93a] Progesterone therapy in women may cause hormonal effects such as breakthrough vaginal bleeding and breast tenderness as well as weight gain, sedation, and emotional depression. Neurosteroids, such as ganaxolone, have not been associated with such side effects and may ultimately prove to be superior as a treatment approach.

Neurosteroids and Stress-Induced Seizure Fluctuations

The availability of neurosteroids is increased during physiological stress. Stress results in the hypothalamic release of corticotropin-releasing hormone (CRH), which liberates adrenocorticotropic hormone (ACTH) from the anterior pituitary. Along with cortisol, ACTH also enhances the synthesis of adrenal DOC,[94,95] which is released into the circulation and can serve as a precursor for synthesis of the neurosteroid DOC (Fig. 77–1). In contrast to allopregnanolone, which is present in the brain even after adrenalectomy and gonadectomy, THDOC appears to be derived nearly exclusively from adrenal sources.[96] Plasma and brain levels of THDOC and allopregnanolone rise rapidly following acute stress.[52,97] Acute stressors such as swimming, foot shock, or carbon dioxide exposure elicit an increase in allopregnanolone and THDOC concentrations in plasma and in brain.[98,99] Plasma levels of THDOC normally fluctuate between 1 and 5 nM, but increase to 15–30 nM following acute stress and might reach 40–60 nM during pregnancy.[97,100] In contrast, allopregnanolone levels during the third trimester of pregnancy typically reach 70–160 nM and have been measured as high as 220 nM.[101]

Stress-induced neurosteroids have been demonstrated to elevate the seizure threshold.[52] Stress-induced seizure protection could be due to circulating neurosteroids synthesized in peripheral tissues or to those produced locally in the brain. However, the effects of swim stress-induced increases in seizure threshold and THDOC levels in rats were abolished in adrenalectomized animals, implicating adrenal-derived THDOC. Despite stress-induced seizure protection in animals,[52,102] patients and clinicians are not likely to recognize a reduction in seizure frequency associated with stress. Indeed, stress has been reported to trigger seizure activity in persons with epilepsy.[103,104] During stressful episodes adrenal hormone levels are expected to fluctuate, and it may simply be the withdrawal of THDOC during such fluctuations that is associated with seizure provocation. Alternatively, other unidentified hormonal factors with proconvulsant activity may be responsible for stress-induced increases in seizures. However, chronic stress of the type experienced by patients with epilepsy likely has different endocrinological consequences than acute stress. The effects on seizures of fluctuations in neurosteroid levels in chronic stress remain to be studied.

Neurosteroids in Temporal Lobe Epilepsy

Sexual and reproductive dysfunction are common among persons with epilepsy.[105] In particular, men with temporal lobe epilepsy (TLE) often have diminished libido and sexual potency that is associated with low testosterone levels.[106–108] This hypogonadal state has been attributed to the effects of certain hepatic enzyme-inducing antiepileptic drugs, or alternatively—given the extensive connections between temporal lobe structures such as the amygdala and hypothalamic nuclei that

govern the production and secretion of gonadotropin releasing hormone—to suppression of the hypothalamic-pituitary-gonadal axis by limbic seizures. There is evidence that serum androgen levels normalize after temporal lobe surgery that results in successful seizure control but not in patients who continue to have seizures, supporting the view that seizures are responsible for the hypoandrongenic state.[109] Testosterone, as noted previously, is a precursor for at least three neurosteroids with anticonvulsant properties: 5α-androstanediol, androsterone, and etiocholanolone.[11,12,110,111] There is evidence that serum levels of at least two of these steroids (androsterone and etiocholanolone) are reduced in men with epilepsy compared with control subjects.[106] It is conceivable that reduced levels of such anticonvulsant neurosteroids lead to an enhanced propensity for seizures and that neurosteroid replacement might be a useful therapeutic approach.

Certain biological factors in TLE may influence the sensitivity to endogenous neurosteroids and could have an impact on the efficacy of exogenous neurosteroids used in epilepsy therapy. Studies in a status epilepticus model of TLE have shown a striking reduction in δ subunit-containing $GABA_A$ receptors in the dentate gyrus,[112,113] suggesting that neurosteroid effects on nonsynaptic $GABA_A$ receptors may be reduced. In addition, in dentate gyrus granule cells, neurosteroid modulation of synaptic currents is diminished and α4 subunit-containing receptors are present at synapses.[114] All of these changes may facilitate seizures in epileptic animals but may reduce the efficacy of endogenous neurosteroids. The expression of neurosteroidogenic enzymes such as P450scc[73] and 3α-HSOR[17,19] appears to be elevated in the hippocampus in animals and human subjects affected by TLE. If local neurosteroidogenesis is enhanced, this may counteract in part the epileptogenesis-induced changes. However, the effect of withdrawal of neurosteroids, as might occur in catamenial epilepsy or with stress, could be enhanced.

Neurosteroids and Alcohol Withdrawal Seizures

Systemic administration of moderate doses (1–2.5 g/kg) of ethanol causes increases in plasma and brain neurosteroids that may contribute to many of the behavioral effects of ethanol in rodents.[115] This effect of ethanol is believed to be due to activation of the hypothalamic-pituitary-adrenal axis. As is the case in the catamenial epilepsy model, chronic ethanol-induced elevations in neurosteroids lead to an enhancement in the anticonvulsant actions of the neurosteroids allopregnanolone and THDOC.[116] These effects are associated with increases in the sensitivity of $GABA_A$ receptors to neurosteroids.[115] Endogenous neurosteroids may protect against ethanol withdrawal seizures. However, ethanol induction of allopregnanolone is diminished in tolerant and dependent animals. Reduced availability of allopregnanolone under such circumstances may be a factor that predisposes to alcohol withdrawal seizures. As is the case with catamenial epilepsy, neurosteroid replacement could conceivably be useful in the treatment of alcohol withdrawal seizures, given that current pharmacological approaches are not entirely satisfactory.[117]

GANAXOLONE AS A NOVEL NEUROSTEROID-BASED ANTIEPILEPTIC DRUG

Ganaxolone, the synthetic 3β-methyl derivative of allopregnanolone,[118] is the only neurosteroid that has been evaluated for the treatment of epilepsy in humans.[75,119] Allopregnanolne itself has been administered to humans at low doses intravenously (0.05–0.09 mg/kg) and found to be largely free of side effects except for sedation.[120,121] However, it has been proposed that allopregnanolone can undergo back conversion by 3α-HSOR isoenzymes to a hormonally active intermediate (dihydroprogesterone).[122] The 3β-methyl substituent of ganaxolone eliminates this back conversion, potentially avoiding hormonal side effects. Other than this theoretical advantage, ganaxolone has pharmacological properties similar to those of the natural neurosteroid from which it is derived.

Preclinical Studies

Ganaxolone has protective activity in diverse rodent seizure models, including clonic seizures induced by the chemoconvulsants pentylenetetrazol, bicuculline, flurothyl,

t-butylbicycloorthobenzoate, and aminophylline; limbic seizures in the 6 Hz model; amygdala- and cocaine-kindled seizures; and corneal kindled seizures (Table 77–1).[62,67,123–126] In chronically treated rats, tolerance does not occur to the anticonvulsant activity of ganaxolone.[67] In addition, a recent study in female amygdala-kindled mice demonstrated suppression of behavioral and electrographic seizures with a median effective dose (ED_{50}) of 6.6 mg/kg.[127]

Animal pharmacokinetic studies have found that ganaxolone has a large steady-state volume of distribution (6.5, 7.0, 19.5, and 3.5 L/kg in mice, rats, rabbits, and dogs, respectively), indicating that it distributes extensively into tissues.[75] Studies with radioactive ganaxolone in rats have found that ganaxolone (and its metabolites) are concentrated in tissues including the brain (brain-to-plasma concentration ratio between 5 and 10). Ganaxalone is highly bound to human plasma proteins (>99%). It is extensively metabolized to at least 16 different compounds; the primary metabolite is 16α-hydroxyganaxolone, which likely results from the action of CYP3A4. This primary metabolite is inactive in the PTZ seizure model and is 25-fold weaker than ganaxolone in inhibiting [^{35}S]TBPS binding. Ganaxolone is a CYP3A4 autoinducer in rodents but not in dogs or humans; chronic exposure to high doses in female rats does cause liver hypertrophy. Metabolites of ganaxolone are eliminated in the urine (13%–23%) and feces (65%–76%) in rats and dogs; the corresponding values in male healthy volunteers are 25% and 69%, respectively. Because of its aqueous insolubility, orally administered ganaxolone is poorly absorbed. To provide more consistent bioavailability, the steroid has been administered as a submicron particulate suspension and in a proprietary solid formulation.

Animal safety studies have demonstrated little evidence of target organ or systemic toxicity with either single-dose or multiple-dose ganaxolone treatment. In studies on pre- and postnatal development in mice, rats, and dogs, ganaxolone did not affect fetal implantation, viability, or growth and development from birth to weaning and was not teratogenic. Genotoxicity tests have not demonstrated any mutagenic or clastogenic potential for ganaxolone. Oral administration of ganaxolone to conscious dogs at a dose of 10 mg/kg did not reveal changes in cardiovascular hemodynamics.

Clinical Safety and Efficacy Studies

Over the past decade, ganaxolone has been studied in various clinical trials to assess its efficacy and safety in the treatment of epilepsy. More than 900 subjects have received the drug at doses of up to 1875 mg/day in adults and up to 54 mg/kg/day in children in Phase 1 normal volunteer studies, epilepsy trials, and also clinical trials for migraine. Single oral doses of 50–1600 mg in healthy volunteers results in peak plasma concentrations of 14 to as high as 460 ng/mL. Overall, the drug is safe and well tolerated. The most common side effect is

Table 77–1 **Anticonvulsant Profile of Ganaxolone in Mouse Seizure Models**

Seizure Model	ED_{50} Value (mg/kg)	Reference
Pentylenetetrazol	3.5 (2.1–5.8)	Reddy and Rogawski[67]
Pentylenetetrazol kindling	4.1 (2.7–6.4)	Gasior et al.[125]
Bicuculline	4.6 (3.2–6.8)	Carter et al.[118]
Flurothyl	5.0 (ND)	Liptáková et al.[123]
6 Hz	6.3 (4.0–9.8)	Kaminski et al.[62]
Amygdala kindling	6.6 (5.1–9.7)	Reddy and Rogawski[127]
t-Butylbicycloorthobenzoate	11.7 (8.8–15.7)	Carter et al.[118]
Aminophylline	11.5 (8.1–16.3)	Carter et al.[118]
Cocaine kindling	17.0 (ND)	Kaminski et al.[126]
Maximal electroshock	29.7 (25.3–34.8)	Carter et al.[118]
N-methyl-D-aspartate	>30 (ND)	Carter et al.[118]
Strychnine	>40 (ND)	Carter et al.[118]

Numbers in parentheses are 95% confidence intervals. ND, not determined. ED_{50} is the dose estimated to produce seizure protection in 50% of mice. The ED_{50} value in the rotarod test of motor toxicity in mice was 33.4 (30.9–39.4) mg/kg.[118]

reversible dose-related sedation. One epilepsy trial used the inpatient presurgical study design in adults with partial seizures.[128] A second study was an open-label, add-on trial in pediatric patients with a history of infantile spasms.[129] A third study was an open-label nonrandomized, dose-escalation add-on trial in highly refractory pediatric and adolescent patients; three patients in this study were followed in an extension phase over 3.5 years.[130] As discussed previously, there is limited anecdotal information supporting the efficacy of ganaxolone in the treatment of catamenial seizure exacerbations.[92] Recently, a double-blind, randomized, placebo-controlled study was completed in adults with partial seizures.[75] A separate trial was completed in infants with spasms. In this study, there was no clear statistically significant treatment effect, although some subjects did appear to demonstrate a treatment-related reduction in spasm clusters as assessed by 24 h video-electroencephalographic recordings.

The adult trial included 147 subjects (100 females, 47 males), aged 18 to 69 years, with partial-onset seizures with or without secondary generalization who were refractory to conventional antiepileptic drugs. Subjects were randomized in a 2:1 ratio to ganaxolone (1500 mg/day in three divided doses) or placebo. Ganaxolone treatment produced an 18% decrease in mean weekly seizure frequency compared with a 2% increase for placebo over the 10 week treatment period ($p = .025$). Responder rates (proportions of subjects with greater than 50% reduction in seizures during the maintenance phase) were 26% for the ganaxolone group versus 13% for the placebo group. Results from the open-label extension phase indicated that ganaxolone maintains its efficacy over time. Adverse events reported by at least 5% of patients, and at least twice as common in the ganaxolone group compared to the placebo group, were dizziness, fatigue (both 16% versus 8%), and somnolence (13% versus 2%). Seven percent of the subjects in the ganaxolone treatment group and 6% of those in the placebo group discontinued treatment due to adverse events.

CONCLUSIONS

Neurosteroids are endogenous modulators of neural excitability that are believed to have a role in the regulation of seizure susceptibility in the setting of preexisting epilepsy. Menstrual and stress-related fluctuations in seizures may in part be related to changes in brain neurosteroid levels. In addition, men with TLE who have suppression of the hypothalamic-pituitary-gonadal axis may have a reduction in testosterone-derived neurosteroids that could worsen seizures.

Treatment with exogenously administered natural neurosteroids or synthetic analogs such as ganaxolone may be beneficial to treat partial seizures. Further studies are required to determine if neurosteroid replacement is a useful therapeutic approach for seizure exacerbations related to endogenous neurosteroid fluctuations, such as in catamenial epilepsy and stress. In the future, agents that influence the endogenous synthesis of neurosteroids, such as TSPO ligands, may find utility as an alternative to neurosteroids themselves in the treatment of epilepsy.[24,131]

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REFERENCES

1. Baulieu E-E. Steroid hormones in the brain: several mechanisms? In: Fuxe F, Gustafsson JA, Wetterberg L, eds. *Steroid Hormone Regulation of the Brain*. Oxford: Pergamon Press; 1981:3–14.
2. Paul SM, Purdy RH. Neuroactive steroids. *FASEB J*. 1992;6:2311–2322.
3. Selye H. Anesthetics of steroid hormones. *Proc Soc Exp Biol Med*. 1941;46:116–121.
4. Clarke RS, Dundee JW, Carson IW. Proceedings: a new steroid anaesthetic-althesin. *Proc R Soc Med*. 1973;66:1027–1030.

5. Scholfield CN. Potentiation of inhibition by general anaesthetics in neurones of the olfactory cortex in vitro. *Pflugers Arch*. 1980;383:249–255.
6. Harrison NL, Simmonds MA. Modulation of the GABA receptor complex by a steroid anaesthetic. *Brain Res*. 1984;323:287–292.
7. Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science*. 1986;232(4753):1004–1007.
8. Do Rego JL, Seong JY, Burel D, Leprince J, Luu-The V, Tsutsui K, Tonon MC, Pelletier G, Vaudry H. Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. *Front Neuroendocrinol*. 2009;30:259–301.
9. Kokate TG, Svensson BE, Rogawski MA. Anticonvulsant activity of neuroactive steroids: correlation with γ-aminobutyric acid-evoked chloride current potentiation. *J Pharmacol Exp Ther*. 1994;270:1223–1229.
10. Langlois VS, Zhang D, Cooke GM, Trudeau VL. Evolution of steroid-5α-reductases and comparison of their function with 5β-reductase. *Gen Comp Endocrinol*. 2010;166:489–497.
11. Reddy DS, Jian K. The testosterone-derived neurosteroid androstanediol is a positive allosteric modulator of $GABA_A$ receptors. *J Pharmacol Exp Ther*. 2010;334: 1031–1041.
12. Kaminski RM, Marini H, Kim WJ, Rogawski MA. Anticonvulsant activity of androsterone and etiocholanolone. *Epilepsia*. 2005;46:819–827.
13. Kaminski RM, Marini H, Ortinski PI, Vicini S, Rogawski MA. The pheromone androstenol (5α-androst-16-en-3α-ol) is a neurosteroid positive modulator of $GABA_A$ receptors. *J Pharmacol Exp Ther*. 2006;317:694–703.
14. Melcangi RC, Poletti A, Cavarretta I, Celotti F, Colciago A, Magnaghi V, Motta M, Negri-Cesi P, Martini L. The 5α-reductase in the central nervous system: expression and modes of control. *J Steroid Biochem Mol Biol*. 1998;65:295–299.
15. Petratos S, Hirst JJ, Mendis S, Anikijenko P, Walker DW. Localization of p450scc and 5α-reductase type-2 in the cerebellum of fetal and newborn sheep. *Dev Brain Res*. 2000;123:81–86.
16. Khanna M, Qin KN, Cheng KC. Distribution of 3α-hydroxysteroid dehydrogenase in rat brain and molecular cloning of multiple cDNAs encoding structurally related proteins in humans. *J Steroid Biochem Mol Biol*. 1995;53:41–46.
17. Stoffel-Wagner B, Beyenburg S, Watzka MS, Blumcke I, Bauer J, Schramm J, Bidlingmaier F, Elger CE. Expression of 5α-reductase and 3α-hydroxysteroid oxidoreductase in the hippocampus of patients with chronic temporal lobe epilepsy. *Epilepsia*. 2000;41:140–147.
18. Stoffel-Wagner B. Neuroactive steroid metabolism in the human brain. *Eur J Endocrinol*. 2001;145: 669–679.
19. Stoffel-Wagner B, Watzka M, Steckelbroeck S, Ludwig M, Clusmann H, Bidlingmaier F, Casarosa E, Luisi S, Elger CE, Beyenburg S. Allopregnanolone serum levels and expression of 5α-reductase and 3α-hydroxysteroid dehydrogenase isoforms in hippocampal and temporal cortex of patients with epilepsy. *Epilepsy Res*. 2003;54:11–19.
20. Jefcoate C. High-flux mitochondrial cholesterol trafficking, a specialized function of the adrenal cortex. *J Clin Invest*. 2002;110:881–890.
21. Korneyev A, Pan BS, Polo A, Romeo E, Guidotti A, Costa E. Stimulation of brain pregnenolone synthesis by mitochondrial diazepam binding inhibitor receptor ligands in vivo. *J Neurochem*. 1993;61:1515–1524.
22. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapère JJ, Lindemann P, Norenberg MD, Nutt D, Weizman A, Zhang MR, Gavish M. Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci*. 2006;27:402–409.
23. Kita A, Furukawa K. Involvement of neurosteroids in the anxiolytic-like effects of AC-5216 in mice. *Pharmacol Biochem Behav*. 2008;89:171–178.
24. Rupprecht R, Rammes G, Eser D, Baghai TC, Schüle C, Nothdurfter C, Troxler T, Gentsch C, Kalkman HO, Chaperon F, Uzunov V, McAllister KH, Bertaina-Anglade V, La Rochelle CD, Tuerck D, Floesser A, Kiese B, Schumacher M, Landgraf R, Holsboer F, Kucher K. Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. *Science*. 2009;325(5939):490–493.
25. Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat Rev Drug Discov*. 2010;9:971–988.
26. Guennoun R, Fiddes RJ, Gouezou M, Lombes M, Baulieu EE. A key enzyme in the biosynthesis of neuroactive steroids, 3β-hydroxysteroid dehydrogenase/ Δ^5-Δ^4-isomerase (3β-HSD), is expressed in rat brain. *Mol Brain Res*. 1995;30:287–300.
27. Kishimoto W, Hiroi T, Shiraishi M, Osada M, Imaoka S, Kominami S, Igarashi T, Funae Y. Cytochrome P450 2D catalyze steroid 21-hydroxylation in the brain. *Endocrinology*. 2004;145:699–705.
28. Purdy RH, Morrow AL, Moore PH Jr, Paul SM. Stress-induced elevations of γ-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc Natl Acad Sci USA*. 1991;88:4553–4557.
29. Corpechot C, Young J, Calvel M, Wehrey C, Veltz JN, Touyer G, Mouren M, Prasad VV, Banner C, Sjovall J. Neuroactive steroids: 3α-hydroxy-5α-pregnan-20-one and its precursors in the brain, plasma and steroidogenic glands of male and female rats. *Endocrinology*. 1993;133:1003–1009.
30. Agís-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, Guidotti A. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *Proc Natl Acad Sci USA*. 2006;103: 14602–14607.
31. Saalmann YB, Kirkcaldie MT, Waldron S, Calford MB. Cellular distribution of the $GABA_A$ receptor-modulating 3α-hydroxy, 5α-reduced pregnane steroids in the adult rat brain. *J Neuroendocrinol*. 2007;19:272–284.
32. Chisari M, Eisenman LN, Covey DF, Mennerick S, Zorumski CF. The sticky issue of neurosteroids and $GABA_A$ receptors. *Trends Neurosci*. 2010;33:299–306.
33. Harrison NL, Majewska MD, Harrington JW, Barker JL. Structure–activity relationships for steroid interactions with the γ-aminobutyric $acid_A$ receptor complex. *J Pharmacol Exp Ther*. 1987;241:346–353.
34. Gee KW, Bolger MB, Brinton RE, Coirini H, McEwen BS. Steroid modulation of the chloride ionophore in rat brain: structure–activity require-

ments, regional dependence and mechanism of action. *J Pharmacol Exp Ther*. 1988;246:803–812.
35. Lan NC, Gee KW, Bolger MB, Chen JS. Differential responses of expressed recombinant human γ-aminobutyric acid$_A$ receptors to neurosteroids. *J Neurochem.* 1991;57:1818–1821.
36. Twyman RE, Macdonald RL. Neuroactive steroid regulation of GABA$_A$ receptor single-channel kinetic properties of mouse spinal cord neurons in culture. *J Physiol.* 1992;456:215–245.
37. Lambert JJ, Belelli D, Peden DR, Vardy AW, Peters JA. Neurosteroid modulation of GABA$_A$ receptors. *Prog Neurobiol*. 2003;71:67–80.
38. Hosie AM, Wilkins ME, Smart TG. Neurosteroid binding sites on GABA$_A$ receptors. *Pharmacol Ther.* 2007;116:7–19.
39. Hosie AM, Clarke L, da Silva H, Smart TG. Conserved site for neurosteroid modulation of GABA$_A$ receptors. *Neuropharmacology.* 2009;56:149–154.
40. Akk G, Li P, Bracamontes J, Reichert DE, Covey DF, Steinbach JH. Mutations of the GABA-A receptor α1 subunit M1 domain reveal unexpected complexity for modulation by neuroactive steroids. *Mol Pharmacol*. 2008;74:614–627.
41. Li G-D, Chiara DC, Cohen JB, Olsen RW. Neurosteroids allosterically modulate binding of the anesthetic etomidate to γ-aminobutyric acid type A receptors. *J Biol Chem*. 2009;284:11771–11775.
42. Purdy RH, Morrow AL, Blinn JR, Paul SM. Synthesis, metabolism, and pharmacological activity of 3α-hydroxy steroids which potentiate GABA-receptor-mediated chloride ion uptake in rat cerebral cortical synaptoneurosomes. *J Med Chem*. 1990;33:1572–1581.
43. Puia G, Santi M, Vicini S, Pritchett DB, Purdy RH, Paul SM, Seeburg PH, Costa E. Neuroactive steroids act on recombinant human GABA$_A$ receptors. *Neuron.* 1990;4:759–765.
44. Glykys J, Mody I. Activation of GABA$_A$ receptors: views from outside the synaptic cleft. *Neuron*. 2007;56: 763–770.
45. Belelli D, Casula A, Ling A, Lambert JJ. The influence of subunit composition on the interaction of neurosteroids with GABA$_A$ receptors. *Neuropharmacology*. 2002;43:651–661.
46. Wohlfarth KM, Bianchi MT, Macdonald RL. Enhanced neurosteroid potentiation of ternary GABA$_A$ receptors containing the δ subunit. *J Neurosci*. 2002;22: 1541–1549.
47. Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen RW, Homanics GE. Attenuated sensitivity to neuroactive steroids in γ-aminobutyrate type A receptor δ subunit knockout mice. *Proc Natl Acad Sci USA*. 1999;96:12905–12910.
48. Spigelman I, Li Z, Banerjee PK, Mihalek RM, Homanics GE, Olsen RW. Behavior and physiology of mice lacking the GABA$_A$-receptor δ subunit. *Epilepsia.* 2002;43(suppl 5):3–8.
49. Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by δ subunit-containing GABA$_A$ receptors. *Proc Natl Acad Sci USA*. 2003;100:14439–14444.
50. Chisari M, Eisenman LN, Krishnan K, Bandyopadhyaya AK, Wang C, Taylor A, Benz A, Covey DF, Zorumski CF, Mennerick S. The influence of neuroactive steroid lipophilicity on GABA$_A$ receptor modulation: evidence for a low-affinity interaction. *J Neurophysiol*. 2009;102:1254–1264.
51. Lambert JJ, Cooper MA, Simmons RD, Weir CJ, Belelli D. Neurosteroids: endogenous allosteric modulators of GABA$_A$ receptors. *Psychoneuroendocrinology.* 2009;34(suppl 1):S48–S58.
52. Reddy DS, Rogawski MA. Stress-induced deoxycorticosterone-derived neuroactive steroids modulate GABA$_A$ receptor function and seizure susceptibility. *J Neurosci*. 2002;42: 3795–3805.
53. Rho JM, Donevan SD, Rogawski MA. Direct activation of GABA$_A$ receptors by barbiturates in cultured rat hippocampal neurons. *J Physiol* (*Lond*). 1996;497(pt 2): 509–522.
54. Belelli D, Bolger MB, Gee KW. Anticonvulsant profile of the progesterone metabolite 5α-pregnan-3α-ol-20-one. *Eur J Pharmacol*. 1989;166:325–329.
55. Frye CA. The neuroactive steroid 3α,5α-THP has anti-seizure and possible neuroprotective effects in an animal model of epilepsy. *Brain Res*. 1995;696:113–120.
56. Wieland S, Belluzzi JD, Stein L, Lan NC. Comparative behavioral characterization of the neuroactive steroids 3α-OH, 5α-pregnan-20-one and 3α-OH,5β-pregnan-20-one in rodents. *Psychopharmacology.* 1995;118:65–71.
57. Reddy DS, Castenada DA, O'Malley BW, Rogawski MA. Antiseizure activity of progesterone and neurosteroids in progesterone receptor knockout mice. *J Pharmacol Exp Ther.* 2004;310:230–239.
58. Snead OC 3rd. Ganaxolone, a selective, high-affinity steroid modulator of the γ-aminobutyric acid-A receptor, exacerbates seizures in animal models of absence. *Ann Neurol*. 1998;44:688–691.
59. Citraro R, Russo E, Di Paola ED, Ibbadu GF, Gratteri S, Marra R, De Sarro G. Effects of some neurosteroids injected into some brain areas of WAG/Rij rats, an animal model of generalized absence epilepsy. *Neuropharmacology*. 2006;50:1059–1071.
60. Reddy DS. Anticonvulsant activity of the testosterone-derived neurosteroid 3α-androstanediol. *Neuroreport.* 2004;15:515–518.
61. Reddy DS. Testosterone modulation of seizure susceptibility is mediated by neurosteroids 3α-androstanediol and 17β-estradiol. *Neuroscience*. 2004;129:195–207.
62. Kaminski RM, Livingood MR, Rogawski MA. Allopregnanolone analogs that positively modulate GABA receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. *Epilepsia.* 2004;45:864–867.
63. Reddy DS, Rogawski MA. Enhanced anticonvulsant activity of neuroactive steroids in a rat model of catamenial epilepsy. *Epilepsia*. 2001;42:303–310.
64. Tsuda M, Suzuki T, Misawa M. Modulation of the decrease in the seizure threshold of pentylenetetrazole in diazepam-withdrawn mice by the neuroactive steroid 5α-pregnan-3α,21-diol-20-one (alloTHDOC). *Addiction Biol*. 1997;2:455–460.
65. Devaud LL, Purdy RH, Finn DA, Morrow AL. Sensitization of γ-aminobutyric acid$_A$ receptors to neuroactive steroids in rats during ethanol withdrawal. *J Pharmacol Exp Ther.* 1996;278:510–517.

66. Kokate TG, Yamaguchi S, Pannell LK, Rajamani U, Carroll DM, Grossman AB, Rogawski MA. Lack of anticonvulsant tolerance to the neuroactive steroid pregnanolone in mice. *J Pharmacol Exp Ther*. 1998;287:553–558.
67. Reddy DS, Rogawski MA. Chronic treatment with the neuroactive steroid ganaxolone in the rat induces anticonvulsant tolerance to diazepam but not to itself. *J Pharmacol Exp Ther*. 2000;295:1241–1248.
68. Bateson AN. Basic pharmacologic mechanisms involved in benzodiazepine tolerance and withdrawal. *Curr Pharmaceut Design*. 2002;8:5–21.
69. Kokate TG, Juhng KN, Kirkby RD, Llamas J, Yamaguchi S, Rogawski MA. Convulsant actions of the neuroactive steroid pregnenolone sulfate in mice. *Brain Res.* 1999;831:119–124.
70. Williamson J, Mtchedlishvili Z, Kapur J. Characterization of the convulsant action of pregnenolone sulfate. *Neuropharmacology*. 2004;46:856–864.
71. Reddy DS, Kulkarni SK. Proconvulsant effects of neurosteroid pregnenolone sulfate and dehydroepiandrosterone sulfate in mice. *Eur J Pharmacol*. 1998;345: 55–59.
72. Biagini G, Baldelli E, Longo D, Pradelli L, Zini I, Rogawski MA, Avoli M. Endogenous neurosteroids modulate epileptogenesis in a model of temporal lobe epilepsy. *Exp Neurol*. 2006;201: 519–524.
73. Biagini G, Longo D, Baldelli E, Zoli M, Rogawski MA, Bertazzoni G, Avoli M. Neurosteroids and epileptogenesis in the pilocarpine model: evidence for a relationship between P450scc induction and length of the latent period. *Epilepsia.* 2009;50(suppl 1):53–58.
74. Biagini G, Panuccio G, Avoli M. Neurosteroids and epilepsy. *Curr Opin Neurol*. 2010;23:170–176.
75. NohriaV, Tsai J, Shaw K, Rogawski MA, Pieribone VA, Farfel G. Ganaxolone. In: Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS. Progress report on new antiepileptic drugs: a summary of the Tenth Eilat Conference (EILAT X). *Epilepsy Res.* 2010;92:89–124.
76. Edwards HE, Mo V, Burnham WM, MacLusky NJ. Gonadectomy unmasks an inhibitory effect of progesterone on amygdala kindling in male rats. *Brain Res*. 2001;889:260–263.
77. Reddy DS, Gangisetty O, Briyal S. Disease-modifying activity of progesterone in the hippocampus kindling model of epileptogenesis. *Neuropharmacology*. 2010;59:573–581.
78. Herzog AG, Harden CL, Liporace J, Pennell P, Schomer DL, Sperling M, Fowler K, Nikolov B, Shuman S, Newman M. Frequency of catamenial seizure exacerbation in women with localization-related epilepsy. *Ann Neurol.* 2004;56:431–434.
79. Bazan AC, Montenegro MA, Cendes F, Min LL, Guerreiro CA. Menstrual cycle worsening of epileptic seizures in women with symptomatic focal epilepsy. *Arq Neuro-Psiquiatr*. 2005;63:751–756.
80. Reddy DS. The role of neurosteroids in the pathophysiology and treatment of catamenial epilepsy. *Epilepsy Res.* 2009;85:1–30.
81. Tuveri A, Paoletti AM, Orrù M, Melis GB, Marotto MF, Zedda P, Marrosu F, Sogliano C, Marra C, Biggio G, Concas A. Reduced serum level of THDOC, an anticonvulsant steroid, in women with perimenstrual catamenial epilepsy. *Epilepsia.* 2008;49:1221–1229.
82. Gulinello M, Gong QH, Li X, Smith SS. Short-term exposure to a neuroactive steroid increases α4 $GABA_A$ receptor subunit levels in association with increased anxiety in the female rat. *Brain Res.* 2001;910:55–66.
83. Gangisetty O, Reddy DS. Neurosteroid withdrawal regulates $GABA_A$ receptor α4-subunit expression and seizure susceptibility by activation of progesterone receptor-independent early growth response factor-3 pathway. *Neuroscience.* 2010;170:865–880.
84. Shen H, Gong QH, Yuan M, Smith SS. Short-term steroid treatment increases δ $GABA_A$ receptor subunit expression in rat CA1 hippocampus: pharmacological and behavioral effects. *Neuropharmacology.* 2005;49: 573–586.
85. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in $GABA_A$ receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci*. 2005;8:797–804.
86. Smith SS, Gong QH. Neurosteroid administration and withdrawal alter $GABA_A$ receptor kinetics in CA1 hippocampus of female rats. *J Physiol.* 2005;564: 421–436.
87. Reddy DS, Kim HY, Rogawski MA. Neurosteroid withdrawal model of perimenstrual catamenial epilepsy. *Epilepsia.* 2001;42:328–336.
88. Reddy DS, Rogawski MA. Neurosteroid replacement therapy for catamenial epilepsy. *Neurotherapeutics.* 2009;6:392–401.
89. Reddy DS, Rogawski MA. Enhanced anticonvulsant activity of ganaxolone after neurosteroid withdrawal in a rat model of catamenial epilepsy. *J Pharmacol Exp Ther*. 2000;294:909–915.
90. Lawrence C, Martin BS, Sun C, Williamson J, Kapur J. Endogenous neurosteroid synthesis modulates seizure frequency. *Ann Neurol*. 2010;67:689–693.
91. Herzog AG, Frye CA. Seizure exacerbation associated with inhibition of progesterone metabolism. *Ann Neurol*. 2003;53:390–391.
92. McAuley JW, Reeves Al, Flyak J, Monaghan EP, Data J. A pilot study of the neurosteroid ganaxolone in catamenial epilepsy: clinical experience in two patients. *Epilepsia.* 2001;42(suppl 7):85.
93. Herzog AG. Hormonal therapies: progesterone. *Neurotherapeutics.* 2009;6:383–391.
93a. Herzog AG, Fowler KM, Massaro JM, Pennell PB, Sperling MR, Liporace JD, Kalayjian LA, Heck CN, Harden CL, Dworetzky BA. Progesterone therapy for women with epilepsy: Results of the Phase 3 NIH progesterone trial. *American Epilepsy Society Abst*. 2011;3,191.
94. Tan SY, Mulrow PJ. The contribution of the zona fasciculata and glomerulosa to plasma 11-deoxycorticosterone levels in man. *J Clin Endocrinol Metab*. 1075;41:126–130.
95. Kater CE, Biglieri EG, Brust N, Chang B, Hirai J, Irony I. Stimulation and suppression of the mineralocorticoid hormones in normal subjects and adrenocortical disorder. *Endocrine Rev.* 1989;10:149–164.
96. Purdy RH, Morrow AL, Blinn JR, Paul SM. Synthesis, metabolism, and pharmacological activity of 3α-hydroxy steroids which potentiate GABA-receptor-mediated chloride ion uptake in rat cerebral cortical synaptoneurosomes. *J Med Chem.* 1990;33: 1572–1581.

97. Concas A, Mostallino MC, Porcu P, Folesa P, Barbaccia ML, Trabucchi M, Purdy RH, Grisenti P, Biggio G. Role of brain allopregnanolone in the plasticity of γ-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. *Proc Natl Acad Sci USA*. 1998;95:13284–13289.
98. Barbaccia ML, Roscetti G, Trabucchi M, Mostallino MC, Concas A, Purdy RH, Biggio G. Time-dependent changes in rat brain neuroactive steroid concentrations and $GABA_A$ receptor function after acute stress. *Neuroendocrinology*. 1996;63:166–172.
99. Barbaccia ML, Roscetti G, Trabucchi M, Purdy RH, Mostallino MC, Concas A, Biggio G. The effects of inhibitors of GABAergic transmission and stress on brain and plasma allopregnanolone concentrations. *Br J Pharmacol*. 1997;120:1582–1588.
100. Vallee M, Rivera JD, Koob GF, Purdy RH, Fitzgerald RL. Quantification of neurosteroids in rat plasma and brain following swim stress and allopregnanolone administration using negative chemical ionization gas chromatography/mass spectrometry. *Anal Biochem*. 2000;287:153–166.
101. Pařízek A, Hill M, Kancheva R, Havlíková H, Kancheva L, Cindr J, Pašková A, Pouzar V, Černý I, Drbohlav P, Hájek Z, Stárka L. Neuroactive pregnanolone isomers during pregnancy. *J Clin Endocrinol Metab*. 2005;90:395–403.
102. Perišić D, Švob D, Jazvinšćak M, Mirković K. Anticonvulsive effect of swim stress in mice. *Pharmacol Biochem Behav*. 2000;66:879–886.
103. Temkin NR, Davis GR. Stress as a risk factor for seizures among adults with epilepsy. *Epilepsia*. 1984;25:450–456.
104. Frucht MM, Quigg M, Schwaner C, Fountain, NB. Distribution of seizure precipitants among epilepsy syndromes. *Epilepsia*. 2000;41:1534–1539.
105. Edwards HE, MacLusky NJ, Burnham WM. Epileptic seizures: do they cause reproductive dysfunction? *Univ Toronto Med J*. 2000;77:104–111.
106. Brunet M, Rodamilans M, Martinez-Osaba MJ, Santamaria J, To-Figueras J, Torra M, Corbella J, Rivera F. Effects of long-term antiepileptic therapy on the catabolism of testosterone. *Pharmacol Toxicol*. 1995;76:371–375.
107. Herzog AG, Seibel MM, Schomer DL, Vaitukaitis JL, Geschwind N. Reproductive endocrine disorders in men with partial seizures of temporal lobe origin. *Arch Neurol*. 1986;43:347–350.
108. Herzog AG. Altered reproductive endocrine regulation in men with epilepsy: implications for reproductive function and seizures. *Ann Neurol*. 2002;51: 539–542.
109. Bauer J, Stoffel-Wagner B, Flügel D, Kluge M, Schramm J, Bidlingmaier F, Elger CE. Serum androgens return to normal after temporal lobe epilepsy surgery in men. *Neurology*. 2000;55:820–824.
110. Reddy DS. Anticonvulsant activity of the testosterone-derived neurosteroid 3α-androstanediol. *Neuroreport*. 2004;15:515–518.
111. Reddy DS. Testosterone modulation of seizure susceptibility is mediated by neurosteroids 3α-androstanediol and 17β-estradiol. *Neuroscience*. 2004;129:195–207.
112. Peng Z, Huang CS, Stell BM, Mody I, Houser CR. Altered expression of the δ subunit of the $GABA_A$ receptor in a mouse model of temporal lobe epilepsy. *J Neurosci*. 2004;24:8629–8639.
113. Zhang N, Wei W, Mody I, Houser CR. Altered localization of $GABA_A$ receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *J Neurosci*. 2007;27:7520–7531.
114. Sun C, Mtchedlishvili Z, Erisir A, Kapur J. Diminished neurosteroid sensitivity of synaptic inhibition and altered location of the α4 subunit of $GABA_A$ receptors in an animal model of epilepsy. *J Neurosci*. 2007;27:12641–12650.
115. Morrow AL, Porcu P, Boyd KN, Grant KA. Hypothalamic-pituitary-adrenal axis modulation of GABAergic neuroactive steroids influences ethanol sensitivity and drinking behavior. *Dialogues Clin Neurosci*. 2006;8:463–477.
116. Devaud LL, Purdy RH, Finn DA, Morrow AL. Sensitization of γ-aminobutyric $acid_A$ receptors to neuroactive steroids in rats during ethanol withdrawal. *J Pharmacol Exp Ther*. 1996;278: 510–517.
117. N'Gouemo P, Rogawski MA. Alcohol withdrawal seizures. In: Pitkänen A, Schwartzkroin PA, Moshé SL, eds. *Models of Seizures and Epilepsy*. Amsterdam: Elsevier Academic Press; 2006;161–177.
118. Carter RB, Wood PL, Wieland S, Hawkinson JE, Belelli D, Lambert JJ, White HS, Wolf HH, Mirsadeghi S, Tahir SH, Bolger MB, Lan NC, Gee KW. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3α-hydroxy-3β-methyl-5α-pregnan-20-one), a selective, high-affinity, steroid modulator of the γ-aminobutyric $acid_A$ receptor. *J Pharmacol Exp Ther*. 1997;280:1284–1295.
119. Monaghan EP, McAuley JW, Data JL. Ganaxolone: a novel positive allosteric modulator of the $GABA_A$ receptor complex for the treatment of epilepsy. *Expert Opin Investig Drugs*. 1999;8:1663–1671.
120. Timby E, Balgård M, Nyberg S, Spigset O, Andersson A, Porankiewicz-Asplund J, Purdy RH, Zhu D, Bäckström T, Poromaa IS. Pharmacokinetic and behavioral effects of allopregnanolone in healthy women. *Psychopharmacology* (*Berl*). 2006;186: 414–424.
121. Kask K, Bäckström T, Lundgren P, Sundström Poromaa I. Allopregnanolone has no effect on startle response and prepulse inhibition of startle response in patients with premenstrual dysphoric disorder or healthy controls. *Pharmacol Biochem Behav*. 2009;92: 608–613.
122. Rupprecht R, Reul JM, Trapp T, van Steensel B, Wetzel C, Damm K, Zieglgansberger W, Holsboer F. Progesterone receptor-mediated effects of neuroactive steroids. *Neuron*. 1993;11:523–530.
123. Liptáková S, Velísek L, Velísková J, Moshé SL. Effect of ganaxolone on flurothyl seizures in developing rats. *Epilepsia*. 2000;41:788–793.
124. Rogawski MA, Reddy DS. Neurosteroids: endogenous modulators of seizure susceptibility. In: Rho JM, Sankar R, Cavazos J, eds. *Epilepsy: Scientific Foundations of Clinical Practice*. New York: Marcel Dekker; 2004:319–355.
125. Gasior M, Ungard JT, Beekman M, Carter RB, Witkin JM. Acute and chronic effects of the synthetic neuroactive steroid, ganaxolone, against the convulsive and lethal effects of pentylenetetrazol in

seizure-kindled mice: comparison with diazepam and valproate. *Neuropharmacology*. 2000;39:1184–1196.
126. Kaminski RM, Gasior M, Carter RB, Witkin JM. Protective efficacy of neuroactive steroids against cocaine kindled-seizures in mice. *Eur J Pharmacol*. 2003;474:217–222.
127. Reddy DS, Rogawski MA. Ganaxolone suppression of behavioral and electrographic seizures in the mouse amygdala kindling model. *Epilepsy Res*. 2010;89: 254–260.
128. Laxer K, Blum D, Abou-Khalil BW, Morrell MJ, Lee DA, Data JL, Monaghan EP. Assessment of ganaxolone's anticonvulsant activity using a randomized, double-blind, presurgical trial design. Ganaxolone Presurgical Study Group. *Epilepsia*. 2000;41: 1187–1194.
129. Kerrigan JF, Shields WD, Nelson TY, Bluestone DL, Dodson WE, Bourgeois BF, Pellock JM, Morton LD, Monaghan EP. Ganaxolone for treating intractable infantile spasms: a multicenter, open-label, add-on trial. *Epilepsy Res*. 2000;42:133–139.
130. Pieribone VA, Tsai J, Soufflet C, Rey E, Shaw K, Giller E, Dulac O. Clinical evaluation of ganaxolone in pediatric and adolescent patients with refractory epilepsy. *Epilepsia*. 2007;48:1870–1874.
131. Dhir A, Rogawski MA. Role of neurosteroids in the anticonvulsant activity of midazolam. *Brit J Pharmacol*. 2011; doi: 10.1111/j.1476-5381.2011.01733.x.

Chapter 78

Mechanisms of Ketogenic Diet Action

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INTRODUCTION

The ketogenic diet (KD) is a high-fat, low-carbohydrate, adequate-protein diet that has been employed as a treatment for medically refractory epilepsy for over 90 years.[1] This alternative therapy was originally designed to mimic the biochemical changes associated with fasting, a treatment reported anecdotally over millennia to control seizure activity. The hallmark features of KD treatment are the production of ketone bodies (principally β-hydroxybutyrate, acetoacetate, and acetone)—products of fatty acid oxidation in the liver—and reduced blood glucose levels (Fig. 78–1). Ketone bodies provide an alternative substrate to glucose for energy utilization and, in the developing brain, also constitute essential building blocks for the biosynthesis of cell membranes and lipids.

Throughout much of the past century, the popularity of the KD waxed and waned. Initial enthusiasm was fueled by dramatic success rates, reported entirely in uncontrolled studies, but the KD was quickly supplanted by new antiepileptic drugs (such as phenytoin) that became available in the 1930s. Clinicians found it more convenient to administer a drug than to supervise a regimen requiring scrupulous attention to foodstuffs and avoidance of anti-ketogenic carbohydrates. Notwithstanding the prolonged stigma of being a fad therapy and one without a credible scientific basis, the KD experienced a major resurgence in the late 1990s, mostly as a consequence of serendipitous media attention and the continued failure of even newer antiepileptic drugs to offer significantly enhanced clinical efficacy.

Today, the KD is acknowledged as a proven therapy for epilepsy.[2] The growing number of clinical KD treatment centers throughout the world serves as a testament to the notion that irrespective of cultural and ethnic differences that define dietary and nutritional practices, a fundamental shift from carbohydrate-based

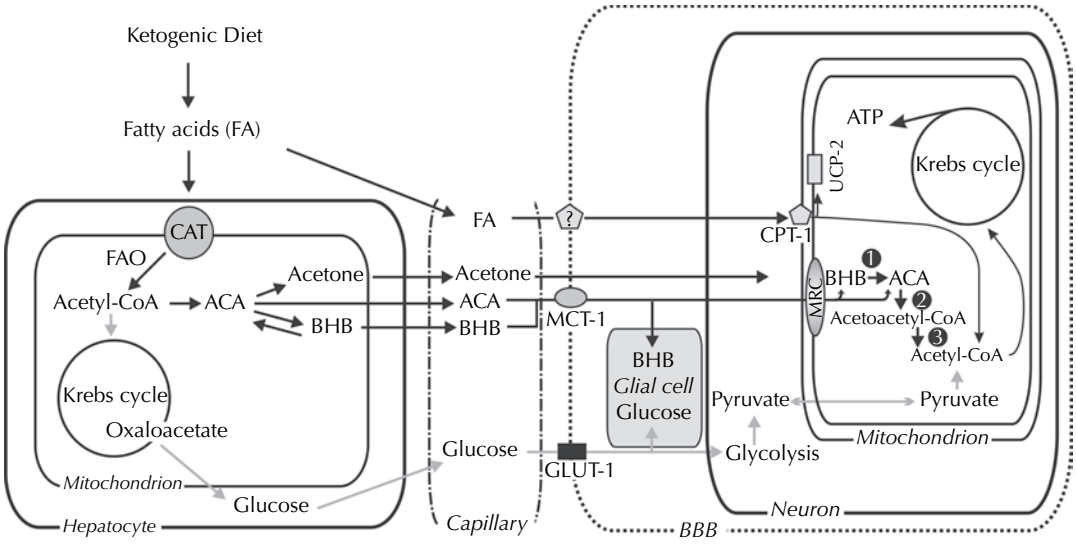


Figure 78–1. Metabolic pathways involved in KD treatment. In the liver, fatty acids are ordinarily converted into acetyl-coenzyme A (acetyl-CoA), which enters the tricarboxylic acid (TCA) cycle. When fatty acid levels are elevated and exceed the metabolic capacity of the TCA cycle, acetyl-CoA is shunted to ketogenesis. Two acetyl-CoAs can combine through a thiolase enzyme to produce acetoacetyl-CoA, which is a precursor for the synthesis of acetoacetate (ACA) and β-hydroxybutyrate (BHB). Acetone, the other major ketone body, is produced primarily from spontaneous decarboxylation of ACA and can be eliminated as a volatile substrate through the lungs and kidneys. In the blood, ACA and BHB are transported from the vascular lumen to the brain interstitial space, and to both glia and neurons, by monocarboxylic acid transporters (MCTs). Monocarboxylate transporter-1 is the principal carrier localized to the vascular endothelium. Within neurons, both ACA and BHB are transported directly into mitochondria and then converted to acetyl-CoA through several enzymatic steps. β-Hydroxybutyrate is converted to ACA through D-β-hydroxybutyrate dehydrogenase, and ACA undergoes subsequent conversion to acetoacetyl-CoA through a succinyl-CoA transferase enzyme. Finally, acetoacetyl-CoA-thiolase converts acetoacetyl-CoA to two acetyl-CoA moieties that then enter the TCA cycle. CAT, carnitine-acylcarnitine translocase; FAO, fatty acid oxidation; ACA, acetoacetate; BHB, β-hydroxybutyrate; MCT-1, monocarboxylate transporter-1; GLUT-1, glucose transporter-1; BBB, blood-brain barrier; CPT-1, carnitine palmitoyl transferase; UCP, uncoupling protein; ATP, adenosine triphosphate; ❶, 3-hydroxybutyrate dehydrogenase; ❷, succinyl-CoA3-oxoacid CoA transferase; ❸, mitochondrial acetoacetyl-CoA thiolase; MRC, mitochondrial respiratory complex. From Kim DY, Rho JM. The ketogenic diet and epilepsy. *Curr Opin Clin Nutr Metab Care*. 2008;11:113–120.

consumption to fatty acid oxidation results in similar clinical effects. Despite such widespread use of the KD, surprisingly little is understood about its underlying mechanisms of action. This may be due to the inherently complex interplay between the network dynamics of the human brain (particularly in the disease state and during development) and the myriad biochemical and physiological changes evoked by consumption of dietary substrates. It has not been straightforward to determine cause-and-effect relationships in this bewildering context, and one cannot be certain whether specific molecular and cellular alterations observed are relevant or simply represent epiphenomena. This knowledge gap has hindered efforts to develop improved or simplified treatments (such as a "KD in a pill"[3]) that obviate the strict adherence to protocol that the KD requires. However, research efforts have been intensifying over the past decade, and recent investigations have provided new insights and molecular targets.

Herein we outline the most prominent mechanisms underlying KD action. We introduce these mechanisms chronologically as they were proposed, integrate these ideas with more recent findings, and examine the evidence for the broad neuroprotective properties of the KD; these properties, if validated, would highlight the clinical potential of the KD as a broadly encompassing disease-modifying intervention.[4–7] Whether the cellular mechanisms responsible for the clinical utility of the KD for human epilepsies are identical to those observed in animal models, or whether they overlap with the mechanisms that afford clinical benefits for other neurological conditions,

remains to be determined. An integration of older and newer ideas regarding the underlying mechanisms of KDs might represent the ideal scientific strategy to eventually unlock the secrets of this metabolism-based therapy.

HISTORICAL AND CLINICAL PERSPECTIVES

Early Hypotheses of KD Action

Initial studies into the mechanisms underlying KD action focused on concepts of acidosis, dehydration, and increased ketone concentrations—largely because these were the readily apparent ideas stemming from clinical implementation and observations.[8] Mild dehydration was postulated as necessary, possibly to maximize concentrations of ketones that were believed to provide anti-convulsant effects. As discussed later, however, peripheral ketone concentrations alone (whether measured in urine or in blood) are not tightly correlated with seizure control, and there is no evidence that dehydration or fluid restriction is necessary for clinical efficacy.[9] Nonetheless, because ketone metabolism generates protons and pH-lowering metabolic products, decreased pH (i.e., acidosis) was also considered initially to be a key aspect of the KD. However, there is no clear evidence that a KD significantly lowers brain pH, let alone that a decrease in pH-however small—may be associated with its anti-convulsant activity.[10]

Despite these negative results, however, it is possible that the KD may induce dynamic and differential pH changes in local microdomains; this possibility has yet to be assayed during KD treatment. Indeed, local compartments have been shown to exhibit differential pH regulation during neuronal activity,[11,12] and recent work has highlighted novel pH-related anti-convulsant mechanisms. For example, acidosis in vivo reduced seizure activity via activation of a depolarizing acid-sensing ion channel 1a (ASIC1a) localized to hippocampal interneurons.[13] Other studies showed that decreased intracellular pH during periods of increased neuronal excitability releases adenosine and decreases excitatory synaptic transmission and bursting in in vitro hippocampal slices.[14] Thus, pH-related mechanisms may offer new prospects for anti-convulsant therapies, and techniques enabling higher-resolution studies of pH dynamics in vivo and within neuron-glia microdomains may resolve permanently whether the KD acts in part by changing pH-which can influence proton-sensitive ion channels such as *N*-methyl-D-aspartate (NMDA) receptors[15,16] and specific gamma-aminobutyric acid A ($GABA_A$) receptor isoforms.[17–19]

Clinical Insights

From decades of clinical experience, it is been observed that almost any diet resulting in ketonemia and/or reduced blood glucose levels can produce an anti-convulsant effect. Comparable clinical efficacy has been seen using KDs comprised of either long-chain triglycerides (LCTs)[20,21] or medium-chain triglycerides (MCTs).[22–25] Within the last decade, two additional variations of these KDs have emerged[26]: the modified Atkins diet (MAD)[27–30] and the low-glycemic index treatment (LGIT).[31–33] The former allows for more liberal carbohydrate consumption and does not significantly restrict protein intake compared to the classic KD, whereas the latter was developed to mirror reduced glucose levels during KD treatment, and as such is based on a fundamental adherence to foods with low glycemic indices. The glycemic index is a value that describes the extent to which a carbohydrate is absorbed and elevates blood glucose, and lower indices correlate with slower insulin responses compared to glucose. Interestingly, LGIT does not induce the prominent ketosis seen with classic KDs and the Atkins diet.

Thus, at present, there are three primary dietary therapies for epilepsy: the traditional KD (a variation of which is the MCT diet), the MAD, and the LGIT. All three diets have been increasingly studied and are being used currently for both children and adults in centers worldwide. Importantly, available evidence indicates that dietary composition per se does not appear to affect the anti-convulsant efficacy of the diet as long as there is a degree of sustained ketosis and/or calorie restriction.[34–39] And although there are patients with epilepsy who respond dramatically within days of initiating the KD, maximum efficacy is not generally achieved for several days or weeks after initiation, suggesting that longer-term adaptive

Table 78–1 **Ketogenic Diet: Clinical Correlates and Experimental Observations**

	Clinical Correlate	Observation in Animal Models
Seizure type	KD is effective against many seizure types and epilepsy syndromes	KD is effective in models employing a wide variety of seizure paradigms
Age range	Children extract and utilize ketones from blood more efficiently than older individuals	Younger animals respond better to the KD
Caloric restriction	Associated with seizure reduction	Increases the seizure threshold
Diet type	Classic and MCT KDs are equally efficacious	Classic and MCT diets both increase the seizure threshold
Ketosis	Ketosis is necessary but not sufficient for seizure control	A threshold level of ketosis is necessary but not sufficient to explain antiseizure effects
Fat	Practical concerns limit the ketogenic ratio; possible role of fat chain length and degree of saturation (e.g., PUFAs)	Improved efficacy with higher ketogenic ratios; uncertain if the type of fat is a critical variable
Latency to KD effectiveness	Seizures may be seen during the prediet fast or after a latency of days to weeks	Several days
Reversal of protective effect when KD is discontinued	Rapid (hours)	Rapid (hours)

Abbreviations: KD, ketogenic diet; MCT, medium-chain triglycerides; PUFAs, polyunsaturated fatty acids.
Source: Adapted from ref. 59.

metabolic and/or genetic mechanisms may be recruited.[40] These adaptations are likely generalized throughout the epileptic brain, irrespective of the underlying pathology or the genetic predisposition to seizures, because the KD is an effective treatment for diverse epileptic conditions.[41,42] Table 78–1 provides a general context for the subsequent discussion, comparing and contrasting key parameters observed clinically and in experimental models.

ANIMAL MODELS

Acute Models

The bulk of the existing experimental literature pertaining to the KD involves studies in which various high-fat treatments are implemented prior to acute provocation with either electrical or chemoconvulsant stimulation in rodents. In these studies, animal diets have modeled the classic LCT diet and conform closely to either a 4:1 or approximately a 6:1 ketogenic ratio of fats to carbohydrates plus protein (by weight). In general, irrespective of the precise dietary formulation—as long as ketosis is seen, reflecting a shift from primarily glycolysis to intermediary metabolism—anti-convulsant effects have been observed. Indeed, whether seizures are provoked by corneal electroshock, hydration electroshock, maximal electroshock, pentylenetetrazol (PTZ), bicuculline, semicarbazide, kainate, flurothyl, or 6 Hz stimulation, chronic pretreatment with a KD appears to render anticonvulsant effects.[43–53] It should be noted that the KD is not anti-convulsant in all acute animal models, particularly in mice,[54–56] and its effects may be of limited duration[44] or can even exacerbate (maximal) seizures.[37,46,47,57,58] But while such results may raise concerns for the validity of the studies as a whole, one must recall that the KD is not universally effective in patients with medically refractory epilepsy.[20,21,41]

Further complicating interpretation of the animal literature, the highly variable methodologies used (e.g., use of calorie restriction, age at initiation and duration of therapy, dietary ratios and formulations, timing of treatment, mode of seizure induction) have made cross-comparisons nearly impossible.[59,60] Regarding observations in rodent models, the anticonvulsant efficacy of 4:1 or 6:1 KDs may be

confounded by the fact that they did not control for intake of vitamins, minerals, and antioxidants. Highlighting this cautionary note, when a balanced KD was utilized in the PTZ, kainate, or flurothyl models, anti-convulsant efficacy was not actually observed.[55] Thus, acute animal studies highlight a number of problems with these experimental approaches and have challenged our ability to directly translate animal research to the human epileptic condition.

Chronic Animal Models

Published animal studies, in attempting to replicate the clinical experience, suggest that dietary effects in controlling brain excitability extend beyond species boundaries, but they have done little to enhance our knowledge of underlying mechanisms of action. Rather, they raise more issues and highlight the necessity of investigating KD effects in a more clinically relevant model—namely, a chronic model characterized by early-onset, medically refractory epilepsy that is responsive to a clinically validated formulation of the KD.[61] That said, it is also important to recognize that species differences, particularly with regard to fatty acid metabolism and blood-brain-barrier properties, may dictate incongruency of experimental results.

Muller-Schwarze and colleagues[62] provided the first evidence that a KD can retard epileptogenesis in a chronic animal model. In this study, rats were first subjected to kainate-induced status epilepticus and then treated with a KD. Seizure frequency and duration, recorded after the latent period, were both significantly lower in the KD-treated group compared to controls. Further, there was a significant reduction in the extent of mossy fiber sprouting in the KD-fed group. In another model of chronic epilepsy, the KD was shown to prolong the lifespan in succinic semialdehyde dehydrogenase (SSADH)-deficient (*Aldh5a1*$^{-/-}$) mice, which are characterized by GABA deficiency, recurrent seizures, and early demise.[63] Interestingly, KD treatment restored spontaneous inhibitory synaptic currents to control levels, effects that were later attributed to an increase in the number of mitochondria in hippocampus, as well as a restoration of reduced hippocampal adenosine triphosphate (ATP) levels compared to controls.[64]

Comparable to induced models, the KD has also been shown to be effective in genetically determined epilepsy models. The EL mouse is a seizure-susceptible inbred strain believed to represent a model of multifactorial idiopathic partial epilepsy with secondary generalization.[65] Environmental stimulation such as repetitive handling can induce seizures in EL mice and facilitate epileptogenesis beginning at postnatal day 30 (P30). Generalized seizures generally manifest by the second postnatal month and persist throughout life.[66] When mice were fed a 4.75:1 KD formula over a 10-week period, seizure susceptibility scores were significantly reduced compared to those of controls after 3 weeks, but this difference disappeared by week 7.[67] These transient results were similar to those reported earlier in the kindling model.[44]

More recently, effects of a 6.3:1 KD were investigated in *Kcna1*-null mice lacking the gene encoding the delayed rectifier potassium channel α subunit, Kv1.1. The frequency of spontaneous recurrent seizures was significantly reduced in KD-fed mice compared to wild types.[68] This observation is of particular interest due to the facts that (1) *Kcna1*-null mice exhibit progressive histological changes in the hippocampus similar to those observed in human epileptic tissues and in many animal models of temporal lobe epilepsy[69] and (2) the *Kcna1* gene is one of only a few epilepsy genes in a developmental animal model that has a homologue in a human epileptic condition.[70,71]

MECHANISTIC STUDIES IN THE EARLY RENAISSANCE ERA

Ultimately, to produce anti-convulsant effects, the KD must reduce neuronal excitability and/or synchrony. The essential currency of neuronal excitability—both normal and aberrant—is the complex array of primarily voltage-gated and ligand-gated ion channels that determine the firing properties of neurons and mediate synaptic transmission. At present, there appear to be at least six important mechanisms through which the currently available antiepileptic drugs exert their anti-convulsant action,[72–74] and the vast majority of molecular targets are ion channels and transporters localized to plasmalemmal membranes. In this

light, a fundamental question pertaining to KD effects at the cellular and molecular levels has been whether any of the metabolic substrates (e.g., ketone bodies) elaborated by this non-pharmacological intervention can interact with ion channels known to regulate neuronal excitability. Broadly speaking, this does not appear to be the case. Given this tantalizing situation, there has been intense recent interest in how metabolic changes induced by a KD translate in a causal manner into a reduction in neuronal excitability and/or synchrony. Most of the postulated mechanisms discussed below are considered in the context of multiple lines of evidence, from human data as well as in vivo and in vitro model systems.

Ketone Bodies

Perhaps the most obvious and potentially most important clinical observation pertaining to the mechanistic underpinnings of the KD is the prominent ketonemia seen in patients. At face value, it would seem to be a relatively straightforward matter to establish a cause-and-effect relationship between the degree of ketonemia and seizure control. Further, it should be no surprise that since the 1930s, researchers have asked time and again whether ketone bodies might themselves exert anti-convulsant effects.[49,75–78] Despite intense scrutiny, this matter remains unresolved. It is well known that seizure control gradually improves within the first few weeks of initiating the KD, as serum ketone levels steadily increase. Interestingly, seizure control can be lost abruptly when ketosis is broken, usually through ingestion of carbohydrates,[22] again implicating ketone body action. Further, blood β-hydroxybutyrate (BHB) levels can appear to correlate directly with seizure control in children placed on a KD,[20,21,79] but the relationship is inconsistent.[41] The threshold BHB level for seizure control appears to be a blood concentration of 4 mmol/L for children successfully maintained on the KD for 3 or 6 months,[79] and investigators have used such clinical observations to model KD conditions in both animal models and in vitro studies. In general, blood levels are better correlated with seizure control than urinary levels; the latter are obtained through dipstick assessments that grossly reflect only acetoacetate levels.[79,80]

Keith first reported that acetoacetate (ACA) protected against thujone-induced seizures in rabbits,[75] an observation that was later confirmed in an audiogenic seizure-susceptible mouse model.[49] Later, Likhodii and colleagues provided direct evidence that acetone could block induced seizures in multiple animal models of seizures and epileptogenesis.[76] They demonstrated that acetone, when injected intraperitoneally, yielded plasma and cerebrospinal fluid (CSF) concentrations consistent with doses used to suppress seizures. In support of this, clinical investigators found that acetone was detectable in the brains of fully controlled KD-treated epileptic patients using proton magnetic resonance spectroscopy and was estimated to be present in concentrations of approximately 0.7 mM.[81] Curiously, while in vivo experiments have demonstrated the acute anticonvulsant properties of ACA and acetone, there are as yet no convincing data indicating that the major ketone body, BHB, can exert similar effects. Intriguingly, however, recent work suggests that metabolizing BHB rather than glucose reduces the availability of glutamate, which could then contribute to anticonvulsant and potentially neuroprotective effects.[82]

Considering the other ketone bodies, the link between ACA and acetone is particularly tight; ACA is spontaneously decarboxylated to acetone, and as such, it was speculated that ACA's acute anticonvulsant effects might be due to immediate conversion to acetone and subsequently to its many downstream derivatives.[83] But more recent work has clearly demonstrated that the anticonvulsant activity of acetone is not dependent upon its metabolites,[84] so the exact mechanism of acetone's rather broad-spectrum anticonvulsant activity remains unknown. It has been proposed that S-D-lactoylglutathione, an intermediate of acetone metabolism, might activate voltage-gated potassium channels and thereby hyperpolarize the neuronal cell membrane,[85] but there is as yet no direct evidence. And there is yet another report that acetone and BHB enhanced inhibitory glycine receptors, whereas BHB alone was able to enhance $GABA_A$ receptor-mediated currents—but all of these actions were observed at highly supratherapeutic (i.e., anesthetic) concentrations, not seen during KD treatment.[86]

Investigations involving potential anticonvulsant compounds would not be complete

without the use of cellular electrophysiological techniques, and thus it was of interest whether ketone bodies would affect any of the principal ion channels that are the primary targets of clinically used antiepileptic drugs. Surprisingly, such studies were initiated little more than a decade ago. Thio and colleagues[87] reported that acute application of low millimolar concentrations of BHB or ACA did not affect synaptic transmission in normal rat hippocampus and did not affect $GABA_A$ receptors, ionotropic glutamate receptors, or voltage-gated sodium channels over a wide concentration range (300 μM to 10 mM). The fact that ACA was ineffective in their hands was indeed surprising, especially given its clear anti-convulsant effects when administered in vivo.[49,76]

Recently, however, Ma and colleagues[77] found that BHB and ACA—at physiological concentrations—reduced the spontaneous firing of GABAergic neurons in rat substantia nigra pars reticulata (SNr; a subcortical structure that influences seizure propagation) by opening cellular membrane-bound, ATP-sensitive potassium (K_{ATP}) channels. K_{ATP} channels, a type of inwardly rectifying potassium channel (Kir6) that is activated when intracellular ATP levels fall, were long considered logical candidates for linking metabolic changes to cellular membrane excitability.[88] Despite the intuitive appeal of this observation, an inherent discrepancy remains to be reconciled. Consistently, studies have shown that the KD can increase levels of ATP and other bioenergetic substrates through enhanced mitochondrial respiration.[89–93] Because high ATP levels block K_{ATP} channel activity, it is unclear how opening of these channels is achieved by infusion of ketone bodies in the SNr.

Yet another intriguing link between ketone bodies and neuronal excitability was recently reported. Juge and colleagues demonstrated that ACA inhibits vesicular glutamate transporters (VGLUTs), which are required for exocytotic release of the excitatory neurotransmitter glutamate, specifically by competing with an anion-dependent regulatory site on presynaptic vesicles.[78] These investigators demonstrated that ACA decreased the quantal size of excitatory neurotransmission at hippocampal synapses, and suppressed glutamate release and seizures evoked by the convulsant 4-aminopyridine in rats. This novel finding may be a plausible explanation for the acute in vivo effects of ACA observed nearly eight decades earlier,[75] but ACA is reported to have other actions as well, notably on mitochondria.[92,94] The other caveat is that ACA is highly unstable and undergoes spontaneous decarboxylation to acetone, which may have other actions. Further, in the presence of BHB dehydrogenase, ACA is interconverted to the major ketone body BHB. Thus, it appears that seemingly straightforward metabolic substrates are players in a more complex arena.

In summary, the available evidence thus far fails to strongly support a primary mechanistic role for ketone bodies in the clinical efficacy of the KD, as no compelling molecular target has been identified and linked to attenuation of spontaneous seizures in a chronic epilepsy model. At this juncture, it is unlikely that there is only one relevant action of any of the primary ketone bodies on neuronal activity. If ketones are indeed fundamentally required for the anticonvulsant efficacy of the KD, they are more likely contributory to other parallel (and possibly synergistic) effects of the diet. Of the various hypotheses proposed, the most likely candidate mechanisms are membrane hyperpolarization through activation of potassium channels, increased GABAergic neurotransmission, or a reduction in vesicular glutamate release. Ketones have been shown to reduce brain glucose consumption,[95] and reduced glucose is another key hallmark of a KD discussed in more detail below.

Age Dependence of Ketone Utilization

Multiple studies and anecdotal observations have suggested that the KD is most effective in immature animals or infants and children,[34,45,54,58,96] perhaps due to greater fatty acid oxidation of breast milk (which is high in fats), more efficient extraction of ketone bodies from the blood, and an early age-dependent surge in the expression of the monocarboxylic acid transporters, MCT1 and MCT2.[97,98] If indeed the degree of ketonemia is a major determinant of seizure control, one would predict that younger patients would respond better than older ones. Clinical studies to date tend to support this notion, although it is becoming clearer that adolescents and adult patients with

epilepsy also benefit from KD treatment[99,100] In addition, there is abundant laboratory evidence suggesting that the anticonvulsant effects of KDs are not age-dependent.[40]

There is recent controversy involving ketone bodies aimed straight at the heart of a scientific dogma—that is, the notion that GABA-mediated responses in the early developing brain are excitatory, not inhibitory.[101,102] Zilberter and colleagues reported that the addition of ketones and other metabolic substrates such as lactate to artificial cerebrospinal fluid (aCSF)—which traditionally contains glucose as the sole energy substrate—resulted in an age-dependent hyperpolarizing shift of the GABA reversal potential in both hippocampus and neocortex and a significant reduction in the generation of giant depolarizing potentials (GDPs), which have long been regarded as the hallmark of spontaneous neonatal network activity in vitro.[103,104] This striking finding suggests that in the immature brain, GABAergic neurotransmission might actually be inhibitory in situ, as there is a greater preponderance of energy substrates (such as ketone bodies, lactate, and pyruvate) than at later ages.[105–107] However, these provocative findings have quickly been challenged by other groups who found that (1) the depolarizing GABA action in neonatal hippocampal slices is not due to deficiencies in energy metabolism[108] and (2) physiological plasma concentrations of BHB, lactate, and pyruvate failed to affect the depolarizing actions of GABA in immature rat pups (P4–7), and only nonphysiological concentrations of pyruvate (5 mM) reduced the driving force for $GABA_A$ receptor-mediated currents and blocked GDPs.[109] Clearly, while this controversy remains unsettled at present, the general notion that bioenergetic substrates may exert profound cellular electrophysiological effects is becoming increasingly appreciated.

GABAergic Inhibition

An enduring hypothesis regarding the mechanisms of KD action involves enhancement of GABA-mediated inhibition. Facilitation of GABAergic neurotransmission has long been accepted as a critical mechanism of action for a variety of clinically effective antiepileptic drugs, and thus there is an intrinsic appeal to invoking this mechanism. With respect to ketone bodies, however, $GABA_A$ receptors do not appear to be the primary targets in this regard. Indirect evidence comes from acute animal studies in which the KD is found to be most effective against seizures evoked by the GABAergic antagonists (i.e., PTZ, bicuculline, picrotoxin and gamma-butyrolactone), whereas it fails to block acute seizures provoked by kainic acid, strychnine (a glycine receptor antagonist), and maximal electroshock (MES; involving voltage-dependent sodium channels).[48]

More direct evidence comes from electrophysiological recordings conducted in vivo, demonstrating that the KD increased paired-pulse inhibition and elevated the maximal dentate activation threshold in rats, consistent with an elevated seizure threshold via enhancement of GABAergic inhibition.[37] In a related study, caloric restriction—which results in mild ketosis—enhanced the expression of both isoforms of glutamic acid decarboxylase (GAD65 and GAD67, the biosynthetic enzymes for GABA) in the tectum, cerebellum, and temporal cortex of rats, suggesting increased GABA levels.[110] However, increased GAD expression might actually reflect decreased GABA production,[111–113] so the ramifications of these findings to neuronal inhibition remain unclear.

At a neurochemical level, Yudkoff and colleagues have proposed that in the ketotic state, a major shift in brain amino acid handling results in a reduction of aspartate relative to glutamate (the precursor to GABA synthesis) and a shift in the equilibrium of the aspartate aminotransferase reaction.[114–117] This adaptation in the metabolism of the excitatory neurotransmitter glutamate (i.e., a decrease in the rate of glutamate transamination to aspartate) would be predicted to increase the rate of glutamate decarboxylation to GABA, the major inhibitory neurotransmitter, because more glutamate would be available for the synthesis of both GABA and glutamine.[114,115,118] An increase in brain GABA levels would then be expected to dampen seizure activity (Fig. 78–2). But is there any evidence that the KD increases GABA levels in seizure-prone areas of the brain? The experimental data thus far are inconsistent or are reflective of changes outside of critical areas such as the hippocampus, thalamus, and neocortex.[10,91,114,119] Two clinical studies have reported significant increases in GABA levels following KD treatment,[120,121] however, further substantiating this view. More recent work

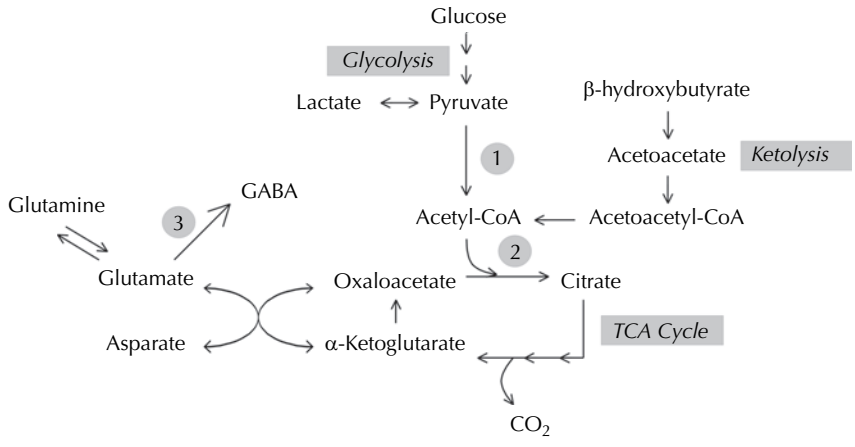


Figure 78–2. The metabolic interrelationships between brain metabolism of glutamate, ketone bodies, and glucose. In ketosis, 3-OH-butyrate (β-hydroxybutyrate) and acetoacetate contribute heavily to brain energy needs. A variable fraction of pyruvate (**1**) is ordinarily converted to acetyl-CoA via pyruvate dehydrogenase. In contrast, all ketone bodies generate acetyl-CoA, which enters the TCA cycle via the citrate synthetase pathway (**2**). This step involves the consumption of oxaloacetate, which is necessary for the transamination of glutamate to aspartate. Oxaloacetate is then less available as a reactant of the aspartate aminotransferase pathway, which couples the glutamate-aspartate interchange via transamination to the metabolism of glucose through the TCA cycle. Less glutamate is converted to aspartate, and thus more glutamate is available for synthesis of GABA (**3**) through glutamic acid decarboxylase (GAD). Adapted from Yudkoff M, Daiken Y, Nissim, I, Nissim I. The ketogenic diet: interactions with brain amino acid handling. In: Stafstrom CE, Rho JM, eds. *Epilepsy and the Ketogenic Diet*. Totowa, NJ: Humana Press; 2004:186.

has demonstrated that BHB decreases GABA degradation and thus could increase the available pool of GABA.[122] Collectively, whereas both laboratory and clinical data support a role for increases in GABA levels and presumably increased inhibition via GABA receptors as a potential mechanism underlying KD action, it remains unclear why a KD can be effective in stopping seizures in patients who have failed to respond to GABAergic drugs.

Norepinephrine and Neuropeptides

One of the more intriguing observations regarding KD action involves the noradrenergic system. Several lines of evidence support the notion that increases in noradrenergic tone result in anticonvulsant activity. For example, norepinephrine (NE) reuptake inhibitors prevent seizures in genetically epilepsy-prone rats (GEPRs),[123] agonists of noradrenergic receptors are generally anticonvulsant,[124] and ablation of the locus coeruleus (the origin of both ascending and descending noradrenergic innervation) contributes to the ontogeny of self-sustaining status epilepticus (SSSE) in rats.[125] Along these lines, it is of significant interest that mice lacking the ability to produce NE (i.e., dopamine β-hydroxylase-deficient mice) do not exhibit increased resistance to flurothyl seizures when treated with a KD,[126] whereas similar treatment renders protective effects in control wild types. These data indicate that NE is required for the anticonvulsant effect of KD, at least in the flurothyl seizure threshold model. It was later shown that a KD increased basal NE levels in hippocampus nearly two-fold,[124] further supporting this mechanistic hypothesis. Increased NE release would also be predicted to promote co-release of anticonvulsant orexigenic peptides such as neuropeptide Y (NPY) and galanin.[124] However, there was is no evidence for enhanced transcription of either of these peptides in the brain after KD treatment, suggesting that neither NPY nor galanin contributes significantly to the anticonvulsant actions of a KD.[127]

Another peptide potentially important in mediating KD effects is leptin, an endogenous substrate that helps regulate energy homeostasis. Leptin is part of the hormonal system that limits energy intake and expenditure and is intimately involved in appetite regulation.[128] Less appreciated, however, is the fact that it can exert modulatory effects on neuronal

excitability and suppress seizure activity.[129,130] In an important translational study, leptin was shown to attenuate focal or generalized seizures in rodents, possibly through alteration of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptor-mediated synaptic transmission.[131] Since the KD causes a rise in leptin levels,[132] it is possible that the KD's mechanism, at least in part, may relate to a leptin-associated reduction in synaptic excitability.

Polyunsaturated Fatty Acids

Of the various fats that constitute KDs, polyunsaturated fatty acids (PUFAs) have received by far the most attention. Docosahexaenoic acid (DHA, C22:6ω3), arachidonic acid (AA, C20:4ω6), and eicosapentaenoic acid (EPA, C20:5ω3) are the main PUFAs believed to profoundly affect cardiovascular function and health. It is well known that PUFAs can directly inhibit voltage-gated sodium channels and L-type calcium channels[133–135] and can activate certain classes of potassium channels.[136,137] Further, DHA and EPA have been shown to decrease neuronal excitability and bursting in hippocampus.[138]

Intriguingly, there is clinical evidence that the KD elevates key PUFA levels in the blood.[139] In this study—in which each patient served as his or her own control—AA increased 1.6- to 2.9-fold, DHA increased 1.5- to 4.0-fold, and the rise in total serum AA correlated with improved seizure control. A later thought-provoking study revealed that CSF taken from epileptic patients on the KD promoted opening of voltage-gated Shaker-type potassium channels expressed in *Xenopus* oocytes, and these authors surmised that the DHA, EPA, and linoleic acid might be responsible for this effect.[140]

Laboratory investigations linking PUFAs to KD action appear discordant. Several studies have found that PUFAs are anticonvulsant in rodents,[133,137,141–143] yet other published investigations report no such effects.[144,145] Interestingly, KDs with widely differing effects on tissue lipids and fatty acid profiles can confer similar degrees of seizure protection,[146] suggesting that specific fatty acids might not be the key mediators of the KD's clinical effects. This latter study is consistent with the notion that a general metabolic shift toward fatty acid oxidation is a fundamental requirement.

Clinical studies are similarly inconclusive. One early study of five patients found that dietary supplementation with a "PUFA spread" (specifically, 5 gm of 65% omega-3 PUFAs once daily) suppressed seizures in those who tolerated the treatment.[147] Later, a randomized, placebo-controlled study involving a larger sample size failed to find similar effects when a PUFA supplement consisting of EPA plus DHA (2.2 mg/day in a 3:2 ratio) was administered over a 12-week treatment period.[148] Another study found no correlation between changes in fatty acid levels and seizure response when a KD was supplemented with omega-3 fatty acids in 25 children with epilepsy.[149] More recently, a retrospective analysis revealed that AA levels were significantly decreased in epileptic patients treated successfully with the KD compared to nonresponders.[150] Although it would be reasonable to dismiss the PUFA hypothesis given the clinical data thus far, it is likely that a proper controlled trial design has yet to be implemented. Further, while there are a number of clinical research variables that were not considered, factors such as PUFA load, duration of treatment, and, potentially, degree of ketosis might be highly important in demonstrating significant effects.

There are a few additional aspects of PUFA biology worth mentioning. Polyunsaturated fatty acids are natural activators of fatty acid receptors, specifically peroxisome proliferator-activated receptors (PPARs), and there is evidence that activation of brain-localized PPARs can influence seizure activity.[151–153] In this regard, PPARα could act as both a sensor and an effector of KD action.[154] Further, nuclear translocation of activated PPARs inhibits pro-inflammatory transcription factors,[151] and, as inflammation is increasingly recognized as a core contributor to seizures and epileptogenesis,[155] a KD (or a similar metabolic formulation) might constitute a rational treatment approach.[156]

METABOLIC MECHANISMS

The earliest demonstration that the KD enhances energy substrates was provided by DeVivo and colleagues,[119] who showed significant increases in brain bioenergetic substrates such

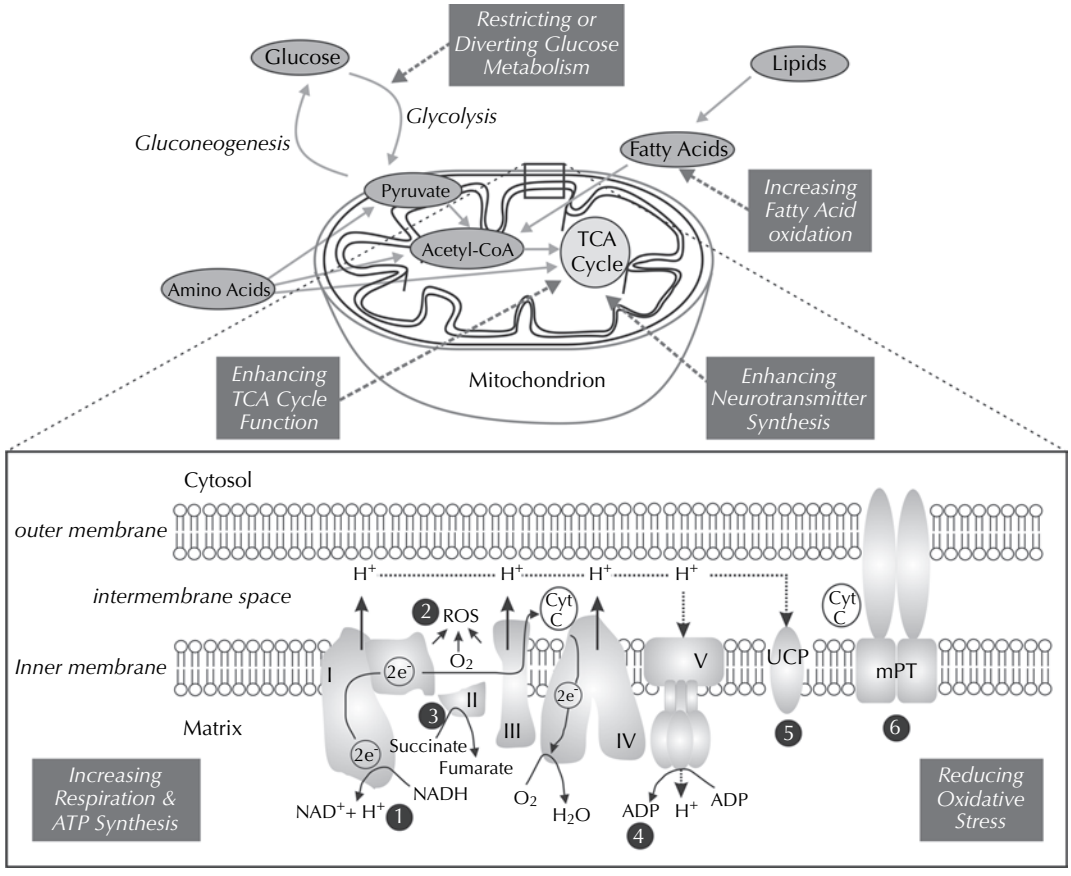
as ATP, creatine, and phosphocreatine in normal adult rats fed a high-fat, low-carbohydrate diet for 3 weeks. The metabolic data examined in this study indicated an overall increase in the cerebral energy reserve and energy charge that the authors believed could account for the neuronal stability that accompanied the chronic ketosis. A later study confirmed the elevation in brain adenine nucleotides as a consequence of KD treatment.[157] However, the most compelling demonstration that the KD profoundly affects energy metabolism was provided by Bough and colleagues, who used microarray and electron microscopic techniques to examine patterns of gene expression in the hippocampus of rats fed either a KD or a control diet.[91] They quantified a robust upregulation of transcripts encoding metabolism enzymes and mitochondrial proteins and an increased number of mitochondrial profiles. Importantly, and consistent with increased energy reserves, hippocampal slices from KD-fed animals were highly resistant to the metabolic stress induced by low-glucose conditions. Additional studies support the beneficial effects of a KD on mitochondrial energy metabolism.[64,158] Figure 78–3 highlights some of the effects of the KD on mitochondrial function.

As intriguing as these studies are, how exactly would enhanced energy reserves lead to a stabilization of synaptic functioning, membrane potential, and diminished seizure activity? One interpretation of the neuronal consequences of these bioenergetic changes is that neurons are better able to maintain ionic gradients and the resting membrane potential and thus resist depolarizing influences. The most likely way this stabilization is accomplished is through the Na^+/K^+ adenosine triphosphatase (ATPase) pump; specifically, KD-induced elevations in ATP concentrations might enhance and/or prolong the activation of the Na^+/K^+-ATPase, perhaps via an increase in the delta-G′ of ATP hydrolysis.[159] To date, however, there are no published studies directly testing this sodium-pump hypothesis in epileptic brain.

There is an alternative and complementary hypothesis linking increased energy substrate availability with membrane hyperpolarization. Kawamura and colleagues evaluated the electrophysiological effects of reduced glucose (a consistent finding in patients successfully treated with the KD) in CA3 hippocampal pyramidal neurons—importantly, under conditions of adequate or enhanced ATP levels, but without the addition of ketone bodies—using whole-cell recording techniques.[160] These investigators found that glucose restriction led to ATP release through pannexin hemichannels localized on CA3 neurons. The increased extracellular ATP, upon rapid degradation by ectonucleotidases to adenosine, resulted in activation of adenosine A_1 receptors that were shown to be coupled to opening of plasmalemmal K_{ATP} channels. This study highlights a novel mechanism of metabolic autocrine regulation, involving close cooperation among pannexin hemichannels, adenosine receptors, and K_{ATP} channels, and provides an elegant connection to an earlier study demonstrating ketone-mediated attenuation of spontaneous neuronal discharge in SNr.[77] It should be noted that the potential contribution of adenosine is plausible, given the well-substantiated role of this endogenous purine in suppressing cellular excitability.[161] Consistent with the involvement of adenosine, this latter group recently found evidence that the KD suppresses electrographic seizures in vivo in mice with spontaneous seizures through a mechanism involving A_1 receptors.[162]

It is important to note that the observation that reduced glucose might contribute to seizure control is not recent. Caloric restriction in rodents was shown to reduce seizure susceptibility, and lowered levels of blood glucose correlated with inhibition of epileptogenesis in a genetic model.[35,163] When the interplay of glucose levels and ketosis is examined, metabolic control theory would argue that glucose restriction might be more important than ketonemia.[164] Clinically, the link between blood glucose levels and seizure control has yet to be proven, and skepticism regarding relative hypoglycemia as the major contributor to KD action is supported by the incongruency of animal data.[165]

Another potentially important consequence of reduced glucose has been highlighted by the use of 2-deoxy-D-glucose (2DG), a glucose analog that inhibits glycolysis by blocking phosphoglucose isomerase. 2-Deoxy-D-glucose has been shown to be a potent anti-convulsant and antiepileptic agent in several animal models, including kindling, audiogenic seizures in Fring's mice, and 6 Hz corneal stimulation,[6,166] as well as in vitro,[6] and decreases synaptic transmission via adenosine in vitro.[167] The acute effects



Electron Transport and Oxidative Phosphorylation

Figure 78–3. Major changes in important biochemical pathways reported to exert anti-convulsant and antiepileptogenic effects in experimental models (*shadowed boxes*; also see text). *Below*: Putative interactions between mitochondrial respiratory complexes (MRCs) and KD-related metabolites. First ❶, either acetoacetate (ACA) or β-hydroxybutyrate (BHB) can oxidize the NADH couple. Second ❷, ketone bodies (KB) can decrease mitochondrial reactive oxygen species (ROS) generation. Third ❸, KB can protect neurons against MRC I and II inhibitors. Also, the KD elevates the seizure threshold in epileptic patients with impaired MRC function. Fourth ❹, either the KD or KB can enhance ATP production. Fifth ❺, fatty acids can activate mitochondrial uncoupling proteins (UCPs). Finally ❻, KB can elevate the threshold for mitochondrial permeability transition (mPT) activation. The bulk of experimental evidence supports the hypothesis that activation of mitochondrial K_{ATP} (mKATP) channels decreases ROS formation, likely by diminishing the proton-motive force ($\Delta\psi$) across the mitochondrial inner membrane (via transmembrane flux of potassium), and subsequently attenuating electron flux across the MRC, in a manner similar to mitochondrial uncoupling. Cyt C, cytochrome c.

of 2DG may be mediated through a number of different downstream mechanisms, but one intriguing possibility is a decrease in the endogenous phosphorylation of $GABA_A$ receptors (which renders them dysfunctional) by the glycolytic enzyme glyceradeldehyde-3-phosphate dehydrogenase.[168,169] Perhaps the most compelling effect of 2DG in the kindling model is decreasing the expression of brain-derived neurotrophic factor (BDNF) and its principal receptor, neurotrophic tyrosine kinase receptor, type 2 TrkB, via induction of the transcription factor NRSF (neuron restrictive silencing factor), a master negative regulator of neuronal genes.[166] This fascinating study reveals a biochemical interruption of glycolysis that can result in downstream physicochemical modulation of transcription, yielding a powerful effect in retarding epileptogenesis.

While glucose inhibition may exert pleiotropic effects in animal seizure models, similar anticonvulsant actions have been achieved by diversion of glucose to the pentose phosphate pathway (PPP). Fructose-1,6-diphosphate (FDP), a glycolytic intermediate, has been shown to exert acute anticonvulsant activity in

several seizure models in adult rats including kainate, pilocarpine, PTZ, and kindling.[170,171] Indeed, FDP was more effective as an anticonvulsant than 2DG, KD, or valproate in these studies. The precise mechanisms through which FDP produces anticonvulsant effects remain unclear, but it is conceivable that this substrate may exert an antioxidant action because the reduced nicotinamide adenine dinucleotide phosphate (NADPH) generated through the PPP reduces glutathione.[172]

Another fascinating dietary approach to epilepsy treatment is based on the observation that seizures cause a deficiency in tricarboxylic acid cycle (TCA) intermediates (especially α-ketoglutarate and oxaloacetate). It has been hypothesized that "refilling" these deficient compounds (a process called *anaplerosis*) might oppose seizure generation. In support of this idea, the anaplerotic substrate triheptanoin was recently studied in both acute and chronic seizure models.[173] Mice fed triheptanoin exhibited delayed development of corneal kindled seizures, and triheptanoin feeding increased the PTZ seizure threshold in chronically epileptic mice that had undergone status epilepticus 3 weeks before PTZ testing.[173] Therefore, like 2DG, anaplerotic compounds appear to alter both acute and chronic seizure susceptibility. Anaplerosis represents a novel approach that expands the potential metabolic modifications that could be anticonvulsant or antiepileptic. Taken together, results from studies of KDs, 2DG, FDP, and anaplerosis suggest that shifts in the activity of certain metabolic pathways might be exploited to develop novel treatments for epilepsy.

KD AND NEUROPROTECTION

While a detailed discussion of the expanding literature on the neuroprotective properties of the KD is beyond the scope of this chapter, a brief discussion is warranted, as such actions are intimately related to epileptogenesis and seizure propensity. Further, the neuroprotective potential of the KD is increasingly being explored in a number of neurological disorders (see below). The reader is referred to recent reviews for more details on this subject.[4,174–176]

Ketone bodies and PUFAs—metabolic substrates that are both elevated in epileptic patients treated with the KD—have been shown to exert neuroprotective activity in neurodegenerative conditions associated with impaired mitochondrial function.[175] Ketone bodies appear not only to raise ATP levels in seizure-prone areas such as hippocampus,[177] but also diminish reactive oxygen species (ROS) production through increases in reduced nicotinamide adenine dinucleotide (NADH) oxidation[92] and inhibition of mitochondrial permeability transition.[178]

Other than their effects on voltage-gated ion channels, PUFAs—through induction of PPARα and its co-activator PGC-1 (peroxisome proliferator-activated receptor γ coactivator-1)—induce the expression of mitochondrial uncoupling proteins (UCPs), which are homodimers spanning the inner mitochondrial membrane that enable a proton leak from the intermembrane space to the mitochondrial matrix. The net effect of this action is to reduce ATP synthesis, reduce calcium influx into the mitochondrial matrix, dissipate heat, and reduce ROS production.[179,180] The protective consequences of UCP activation have been detailed in a number of published reports.[180] One study demonstrated that dietary enhancement of UCP expression and function in immature rats protected against kainate-induced excitotoxicity, most likely by decreasing ROS generation.[181] Further work demonstrated that mice maintained on a high-fat KD demonstrated an increase in the hippocampal expression and activity of all three brain-localized mitochondrial UCPs (UCP2, UCP4, and UCP5) and exhibited a significant reduction in ROS generation in mitochondria isolated from the same brain region.[90] Thus, it appears that a prominent neuroprotective mechanism of KD action involves a reduction in mitochondrial free radical production, which would decrease oxidative stress and potentially neuronal injury.

FUTURE DIRECTIONS

To date, research efforts aimed at elucidating the mechanisms of KD action have not yielded simple answers. It is becoming increasingly apparent that the relevant mechanisms are likely diverse and operate in a coordinated and potentially synergistic fashion. The ongoing quest is challenged by the inherent difficulties in understanding neuronal network activity, let alone metabolism in the context of disease

states, and importantly, in the intact patient or animal. Despite these issues, the research reviewed herein provides a clearer link between metabolism and neuronal excitability and further validates the emerging field of neurometabolism, especially as it relates to epilepsy.

Based on the foregoing discussion, there are broader clinical implications posed by the KD. As the mechanisms underlying the neuroprotective activity of the KD are fundamental to many disease processes, it should be no surprise that diet can profoundly influence brain function and, in a growing number of instances, exert protective and potentially disease-modifying effects.[4,175,182] As examples, the KD (or various formulations of its key metabolic substrates) have been found to ameliorate a range of clinical disorders and/or experimental models such as autism and Rett syndrome,[183,184] pain and inflammation,[185] traumatic brain injury,[186–188] neurodegenerative diseases such as Alzheimer's and Parkinson's diseases,[189,190] brain cancer,[191–193] prostate cancer,[194] diabetes,[195,196] and obesity.[197] It should be noted that some of these disorders are comorbid with each other, thus presenting the opportunity to help ameliorate multiple diseases with a single therapy.

Of the many potential uses of the KD, brain cancer is perhaps one of the most compelling targets given the rising interest in identifying metabolic targets for intervention.[198] The theoretical basis for using the KD to treat cancer is that tumorigenesis relies heavily on glucose, whereas normal cells retain metabolic flexibility and can use ketones for fuel.[192,198,199] A calorically restricted KD or other glycolysis-limiting treatment forces higher ketone production, putting maximal stress on the tumor cells and minimal stress on the normal cells.[191,192,198] Paradoxically, some aspects of the current standard of care for brain cancer may support tumor growth.[200]

Neurodegenerative diseases are universally associated not only with mitochondrial dysfunction but also with inflammation, and inflammation is a hallmark of virtually every chronic disease process throughout the body. In addition to reducing pain and inflammation,[185] the KD appears to enhance motor and cognitive functioning in a model of multiple sclerosis.[201] Further evidence is provided by recent reports of the KD mitigating 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity and microglial activation,[190] as well as encephalopathy and seizures in some forms of fever-induced epilepsy.[202] Ultimately, reducing inflammation could be one of the most important disease-modifying effects of a KD.

In addition to inflammation, disrupted sleep and circadian rhythms are common comorbidities with many diseases. Recent evidence in humans and in animal models shows that a KD can improve sleep in children with epilepsy[203] and circadian rhythmicity in epileptic *Kcna1*-null mice.[68] It is well known that circadian rhythm disruption is associated with epilepsy, psychiatric disorders, and the prevalent metabolic syndrome.[204–207] Normalization of circadian rhythms alone could yield enormous clinical benefits.[208]

CONCLUSIONS

The evidence for a KD as a successful epilepsy treatment is clear. Multiple retrospective, multi-center, and randomized prospective studies document consistent and significant clinical benefits. The true efficacy of dietary treatments for epilepsy may be underestimated, as the KD is rarely used as a first-line therapy. Certainly, by the time the KD is initiated to thwart medically refractory epilepsy, in some instances the severity of the epileptic condition may be too difficult to overcome. But remarkably, the KD works in the majority of patients who failed to respond to numerous antiepileptic drugs. A detailed understanding of key KD mechanisms could offer a meaningful adjuvant or ultimately the development of a "diet in a pill."[3] But while clinical applications of metabolism-based therapy appear to

(*continued from page 1017*)

(BDNF) and TrkB signaling in brain. As activation of TrkB pathways by BDNF has been shown to promote hyperexcitability and kindling, these potential KD-induced effects would be expected to limit the symptom (seizures) as well as epileptogenesis. Boxed variables depict findings described from KD studies; up (↑) or down (↓) arrows indicate the direction of the relationship between variables as a result of KD treatment. Dashed lines are used to clarify linkages and are not meant to suggest either magnitude or relative importance compared to solid lines. NE, Norepinephrine; VGLUT, vesicular glutamate transporter; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; Nrf1, nuclear respiratory factor 1. Adapted from ref. 40.

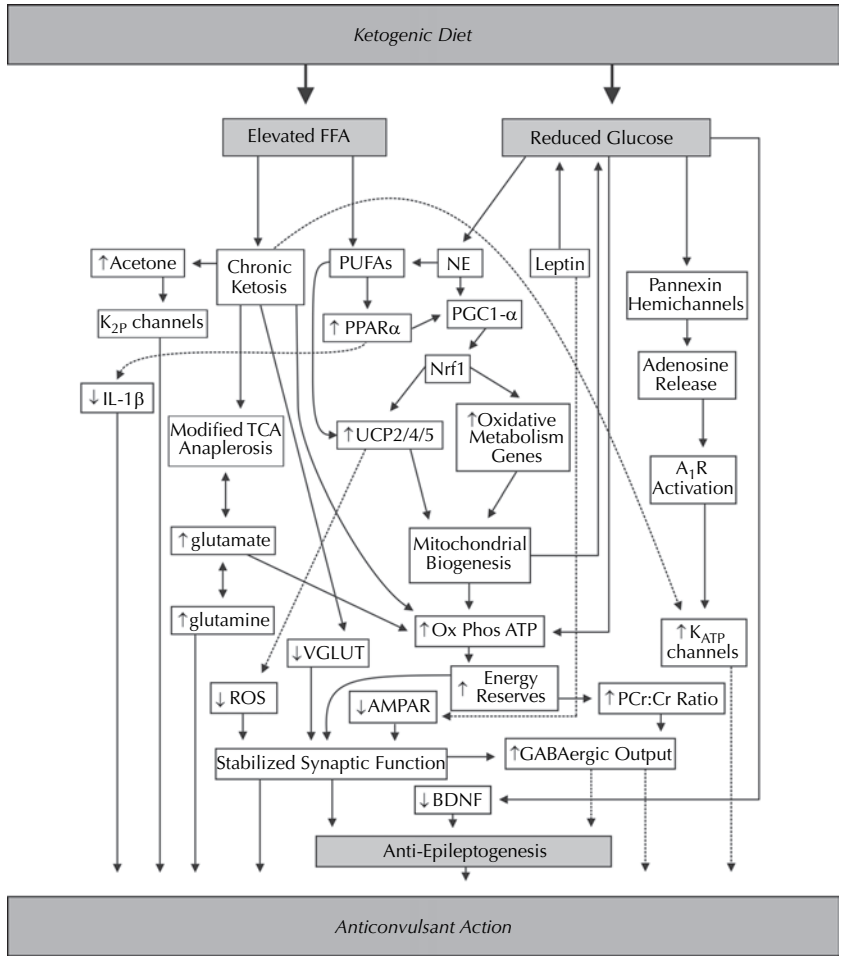


Figure 78–4. Hypothetical pathways leading to the anti-convulsant effects of the KD. Elevated free fatty acids (FFA) lead to chronic ketosis and increased concentrations of polyunsaturated fatty acids (PUFAs) in the brain. Chronic ketosis is predicted to lead to increased levels of acetone; this might activate K_{2P} channels to hyperpolarize neurons and limit neuronal excitability. Chronic ketosis is also anticipated to modify the tricarboxcylic acid (TCA) cycle, as would the presence of anaplerotic substrates such as triheptanoin. This would increase glutamate and, subsequently, GABA synthesis in brain. Among several direct inhibitory actions, PUFAs boost the activity of brain-specific uncoupling proteins (UCPs). This is expected to limit ROS generation, neuronal dysfunction, and resultant neurodegeneration. Acting via the nuclear transcription factor peroxisome proliferator-activated receptor-α (PPARα) and its coactivator peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1α), PUFAs would induce the expression of UCPs and coordinately upregulate several dozen genes related to oxidative energy metabolism. Expression of PPARα is inversely correlated with interleukin 1β (IL-1β) cytokine expression; given the role of IL-1β in hyperexcitability and seizure generation, diminished expression of IL-β cytokines during KD treatment could lead to improved seizure control. Ultimately, PUFAs would stimulate mitochondrial biogenesis. Mitochondrial biogenesis is predicted to increase adenosine triphosphate (ATP) production capacity and enhance energy reserves, leading to stabilized synaptic function and improved seizure control. In particular, an elevated phosphocreatine:creatine (PCr:Cr) energy-reserve ratio is predicted to enhance GABAergic output, perhaps in conjunction with the ketosis-induced elevated GABA production, leading to diminished hyperexcitability. Reduced glucose coupled with elevated FFA is proposed to reduce glycolytic flux during KD, which would further be feedback inhibited by high concentrations of citrate and ATP produced during KD treatment. This would activate metabolic K_{ATP} channels. Ketones may also directly activate K_{ATP} channels. Reduced glucose alone, under conditions of adequate or enhanced energy levels, activates pannexin hemichannels on CA3 pyramidal neurons, releasing ATP into the extracellular space; ATP is converted via ectonucleotidases to adenosine, which subsequently activates adenosine receptors (A_1R). Activation of A_1R is also coupled to K_{ATP} channels. Ultimately, opening of K_{ATP} channels would hyperpolarize neurons and diminish neuronal excitability to contribute to the anti-convulsant (and perhaps neuroprotective) actions of the KD. Increased leptin, seen with KD treatment, can reduce glucose levels and inhibit AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptor-mediated synaptic excitation. Reduced glucose is also expected to downregulate brain-derived neurotrophic factor

(*continues on page 1016*)

be growing rapidly, there is a continuing need to develop modified diet formulations with improved efficacy and tolerability (as well as palatability) and to identify new pharmacological targets for drug discovery.

It is often stated that no single mechanism is likely to explain the clinical effects of antiepileptic drugs, and certainly the same could be said for the KD. The challenge of finding key mediators of KD action is made even more difficult by the intrinsic complexity of metabolic activity within neurons and glia, which, to be relevant to the epileptic condition, must be interpreted at network levels. In this chapter, we have reviewed a number of seemingly disparate variables proposed to collectively exert anti-convulsant (and potentially neuroprotective) effects. The important interrelationships are summarized in Fig. 78–4. The fact that a fundamental modification in diet can have such profound therapeutic effects on neurological disease underscores the importance of elucidating the mechanisms of KD action. In summary, mounting interest in and insight into the mechanisms of KD action have laid a promising foundation for metabolic therapy as an emerging strategy for neurological disorders.

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DISCLOSURE STATEMENT

The authors have no conflicts to disclose.

REFERENCES

1. Wilder RM. The effects of ketonemia on the course of epilepsy. *Mayo Clin Proc*. 1921;2:307–308.
2. Neal EG, Chaffe H, Schwartz RH, Lawson MS, Edwards N, Fitzsimmons G, Whitney A, Cross JH. A randomized trial of classical and medium-chain triglyceride ketogenic diets in the treatment of childhood epilepsy. *Epilepsia*. 2009;50:1109–1117.
3. Rho J, Sankar R. The ketogenic diet in a pill: is this possible? *Epilepsia.* 2008;49(suppl 8):127–133.
4. Gasior M, Rogawski MA, Hartman AL. Neuroprotective and disease-modifying effects of the ketogenic diet. *Behav Pharmacol*. 2006;17:431–439.
5. Masino SA, Kawamura M Jr, Wasser CD, Pomeroy LT, Ruskin DN. Adenosine, ketogenic diet and epilepsy: the emerging therapeutic relationship between metabolism and brain activity. *Curr Neuropharmacol*. 2009;7:257–268.
6. Stafstrom CE, Ockuly JC, Murphree L, Valley MT, Roopra A, Sutula TP. Anticonvulsant and antiepileptic actions of 2-deoxy-D-glucose in epilepsy models. *Ann Neurol*. 2009;65:435–447.
7. Balietti M, Casoli T, Di Stefano G, Giorgetti B, Aicardi G, Fattoretti P. Ketogenic diets: an historical antiepileptic therapy with promising potentialities for the aging brain. *Ageing Res Rev*. 2010;9: 273–279.
8. Withrow CD. The ketogenic diet: mechanism of anticonvulsant action. *Adv Neurol*. 1980;27:635–642.
9. Wirrell E. Ketogenic ratio, calories, and fluids: do they matter? *Epilepsia.* 2008;49(suppl 8):17–19.
10. Al-Mudallal AS, LaManna JC, Lust WD, Harik SI. Diet-induced ketosis does not cause cerebral acidosis. *Epilepsia*. 1996;37:258–261.
11. Kraig RP, Ferreira-Filho CR, Nicholson C. Alkaline and acid transients in cerebellar microenvironment. *J Neurophysiol*. 1983;49:831–850.
12. DeVries S. Exocytosed protons feedback to suppress the Ca^{2+} current in mammalian cone photoreceptors. *Neuron.* 2001;32:1107–1117.
13. Ziemann AE, Schnizler MK, Albert GW, Severson MA, Howard MA 3rd, Welsh MJ, Wemmie JA. Seizure termination by acidosis depends on ASIC1a. *Nat Neurosci*. 2008;11:816–822.
14. Dulla CG, Frenguelli BG, Staley KJ, Masino SA. Intracellular acidification causes adenosine release during states of hyperexcitability in the hippocampus. *J Neurophysiol*. 2009;102:1984–1993.
15. Traynelis SF, Cull-Candy SG. Proton inhibition of *N*-methyl-D-aspartate receptors in cerebellar neurons. *Nature.* 1990;345:347–350.
16. Gielen M, Le Goff A, Stroebel D, Johnson J, Neyton J, Paoletti P. Structural rearrangements of NR1/NR2A NMDA receptors during allosteric inhibition. *Neuron.* 2008;57:80–93.
17. Mercik K, Pytel M, Cherubini E, Mozrzymas J. Effect of extracellular pH on recombinant $\alpha1\beta2\gamma2$ and $\alpha1\beta2$ $GABA_A$ receptors. *Neuropharmacology.* 2006;51: 305–314.
18. Pasternack M, Smirnov S, Kaila K. Proton modulation of functionally distinct $GABA_A$ receptors in acutely isolated pyramidal neurons of rat hippocampus. *Neuropharmacology.* 1996;35:1279–1288.
19. Feng H-J, Macdonald R. Proton modulation of $\alpha1\beta3\delta$ $GABA_A$ receptor channel gating and desensitization. *J Neurophysiol*. 2004;92:1577–1585.
20. Vining EP, Freeman JM, Ballaban-Gil K, Camfield CS, Camfield PR, Holmes GL, Shinnar S, Shuman R, Trevathan E, Wheless JW. A multicenter study of the efficacy of the ketogenic diet. *Arch Neurol.* 1998;55: 1433–1437.

21. Freeman JM, Vining EP, Pillas DJ, Pyzik PL, Casey JC, Kelly LM. The efficacy of the ketogenic diet—1998: a prospective evaluation of intervention in 150 children. *Pediatrics.* 1998;102:1358–1363.
22. Huttenlocher PR. Ketonemia and seizures: metabolic and anticonvulsant effects of two ketogenic diets in childhood epilepsy. *Pediatr Res*. 1976;10:536–540.
23. Sills M, Forsythe W, Haidukewych D, MacDonald A, Robinson M. The medium-chain triglyceride diet and intractable epilepsy. *Arch Dis Child*. 1986;61: 1168–1172.
24. Schwartz R, Eaton J, Bower B, Aynsley-Green A. Ketogenic diets in the treatment of epilepsy: short-term clinical effects. *Dev Med Child Neurol*. 1989;31: 145–151.
25. Mak S, Chi C, Wan C. Clinical experience of ketogenic diet on children with refractory epilepsy. *Acta Paediatr Taiwan*. 1999;40:97–100.
26. Kossoff EH, Zupec-Kania BA, Rho JM. Ketogenic diets: an update for child neurologists. *J Child Neurol*. 2009;24:979–988.
27. Weber S, Mølgaard C, Taudorf K, Uldall P. Modified Atkins diet to children and adolescents with medically intractable epilepsy. *Seizure*. 2009;18:237–240.
28. Kossoff E, Rowley H, Sinha S, Vining E. A prospective study of the modified Atkins diet for intractable epilepsy in adults. *Epilepsia.* 2008;49:316–319.
29. Kossoff E, McGrogan J, Bluml R, Pillas D, Rubenstein J, Vining E. A modified Atkins diet is effective for the treatment of intractable pediatric epilepsy. *Epilepsia.* 2006;47:421–424.
30. Kang H-C, Lee HS, You SJ, Kang DC, Ko T-S, Kim HD. Use of a modified Atkins diet in intractable childhood epilepsy. *Epilepsia*. 2007;48:182–186.
31. Pfeifer H, Lyczkowski D, Thiele E. Low glycemic index treatment: implementation and new insights into efficacy. *Epilepsia.* 2008;49(suppl 8):42–45.
32. Pfeifer H, Thiele E. Low-glycemic-index treatment: a liberalized ketogenic diet for treatment of intractable epilepsy. *Neurology.* 2005;65:1810–1812.
33. Muzykewicz D, Lyczkowski D, Memon N, Conant K, Pfeifer H, Thiele E. Efficacy, safety, and tolerability of the low glycemic index treatment in pediatric epilepsy. *Epilepsia*. 2009;50:1118–1126.
34. Bough KJ, Valiyil R, Han FT, Eagles DA. Seizure resistance is dependent upon age and calorie restriction in rats fed a ketogenic diet. *Epilepsy Res*. 1999;35: 21–28.
35. Greene AE, Todorova MT, McGowan R, Seyfried TN. Caloric restriction inhibits seizure susceptibility in epileptic EL mice by reducing blood glucose. *Epilepsia.* 2001;42:1371–1378.
36. Likhodii SS, Musa K, Mendonca A, Dell C, Burnham WM, Cunnane SC. Dietary fat, ketosis, and seizure resistance in rats on the ketogenic diet. *Epilepsia*. 2000;41:1400–1410.
37. Bough KJ, Schwartzkroin PA, Rho JM. Caloric restriction and ketogenic diet diminish neuronal excitability in rat dentate gyrus in vivo. *Epilepsia*. 2003;44:752–760.
38. Eagles D, Boyd S, Kotak A, Allan F. Calorie restriction of a high-carbohydrate diet elevates the threshold of PTZ-induced seizures to values equal to those seen with a ketogenic diet. *Epilepsy Res*. 2003;54:41–52.
39. Hamdy R, Turner Z, Pyzik P, Kossoff E. Lack of influence of body mass index on the efficacy of the ketogenic diet. *J Child Neurol*. 2007;22:1167–1171.
40. Bough KJ, Rho JM. Anticonvulsant mechanisms of the ketogenic diet. *Epilepsia*. 2007;48:43–58.
41. Freeman J, Veggiotti P, Lanzi G, Tagliabue A, Perucca E. The ketogenic diet: from molecular mechanisms to clinical effects. *Epilepsy Res*. 2006;68:145–180.
42. Kossoff EH, Zupec-Kania BA, Amark PE, Ballaban-Gil KR, Bergqvist AGC, Blackford R, Buchhalter JR, Caraballo RH, Cross JH, Dahlin MG, Donner EJ, Klepper J, Jehle RS, Kim HD, Liu YMC, Nation J, Nordli DR Jr, Pfeifer HH, Rho JM, Stafstrom CE, Thiele EA, Turner Z, Wirrell EC, Wheless JW, Veggiotti P, Vining EPG, Charlie Foundation, Practice Committee of the Child Neurology Society, International Ketogenic Diet Study Group. Optimal clinical management of children receiving the ketogenic diet: recommendations of the International Ketogenic Diet Study Group. *Epilepsia*. 2009;50:304–317.
43. Appleton DB, DeVivo DC. An animal model for the ketogenic diet. *Epilepsia*. 1974;15:211–227.
44. Hori A, Tandon P, Holmes GL, Stafstrom CE. Ketogenic diet: effects on expression of kindled seizures and behavior in adult rats. *Epilepsia.* 1997;38:750–758.
45. Rho JM, Kim DW, Robbins CA, Anderson GD, Schwartzkroin PA. Age-dependent differences in flurothyl seizure sensitivity in mice treated with a ketogenic diet. *Epilepsy Res.* 1999;37:233–240.
46. Bough KJ, Matthews PJ, Eagles DA. A ketogenic diet has different effects upon seizures induced by maximal electroshock and by pentylenetetrazole infusion. *Epilepsy Res*. 2000;38:105–114.
47. Thavendiranathan P, Mendonca A, Dell C, Likhodii S, Musa K, Iracleous C, Cunnane S, Burnham W. The MCT ketogenic diet: effects on animal seizure models. *Exp Neurol.* 2000;161:696–703.
48. Bough KJ, Gudi K, Han FT, Rathod AH, Eagles DA. An anticonvulsant profile of the ketogenic diet in the rat. *Epilepsy Res.* 2002;50:313–325.
49. Rho JM, Anderson GD, Donevan SD, Steve HS. Acetoacetate, acetone, and dibenzylamine (a contaminant in L-(+)-β-hydroxybutyrate) exhibit direct anticonvulsant actions in vivo. *Epilepsia.* 2002;43:358–361.
50. Noh HS, Kim YS, Lee HP, Chung KM, Kim DW, Kang SS, Cho GJ, Choi WS. The protective effect of a ketogenic diet on kainic acid-induced hippocampal cell death in the male ICR mice. *Epilepsy Res.* 2003;53:119–128.
51. Zhao Q, Stafstrom CE, Fu DD, Hu Y, Holmes GL. Detrimental effects of the ketogenic diet on cognitive function in rats. *Pediatr Res.* 2004;55:498–506.
52. Kwon Y, Jeong S, Kim D, Choi E, Son B. Effects of the ketogenic diet on neurogenesis after kainic acid-induced seizures in mice. *Epilepsy Res*. 2008;78:186–194.
53. Hartman AL, Lyle M, Rogawski MA, Gasior M. Efficacy of the ketogenic diet in the 6-Hz seizure test. *Epilepsia.* 2008;49:334–339.
54. Uhlemann ER, Neims AH. Anticonvulsant properties of the ketogenic diet in mice. *J Pharmacol Exp Ther*. 1972;180:231–238.
55. Samala R, Willis S, Borges K. Anticonvulsant profile of a balanced ketogenic diet in acute mouse seizure models. *Epilepsy Res.* 2008;81:119–127.
56. Borges K. Mouse models: the ketogenic diet and polyunsaturated fatty acids. *Epilepsia.* 2008;49(suppl 8): 64–66.
57. Mahoney A, Hendricks D, Bernhard N, Sisson D. Fasting and ketogenic diet effects on audiogenic

seizures susceptibility of magnesium deficient rats. *Pharmacol Biochem Behav.* 1983;18:683–687.
58. Otani K, Yamatodani A, Wada H, Mimaki T, Yabuuchi H. [Effect of ketogenic diet on convulsive threshold and brain monoamine levels in young mice]. *No To Hattatsu*. 1984;16:196–204.
59. Stafstrom CE. Dietary approaches to epilepsy treatment: old and new options on the menu. *Epilepsy Curr*. 2004;4:215–222.
60. Stafstrom CE, Wang C, Jensen FE. Electrophysiological observations in hippocampal slices from rats treated with the ketogenic diet. *Dev Neurosci*. 1999;21: 393–399.
61. Holmes G. What constitutes a relevant animal model of the ketogenic diet? *Epilepsia.* 2008;49(suppl 8): 57–60.
62. Muller-Schwarze A, Tandon P, Liu Z, Yang Y, Holmes G, Stafstrom C. Ketogenic diet reduces spontaneous seizures and mossy fiber sprouting in the kainic acid model. *Neuroreport.* 1999;10:1517–1522.
63. Nylen K, Velazquez JLP, Likhodii SS, Cortez MA, Shen L, Leshchenko Y, Adeli K, Gibson KM, Burnham WM, Snead OC III. A ketogenic diet rescues the murine succinic semialdehyde dehydrogenase deficient phenotype. *Exp Neurol*. 2008;210:449–457.
64. Nylen K, Velazquez JLP, Sayed V, Gibson KM, Burnham WM, Snead OC III. The effects of a ketogenic diet on ATP concentrations and the number of hippocampal mitochondria in Aldh5a1$^{-/-}$ mice. *Biochim Biophys Acta.* 2009;1790:208–212.
65. Brigande J, Wieraszko A, Albert M, Balkema G, Seyfried T. Biochemical correlates of epilepsy in the E1 mouse: analysis of glial fibrillary acidic protein and gangliosides. *J Neurochem*. 1992;58:752–760.
66. Todorova M, Burwell T, Seyfried T. Environmental risk factors for multifactorial epilepsy in EL mice. *Epilepsia.* 1999;40:1697–1707.
67. Todorova MT, Tandon P, Madore RA, Stafstrom CE, Seyfried TN. The ketogenic diet inhibits epileptogenesis in EL mice: a genetic model for idiopathic epilepsy. *Epilepsia.* 2000;41:933–940.
68. Fenoglio-Simeone KA, Wilke JC, Milligan HL, Allen CN, Rho JM, Maganti RK. Ketogenic diet treatment abolishes seizure periodicity and improves diurnal rhythmicity in epileptic Kcna1-null mice. *Epilepsia.* 2009;50:2027–2034.
69. Wenzel H, Vacher H, Clark E, Trimmer J, Lee A, Sapolsky R, Tempel B, Schwartzkroin P. Structural consequences of Kcna1 gene deletion and transfer in the mouse hippocampus. *Epilepsia.* 2007;48:2023–2046.
70. Zuberi S, Eunson L, Spauschus A, De Silva R, Tolmie J, Wood N, McWilliam R, Stephenson J, Kullmann D, Hanna M. A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain*. 1999;122:817–825.
71. Catterall W, Kalume F, Oakley J. $Na_v1.1$ channels and epilepsy. *J Physiol*. 2010;588:1849–1859.
72. Rogawski M, Löscher W. The neurobiology of antiepileptic drugs. *Nat Rev Neurosci*. 2004;5:553–564.
73. White H, Smith M, Wilcox K. Mechanisms of action of antiepileptic drugs. *Int Rev Neurobiol*. 2007;81: 85–110.
74. White H, Rho J. *Mechanisms of Action of Antiepileptic Drugs*. West Islip, NY: Professional Communications; 2010.
75. Keith HM. Factors influencing experimentally produced convulsions. *Arch Neurol Psychiatry*. 1933;29: 148–154.
76. Likhodii SS, Serbanescu I, Cortez MA, Murphy P, Snead OC III, Burnham WM. Anticonvulsant properties of acetone, a brain ketone elevated by the ketogenic diet. *Ann Neurol*. 2003;54:219–226.
77. Ma W, Berg J, Yellen G. Ketogenic diet metabolites reduce firing in central neurons by opening K_{ATP} channels. *J Neurosci*. 2007;27:3618–3625.
78. Juge N, Gray JA, Omote H, Miyaji T, Inoue T, Hara C, Uneyama H, Edwards RH, Nicoll RA, Moriyama Y. Metabolic control of vesicular glutamate transport and release. *Neuron*. 2010;68:99–112.
79. Gilbert D, Pyzik P, Freeman J. The ketogenic diet: seizure control correlates better with serum β-hydroxybutyrate than with urine ketones. *J Child Neurol*. 2000;15:787–790.
80. van Delft R, Lambrechts D, Verschuure P, Hulsman J, Majoie M. Blood beta-hydroxybutyrate correlates better with seizure reduction due to ketogenic diet than do ketones in the urine. *Seizure*. 2010;19:36–39.
81. Seymour KJ, Bluml S, Sutherling J, Sutherling W, Ross BD. Identification of cerebral acetone by ^{1}H-MRS in patients with epilepsy controlled by ketogenic diet. *MAGMA.* 1999;8:33–42.
82. Lund TM, Risa O, Sonnewald U, Schousboe A, Waagepetersen HS. Availability of neurotransmitter glutamate is diminished when β-hydroxybutyrate replaces glucose in cultured neurons. *J Neurochem*. 2009;110:80–91.
83. Likhodii S, Nylen K, Burnham W. Acetone as an anticonvulsant. *Epilepsia.* 2008;49(suppl 8):83–86.
84. Gasior M, Hartman AR, Rogawski MA. The anticonvulsant activity of acetone does not depend upon its metabolites. *Epilepsia*. 2008;49:936–937.
85. Kalapos M. Possible mechanism for the effect of ketogenic diet in cases of uncontrolled seizures. The reconsideration of acetone theory. *Med Hypotheses*. 2007;68:1382–1388.
86. Yang L, Zhao J, Milutinovic PS, Brosnan RJ, Eger EI II, Sonner JM. Anesthetic properties of the ketogenic bodies β-hydroxybutyric acid and acetone. *Anesth Analg*. 2007;105:673–679.
87. Thio LL, Wong M, Yamada KA. Ketone bodies do not directly alter excitatory or inhibitory hippocampal synaptic transmission. *Neurology*. 2000;54:325–331.
88. Schwartzkroin PA. Mechanisms underlying the antiepilepsy efficacy of the ketogenic diet. *Epilepsy Res*. 1999;37:171–180.
89. Cullingford TE, Eagles DA, Sato H. The ketogenic diet upregulates expression of the gene encoding the key ketogenic enzyme mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase in rat brain. *Epilepsy Res*. 2002;49:99–107.
90. Sullivan PG, Rippy NA, Dorenbos K, Concepcion RC, Agarwal AK, Rho JM. The ketogenic diet increases mitochondrial uncoupling protein levels and activity. *Ann Neurol*. 2004;55:576–580.
91. Bough KJ, Wetherington J, Hassel B, Pare JF, Gawryluk JW, Greene JG, Shaw R, Smith Y, Geiger JD, Dingledine RJ. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Ann Neurol*. 2006;60:223–235.
92. Maalouf M, Sullivan PG, Davis L, Kim DY, Rho JM. Ketones inhibit mitochondrial production of reactive

oxygen species production following glutamate excitotoxicity by increasing NADH oxidation. *Neuroscience*. 2007;145:256–264.
93. Jarrett SG, Milder JB, Liang L-P, Patel M. The ketogenic diet increases mitochondrial glutathione levels. *J Neurochem*. 2008;106:1044–1051.
94. Bentourkia M, Tremblay S, Pifferi F, Rousseau J, Lecomte R, Cunnane S. PET study of ^{11}C-acetoacetate kinetics in rat brain during dietary treatments affecting ketosis. *Am J Physiol Endocrinol Metab*. 2009;296:E796–E801.
95. LaManna JC, Salem N, Puchowicz M, Erokwu B, Koppaka S, Flask C, Lee Z. Ketones suppress brain glucose consumption. *Adv Exp Biol Med*. 2009;645: 301–306.
96. Livingston S. Dietary treatment of epilepsy. In: Livingston S, ed. *Comprehensive Management of Epilepsy in Infancy, Childhood and Adolescence*. Springfield, IL: Charles C Thomas; 1972:378–405.
97. Morris A. Cerebral ketone body metabolism. *J Inherit Metab Dis*. 2005;28:109–121.
98. Prins ML. Cerebral metabolic adaptation and ketone metabolism after brain injury. *J Cereb Blood Flow Metab*. 2008;28:1–16.
99. Sirven J, Whedon B, Caplan D, Liporace J, Glosser D, O'Dwyer J, Sperling MR. The ketogenic diet for intractable epilepsy in adults: preliminary results. *Epilepsia*. 1999;40:1721–1726.
100. Klein P, Janousek J, Barber A, Weissberger R. Ketogenic diet treatment in adults with refractory epilepsy. *Epilepsy Behav*. 2010;19:575–579.
101. Ben-Ari Y. Excitatory actions of GABA during development: the nature of the nurture. *Nat Rev Neurosci*. 2002;3:728–739.
102. Ben-Ari Y, Gaiarsa J, Tyzio R, Khazipov R. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev*. 2007;87:1215–1284.
103. Rheims S, Holmgren CD, Chazal G, Mulder J, Harkany T, Zilberter T, Zilberter Y. GABA action in immature neocortical neurons directly depends on the availability of ketone bodies. *J Neurochem*. 2009;110:1330–1338.
104. Holmgren C, Mukhtarov M, Malkov A, Popova I, Bregestovski P, Zilberter Y. Energy substrate availability as a determinant of neuronal resting potential, GABA signaling and spontaneous network activity in the neonatal cortex in vitro. *J Neurochem*. 2010;112: 900–912.
105. Dombrowski G Jr, Swiatek K, Chao K. Lactate, 3-hydroxybutyrate, and glucose as substrates for the early rat brain. *Neurochem Res*. 1989;14:667–675.
106. Nehlig A. Cerebral energy metabolism, glucose transport and blood flow: changes with maturation and adaptation to hypoglycemia. *Diabetes Metab*. 1997;23:18–29.
107. Nehlig A. Brain uptake and metabolism of ketone bodies in animal models. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70:265–275.
108. Ruusuvuori E, Kirilkin I, Pandya N, Kaila K. Spontaneous network events driven by depolarizing GABA action in neonatal hippocampal slices are not attributable to deficient mitochondrial energy metabolism. *J Neurosci*. 2010;30:15638–15642.
109. Tyzio R, Allene C, Nardou R, Picardo M, Yamamoto S, Sivakumaran S, Caiati M, Rheims S, Minlebaev M, Milh M, Ferré P, Khazipov R, Romette J, Lorquin J, Cossart R, Khalilov I, Nehlig A, Cherubini E, Ben-Ari Y. Depolarizing actions of GABA in immature neurons depend neither on ketone bodies nor on pyruvate. *J Neurosci*. 2011;31:34–45.
110. Cheng CM, Hicks K, Wang J, Eagles DA, Bondy CA. Caloric restriction augments brain glutamic acid decarboxylase-65 and -67 expression. *J Neurosci Res*. 2004;77:270–276.
111. Rimvall K, Martin DL. Increased intracellular gamma-aminobutyric acid selectively lowers the level of the larger of two glutamate decarboxylase proteins in cultured GABAergic neurons from rat cerebral cortex. *J Neurochem*. 1992;58:158–166.
112. Rimvall K, Martin DL. The level of GAD67 protein is highly sensitive to small increases in intraneuronal gamma-aminobutyric acid levels. *J Neurochem*. 1994;62:1375–1381.
113. Rimvall K, Sheikh SN, Martin DL. Effects of increased gamma-aminobutyric acid levels on GAD67 protein and mRNA levels in rat cerebral cortex. *J Neurochem*. 1993;60:714–720.
114. Yudkoff M, Daikhin Y, Nissim I, Lazarow A, Nissim I. Brain amino acid metabolism and ketosis. *J Neurosci Res*. 2001;66:272–281.
115. Yudkoff M, Daikhin Y, Nissim I, Horyn O, Lazarow A, Luhovyy B, Wehrli S, Nissim I. Response of brain amino acid metabolism to ketosis. *Neurochem Int*. 2005;47:119–128.
116. Yudkoff M, Daikhin Y, Horyn O, Nissim I, Nissim I. Ketosis and brain handling of glutamate, glutamine, and GABA. *Epilepsia*. 2008;49(suppl 8):73–75.
117. Yudkoff M, Daikhin Y, Nissim I, Lazarow A, Nissim I. Ketogenic diet, amino acid metabolism, and seizure control. *J Neurosci Res*. 2001;66:931–940.
118. Erecińska M, Nelson D, Daikhin Y, Yudkoff M. Regulation of GABA level in rat brain synaptosomes: fluxes through enzymes of the GABA shunt and effects of glutamate, calcium, and ketone bodies. *J Neurochem*. 1996;67:2325–2334.
119. DeVivo DC, Leckie MP, Ferrendelli JS, McDougal DB Jr. Chronic ketosis and cerebral metabolism. *Ann Neurol*. 1978;3:331–337.
120. Wang ZJ, Bergqvist C, Hunter JV, Jin D, Wang D-J, Wehrli S, Zimmerman RA. In vivo measurement of brain metabolites using two-dimensional double-quantum MR spectroscopy—exploration of GABA levels in a ketogenic diet. *Magn Reson Med*. 2003;49: 615–619.
121. Dahlin M, Elfving Å, Ungerstedt U, Åmark P. The ketogenic diet influences the levels of excitatory and inhibitory amino acids in the CSF in children with refractory epilepsy. *Epilepsy Res*. 2005;64:115–125.
122. Suzuki Y, Takahashi H, Fukuda M, Hino H, Kobayashi K, Tanaka J, Ishii E. β-Hydroxybutyrate alters GABA-transaminase activity in cultured astrocytes. *Brain Res*. 2009;1268:17–23.
123. Yan Q, Jobe P, Dailey J. Noradrenergic mechanisms for the anticonvulsant effects of desipramine and yohimbine in genetically epilepsy-prone rats: studies with microdialysis. *Brain Res*. 1993;610:24–31.
124. Weinshenker D, Szot P. The role of catecholamines in seizure susceptibility: new results using genetically engineered mice. *Pharmacol Ther*. 2002;94:213–233.
125. Giorgi F, Pizzanelli C, Biagioni F, Murri L, Fornai F. The role of norepinephrine in epilepsy: from the

bench to the bedside. *Neurosci Biobehav Rev*. 2004;28:507–524.
126. Szot P, Weinshenker D, Rho JM, Storey TW, Schwartzkroin PA. Norepinephrine is required for the anticonvulsant effect of the ketogenic diet. *Dev Brain Res*. 2001;129:211–214.
127. Tabb K, Szot P, White S, Liles L, Weinshenker D. The ketogenic diet does not alter brain expression of orexigenic neuropeptides. *Epilepsy Res*. 2004;62:35–39.
128. Gao Q, Horvath T. Neurobiology of feeding and energy expenditure. *Annu Rev Neurosci*. 2007;30:367–398.
129. Harvey J. Leptin regulation of neuronal excitability and cognitive function. *Curr Opin Pharmacol*. 2007;7:643–647.
130. Obeid M, Frank J, Medina M, Finckbone V, Bliss R, Bista B, Majmudar S, Hurst D, Strahlendorf H, Strahlendorf J. Neuroprotective effects of leptin following kainic acid-induced status epilepticus. *Epilepsy Behav*. 2010;19:278–283.
131. Xu L, Rensing N, Yang X, Zhang H, Thio L, Rothman S, Weisenfeld A, Wong M, Yamada K. Leptin inhibits 4-aminopyridine- and pentylenetetrazole-induced seizures and AMPAR-mediated synaptic transmission in rodents. *J Clin Invest*. 2008;118:272–280.
132. Thio LL, Erbayat-Altay E, Rensing N, Yamada KA. Leptin contributes to slower weight gain in juvenile rodents on a ketogenic diet. *Pediatr Res*. 2006;60: 413–417.
133. Leaf A, Kang J, Xiao Y, Billman G, Voskuyl R. Functional and electrophysiologic effects of polyunsaturated fatty acids on excitable tissues: heart and brain. *Prostaglandins Leukot Essent Fatty Acids*. 1999;60:307–312.
134. Xiao YG, Gomez AM, Morgan J, Lederer W, Leaf A. Suppression of voltage-gated L-type Ca^{2+} currents by polyunsaturated fatty acids in adult and neonatal rat ventricular myocytes. *Proc Natl Acad Sci USA*. 1997;94:4182–4187.
135. Xiao Y, Wright S, Wang G, Morgan J, Leaf A. Fatty acids suppress voltage-gated Na^+ currents in HEK293t cells transfected with the α-subunit of the human cardiac Na^+ channel. *Proc Natl Acad Sci USA*. 1998;95:2680–2685.
136. Börjesson S, Hammarström S, Elinder F. Lipoelectric modification of ion channel voltage gating by polyunsaturated fatty acids. *Biophys J*. 2008;95:2242–2253.
137. Lauritzen I, Blondeau N, Heurteaux C, Widmann C, Romey G, Lazdunski M. Polyunsaturated fatty acids are potent neuroprotectors. *EMBO J*. 2000;19: 1784–1793.
138. Xiao Y, Li X. Polyunsaturated fatty acids modify mouse hippocampal neuronal excitability during excitotoxic or convulsant stimulation. *Brain Res*. 1999;846:112–121.
139. Fraser DD, Whiting S, Andrew RD, Macdonald EA, Musa-Veloso K, Cunnane SC. Elevated polyunsaturated fatty acids in blood serum obtained from children on the ketogenic diet. *Neurology*. 2003;60: 1026–1029.
140. Xu X-P, Erichsen D, Börjesson SI, Dahlin M, Åmark P, Elinder F. Polyunsaturated fatty acids and cerebrospinal fluid from children on the ketogenic diet open a voltage-gated K channel: a putative mechanism of antiseizure action. *Epilepsy Res*. 2008;80:57–66.
141. Yehuda S, Carasso RL, Mostofsky DI. Essential fatty acid preparation (SR-3) raises the seizure threshold in rats. *Eur J Pharmacol*. 1994;254:193–198.
142. Taha A, Huot P, Reza-López S, Prayitno N, Kang J, Burnham W, Ma D. Seizure resistance in fat-1 transgenic mice endogenously synthesizing high levels of omega-3 polyunsaturated fatty acids. *J Neurochem*. 2008;105:380–388.
143. Porta N, Bourgois B, Galabert C, Lecointe C, Cappy P, Bordet R, Vallee L, Auvin S. Anticonvulsant effects of linolenic acid are unrelated to brain phospholipid cell membrane compositions. *Epilepsia*. 2009;50:65–71.
144. Willis S, Samala R, Rosenberger T, Borges K. Eicosapentaenoic and docosahexaenoic acids are not anticonvulsant or neuroprotective in acute mouse seizure models. *Epilepsia*. 2009;50:138–142.
145. Taha A, Baghiu B, Lui R, Nylen K, Ma D, Burnham W. Lack of benefit of linoleic and alpha-linolenic polyunsaturated fatty acids on seizure latency, duration, severity or incidence in rats. *Epilepsy Res*. 2006;71: 40–46.
146. Dell CA, Likhodii SS, Musa K, Ryan MA, Burnham WC, Cunnane SC. Lipid and fatty acid profiles in rats consuming different high-fat ketogenic diets. *Lipids*. 2001;36:373–378.
147. Schlanger S, Shinitzky M, Yam D. Diet enriched with omega-3 fatty acids alleviates convulsion symptoms in epilepsy patients. *Epilepsia*. 2002;43:103–104.
148. Bromfield E, Dworetzky B, Hurwitz S, Eluri Z, Lane L, Replansky S, Mostofsky D. A randomized trial of polyunsaturated fatty acids for refractory epilepsy. *Epilepsy Behav*. 2008;12:187–190.
149. Dahlin M, Hjelte L, Nilsson S, Åmark P. Plasma phospholipid fatty acids are influenced by a ketogenic diet enriched with n-3 fatty acids in children with epilepsy. *Epilepsy Res*. 2007;73:199–207.
150. Porta N, Vallée L, Boutry E, Fontaine M, Dessein A, Joriot S, Cuisset J, Cuvellier J, Auvin S. Comparison of seizure reduction and serum fatty acid levels after receiving the ketogenic and modified Atkins diet. *Seizure*. 2009;18:359–364.
151. Cullingford TE. The ketogenic diet; fatty acids, fatty acid-activated receptors and neurological disorders. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70: 253–264.
152. Porta N, Vallee L, Lecointe C, Bouchaert E, Staels B, Bordet R, Auvin S. Fenofibrate, a peroxisome proliferator-activated receptor-α agonist, exerts anticonvulsive properties. *Epilepsia*. 2009;50:943–948.
153. Abdallah D. Anticonvulsant potential of the peroxisome proliferator-activated receptor gamma agonist pioglitazone in pentylenetetrazole-induced acute seizures and kindling in mice. *Brain Res*. 2010;1351: 246–253.
154. Cullingford T. Peroxisome proliferator-activated receptor alpha and the ketogenic diet. *Epilepsia*. 2008;49(suppl 8):70–72.
155. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Clin Neurol*. 2011;7:31–40.
156. Nabbout R, Vezzani A, Dulac O, Chiron C. Acute encephalopathy with inflammation-mediated status epilepticus. *Lancet Neurol*. 2011;10:99–108.
157. Nakazawa M, Kodama S, Matsuo T. Effects of ketogenic diet on electroconvulsive threshold and brain contents of adenosine nucleotides. *Brain Dev*. 1983;5: 375–380.
158. Noh HS, Lee HP, Kim DW, Kang SS, Cho GJ, Rho JM, Choi WS. A cDNA microarray analysis of

gene expression profiles in rat hippocampus following a ketogenic diet. *Mol Brain Res*. 2004;129: 80–87.
159. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70: 309–319.
160. Kawamura M Jr, Ruskin DN, Masino SA. Metabolic autocrine regulation of neurons involves cooperation among pannexin hemichannels, adenosine receptors and K_{ATP} channels. *J Neurosci*. 2010;30:3886–3895.
161. Boison D. Adenosine augmentation therapies (AATs) for epilepsy: prospect of cell and gene therapies. *Epilepsy Res*. 2009;85:131–141.
162. Masino SA, Li T, Theofilas P, Sandau US, Ruskin DN, Fredholm BB, Geiger JD, Aronica E, Boison D. A ketogenic diet suppresses seizures in mice through adenosine A_1 receptors. *J Clin Invest*. 2011;121: 2679–2683.
163. Mantis JG, Centeno NA, Todorova MT, McGowan R, Seyfried TN. Management of multifactorial idiopathic epilepsy in EL mice with caloric restriction and the ketogenic diet: role of glucose and ketone bodies. *Nutr Metab*. 2004;1:11.
164. Greene AE, Todorova MT, Seyfried TN. Perspectives on the metabolic management of epilepsy through dietary reduction of glucose and elevation of ketone bodies. *J Neurochem*. 2003;86:529–537.
165. Hartman AL, Zheng X, Bergbower E, Kennedy M, Hardwick JM. Seizure tests distinguish intermittent fasting from the ketogenic diet. *Epilepsia*. 2010;51: 1395–1402.
166. Garriga-Canut M, Schoenike B, Qazi R, Bergendahl K, Daley TJ, Pfender RM, Morrison JF, Ockuly J, Stafstrom C, Sutula T, Roopra A. 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. *Nat Neurosci*. 2006;9:1382–1387.
167. Zhao YT, Tekkök S, Krnjević K. 2-Deoxy-D-glucose-induced changes in membrane potential, input resistance, and excitatory postsynaptic potentials of CA1 hippocampal neurons. *Can J Physiol Pharmacol*. 1997;75:368–374.
168. Laschet J, Minier F, Kurcewicz I, Bureau M, Trottier S, Jeanneteau F, Griffon N, Samyn B, Van Beeumen J, Louvel J, Sokoloff P, Pumain R. Glyceraldehyde-3-phosphate dehydrogenase is a $GABA_A$ receptor kinase linking glycolysis to neuronal inhibition. *J Neurosci*. 2004;24:7614–7622.
169. Laschet J, Kurcewicz I, Minier F, Trottier S, Khallou-Laschet J, Louvel J, Gigout S, Turak B, Biraben A, Scarabin J, Devaux B, Chauvel P, Pumain R. Dysfunction of $GABA_A$ receptor glycolysis-dependent modulation in human partial epilepsy. *Proc Natl Acad Sci USA*. 2007;104:3472–3477.
170. Lian X, Khan F, Stringer J. Fructose-1,6-bisphosphate has anticonvulsant activity in models of acute seizures in adult rats. *J Neurosci*. 2007;27:12007–12011.
171. Ding Y, Wang S, Zhang M, Guo Y, Yang Y, Weng S, Wu J, Qiu X, Ding M. Fructose-1,6-diphosphate inhibits seizure acquisition in fast hippocampal kindling. *Neurosci Lett*. 2010;477:33–36.
172. Stringer J, Xu K. Possible mechanisms for the anticonvulsant activity of fructose-1,6-diphosphate. *Epilepsia*. 2008;49(suppl 8):101–103.
173. Willis S, Stoll J, Sweetman L, Borges K. Anticonvulsant effects of a triheptanoin diet in two mouse chronic seizure models. *Neurobiol Dis*. 2010;40:565–572.
174. Masino SA, Geiger JD. Are purines mediators of the anticonvulsant/neuroprotective effects of ketogenic diets? *Trends Neurosci*. 2008;31:273–278.
175. Maalouf M, Rho JM, Mattson MP. The neuroprotective properties of calorie restriction, the ketogenic diet, and ketone bodies. *Brain Res Rev*. 2009;59: 293–315.
176. Hartman A. Neuroprotection in metabolism-based therapy. *Epilepsy Res*. 2011. [Epub ahead of print.]
177. Kim DY, Vallejo J, Rho JM. Ketones prevent synaptic dysfunction induced by mitochondrial respiratory complex inhibitors. J Neurochem. 2010;114: 130–141.
178. Kim DY, Davis LM, Sullivan PG, Maalouf M, Simeone TA, van_Brederode J, Rho JM. Ketone bodies are protective against oxidative stress in neocortical neurons. *J Neurochem*. 2007;101:1316–1326.
179. Mattson M, Liu D. Mitochondrial potassium channels and uncoupling proteins in synaptic plasticity and neuronal cell death. *Biochem Biophys Res Commun*. 2003;304:539–549.
180. Andrews Z, Diano S, Horvath T. Mitochondrial uncoupling proteins in the CNS: in support of function and survival. *Nat Rev Neurosci*. 2005;6: 829–840.
181. Sullivan P, Dubé C, Dorenbos K, Steward O, Baram T. Mitochondrial uncoupling protein-2 protects the immature brain from excitotoxic neuronal death. *Ann Neurol*. 2003;53:711–717.
182. Freeman J, Kossoff E. Ketosis and the ketogenic diet, 2010: advances in treating epilepsy and other disorders. *Adv Pediatr*. 2010;57:315–329.
183. Evangeliou A, Vlachonikolis I, Mihailidou H, Spilioti M, Skarpalezou A, Makaronas N, Prokopiou A, Christodoulou P, Liapi-Adamidou G, Helidonis E, Sbyrakis S, Smeitink J. Application of a ketogenic diet in children with autistic behavior: pilot study. *J Child Neurol*. 2003;18:113–118.
184. Mantis JG, Fritz CL, Marsh J, Heinrichs SC, Seyfried TN. Improvement in motor and exploratory behavior in Rett syndrome mice with restricted ketogenic and standard diets. *Epilepsy Behav*. 2009;15:133–141.
185. Ruskin DN, Kawamura M Jr, Masino SA. Reduced pain and inflammation in juvenile and adult rats fed a ketogenic diet. *PLoS One*. 2009;4:e8349.
186. Prins ML, Fujima LS, Hovda DA. Age-dependent reduction of cortical contusion volume by ketones after traumatic brain injury. *J Neurosci Res*. 2005;82: 413–420.
187. Appelberg KS, Hovda DA, Prins ML. The effects of a ketogenic diet on behavioral outcome after controlled cortical impact injury in the juvenile and adult rat. *J Neurotrauma*. 2009;26:497–506.
188. Hu Z-G, Wang H-D, Qiao L, Yan W, Tan Q-F, Yin H-X. The protective effect of the ketogenic diet on traumatic brain injury-induced cell death in juvenile rats. *Brain Inj*. 2009;23:459–465.
189. Kashiwaya Y, Takeshima T, Mori N, Nakashima K, Clarke K, Veech RL. D-β-Hydroxybutyrate protects neurons in models of Alzheimer's and Parkinson's disease. *Proc Natl Acad Sci USA*. 2000;97:5440–5444.
190. Yang X, Cheng B. Neuroprotective and anti-inflammatory activities of ketogenic diet on MPTP-induced neurotoxicity. *J Mol Neurosci*. 2010;42:145–153.

191. Marsh J, Mukherjee P, Seyfried T. Drug/diet synergy for managing malignant astrocytoma in mice: 2-deoxy-D-glucose and the restricted ketogenic diet. *Nutr Metab*. 2008;5:33.
192. Seyfried T, Kiebish M, Marsh J, Shelton L, Huysentruyt L, Mukherjee P. Metabolic management of brain cancer. *Biochim Biophys Acta*. 2011;1807: 577–594.
193. Stafford P, Abdelwahab M, Kim D, Preul M, Rho J, Scheck A. The ketogenic diet reverses gene expression patterns and reduces reactive oxygen species levels when used as an adjuvant therapy for glioma. *Nutr Metab*. 2010;7:74.
194. Mavropoulos J, Buschemeyer W 3rd, Tewari A, Rokhfeld D, Pollak M, Zhao Y, Febbo P, Cohen P, Hwang D, Devi G, Demark-Wahnefried W, Westman E, Peterson B, Pizzo S, Freedland S. The effects of varying dietary carbohydrate and fat content on survival in a murine LNCaP prostate cancer xenograft model. *Cancer Prev Res*. 2009;2: 557–565.
195. Westman EC, Yancy WS Jr, Mavropoulos JC, Marquart M, McDuffie JR. The effect of a low-carbohydrate, ketogenic diet versus a low-glycemic index diet on glycemic control in type 2 diabetes mellitus. *Nutr Metab* (*Lond*) 2008;5:36.
196. Dressler A, Reithofer E, Trimmel-Schwahofer P, Klebermasz K, Prayer D, Kasprian G, Rami B, Schober E, Feucht M. Type 1 diabetes and epilepsy: efficacy and safety of the ketogenic diet. *Epilepsia*. 2010;51:1086–1089.
197. Mobbs C, Mastaitis J, Yen K, Schwartz J, Mohan V, Poplawski M, Isoda F. Low-carbohydrate diets cause obesity, low-carbohydrate diets reverse obesity: a metabolic mechanism resolving the paradox. *Appetite*. 2007;48:135–138.
198. Seyfried B, Kiebish M, Marsh J, Mukherjee P. Targeting energy metabolism in brain cancer through calorie restriction and the ketogenic diet. *J Cancer Res Ther*. 2009;5(suppl 1):S7–S15.
199. Fearon K. Nutritional pharmacology in the treatment of neoplastic disease. *Baillieres Clin Gastroenterol*. 1988;2:941–949.
200. Seyfried T, Shelton L, Mukherjee P. Does the existing standard of care increase glioblastoma energy metabolism? *Lancet Oncol*. 2010;11:811–813.
201. Kim D, Hao J, Liu R, Turner G, Shi F, Rho J. Inflammation-mediated memory dysfunction and effects of a ketogenic diet in a murine model of multiple sclerosis. *Soc Neurosci Abstr*. 2009;830.12.
202. Nabbout R, Vezzani A, Dulac O, Chiron C. Acute encephalopathy with inflammation-mediated status epilepticus. *Lancet Neurol*. 2011;10:99–108.
203. Hallböök T, Lundgren J, Rosén I. Ketogenic diet improves sleep quality in children with therapy-resistant epilepsy. *Epilepsia*. 2007;48:59–65.
204. Malow B. Sleep and epilepsy. *Neurol Clin*. 2005;23: 1127–1147.
205. Chokroverty S. Sleep and neurodegenerative diseases. *Semin Neurol*. 2009;29:446–467.
206. Benca R, Duncan M, Frank E, McClung C, Nelson R, Vicentic A. Biological rhythms, higher brain function, and behavior: gaps, opportunities, and challenges. *Brain Res Rev*. 2009;62:57–70.
207. Garaulet M, Madrid J. Chronobiology, genetics and metabolic syndrome. *Curr Opin Lipidol*. 2009;20: 127–134.
208. Allen C. Circadian rhythms, diet, and neuronal excitability. Epilepsia. 2008;49(suppl 8):124–126.

Chapter 79

Deep Brain Stimulation for Epilepsy

Animal Models

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Epilepsy afflicts approximately 50 million individuals globally.[1] Approximately 40% of patients with symptomatic epilepsy continue to have seizures despite aggressive medical therapy,[2] and about one-third are less than satisfied with their antiepileptic medicine.[3] Surgical resection of the epileptogenic zone is an option for only a minority of patients.[4] Thus, there is great need for new and better treatments for various epilepsy syndromes. A recent clinical trial showed electrical stimulation of the anterior nucleus of the thalamus to be effective in refractory patients with partial and secondarily generalized seizures.[5] Clinical trials have proceeded with only limited understanding of the basic mechanisms of brain stimulation. The most suitable animal models to use can be disputed, and occasionally the results are contradictory. The outcome depends exquisitely upon detailed parameters of stimulation and other unknown factors.

Electrical brain stimulation in laboratory animals dates to the 1860s, with the discovery of involuntary motor phenomena in dogs with acute focal currents applied to the cortical surface.[6] Early studies of high-intensity stimulation of motor cortices of monkeys and dogs produced repetitive movements reminiscent of epileptic seizures.[7] Much earlier work over the last century focused on behavioral effects of brain stimulation in animals. An early dramatic presentation of the effects of chronically implanted electrodes involved stopping a charging bull via a remotely controlled caudate and internal capsule stimulation.[8,9] There is a dichotomy between brain stimulation and epilepsy; kindling, a popular model for generation of an epileptogenic focus in laboratory rodents,

involves application of repetitive stimulation typically to limbic circuits, which results in increasing excitability and eventual spontaneous seizures.[10] Although initially epilepsy was thought to be simply pathological learning of a neuronal circuit, extensive studies have demonstrated that the specific stimulation protocols necessary to generate an epileptic focus act in the context of some neuronal injury, axonal changes, synaptogenesis, and receptor changes dependent on *N*-methyl-D-aspartate (NMDA) receptor-mediated activity (see ref. 11 for review). By contrast, this chapter will deal with antiepileptic effects of deep brain stimulation via implantation of electrodes to various regions in laboratory animals, including the cerebellum, locus coeruleus, substantia nigra, caudate, hippocampus, hypothalamus, subthalamus, centromedian thalamus, anterior nucleus of the thalamus, and, while not necessarily "deep brain," cortex. Sites are separated into those that may be part of a wider epileptic network or have a gating effect versus those that constitute the potential seizure focus; however, it should be understood that this is a loose categorization, as epileptogenic zones are perhaps not strictly demarcated and may involve networks of neurons with long projections separated in space.[11]

POTENTIAL MECHANISMS OF ACTION

At the level of an individual nerve fiber, electrical stimulation effects are predictable, but predictability is generally lost when neuronal tissue is stimulated. The fields of distribution of current can only be estimated, and effects cannot be classified simply as excitatory or inhibitory. Stimulation may inhibit inhibitory pathways and may either synchronize or desynchronize the networks participating in seizure generation.[12] Stimulation may have one effect near the electrode and the opposite or a different effect at more distant locations. An applied electrical field in a hippocampal slice produces an increase in extracellular potassium, a negative direct current (DC) shift, depolarization block of sodium channels, and inhibition of penicillin-induced bursting.[13] Acute inhibition of seizures by stimulation is different from either inhibition of seizures in chronic animal models or prevention of epileptogenesis. One clinical puzzle has been the observation of increasing efficacy of nerve stimulation against seizures over time. Laboratory studies show that growth factors increase with stimulation[14] and that stimulation can generate synapses.[15]

Since effects of brain stimulation on seizures cannot be predicted from first principles, much of the little that we do know comes from empirical experiments on in vitro tissue, laboratory animals, or limited numbers of clinical trials. Animal experimentation is of value in proportion to the relevance of the model to the clinical disease. It is important to be clear about whether a treatment is being tested on a model of acute seizures or of spontaneously recurring seizures, such as those of epilepsy. Multiple possible parameters and sites of stimulation also make it difficult to compare and contrast studies in this area. In the following section, we review laboratory studies by site in the nervous system. Clinical reviews may be found elsewhere.[16]

SITES OF STIMULATION: MODULATION OF THE NETWORK

Cerebellum

Intrinsic cerebellar lesions themselves do not seem to generate epileptic seizures, but the cerebellum was an early target for stimulation studies with mixed results. Although a role for modulating epileptic seizures is poorly understood, output from cerebellar cortex inhibits the deep cerebellar nuclei, which have wide projections. The most radical related approach, cerebellectomy, in rodents reduced hindlimb extension following maximal electroshock-induced seizures but had little effect on the threshold for chemically induced convulsions,[17] suggesting the potential for a differential effect of reducing focal seizures but not attenuating diffuse seizures. However, the effect may be highly model dependent, as rodent cerebellectomy enhanced cortical focal hyperexcitability following penicillin injection.[18] On this backdrop, electrical stimulation studies have likewise produced somewhat contradictory data.

Early studies in anesthetized cats show that high-frequency stimulation at 300 Hz, 40 V for 60 s to the paramedian lobule could abort seizures induced by cortical[19] or hippocampal stimulation.[20] Stimulation of the cerebellar cortex and fastigial nucleus was effective, although no systematic evaluation of various stimulation parameters was initially pursued. Dorsal midline cerebellar stimulation was also reported to be effective in reducing the hyperexcitability produced by cobalt injection into anesthetized feline hippocampus.[21] Hutton and colleagues[22] reported that cerebellar stimulation of anesthetized cats reduced spiking or focal penicillin-induced seizures when applied to the vermis and focally to right paramedian lobules, but deep nuclear stimulation (dentate, fastigial, and interpositus) was ineffective, while widespread stimulation across the cerebellar surface worsened seizures. Babb and colleagues[23] reported similar findings, noting that dentate nuclear stimulation worsened seizures. Dow and colleagues[24] reported that both superficial and deep high-frequency cerebellar stimulation reduced chronic cobalt-induced seizures in freely behaving rats. Low-frequency (10 Hz, 1.5 ms, biphasic 3–4 V) stimulation of rabbit cerebellar cortex (simplex or ansiform lobes) reduced seizure activity generated with intravenous pentylenetetrazole (PTZ) or frontal lobe electrical stimulation, similar to the effects of phenytoin and phenobarbital.[25] In this study, 8 and 12 Hz stimulation was less effective and 6 Hz was ineffective. By contrast, more recent studies in freely behaving rats reported that stimulation of the uvula and nodulus at low frequencies (10–12 Hz, 0.5 ms pulse duration, for 4 to 7 s trains) worsened penicillin-induced epileptiform discharges in rats, while higher-frequency stimulation (100–300 Hz, 0.25 ms pulse duration, for 4 to 7 s trains) was beneficial.[26]

Early studies in monkeys were not promising, and deep nuclear stimulation had no measurable effect on cobalt-induced seizures.[27] Hemmy and colleagues[28] found no benefit of 4–100 Hz (1 ms, 10 mA) superficial lateral cerebellar and dentate nuclear stimulation in awake macaques with electrically induced cortical seizures. Some parameters of chronic cerebellar stimulation in rhesus monkeys caused neuronal damage.[29]

Many early studies do not use statistics and simply report examples of findings. Details of the parameters of stimulation often are lacking. Some studies show efficacy and others do not. Stimulation of midline cerebellar cortex and of deep cerebellar nuclei, which are inhibited by cerebellar cortex, both have shown efficacy or lack thereof in different circumstances. Not all studies have been included; see Krauss and Koubeissi[30] and Fountas and colleagues[31] for additional reviews.[32]

There has been interest in cerebellar stimulation for seizure control in humans, although placement of electrodes in the posterior fossa increases the potential for grave consequences associated with hemorrhage, should there be complications with placement. Hematoma after 5 years of implantation has been also reported.[33] Pilot clinical trials were reportedly effective (e.g., Cooper and colleagues[34]), while blinded studies involving small numbers of subjects produced variable results.[35–37]

Basal Ganglia

CAUDATE

Early studies of caudate stimulation found enhancement of hippocampal afterdischarges in rabbit.[38] Administration of pulses at 5–300 Hz of 1–2 ms duration for 10–400 s into various caudate regions caused synchronous changes in dorsal hippocampal electrodes and increased afterdischarges from hippocampal stimulation following caudate stimulation. However, prolonged caudate stimulation reduced evoked hippocampal activity. Electrical stimulation of feline caudate, concomitant with cholinesterase inhibition focally in caudate, caused cortical seizures.[39] Longer trains of caudate stimulation in the cat were effective against seizures induced by topical penicillin in hippocampus or by atropine treatment.[40] Trains of 10 Hz lasting for 180 s and 25 Hz stimulation for 90 s reduced spike frequency, with a greater effect produced by trains of longer duration. Other investigators also found similar results—reduced epileptiform discharges with feline stimulation—with hippocampal penicillin.[41] Trains of 1 ms at 0.5 Hz for 60 s into cat caudate suppressed seizure activity induced by cortical cobalt if stimulation was started during an interictal period; however, no effect was observed if stimulation was applied during ictal discharges.[42] Psatta[43]

studied 1 to 3 s, 0.3 ms pulses at 5–100 Hz in feline caudate with chronic cobalt-induced neocortical epileptogenic foci. They reported that low-frequency stimulation worked better than high-frequency stimulation and abruptly stopped interictal spiking, but occasionally it provoked focal cortical discharges. Others found that 400 Hz feline caudate stimulation reduced spikes from penicillin-induced foci better than did stimulation at several thalamic targets, with return of spiking following cessation of stimuli. There was greater efficacy with several minutes of stimulation, although "very active foci" were driven by prolonged stimulation.[44]

In monkey, continuous stimulation of the caudate head inhibited seizures from alumina gel on cortex,[45] but there was a rebound effect when stimulation was stopped. A small clinical series reported reduced frequency of seizures with 4–8 Hz caudate stimulation[46]; however, additional studies are necessary to clarify the potential benefits. In summary, animal studies of caudate stimulation have reported both pro- and antiseizure effects, depending on the model, the parameters of stimulation, and whether there was a rebound increase in seizures when stimulation stopped.

SUBSTANTIA NIGRA

It has been suggested that GABAergic (gamma-aminobutyric acid receptor mediated) projections from the pars reticulata of the substantia nigra (SNr) may be one site of action for GABA-mediated antiseizure medications[47] in addition to local inhibitory effects. Substantia nigra pars reticulata projections also appear to influence the kindling threshold.[48] Six 0.1 ms pulses at 100 Hz into rat SNr increased afterdischarges in hippocampal dentate gyrus evoked by perforant path stimulation.[49] However, stimulation of SNr in rats generally inhibited seizures provoked by 3-aminopyridine, including decreased propagation, duration, and occurrence of runs, although effects were more marked with lower-frequency seizures, as some high-frequency seizure patterns worsened.[50] Stimulation of the SNr reduced cortical spikes in the feline generalized penicillin model by 80% in contrast to a 19% reduction with stimulation of the substantia nigra pars compacta.[51] In the rat flurothyl model, 130 Hz unilateral and bilateral posterior SNr stimulation was efficacious against clonic but not clonic-tonic seizures in 2-month-old rats, but anterior SNr stimulation was ineffective.[52] In this same study with 2-week-old rats, bilateral stimulation was effective anywhere in the SNr against both seizure types but unilateral stimulation was ineffective, suggesting age-dependent effects. Stimulation of the SNr reduced acquisition of rat amygdala kindling,[53,54] suggesting a possible effect on epileptogenesis. In Genetic Absence Epilepsy Rats from Strasbourg (GAERS rats), Feddersen and associates more recently report that isolated 60 Hz, 60 μs pulse width bilateral SNr stimulation for 5 s was optimal in suppressing individual seizures but that chronic stimulation overall worsened seizures.[55] In summary, stimulation of the SNr has shown both pro- and antiseizure effects in different models and parameters. Concern about movement side effects in patients may also limit any potential clinical utility.

SUBTHALAMIC NUCLEUS

The subthalamic nucleus (STN) has been the target of numerous studies for the treatment of tremor and other movement disorders (see refs. 56 and 57 for recent reviews). In a detailed and controlled study, Lado and colleagues[58] studied the effects of bilateral STN stimulation on the flurothyl seizure thresholds in rats, using pulses of 30–60 μs duration, titrated to a level just below threshold for a non-seizure-related motor response. In this model, clonic seizures occurred at a lower threshold than did tonic-clonic seizures; 130 Hz stimulation reduced seizures by increasing the clonic seizure threshold 28%, but it only slightly increased the threshold for tonic-clonic seizures. Stimulation at 260 Hz had no significant effect on either seizure type, while 800 Hz stimulation increased seizures, reducing the threshold for tonic-clonic seizures 16%, with a tendency to reduce the threshold of clonic seizures. Stimulation of the STN was shown to interrupt spike-wave discharges in GAERS rats,[59] although there had been criticism that the effects may be nonspecific, as various external stimuli can produce the same response.[60] In summary, STN stimulation affects motor control above a certain threshold, and the influence on seizures appears to be variable.

Brainstem

LOCUS COERULEUS

Vagus nerve stimulation is an accepted palliative but effective treatment for medically refractory epilepsy.[61] Norepinephrine released from locus coeruleus (LC) projections may affect the seizure threshold, and LC circuitry has been proposed to be important for efficacy of vagus nerve stimulation for clinical epilepsy.[62] There has been a small number of investigations of LC stimulation in animal models. Trains of up to 1–2 min of 50–200 Hz rectangular constant current pulses (20–200 μs duration modified by 0.01 μF in series capacitor), delivered via monopolar electrodes placed in the vicinity of the LC and grounded to occipital bone, suppressed but sometimes only reduced PTZ-induced epileptiform discharges in young rats.[63] Unilateral stimulation had bilateral suppressive effects. In the penicillin model of focal epilepsy, bipolar stimulation at 1–200 Hz (biphasic current, 0.1–0.5 ms per phase) in rat LC also suppressed epileptiform activity in proportion to its frequency and intensity, with pharmacological manipulations suggesting that the effects were mediated by the alpha-1 adrenoreceptor.[64] Others have suggested effects at beta adrenoreceptors.[65] Similar results were found with cobalt-induced seizure foci in rats,[66] although the effects in all studies were limited to the seconds during and immediately following stimulation. Longer-term effects have been proposed with delayed amygdala kindling via LC stimulation.[67] A few patients have been implanted with LC stimulation electrodes for spasticity and epilepsy; however, the numbers are too small to draw significant conclusions.[68]

RAPHE NUCLEI

The dorsal raphe nucleus provides strong serotonergic input to forebrain, amygdala, and hypothalamus. Sensitivity to inhibitory inputs changes following amygdala kindling.[69] An older study found increased hyperexcitability with stimulation of this region in rats.[70] Based on pharmacological manipulations, it has been suggested that noxious tactile stimuli can abort seizures in some rodent models via serotonergic inputs from the raphe nuclei.[71] Although understudied, there are no good data suggesting any antiseizure effect of the dorsal raphe nucleus with electrical stimulation. The medial raphe nucleus (superior central nucleus) is also rich in neurons containing serotonin. Bipolar 8 Hz stimulation (1 ms pulses) of rat median raphe nucleus for 1 h reduced the severity of PTZ-induced seizures in rats, as well as those induced by amygdala stimulation following full kindling.[72] At present, there is insufficient evidence to promote clinical studies in these nuclei, and some animal studies support a pro-epileptic effect in some circumstances.

NUCLEUS OF THE SOLITARY TRACT

Receiving afferents from the vagus nerve, the nucleus of the solitary tract may also potentially mediate effects of vagus nerve stimulation for epilepsy.[61,73] The neurons from the nucleus of the solitary tract send afferents to amygdala, hypothalamus, and elsewhere. Hypothetically, direct stimulation might provide a mechanism to avoid side effects such as changes in voice with vagus nerve stimulation and/or could potentially increase potency. One group has reported that 0.5 ms pulses at 30 Hz for 1 min four times a day reduced seizure severity in amygdala-kindled cats.[74] Similar stimulation patterns applied more frequently to the feline nucleus of the solitary tract have been suggested to be antiepileptogenic based on delayed kindling in this model.[75] However, effects at this brainstem site have not been widely studied, and mechanisms of any potential benefit have not been elucidated. Without strong evidence of superior efficacy, it is unclear why placement of a brainstem electrode in any specific target would be a preferable treatment to vagus nerve stimulation unless other side effects could be avoided.

Hypothalamus

Hypothalamic circuits may be important for certain epilepsies, most obviously with the less common syndrome of hypothalamic hamartoma.[76] While there is no suitable animal model of this specific syndrome, in the broader scheme hypothalamic connections may play a role in many seizures via the classical circuit of Papez, linking the mammillary bodies of posterior hypothalamus to anterior thalamus,

cingulate, entorhinal cortex, and hippocampus. Low-frequency hypothalamic stimulation in rats was initially found to be pro-convulsive,[77] although antecedent stimulation with subconvulsant currents sometimes appeared to convey resistance to the occurrence of seizures with suprathreshold stimulation.[78] Cullen and Goddard reported that intermittent chronic stimulation of various hypothalamic locations in rats resulted in amygdala kindling.[79] However, others reported that 5 s of 60 Hz sinusoidal bipolar stimulation of the posterior-lateral hypothalamus of rats reduced behavioral convulsions induced by carbachol injections into the amygdala.[80] More rigorous studies employing 100 Hz stimulation of mammillary bodies of the posterior hypothalamus increased the PTZ seizure threshold in rats, likely related to disruption of mammillothalamic connections[81] (see the discussion of the thalamus below). In a very small clinical series of posterior medial hypothalamic stimulation in patients for pain syndromes and behavior, there is anecdotal mention of a 50% reduction of seizures in two patients[82]; however, overall experience with hypothalamic stimulation is too limited to draw strong conclusions. This target may not be desirable in human due to consequences of hemorrhage in this region with electrode implantation.

Thalamus

ANTERIOR NUCLEUS OF THE THALAMUS

The anterior nucleus of the thalamus (ANT) has been categorized with the so-called midline thalamus and the nonspecific thalamus, structures considered to have associative functions rather than direct sensorimotor relay functions.[83] In the 1940s and 1950s, Dempsey and Morison[84] showed that thalamic stimulation could produce waxing and waning cortical electroencephalographic (EEG) potentials called *recruiting rhythms*. Jasper and Droogleever-Fortuyn[85] suggested a role for nonspecific thalamus in primary and secondarily generalized epilepsies and showed that 3 Hz thalamic stimulation in young cats can produce a spike-wave. More recently, the midline thalamus in rat, although not necessarily the ANT, has been shown to demonstrate electrophysiological changes early during temporal lobe seizures.[86] Figure 79–1 shows a correlation of epileptiform activity between ANT and neocortex during a PTZ-induced seizure in a rat. Spectral coherence of the EEG in neocortex and ANT during a seizure exceeds that of neocortex and posterior thalamus.[87]

The ANT is part of the so-called circuit of Papez, which is believed to be involved in memory, emotionality, and epilepsy. In this circuit, hippocampal outflow via the fornix travels to the mammillary bodies of the posterior hypothalamus. Mammillary neurons give rise to the mammillothalamic tract to the ANT, and from there to the mesial frontal cingulate cortex, and via the cingulum bundle back to the entorhinal cortex and hippocampus. Stimulation of the ANT would therefore be expected to influence oligosynaptically both the superior-mesial frontal cortex and the mesial temporal cortex. These regions are intimately involved in the pathoanatomy of refractory epilepsy.

Autoradiography of the brain in guinea pigs made to have seizures with the convulsant drug PTZ demonstrated marked activation of mammillary bodies, the anterior thalamus, and the mammillothalamic tract connecting them.[88–91] Cutting the tract increases the threshold for

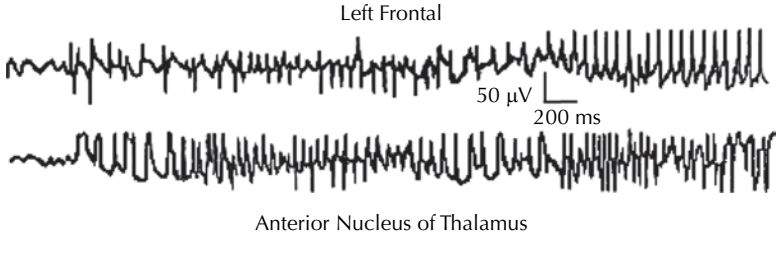

Figure 79–1. Simultaneous EEG recordings from a left frontal cortex bone screw (top trace) and an ANT depth wire (bottom trace) in a rat treated with PTZ. Epileptiform potentials can be seen at both sites and are attenuated (see text) by thalamic electrical stimulation. The EEG tracings were retraced in places for clarity of display.

inducing seizures.[92] Mirski and Fisher[81] used 100 Hz electrical stimulation of mammillary bodies to induce a temporary disruption of the mammillothalamic connections. During times of stimulation, the amount of PTZ required to provoke seizures doubled. The possibility that high-frequency stimulation of mammillary bodies was acting as a local inhibitory stimulus was supported by similar effects produced by injecting the GABAergic drug, muscimol, into posterior hypothalamus during PTZ-induced seizures.[93] Status epilepticus produced by systemic pilocarpine could be delayed two- to threefold, but not prevented, by cannula injection of 160 μmol muscimol bilaterally into the ANT.[94]

The posterior hypothalamus could be a relatively high-risk target for electrode implantation, since local hemorrhage could result in Korsakoff's encephalopathy. The next way station on the circuit of Papez, the ANT, might be a more attractive implantation target. Electrical stimulation of the ANT at frequencies of 100 Hz in rats inhibited PTZ-induced seizures.[95] Stimulation at rates of fewer than Hz was ineffective. High-frequency stimulation delayed the onset of pilocarpine-induced status epilepticus, whereas bilateral ANT lesions delayed seizure onset[96] or prevented status epilepticus.[97] Unilateral stimulation, unilateral lesions, or stimulation initiated after the onset of status epilepticus all were ineffective.

Stimulation of the ANT in a chronic epilepsy model produced by kainic acid injected into rat amygdala[98] decreased the number of total and secondarily generalized seizures. Rearing, falling, and forelimb clonus, associated with seizure spread, were more suppressed than limbic system behaviors such as sniffing, searching, and salivation. Seizures decreased significantly with unilateral lesions of the ANT but were further reduced with bilateral lesions. Stimulation reduced corticothalamic metabolic consumption of glucose, but there was less reduction in temporal regions. Similar reductions of seizures and widespread glucose utilization were seen with seizures induced by kainic acid injection into the rat left sensorimotor cortex.[99]

Several investigations have not documented any efficacy of ANT stimulation in seizure or epilepsy models. A study by Lado[100] of ANT stimulation in a kainic acid-induced chronic epilepsy model in the rat resulted in increased seizure frequency. Another study[101] failed to find any benefit of 100 Hz intermittent ANT stimulation against PTZ-induced seizures, even though benefit could be seen with stimulation in other brain regions. Stimulation of the ANT delayed the acute onset of seizures produced by pilocarpine,[96] but stimulation failed to reduce the number of chronically recurrent seizures after a bout of pilocarpine-induced status epilepticus.[102] The reasons for these differing results are unclear; however, dependence of efficacy upon exact stimulation parameters and experimental conditions was shown earlier by the study of Mirski and colleagues,[95] in which some stimulation parameters induced cortical spike-wave discharges and behavioral activity arrest and others inhibited seizures. No study of electrical stimulation of ANT has documented the anatomical extent of the stimulation effects and how changes in parameters of stimulation might involve different neuronal networks.

How ANT stimulation might inhibit seizures is unknown, but studies have evaluated the release of two neurotransmitters, histamine and serotonin, in relation to efficacy of stimulation. Electrical stimulation of the histaminergic tuberomammillary nucleus delayed and shortened PTZ-induced seizures in rats.[103] The serotonin (HT) metabolite, 5-hydroxyindoleacetic acid (5-HIAA), increases regionally in rat ANT during PTZ-induced seizures.[101] Since ANT is enriched with 5HT-7 receptor subtypes, Mirski and colleagues[104] used a selective 5HT-7 agonist, 5-carboxamidotryptamine, dialyzed into ANT to inhibit PTZ-induced seizures. Rats given control injections had PTZ-induced seizures at a latency of 3120 ± 770 s. Stimulation of ANT at 100 Hz, 150 mA, 0.1 ms pulse duration beginning 40 min prior to PTZ infusion delayed seizure onset to 5018 ± 1100 s ($p < 0.01$). Dialysis of 5-carboxamidotryptamine increased the latency to 4247 ± 528 s ($p < 0.05$). Glutamate, GABA, adenosine, and other neurotransmitters or neuromodulators might play a role in the mechanism of ANT stimulation, but their specific effects have not been elucidated in experiments.

In summary, ANT is a participant in several models of seizures. Lesions, local injection of muscimol, and high-frequency electrical stimulation can inhibit seizures in several chemoconvulsant models, although not all experiments find efficacy. A large, randomized clinical trial

of ANT in patients with refractory partial or secondarily generalized seizures did document efficacy,[5] so further investigation into the mechanisms of stimulation at this nucleus is likely to be clinically relevant.

CENTROMEDIAN NUCLEUS

Other midline thalamic structures can influence seizures in animal models.[105] Injection of $GABA_A$ and $GABA_B$ receptor antagonists into the rodent centromedian nucleus (CM) of the thalamus facilitates some seizure types[106]; however, good animal studies with stimulation are largely lacking. In a controlled pilot study of CM stimulation in seven patients, Fisher and colleagues found a nonsignificant 30% reduction in overall seizure frequency.[107] Nevertheless, the CM of the thalamus has been a further target of considerable clinical interest.[108]

NUCLEUS RETICULARIS THALAMI

A single report suggests that stimulation of the reticular nucleus of rodent thalamus attenuates hippocampal kindling.[109] The reticular nuclei are shells surrounding other thalamic nuclei. They are believed to be critical for widespread network synchrony and important in the physiology of absence seizures and potentially other seizures.[110] However, brain stimulation of this structure has not been sufficiently studied.

SITES OF STIMULATION: THE SEIZURE FOCUS

Hippocampus and Amygdala

The mesial temporal structures are important in the pathophysiology of many clinical focal epilepsies. A popular model for the generation of an epileptogenic focus in laboratory rodents, kindling, involves application of repetitive stimulation to the hippocampus, amygdala, or other limbic structures.[10] Pro- or antiseizure effects appear to depend on the details of stimulation; however, in contrast to studies of many possible targets, these experiments typically deal with current injection directly into the epileptogenic region. Laboratory studies have used both in vitro slice and whole animal models. In rodent hippocampal slices, epileptiform discharges in CA1 provoked with bicuculline, primarily blocking $GABA_A$-mediated inhibition, can be rapidly attenuated with 100 Hz stimulation to Schaeffer collaterals, but the effect is temporary.[111] Stimulation in these studies at 1 Hz had a more persistent effect, possibly mediated by NMDA receptor activity. Albensi and colleagues[112] studied a range of stimulation parameters affecting epileptiform discharges induced by low magnesium, suggesting that 0.5 and 1 Hz for up to 5 min were optimal, although stimulation up 50 Hz was also suppressive; the authors noted that there was no continuum of efficacy from slow to rapid parameters. Although these types of manipulations may provide a platform to better understand some of the mechanisms by which stimulation can abort seizures, truly long-term effects can only be studied in whole animal models. On this note, similar stimulation frequencies of 1 and 50 Hz applied to the hippocampal dentate gyrus or to the entorhinal cortex of behaving rats reduced interictal spikes transiently but did not reduce seizures.[113]

Contralateral rat hilar stimulation reduced the effect of perforant path stimulation-induced population discharges in hippocampal dentate gyrus.[114] In some cases, antiseizure stimulation worked to prevent later seizures, but at the cost of provoking an initial seizure. Early studies of "antecedent" amydgala[115] or hippocampal[116] stimulation to fully kindled rats demonstrated a reduced seizure rate with "test" pulses intended to evoke seizures, although the antecedent current also typically evoked seizures, leaving unanswered the question of whether the effect was due to the applied current or to ictal aftereffects. However, Shao and Valenstein[117] reported success in blocking evocable seizures (at barely suprathreshold parameters) for a week in fully kindled rats after administration of subthreshold constant 60 Hz sinusoidal 1 s current bursts in trains (6 s interpulse interval) using gradually increasing intensities, delivered to the same amydgala electrodes used for kindling and recording. Low-frequency amygdala stimulation of 1 Hz was reported to provide a "quenching" stimulus slowing or arresting the kindling process, later to be attributed to a 5–15 μA direct current leakage of the stimulation.[118] However, more recent studies also report that 1 Hz stimulation of the hippocampus delayed the effects of kindling, suggesting antiepileptogenic properties.[119] Low-frequency stimulation of the perforant path to the hippocampus slowed the kindling process if given immediately after the stimulation for

kindling.[120] High-frequency stimulation also can be effective. Ten days of hippocampal stimulation at 130 Hz increased the afterdischarge threshold in previously kindled rats.[121]

Implantation of electrodes has made kindling a convenient method for creating seizures in many studies, which facilitates additional studies of potential antiseizure stimulation parameters. But given that electrical stimulation drives the processes responsible for epileptogenesis in the kindling model, it is unclear if attempts at *antiseizure* manipulations bring about separate antiepileptic/antiepileptogenic pathways or simply modify the early and intermediate effects of the kindling stimuli. Both 1 and 50 Hz rostral hippocampal and perforant path stimulation trains lasting for 10 min to 2 h reduced interictal epileptiform discharges for 30–60 min in the dentate gyrus of epileptic rats following kainic acid-induced status epilepticus, but there was no measurable effect on the spontaneous seizure rate and no long-term benefit.[113] Nevertheless, Velasco and colleagues[122] and Boon and colleagues[123] found high-frequency hippocampal stimulation to be effective in unblinded pilot patient trials. A small blinded crossover trial of hippocampal stimulation in epilepsy was negative.[124]

Piriform and Entorhinal Cortex

The entorhinal cortex provides strong feedforward excitation to the hippocampus, with strong hippocampal afferents also originating in the piriform cortex in many species, circuits likely involved with limbic epilepsy.[125–127] Because of their mesial temporal location in human anatomy, we have grouped these structures with other epileptogenic foci, possibly a bit tenuous in classification. Stimulation of these parahippocampal structures within certain parameters is also epileptogenic,[128] similar, albeit with varying thresholds, to kindling elsewhere in limbic circuits.[129] Low-frequency stimulation of the central piriform[130,131] and entorhinal[132] cortices has been reported to delay rodent amygdala kindling; however, the relevance of this finding to other epilepsies is uncertain.

Neocortex

Chronically undercut and partially isolated neocortical islands were originally made and studied in cats and monkeys, becoming progressively more hyperexcitable after a latent period of 2–3 weeks in this model.[133,134] Spontaneous epileptiform discharges spread from the area of isolation to adjacent and contralateral cortices and persisted for at least a year,[135,136] a relatively focal model of posttraumatic epileptogenesis and deafferentation. There is a suggestion that focal electrical stimulation may be antiepileptogenic in the undercut feline marginal gyrus.[137] Using an array of dural electrodes placed about 3 mm apart, Rutledge and colleagues[137] applied 20 daily 2 s trains of 50 Hz, 1 ms pulses at 0.6–1 mA, subthreshold for afterdischarges, spaced 1 min apart, for 1 week and starting 1 week postinjury (6 weeks in two animals). At least 1 week following electrical stimulation, 80% of control animals demonstrated evocable epileptiform afterdischarge runs, in contrast to 18% of the stimulation group. In more recent decades, this model has been adapted to rodents for in vitro slice physiology studies. Supramaximal or rapid stimulation acutely also attenuates hyperexcitability in rodent neocortical slices in this model, hypothetically from recruitment of more intense inhibition.[138] However, the mechanisms of direct cortical electrical stimulation have not been explored in this model.

Many studies of direct electrical stimulation of an epileptogenic focus have drawn largely from the human experience. Penfield and Jasper reported that stimulation of exposed cortex flattened the EEG of epileptic patients.[139] There are also examples of electrical stimulation applied through implanted intracranial electrodes both starting and stopping EEG seizures.[140,141] Clinical trials have been performed with direct responsive neurostimulation of a cortical epileptogenic focus based upon analysis of the ongoing EEG.[142] Trials were preceded by safety and feasibility studies of the implanted device in Suffolk sheep using surface cortical and hippocampal depth electrodes with 30 min of stimulation (50 Hz, 2.5 mA, 300 μs phase duration, biphasic) daily; however, limited data are only available in abstract form,[143,144] and the results of planned histological studies are currently unavailable.

LIMITATIONS OF ANIMAL STUDIES

Clinical trials in patients ideally should be based upon a solid foundation of laboratory

experimentation documenting the basis for a therapeutic effect and the optimal parameters for producing such an effect. Neurostimulation for epilepsy, in contrast, often has moved in reverse, from pilot studies in patients back to the laboratory for validation and modification of stimulation methods. Some degree of public hysteria about the possibilities for "mind control" and other ethical controversies several decades ago surrounding brain stimulation may have steered some scientists away from the field in the past.[9]

Some reports are problematic. Many early studies report empirical examples of changes in epileptiform patterns without the use of statistics, and some parameters of stimulation are not mentioned. Often studies do not provide information about equipment calibration or examples of raw stimulation patterns that may test the limits of safety, particularly for older equipment; leakage currents and other technical issues may contribute to the findings.[118] Small differences in parameters can invoke very different neuronal networks with different effects. Spatial and volumetric differences between the human brain and the small rodent brain, differences in electrode size, and numbers of contacts all may produce significant differences in electrical fields, altering the outcome. Most studies are empirical and do not answer many questions concerning the mechanisms of deep brain stimulation, which should be the true strength of laboratory research.

All animal models of epilepsy have inherent limitations. Conclusions from laboratory work extend only as far as the models reflect human epilepsy in the clinical setting. In contrast to clinical experience, seizures in a large number of animal models can be manipulated by external stimuli, such as sound, novel environments, and tactile stimulation (see ref. 145), leaving some uncertainty about the specific effects of localized stimulation in behaving animals. The chronicity of animal studies is often short in comparison to potential treatment of patients lasting for years, resulting in some uncertainty about the long-term risks of prolonged stimulation with concern about potential for kindling (depending on the site and nature of stimulation), gliosis, or neuronal damage.[29] But in contrast to worsening, the pivotal trial of ANT stimulation revealed a trend toward clinical improvement over months,[5] similar to latent improvement with vagus nerve stimulation.[61] Thus, despite their limitations, rigorous laboratory studies have the potential to provide insight into the cellular mechanisms of antiseizure effects of deep brain stimulation that may guide future clinical improvements.

CONCLUSIONS

Studies of deep brain stimulation in laboratory models of seizures and epilepsy are invaluable for understanding the mechanisms of stimulation and for identifying the best targets and parameters of stimulation. Unfortunately, existing laboratory studies of brain stimulation in epilepsy models present an incomplete and often contradictory picture (Table 79–1). Some efficacy for deep brain stimulation has been documented in labora-

Table 79–1 **Examples of Diversity of Effects of Brain Stimulation in Animal Models***

Site of Stimulation	Result		
	Anti-seizure	No effect	Pro-seizure
Cerebellum	Both deep nuclear and superficial in multiple models in cat, rabbit, rat	Monkeys with deep nuclear or superficial stimulation (cobalt and electrically induced seizures) Deep nuclear stimulation in cat, (penicillin model)	Dentate nucleus stimulation in cat (cobalt model) Superficial stimulation in rat, cat (penicillin model)

(*continued*)

Table 79–1 **(Continued)**

Site of Stimulation	Result		
	Anti-seizure	**No effect**	**Pro-seizure**
Caudate	Cat, some circumstances (focal penicillin, atropine, cobalt models) Monkey (alumina cream model)		Enhance evoked hippocampal discharges in rabbit. Robust seizures in cat worsened (penicillin model) Rebound effect in monkey after stimulation stopped (alumina cream model)
Substantia Nigra pars reticulate (see text for differences of unilateral vs. bilateral stimulation)	Rat (3-aminopyridine model) Cat (penicillin model) Rat (flurothyl model)	Older rat, tonic clonic seizures (flurothyl model)	Rats (evoked hippocampal discharges) Rat (GAERS)
Subthalamic Nucleus	Rat (flurothyl model) Rat (GAERS)	Rat (flurothyl model)	
Brainstem	Locus Coeruleus, rat (PTZ, penicillin, delayed kindling) Median raphe nucleus, rat (PTZ, kindled) Nucleus of the Solitary Tract, kindled cat, delayed kindling in cat		Dorsal raphe nucleus, rat (kindled)
Hypothalamus	Rat (focal carbachol, PTZ models)		Low-frequency stimulation, rat.
Anterior Nucleus of Thalamus	Rat (PTZ, hippocampal kainic acid, pilocarpine)	Low-frequency stimulation, rat (PTZ)	Rat (Kainic acid)
Hippocampus/ Amygdala	Some parameters of rodent hippocampal slice stimulation (bicuculline, low magnesium in vitro models) Rat, some parameters of stimulation (kindling, perforant path stimulation)	Rodent hippocampal slices-some stimulation suppresses spikes, not seizures.	Kindling and evoked seizures in multiple species
Piriform Cortex	Rat (delayed kindling with low-frequency stimulation)		Kindling in rodents
Neocortex	Possible antiepileptogenic in cat (undercut model) Rodent brain slice		Evoked seizures in multiple species/models

*See text for references.

tory models involving the cerebellar cortex, caudate, hypothalamus, subthalamus, centromedian and midline thalamus, anterior thalamus, hippocampus, and neocortex. However, many studies are empiric in nature and mechanisms, and our understanding of the optimal parameters remains incomplete. Pivotal clinical trials with the ANT and responsive neural stimulation of the hippocampus and neocortex have been effective in reducing seizures. These recent successes in clinical studies may give more impetus to laboratory investigations, which in turn will potentially further our clinical practice.

DISCLOSURE STATEMENT

K.D.G. has no conflicts of interest to disclose. R.S.F. has no commercial conflicts of interest

to disclose; he is a nonpaid consultant to the Medtronic Corporation.

REFERENCES

1. World Health Organization. Epilepsy. Fact sheet No. 999. January 2009 http://www.who.int/mediacentre/factsheets/fs999/en/print.html. Accessed 5/21/2011.
2. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N Engl J Med*. 2000;342:314–319.
3. Fisher RS, Vickrey BG, Gibson P, Hermann B, Penovich P, Scherer A, Walker S. The impact of epilepsy from the patient's perspective. II. Views about therapy and health care. *Epilepsy Res*. 2000;41: 53–61.
4. Engel J Jr, Wiebe S, French J, Sperling M, Williamson P, Spencer D, Gumnit R, Zahn C, Westbrook E, Enos B. Practice parameter: temporal lobe and localized neocortical resections for epilepsy: Report of the Quality Standards Subcommittee of the American Academy of Neurology, in association with the American Epilepsy Society and the American Association of Neurological Surgeons. *Neurology.* 2003;60;538–547.
5. Fisher R, Salanova V, Witt T, Worth R, Henry T, Gross R, Oommen K, Osorio I, Nazzaro J, Labar D, Kaplitt M, Sperling M, Sandok E, Neal J, Handforth A, Stern J, DeSalles A, Chung S, Shetter A, Bergen D, Bakay R, Henderson J, French J, Baltuch G, Rosenfeld W, Youkilis A, Marks W, Garcia P, Barbaro N, Fountain N, Bazil C, Goodman R, McKhann G, Babu Krishnamurthy K, Papavassiliou S, Epstein C, Pollard J, Tonder L, Grebin J, Coffey R, Graves N, SANTE Study Group. Electrical stimulation of the anterior nucleus of thalamus for treatment of refractory epilepsy. *Epilepsia.* 2010;51:899–908.
6. Fritsch G, Hitzig E. International Classics in Epilepsy and Behavior: 1870. Electric excitability of the cerebrum (Uber die elektrische Erregbarkeit des Grosshirns). *Epilepsy Behav*. 2009;15:123–130.
7. Ferrier D. Experimental researches in cerebral physiology and pathology. *J Anat Physiol.* 1873;8: 152–155.
8. Delgado JMR. *Physical Control of the Mind: Toward a Psychocivilized Society*. New York: Harper and Row; 1969.
9. Horgan J. The forgotten era of brain chips. *Sci Am*. 2005;293:66–73.
10. Goddard GV. Development of epileptic seizures through brain stimulation at low intensity. *Nature*. 1967;214:1020–1021.
11. Gotman J. Epileptic networks studied with EEG-fMRI. *Epilepsia.* 2008;49(suppl 3):42–51.
12. Park EH, Barreto E, Gluckman BJ, Schiff SJ, So P. A model of the effects of applied electric fields on neuronal synchronization. *J Comput Neurosci*. 2005;19: 53–70.
13. Bikson M, Lian J, Hahn PJ, Stacey WC, Sciortino C, Durand DM. Suppression of epileptiform activity by high frequency sinusoidal fields in rat hippocampal slices. *J Physiol.* 2001;531:181–191.
14. Duman RS, Vaidya VA. Molecular and cellular actions of chronic electroconvulsive seizures. *J ECT*. 1998;14:181–193.
15. Keller A, Arissian K, Asanuma H. Synaptic proliferation in the motor cortex of adult cats after long-term thalamic stimulation. *J Neurophysiol*. 1992;68:295–308.
16. Lockman J, Fisher RS. Therapeutic brain stimulation for epilepsy. *Neurol Clin*. 2009; 27:1031–1040.
17. Raines A, Anderson RJ. Effects of acute cerebellectomy on maximal electroshock seizures and anticonvulsant efficacy of diazepam in the rat. *Epilepsia.* 1976;17:177–182.
18. Gartside IB. The effects of cerebellectomy on a penicillin epileptogenic focus in the cerebral cortex of the rat. *Electroencephalogr Clin Neurophysiol.* 1978;44:373–379.
19. Cooke PM, Snider RS. Some cerebellar influences on electrically-induced cerebral seizures. *Epilepsia.* 1955;4:19–28.
20. Iwata K, Snider RS. Cerebello-hippocampal influences on the electroencephalogram. *Electroencephalogr Clin Neurophysiol*. 1958;11:439–446.
21. Mutani R, Bergamini L, Doriguzzi T. Effects of paleocerebellar and caudate stimulation of the activity of experimental rhinencephalic epileptogenic foci. *Electroencephalogr Clin Neurophysiol*. 1968; 25:515.
22. Hutton JT, Frost JD Jr, Foster J. The influence of the cerebellum in cat penicillin epilepsy. *Epilepsia*. 1972;13:401–408.
23. Babb TL, Mitchell AG, Crandall PH. Fastigiobulbar and dentatothalamic influences on hippocampal cobalt epilepsy in the cat. *Electroencephalogr Clin Neurophysiol*. 1974;36:141–154.
24. Dow RS, Manni E, Guardia FA. Influence of cerebellum on experimental epilepsy. *Electroencephalogr Clin Neurophysiol*. 1962;14:383–389.
25. Strain GM, Van Meter WG, Brockman WH. Elevation of seizure thresholds: a comparison of cerebellar stimulation, phenobarbital, and diphenylhydantoin. *Epilepsia.* 1978;19:493–504.
26. Godlevskii LS, Stepanenko KI, Lobasyuk BA, Sarakhan EV, Bobkova LM. The effects of electrical stimulation of the paleocerebellar cortex on penicillin-induced convulsive activity in rats. *Neurosci Behav Physiol.* 2004;34:797–802.
27. Grimm RJ, Frazee JG, Bell CC, Kawasaki T, Dow RS. Quantitative studies in cobalt model epilepsy: the effect of cerebellar stimulation. *Int J Neurol*. 1970;7: 126–140.
28. Hemmy DC, Larson SJ, Sances A Jr, Millar EA. The effect of cerebellar stimulation on focal seizure activity and spasticity in monkeys. *J Neurosurg*. 1977;46: 648–653.
29. Babb TL, Soper HV, Lieb JP, Brown WJ, Ottino CA, Crandall PH. Electrophysiological studies of long-term electrical stimulation of the cerebellum in monkeys. *J Neurosurg*. 1977;47:353–365.
30. Krauss GL, Koubeissi MZ. Cerebellar and thalamic stimulation treatment for epilepsy. *Acta Neurochir Suppl*. 2007;97:347–356.
31. Fountas KN, Kapsalaki E, Hadjigeorgiou G. Cerebellar stimulation in the management of medically intractable epilepsy: a systematic and critical review. *Neurosurg Focus*. 2010;29:E8.
32. Babb TL, Mitchell AG, Crandall PH. Cerebellar influences on the hippocampus. In: Cooper IS, Riklan M, Snider RS, eds. *The Cerebellum, Epilepsy, and Behavior*. New York: Plenum Press; 1974:37–56.

33. Zuccarello M, Sawaya R, Lukin R, deCourten-Myers G. Spontaneous cerebellar hematoma associated with chronic cerebellar stimulation. Case report. *J Neurosurg*. 1986;65:860–862.
34. Cooper IS, Amin I, Riklan M, Waltz JM, Poon TP. Chronic cerebellar stimulation in epilepsy. *Arch Neurol*. 1976;33:559–570.
35. Van Buren JM, Wood JH, Oakley J, Hambrech F. Preliminary evaluation of cerebellar stimulation by double-blinded stimulation and biological criteria in the treatment of epilepsy. *J Neurosurg*. 1978;48:407–416.
36. Wright GD, McLellan DL, Brice JG. A double-blind trial of chronic cerebellar stimulation in twelve patients with severe epilepsy. *J Neurol Neurosurg Psychiatry*. 1984;47:769–174.
37. Velasco F, Carrillo-Ruiz JD, Brito F, Velasco M, Velasco AL, Marquez I, Davis R. Double-blind, randomized controlled pilot study of bilateral cerebellar stimulation for treatment of intractable motor seizures. *Epilepsia*. 2005;46:1071–1081.
38. Costin A, Bergmann F, Gutman J. Relationship between caudate nucleus and dorsal hippocampus in rabbit. *Electroencephalogr Clin Neurophysiol*. 1963;15:997–1011.
39. Rakic L, Buchwald NA, Wyers EJ. Induction of seizures by stimulation of the caudate nucleus. *Electrooencephalogr Clin Neurophysiol*. 1962;14: 809–823.
40. La Grutta V, Sabatino M, Gravante G, Morici G, Ferraro G, La Grutta G. A study of caudate inhibition on an epileptic focus in the cat hippocampus. *Arch Int Physiol Biochim*. 1988;96:113–120.
41. Sabatino M, Gravante G, Ferraro G, Vella N, La Grutta G, La Grutta V. Striatonigral suppression of focal hippocampal epilepsy. *Neurosci Lett*. 1989;98:285–290.
42. Mutani R, Fariello R. Effect of low frequency caudate stimulation on the EEG of epileptic neocortex. *Brain Res*. 1969;14:749–753.
43. Psatta DM. Control of chronic experimental focal epilepsy by feedback caudatum stimulations. *Epilepsia*. 1983;24:444–454.
44. Wagner R 2nd, Feeney DM, Gullotta FP, Cote IL. Suppression of cortical epileptiform activity by generalized and localized ECoG desynchronization. *Electroencephalogr Clin Neurophysiol*. 1975;39: 499–506.
45. Oakley JC, Ojemann GA. Effects of chronic stimulation of the caudate nucleus on a preexisting alumina seizure focus. *Exp Neurol*. 1982;75:360–367.
46. Chkhenkeli SA, Sramka M, Lortkipanidze GS, Rakviashvili TN, Bregvadze ESh, Magalashvili GE, Gagoshidze TSh, Chkhenkeli IS. Electrophysiological effects and clinical results of direct brain stimulation for intractable epilepsy. *Clin Neurol Neurosurg*. 2004;106:318–329.
47. Gale K. Role of the substantia nigra in GABA-mediated anticonvulsant actions. *Adv Neurol*. 1986;44:343–364.
48. McNamara JO, Galloway MT, Rigsbee LC, Shin C. Evidence implicating substantia nigra in regulation of kindled seizure threshold. *J Neurosci*. 1984;4: 2410–2417.
49. Shin C, Scialabba FA, McNamara JO. Stimulation of substantia nigra pars reticulata enhances dentate granule cell excitability. *Brain Res*. 1987;411:21–27.
50. Boda B, Szente MB. Stimulation of substantia nigra pars reticulata suppresses neocortical seizures. *Brain Res*. 1992;574:237–243.
51. Sabatino M, Gravante G, Ferraro G, Savatteri V, La Grutta V. Inhibitory control by substantia nigra of generalized epilepsy in the cat. *Epilepsy Res*. 1988;2: 380–386.
52. Velísêk L, Velísková J, Moshé SL. Electrical stimulation of substantia nigra pars reticulata is anticonvulsant in adult and young male rats. *Exp Neurol*. 2002;73:145–152.
53. Morimoto K, Goddard GV. The substantia nigra is an important site for the containment of seizure generalization in the kindling model of epilepsy. *Epilepsia*. 1987;28:1–10.
54. Shi LH, Luo F, Woodward D, Chang JY. Deep brain stimulation of the substantia nigra pars reticulata exerts long-lasting suppression of amygdala-kindled seizures. *Brain Res*. 2006;1090:202–207.
55. Feddersen B, Vercueil L, Noachtar S, David O, Depaulis A, Deransart C. EEG and evoked potential recording from the subthalamic nucleus for deep brain stimulation of intractable epilepsy. *Clin Neurophysiol*. 2002;113:1391–1402.
56. Benabid AL, Chabardes S, Mitrofanis J, Pollak P. Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. *Lancet Neurol*. 2009;8:67–81.
57. Baunez C, Gubellini P. Effects of GPi and STN inactivation on physiological, motor, cognitive and motivational processes in animal models of Parkinson's disease. *Prog Brain Res*. 2010;183C:235–258.
58. Lado FA, Velísek L, Moshé SL. The effect of electrical stimulation of the subthalamic nucleus on seizures is frequency dependent. *Epilepsia*. 2003;44:157–164.
59. Vercueil L, Benazzouz A, Deransart C, Bressand K, Marescaux C, Depaulis A, Benabid AL. High-frequency stimulation of the subthalamic nucleus suppresses absence seizures in the rat: comparison with neurotoxic lesions. *Epilepsy Res*. 1998;31:39–46.
60. Wyckhuys T, Geerts PJ, Raedt R, Vonck K, Wadman W, Boon P. Deep brain stimulation for epilepsy: knowledge gained from experimental animal models. *Acta Neurol Belg*. 2009;109:63–80.
61. Ben-Menachem E. Vagus-nerve stimulation for the treatment of epilepsy. *Lancet Neurol*. 2002;1: 477–482.
62. Krahl SE, Clark KB, Smith DC, Browning RA. Locus coeruleus lesions suppress the seizure-attenuating effects of vagus nerve stimulation. *Epilepsia*. 1998;39: 709–714.
63. Libet B, Gleason CA, Wright EW Jr, Feinstein B. Suppression of an epileptiform type of electrocortical activity in the rat by stimulation in the vicinity of locus coeruleus. *Epilepsia*. 1977;18:451–462.
64. Neuman RS. Suppression of penicillin-induced focal epileptiform activity by locus coeruleus stimulation: mediation by an alpha 1-adrenoceptor. *Epilepsia*. 1986;27:359–366.
65. Ferraro G, Sardo P, Sabatino M, La Grutta V. Locus coeruleus noradrenaline system and focal penicillin hippocampal epilepsy: neurophysiological study. *Epilepsy Res*. 1994;19:215–220.
66. Fischer W, Kästner I, Lasek R, Müller M. Effect of stimulation of the locus coeruleus on cobalt-induced epileptiform activity in the rat. *Biomed Biochim Acta*. 1983;42:1179–1187.
67. Weiss GK, Lewis J, Jimenez-Rivera C, Vigil A, Corcoran ME. Antikindling effects of locus coeruleus

stimulation: mediation by ascending noradrenergic projections. *Epilepsia*. 1998;39:709–714.
68. Feinstein B, Gleason CA, Libet B. Stimulation of locus coeruleus in man. Preliminary trials for spasticity and epilepsy. *Stereotact Funct Neurosurg.* 1989;52:26–41.
69. Hernandez TD, Rosen JB, Gallager DW. Long-term changes in sensitivity to GABA in dorsal raphe neurons following amygdala kindling. *Brain Res*. 1990;517: 294–300.
70. Rougerie A, Kästner I, Seidel J. Effect of stimulation of the nucleus raphe dorsalis on cortical hypersynchronous activity in the rat. *Acta Biol Med Ger*. 1978;37:1289–1293.
71. Neuman RS, Thompson PM. Serotonin mediates suppression of focal epileptiform activity induced by noxious stimulation. *Epilepsia*. 1989;30:307–313.
72. Kovacs DA, Zoll JG. Seizure inhibition by median raphe nucleus stimulation in rat. *Brain Res*. 1974;70: 165–169.
73. Walker BR, Easton A, Gale K. Regulation of limbic motor seizures by GABA and glutamate transmission in nucleus tractus solitarius. *Epilepsia*. 1999;40: 1051–1057.
74. Magdaleno-Madrigal VM, Valdés-Cruz A, Martínez-Vargas D, Martínez A, Almazán S, Fernández-Mas R, Fernández-Guardiola A. Effect of electrical stimulation of the nucleus of the solitary tract on the development of electrical amygdaloid kindling in the cat. *Epilepsia*. 2002;43:964–969.
75. Magdaleno-Madrigal VM, Martínez-Vargas D, Valdés-Cruz A, Almazán-Alvarado S, Fernández-Mas R. Preemptive effect of nucleus of the solitary tract stimulation on amygdaloid kindling in freely moving cats. *Epilepsia.* 2010;51:438–444.
76. Fenoglio KA, Wu J, Kim do Y, Simeone TA, Coons SW, Rekate H, Rho JM, Kerrigan JF. Hypothalamic hamartoma: basic mechanisms of intrinsic epileptogenesis. *Semin Pediatr Neurol.* 2007;14:51–59.
77. Herberg LJ, Watkins PJ. Epileptiform seizures induced by hypothalamic stimulation in the rat: resistance to fits following fits. *Nature.* 1966;209:515–516
78. Herberg LJ, Tress KH, Blundell JE. Raising the threshold in experimental epilepsy by hypothalamic and septal stimulation and by audiogenic seizures. *Brain.* 1969;92:313–328.
79. Cullen N, Goddard GV. Kindling in the hypothalamus and transfer to the ipsilateral amygdala. *Behav Biol.* 1975;15:119–131.
80. Dubicka I, Frank JM, Mccutcheon B. Attenuation of a convulsive syndrome in the rat by lateral hypothalamic stimulation. *Physiol Behav*. 1978;20:31–38.
81. Mirski MA, Fisher RS. Electrical stimulation of the mammillary nuclei increases seizure threshold to pentylenetetrazol in rats. *Epilepsia.* 1994;35:1309–1316.
82. Franzini A, Messina G, Cordella R, Marras C, Broggi G. Deep brain stimulation of the posteromedial hypothalamus: indications, long-term results, and neurophysiological considerations. *Neurosurg Focus*. 2010;29:E13.
83. Jasper H, Naquet R, King EV. Thalamocortical recruiting responses in sensory receiving areas in the cat. *Electroencephalogr Clin Neurophysiol*. 1955;7: 99–114.
84. Dempsey EW, Morison RS. The production of rhythmically recurrent cortical potentials after localized thalamic stimulation. *Am J Physiol*. 1942;135:293–300.
85. Jasper HH, Droogleever-Fortuyn J. Experimental studies of the functional anatomy of petit ma1 epilepsy. *Res Publ Assoc Nerv Ment Dis*. 1947;26:272–298.
86. Bertram EH, Mangan PS, Zhang D, Scott CA, Williamson JM. The midline thalamus: alterations and a potential role in limbic epilepsy. *Epilepsia.* 2001;42: 967–978.
87. Mirski MA, Thakor NV, Sherman DL. Anterior thalamic mediation of experimental seizures: selective EEG spectral coherence. *Epilepsia.* 2003;44:355–365.
88. Mirski MA, Ferrendelli JA. Anterior thalamus and substantia nigra: two distinct structures mediating experimental generalized seizures. *Brain Res*. 1986;397: 377–380.
89. Mirski MA, Ferrendelli JA. Anterior thalamic mediation of generalized pentylenetetrazol seizures. *Brain Res.* 1986;399:212–223.
90. Mirski MA, Ferrendelli JA. Selective metabolic activation of the mammillary bodies and their connections during ethosuximide-induced suppression of pentylenetetrazol seizures. *Epilepsia.* 1986;27:194–203.
91. Mirski MA, Ferrendelli JA. Interruption of the connections of the mamillary bodies protect against generalized pentylenetetrazol seizures in guinea pigs. *J Neurosci*. 1987;7:662–670.
92. Mirski MA, Ferrendelli JA. Interruption of the mammillothalamic tracts prevents seizures in guinea pigs. *Science*. 1984;226:72–74.
93. Mirski MA, Fisher RA. Pharmacological inhibition of posterior hypothalamus raises seizure threshold in rats. *Epilepsia.* 1993;34(suppl 6):12–14.
94. Bittencourt S, Dubiela FP, Queiroz C, Covolan L, Andrade D, Lozano A, Mello LE, Hamani C. Microinjection of GABAergic agents into the anterior nucleus of the thalamus modulates pilocarpine-induced seizures and status epilepticus. *Seizure.* 2010;19:242–246.
95. Mirski MA, Rossell LA, Terry JB, Fisher RS. Anticonvulsant effect of anterior thalamic high frequency electrical stimulation in the rat. *Epilepsy Res.* 1997;28:89–100.
96. Hamani C, Hodaie M, Chiang J, del Campo M, Andrade DM, Sherman D, Mirski M, Mello LE, Lozano AM. Deep brain stimulation of the anterior nucleus of the thalamus: effects of electrical stimulation on pilocarpine-induced seizures and status epilepticus. *Epilepsy Res*. 2008;78:117–123.
97. Hamani C, Ewerton FIS, Bonilha SM, Ballester G, Mello LEAM, Lozano A. Bilateral anterior thalamic nucleus lesions and high-frequency stimulation are protective against pilocarpine-induced seizures and status epilepticus. *Neurosurgery.* 2004;54:191–197.
98. Takebayashi S, Hashizume K, Tanaka T, Hodozuka A. Anticonvulsant effect of electrical stimulation and lesioning of the anterior thalamic nucleus on kainic acid-induced focal limbic seizure in rats. *Epilepsy Res*. 2007;74:163–170.
99. Takebayashi S, Hashizume K, Tanaka T, Hodozuka A. The effect of electrical stimulation and lesioning of the anterior thalamic nucleus on kainic acid-induced focal cortical seizure status in rats. *Epilepsia*. 2007;48:348–358.
100. Lado FA. Chronic bilateral stimulation of the anterior thalamus of kainate-treated rats increases seizure frequency. *Epilepsia.* 2006;47:27–32.
101. Ziai W, Sherman DL, Bhardwaj A, Mirski MA. Target-specific catecholamine elevation induced

by anticonvulsant thalamic deep brain stimulation. *Epilepsia.* 2005;46:878–888.
102. Hamani C, Ewerton FI, Marcolin de Almeida F, Bonilha SM, Covolan L, Fantin Cavarsan C, Ballester G, Mello LE, Lozano AM. Bilateral anterior thalamic nucleus lesions are not protective against seizures in chronic pilocarpine epileptic rats. *Stereotact Funct Neurosurg*. 2009;87:143–147.
103. Nishida N, Huang ZL, Mikuni N, Miura Y, Urade Y, Hashimoto N. Deep brain stimulation of the posterior hypothalamus activates the histaminergic system to exert antiepileptic effect in rat pentylenetetrazol model. *Exp Neurol.* 2007;205:132–144.
104. Mirski MA, Ziai WC, Chiang J, Hinich M, Sherman D. Anticonvulsant serotonergic and deep brain stimulation in anterior thalamus. *Seizure*. 2009; 18:64–70.
105. Zhang DX, Bertram EH. Midline thalamic region: widespread excitatory input to the entorhinal cortex and amygdala. *J Neurosci*. 2002;22:3277–3284.
106. Miller JW, Ferrendelli JA. The central medial nucleus: thalamic site of seizure regulation. *Brain Res*. 1990;508:297–300.
107. Fisher RS, Uematsu S, Krauss GL, Cysyk BJ, McPherson R, Lesser RP, Gordon B, Schwerdt P, Rise M. Placebo-controlled pilot study of centromedian thalamic stimulation in treatment of intractable seizures. *Epilepsia*. 1992;33:841–851.
108. Velasco F, Velasco AL, Velasco M, Jiménez F, Carrillo-Ruiz JD, Castro G. Deep brain stimulation for treatment of the epilepsies: the centromedian thalamic target. *Acta Neurochir Suppl*. 2007;97:337–342.
109. Nanobashvili Z, Chachua T, Nanobashvili A, Bilanishvili I, Lindvall O, Kokaia Z. Suppression of limbic motor seizures by electrical stimulation in thalamic reticular nucleus. *Exp Neurol*. 2003;181: 224–230.
110. Huguenard JR, McCormick DA. Thalamic synchrony and dynamic regulation of global forebrain oscillations. *Trends Neurosci*. 2007;30:350–356.
111. Albensi BC, Ata G, Schmidt E, Waterman JD, Janigro D. Activation of long-term synaptic plasticity causes suppression of epileptiform activity in rat hippocampal slices. *Brain Res.* 2004;998:56–64.
112. Albensi BC, Toupin JD, Oikawa K, Oliver DR. Controlled pulse delivery of electrical stimulation differentially reduces epileptiform activity in Mg^{2+}-free-treated hippocampal slices. *Brain Res*. 2008;1226: 163–172.
113. Bragin A, Wilson CL, Engel J Jr. Rate of interictal events and spontaneous seizures in epileptic rats after electrical stimulation of hippocampus and its afferents. *Epilepsia.* 2002;43(suppl 5):81–85.
114. Douglas RM, McNaughton B, Goddard GV. Commissural inhibition and facilitation of granule cell discharge in fascia dentate. *J Comp Neurol.* 1983;219:285–294.
115. Mucha RF, Pinel PJ. Postseizure inhibition of kindled seizures. *Exp Neurol.* 1977;54:266–282.
116. Sainsbury RS, Bland BH, Buchan DH. Electrically induced seizure activity in the hippocampus: time course for postseizure inhibition of subsequent kindled seizures. *Behav Biol*. 1978;22:479–488.
117. Shao J, Valenstein ES. Long-term inhibition of kindled seizures by brain stimulation. *Exp Neurol.* 1982;76:376–392.
118. Weiss SR, Eidsath A, Li XL, Heynen T, Post RM. Quenching revisited: low level direct current inhibits amygdala-kindled seizures. *Exp Neurol*. 1998;154:185–192.
119. Zhang S-H, Sun H-L, Fang Q, Zhong K, Wu D-C, Wang S, Chen Z. Low-frequency stimulation of the hippocampal CA3 subfield is anti-epileptogenic and anti-ictogenic in rat amygdaloid kindling model of epilepsy. *Neurosci Lett.* 2009;455:51–55.
120. Mohammad-Zadeh M, Mirnajafi-Zadeh J, Fathollahi Y, Javan M, Ghorbani P, Sadegh M, Noorbakhsh SM. Effect of low frequency stimulation of perforant path on kindling rate and synaptic transmission in the dentate gyrus during kindling acquisition in rats. *Epilepsy Res.* 2007;75:154–161.
121. Wyckhuys T, Raedt R, Vonck K, Wadman W, Boon P. Comparison of hippocampal deep brain stimulation with high (130 Hz) and low frequency (5 Hz) on afterdischarges in kindled rats. *Epilepsy Res*. 2010;88: 239–246.
122. Velasco AL, Velasco F, Velasco M, Trejo D, Castro G, Carrillo-Ruiz JD. Electrical stimulation of the hippocampal epileptic foci for seizure control: a double-blind, long-term follow-up study. *Epilepsia.* 2007;48: 1895–1903.
123. Boon P, Vonck K, De Herdt V, Van Dycke A, Goethals M, Goossens L, Van Zandijcke M, De Smedt T, Dewaele I, Achten R, Wadman W, Dewaele F, Caemaert J, Van Roost D. Deep brain stimulation in patients with refractory temporal lobe epilepsy. *Epilepsia*. 2007;48:1551–1560.
124. Tellez-Zenteno JF, McLachlan RS, Parrent A, Kubu CS, Wiebe S. Hippocampal electrical stimulation in mesial temporal lobe epilepsy. *Neurology.* 2006;66: 1490–1494.
125. Gale K. Subcortical structures and pathways involved in convulsive seizure generation. *J Clin Neurophysiol*. 1992;9:264–277.
126. McIntyre DC, Gilby KL. Mapping seizure pathways in the temporal lobe. *Epilepsia.* 2008;49(suppl 3): 23–30.
127. Kumar SS, Buckmaster PS. Hyperexcitability, interneurons, and loss of GABAergic synapses in entorhinal cortex in a model of temporal lobe epilepsy. *J Neurosci*. 2006;26:4613–4623.
128. McIntyre DC, Kelly ME. The parahippocampal cortices and kindling. *Ann NY Acad Sci.* 2000;911: 343–354.
129. Löscher W, Ebert U, Wahnschaffe U, Rundfeldt C. Susceptibility of different cell layers of the anterior and posterior part of the piriform cortex to electrical stimulation and kindling: comparison with the basolateral amygdala and "area tempestas." *Neuroscience.* 1995;66:265–276.
130. Yang LX, Jin CL, Zhu-Ge ZB, Wang S, Wei EQ, Bruc IC, Chen Z. Unilateral low-frequency stimulation of central piriform cortex delays seizure development induced by amygdaloid kindling in rats. *Neuroscience.* 2006;138:1089–1096.
131. Ghorbani P, Mohammad-Zadeh M, Mirnajafi-Zadeh J, Fathollahi Y. Effect of different patterns of low-frequency stimulation on piriform cortex kindled seizures. *Neurosci Lett.* 2007;425:162–166.
132. Xu ZH, Wu DC, Fang Q, Zhong K, Wang S, Sun HL, Zhang SH, Chen Z. Therapeutic time window of low-frequency stimulation at entorhinal cortex for

amygdaloid-kindling seizures in rats. *Epilepsia.* 2010; 51:1861–1864.
133. Grafstein B., Sastry PB. Some preliminary electrophysiological studies on chronic neuronally isolated cerebral cortex. *Electroencephalogr Clin Neurophysiol*. 1957;9:723–725.
134. Sharpless SK, Halpern LM. The electrical excitability of chronically isolated cortex studied by means of permanently implanted electrodes. *Electroencephalogr Clin Neurophysiol*. 1962;14:244–255.
135. Echlin FA, Battista AA. Epileptic seizures originating in chronic partially isolated cerebral cortex following peripheral nerve stimulation. *Trans Am Neurol Assoc.* 1961;86:209–211.
136. Echlin FA, Battista AA. Epileptiform seizures from chronic isolated cortex. *Arch Neurol.* 1963;168: 154–170.
137. Rutledge LT, Ranck JB Jr, Duncan JA. Prevention of supersensitivity in partially isolated cerebral cortex. *Electroencephalogr Clin Neurophysiol*. 1967;23: 256–262.
138. Prince DA, Tseng GF. Epileptogenesis in chronically injured cortex: in vitro studies. *J Neurophysiol.* 1993;69:1276–1291.
139. Jasper H. Electrocorticography. In: Penfield W, Jasper H, eds. *Epilepsy and the Functional Anatomy of the Human Brain.* Boston: Little, Brown; 1954: 692–738.
140. Lesser RP, Kim SH, Beyderman L, Miglioretti DL, Webber WR, Bare M, Cysyk B, Krauss G, Gordon B. Brief bursts of pulse stimulation terminate afterdischarges caused by cortical stimulation. *Neurology*. 1999;53:2073–2081.
141. Kinoshita M, Ikeda A, Matsumoto R, Begum T, Usui K, Yamamoto J, Matsuhashi M, Takayama M, Mikuni N, Takahashi J, Miyamoto S, Shibasaki H. Electrical stimulation on human cortex suppresses fast cortical activity and epileptic spikes. *Epilepsia.* 2004;45: 787–791.
142. Skarpaas TL, Morrell MJ. Intracranial stimulation therapy for epilepsy. *Neurotherapeutics*. 2009;6: 238–243.
143. Sweazey R, Munz M, Vinters H, Barrett C, Plenys A, Greene D. Chronic implantation of the responsive neurostimulator (RNS) lead system in the sheep model to demonstrate essential safety prior to a human clinical trial. *Epilepsia.* 2002;43(suppl 7):351.
144. Munz M, Sweazey R, Barrett C, Loftman AP, Potts D, Greene D. Preclinical testing of an implantable responsive neurostimulator system in a sheep model. Program No. 442.8. *2003 Abstract Viewer/Itinerary Planner*. New Orleans: Society for Neuroscience; 2003.
145. Pitkanen A, Schwartzkroin P, Moshe S, eds. *Models of Seizures and Epilepsy*. San Diego, CA: Elsevier; 2006.

Chapter 80

Animal Models for Evaluating Antiepileptogenesis

H. Steve White

INTRODUCTION

Since the first White House-initiated CURE conference in March 2000, the epilepsy research community has placed greater emphasis on finding a cure for epilepsy. Supported by the National Institutes of Health, Citizen's United for Research in Epilepsy (CURE), the Epilepsy Therapy Development Project, the American Epilepsy Society, the Epilepsy Foundation of America, and the pharmaceutical industry, this multifaceted approach has led to several important advances that include (*1*) *a greater understanding of the pathophysiology of epilepsy at the molecular and genetic levels;* (*2*) *the search for surrogate markers of the epileptogenic process;* (*3*) *the development and utilization of model systems (animal and human) for evaluating novel therapeutic approaches; and* (*4*) *productive discussions and collaborations within the scientific, regulatory, and pharmaceutical communities.*

The need to find a therapy that will prevent or delay the development of epilepsy in the susceptible individual is evident, and many of the required tools have already been developed. Nonetheless, there are many practical challenges and hurdles at both the preclinical and clinical levels that must be overcome before a cure can be developed. Prior to embarking on a clinical trial, there should be some evidence that a hypothetical therapy is effective in one or more animal models of epileptogenesis. However, unlike the animal models routinely employed in the search for novel anticonvulsant compounds, the animal models of epileptogenesis that are currently available have not been validated clinically. Unfortunately, clinical validation will not be provided until the first truly antiepileptic or disease-modifying therapy has been found to be effective in an appropriately designed clinical trial. With this in mind, it is still unknown what preclinical evidence of efficacy would be required before one would be

willing to embark on an expensive clinical trial that involves a path not yet paved by past experience. Nevertheless, the scientific community should not be discouraged from pursing this approach but should clearly be aware of the limitations of the existing models and employ caution when designing preclinical studies and interpreting the results obtained.

ANIMAL MODELS OF ACQUIRED EPILEPSY

There are a number of experimental animal models that could be used to test a potential disease-modifying or antiepileptogenic therapy. These include several models of acquired epilepsy (Table 80–1) and an expanding number of genetic models that possess a known mutation found to be associated with a particular genetic form of human epilepsy.[1,2] The models of acquired epilepsy listed in Table 80–1 involve an initial insult followed by a variable latent period that often culminates in the evolution of recurrent, spontaneous seizure activity or, at the very least, measurable hyperexcitability at some later time. In this regard, many of the animal models of epileptogenesis that have been described in recent years recapitulate several of the features of human epilepsy. The use of these and other emerging models will likely play an important role in the discovery of novel treatment strategies for the individual at risk of developing epilepsy as a result of a brain insult or genetic susceptibility. Moreover, their use can help guide clinical trials once an ideal clinical candidate has been identified.

The rodent models of epileptogenesis that have been most often employed in epilepsy research include kindling and status epilepticus (SE).[3] As summarized in Table 80–1, other models of epileptogenesis that have been developed over the years include the tetanus toxin model of partial epilepsy, the neonatal hypoxia-ischemia model of hypoxic-ischemic injury, the neonatal hyperpyrexia model of febrile seizures, models of infantile spasms (IS), models of cortical dysplasia, and models of traumatic brain injury (TBI). Each of these rodent models displays varying degrees of cell loss, synaptic reorganization, network hyperexcitability, and a latent period that is followed by the expression of an altered seizure threshold or the development of spontaneous seizures or spasms in the case of IS, that is, the hallmark of epilepsy. As such, they embody some of the important characteristics of a model of acquired epilepsy.[4] A comprehensive review of all of the models listed in Table 80–1 is beyond the scope of this chapter, and the reader is encouraged to review the primary literature cited within. The remainder of this chapter will review the salient points and utility of the kindling, SE, and TBI models of acquired epilepsy and discuss an emerging model of viral encephalitis wherein the central administration of Theiler's virus to C57BL/6 mice results in acute symptomatic seizures followed by latent hyperexcitability and the development of recurrent spontaneous seizures. Importantly, each of these models has a clear clinical correlate wherein therapies identified at the preclinical level could be quickly translated to the human population at risk for developing epilepsy. As such, they offer something unique for the investigator interested in evaluating a proposed antiepileptogenic therapy.

Kindling

Kindling is the process originally described by Goddard and colleagues[5] whereby an electrical stimulus typically delivered to a limbic brain structure sufficient to evoke a limited afterdischarge duration (ADD) is, with repetition over time, able to evoke an electrographic afterdischarge that is capable of spreading and initiating a generalized convulsive seizure. The change in responsiveness that occurs during kindling acquisition is permanent, and although it is not usually associated with spontaneous seizures, it is associated with a reduced seizure threshold. The fully kindled animal model is routinely employed in the search for novel therapies for the patient with complex partial seizures with secondary generalization. Indeed, the pharmacological profile of the fully kindled animal supports the validity of this model for drug screening (see Loscher and Brandt,[3] Smith et al.,[68] and Bialer and White[69] for review and references).

The process by which an initially subconvulsive stimulation becomes convulsive has provided the experimental epilepsy community with a valuable platform to study the neurobiological substrates that underlie the

Table 80–1 **Animal Models of Epileptogenesis**

Experimental Model	Neuropathology Present	Chronic Hyper-excitability	Latent Period	Spontaneous Seizures	Selected References
Kindling (amygdala, hippocampal, corneal)	Yes	Yes	No	No[a]	5–10
Post-status epilepticus (bicuculline, kainic acid, pilocarpine ± Li^+, electrical models of self-sustaining status, cobalt-homocysteine, fluorothyl)	Yes; age-dependent variability	Yes	Yes (days, weeks, to months)	Yes; marked age-dependent variability	11–26
Tetanus toxin	Yes	Yes	Yes	Yes, but may remit	27–29
Traumatic brain injury					
Cortical undercut	Yes	Yes	Yes	Yes	30–35
Ferric chloride	Yes	Yes	Yes	Yes	36
Fluid percussion	Yes	Yes	Yes	Yes	37–44
Neonatal hypoxia-ischemia	Yes	Yes	Yes	Yes	45–51
Neonatal hyperthermia	No	Yes	Yes	Yes	52–54
Cortical dysplasia models (MAM, freeze lesion, $Otx^{-/-}$)	Yes	Decreased seizure threshold (MAM and freeze lesion models)	?	Yes in $Otx^{-/-}$ but not other models	55, 56
Infantile spasms (multiple models)	Yes	Yes	Variable	Presence of spasms and seizures is age- and model-dependent	See refs. 57 and 58 for review and references
Encephalitis-induced epilepsy (Theiler's mouse encephalitis virus)	Yes	Yes	Yes	Yes	59–63
Genetic models	Model-specific	Yes	Model-specific	Model-specific	1, 2

MAM, methylazoxymethanol.
[a]With repeated stimulation (i.e., overkindling), spontaneous seizures do develop (postkindling acquisition).[66,67]
Source: Adapted from refs. 3, 55, 64, and 65.

epileptogenic process. Unfortunately, the extent to which the acquisition of kindling reflects human epileptogenesis, and the validity of the kindled animal for studying the antiepileptogenic or disease-modifying potential of a novel therapeutic, are still debated (see Loscher and Brandt[3] for review and discussion). For example, most of the studies that use kindled rats do not employ animals that display spontaneous seizures. This is important because it is highly unlikely that the neuropathological alterations at the synaptic and network levels are the same in the rat with an altered threshold as those in an animal that displays spontaneous seizures; as such, the therapies that might be found to be disease-modifying in one model would likely differ from those effective in the other. These and other issues surrounding the validity of the kindled rat as a true model of human epilepsy are nicely summarized in a 2010 review by Loscher and Brandt.[3]

A number of changes at the molecular and structural levels have been associated with kindling. Studies of hippocampal cellular pathology and electrophysiology during the kindling process suggest a role for mossy fiber sprouting involving an increased recurrent excitatory action on both dentate cell dendrites and GABAergic (gamma-aminobutyric acid) interneurons and alterations in the function of GABAergic synapses and glutamatergic synapses within the dentate gyrus and hilar regions.[70–72] The degree to which these changes are causative or represent an epiphenomena of the kindling process is not known, but they do provide some insight into the pathophysiology associated with kindling.

In the kindling model, an intervention study most often assesses the effect of an intervention on limbic or cortical ADD or quantifies the number of stimulations required to reach a particular Racine seizure score;[73,74] for example, does a particular intervention reduce the ADD or delay the time required to reach a grade 5 Racine seizure?

Choosing the appropriate timing for therapeutic intervention in the kindling model is problematic. Conventionally, drugs have been given 0.5 to 2 h prior to the kindling stimulus. When the drug under investigation possesses anticonvulsant activity, interpretation of the results from an intervention study can be complicated. Insult modification; that is, decreasing the duration of the ADD or decreasing the seizure severity through a drug's inherent anticonvulsant action can be easily confused with antiepileptogenesis. Thus, any study that sets out to modify or prevent kindling acquisition needs to be carefully considered and the results interpreted with this caveat in mind. Of the numerous studies that have employed the kindled rat as the test subject to evaluate the antiepileptogenic effect of a number of antiseizure drugs and *N*-methyl-D-aspartate (NMDA) antagonists, the only drugs found to reliably retard kindling acquisition using a treatment paradigm that was designed to separate the anticonvulsant effect from any potential disease-modifying effect of the treatment were levetiracetam,[75,76] phenobarbital,[77,78] and valproate.[77] As discussed by Loscher and Brandt,[3] the time- and labor-intensive nature of the kindled rat is not ideal for even medium- throughput screening of potential antiepileptogenic or disease-modifying treatments. To address this issue, Mazarati and colleagues[79–84] have been actively characterizing the rapid hippocampal kindling model originally described by Lothman et al.[85] as a viable alternative for drug screening. In contrast to the days to weeks that are required to fully kindle an adult rat using the traditional kindling paradigm, they have demonstrated that the kindling process can be contracted to several hours using the rapid hippocampal kindling protocol.[79–84] They have evaluated a number of traditional and investigational antiepileptic drugs (AEDs) using this model and demonstrated that it has potential utility as a high-throughput screening tool for the early evaluation of potential antiepileptogenic or disease-modifying therapies. Moreover, they have demonstrated that the effect of any given drug treatment can be age-dependent.[84] These effects suggest that there are likely to be age-dependent changes in the targets and that the window of treatment opportunity will be different, depending on the age of the patient at the time of a particular brain insult. As discussed above, the kindling model has some limitations, and any findings suggestive of a disease-modifying effect will certainly require confirmation in one or more models of epileptogenesis.

Status Epilepticus

Status epilepticus is a medical emergency that requires prompt recognition and aggressive

treatment. Status epilepticus is not a disease; it is a manifestation of an underlying central nervous system (CNS) insult or systemic pathology that affects CNS function. Status epilepticus results when there is a failure of those inherent factors that would normally function to stop seizures.

Although many people will survive an episode of SE with no, or only limited, untoward effects, SE is life-threatening and is often associated with long-term neurological sequelae that include an elevated risk for developing epilepsy and substantial cognitive decline (see ref. 86 for review and references). The high morbidity and mortality associated with convulsive SE underlie the importance of early recognition and aggressive treatment. Although controversial, nonconvulsive SE[87] is also associated with high morbidity and mortality.

The chemoconvulsant and electrical post-SE models of temporal lobe epilepsy (TLE) that have emerged over the last three decades (Table 80–1) involve prolonged seizure activity within the limbic system sufficient to induce some focal hippocampal damage that is followed, after a latent period of days, weeks, or months, by the appearance of spontaneous limbic seizures.[12,13,18,22,24,25,88–90] In this regard, post-SE models reproduce the clinical features of acquired epilepsy; such as a brain insult that is followed by a variable latent period before spontaneous seizures emerge. As in patients with epilepsy, the seizure frequency of a rodent with epilepsy can also be age-dependent and quite variable, and seizures are often clustered. Variability in seizure frequency and seizure clustering have the disadvantage that seizure monitoring and quantification are technically challenging and require dedicated commitment to rigorous video-electroencephalographic (EEG) monitoring (see below).

Not all models of SE are created equally; the extent of damage to hippocampal and extrahippocampal structures, as well as the length of the latent period, vary greatly among the various SE models. To this point, Sloviter and colleagues have described a model wherein prolonged hippocampal excitation in awake rats results in classic hippocampal sclerosis in the absence of convulsive SE.[22] In two separate models, these investigators were able to demonstrate that a single episode of clinically cryptic excitation without evidence of convulsive seizure activity produced classic hippocampal sclerosis that was followed by the emergence of spontaneous hippocampal-onset seizures. Their results suggest that epileptogenic insults may involve prolonged excitation that can go undetected at the time of the initial insult. This is not to say that models of convulsive SE lack value in therapy discovery, but the availability of an animal model that mimics human patterns of limited pathology with hippocampal-onset seizures that displays minimal variability and lethality and does not require exposure to chemoconvulsant drugs is a potentially important advance. For example, being able to tightly and reproducibly control the outcome in terms of both pathology and seizure origin should lead to a greater understanding of the relationship between neuronal loss, subclinical focal events, and those network processes that contribute to the expression of clinical seizures. As the authors suggest, such a model should help to elucidate the mechanisms that convert focal seizures into clinical seizures and facilitate the development of strategies that might prolong the latent period indefinitely (see ref. 22 for further discussion).

Traumatic Brain Injury

Traumatic brain injury (TBI) is a well-appreciated risk factor for epilepsy.[91,92] *Traumatic brain injury* refers to any biomechanically induced damage to the brain. The greater the initial severity, the higher the risk for developing epilepsy.[91] Traumatic brain injury is a common cause of acquired epilepsy; for example, it accounts for up to 20% of all symptomatic epilepsies and 6% of all epilepsies.[93] In the United States, TBI is also the most common cause of death and disability in persons under 45 years of age and is particularly prevalent in young adult men; the male:female ratio is 2:1 or 3:1 (see ref. 94 for review and references). Causes of TBI include motor vehicle accidents, falls, sports-related concussions, assaults, gunshot wounds, and military-related injuries. Depending on the severity of the insult, TBI can be associated with both early (within the first 7 days postinjury) and late posttraumatic seizures (those occurring after the first 7 days postinjury). The distinction is important because the pathophysiological substrate underlying early versus late seizures and

the onset of epilepsy is thought to be different and as such implies that there could be different therapeutic approaches, depending on the time frame in which an intervention therapy is initiated.[94]

From a pathophysiological perspective, early posttraumatic seizures (PTS) are associated with changes in ion (Na^+, K^+, and Ca^{2+}) flux, glutamate release, spreading depression-like state, secondary injury, metabolic dysfunction, oxidative stress/heme toxicity, inflammation, acute cell death, altered connectivity, excitation, and inhibition. In addition to altered excitatory and inhibitory neurotransmission, late PTS and posttraumatic epilepsy, have been associated with aberrant sprouting, changes in neurotrophin (e.g., brain-derived neurotrophic factor [BDNF]) expression, synaptic structural changes, delayed cell death, chronic inflammatory changes, and progressive disconnection.[94]

Thus, the availability of an animal model of TBI that recapitulates many of the features of trauma-induced human epilepsy could be used for the early identification of neuroprotective and/or disease-modifying therapies that can be quickly transferred to human clinical trials. As summarized in Table 80–1, a number of models of TBI have been described over the last four decades. Collectively, they have all provided a greater understanding of the pathophysiology of PTS and trauma-induced epilepsy.[94]

In recent years, the rostral parasagittal fluid percussion injury (rpFPI) model has been extensively characterized at the electrographic, phenotypic, histological, and pharmacological levels.[37–40,95,96] As early as 2 weeks after FPI delivered to the rostral parasagittal convexity, animals display subtle ictal behaviors that include motion arrest, posturing, and automatisms that are coincident with epileptiform electrographic discharges. Cross-correlograms obtained by analysis of the behavior of the animals while blinded to the electrocorticogram (ECoG) suggest that epileptiform ECoG events as short as ~1 s may satisfy the definition of clinical seizure.[39] However, seizures appearing in the early weeks after FPI present with a wide range of durations, most commonly lasting for 2–15 s but sometimes up to 6 min, and then progress in duration over time postinjury.[40,95] Similar progression is also observed in the frequency of the seizures, severity of the ictal behavior, and underlying pathology.[37,38] A remarkable feature of posttraumatic epilepsy (PTE) induced by rpFPI in the rat is that it presents with both neocortical and subcortical seizures, just as human PTE does.[97,98]

Although debated,[99,100] the results obtained in the rpFPI model support the authors' hypothesis that the focal electrographic and clinical events observed following rpFPI mimic human simple partial and complex partial seizures.[39,95] Furthermore, their results support the earlier hypothesis that chronically recurring spontaneous partial seizures with subtle manifestations occur in patients long before a diagnosis of epilepsy is made.[89,101] If this is true, early and aggressive use of emerging seizure detection technologies following TBI to explore the presence of subtle seizures seems warranted. Minimizing their occurrence and spread could, as suggested previously,[22] facilitate the development of strategies that might prolong the latent period indefinitely.

Viral Encephalitis

The precise mechanisms that underlie the development of epilepsy following an insult to the brain have yet to be clearly defined. Nevertheless, it is becoming increasingly apparent that inflammation plays an important role in some of the damage and reorganization observed following a variety of brain insults including hypoxia/ischemia, SE, TBI, and others.[102] A number of studies have demonstrated that inflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6 can contribute to both acute excitability of neurons and chronic molecular changes that can contribute to the development of epilepsy.[103] Inflammatory cytokines have been shown to increase blood-brain-barrier (BBB) permeability, decrease the seizure threshold, increase expression of the drug transporter protein P-glycoprotein (Pgp), and epileptogenesis.[104–106] Interleukin-1β can increase the permeability of the BBB to immunoglobulin G (IgG) and albumin, which can trigger an immune response.[104] Albumin, which is taken up by astrocytes, impairs astrocytic function and reduces the ability to clear extracellular glutamate. The ability of astrocytes to regulate extracellular ion homeostasis can also be impaired. Both of these effects can contribute to an acute lowering of the seizure threshold and an imbalance between excitation

and inhibition.[103] Moreover, overexpression of inflammatory cytokines can have long-term consequences for brain excitability by inducing structural and functional changes in glial and neuronal networks. For example, IL-1β can activate mitogen-activated protein kinases and (NF-κB)-dependent pathways.[102,103] The resultant transcription of genes can lead to the modification of ion channels and thus contribute to long-term hyperexcitability and epileptogenesis.

Therapies that target inflammatory processes could have a profound effect on both acute seizure susceptibility and the development of epilepsy following an acute insult to the brain.[102,103,107,108] Having an animal model that mimics much of the pathology of CNS inflammation would provide an important platform to study epileptogenesis resulting from inflammatory insults.

Viral infection of the CNS can lead to long-term neurological defects, including an increased risk for the development of epilepsy.[102,103] Common viral infections that have been associated with clinical seizures include human immunodeficiency virus (HIV), West Nile virus, measles, and human herpes simplex virus-6 (HHV6), a virus that infects nearly everyone by 3 years of age.[109,110] Human herpes simplex virus-6 has been found in astrocytes obtained from resected tissue from patients with medial temporal lobe epilepsy, a severe seizure disorder that is often resistant to treatment with existing therapies.[111,112] Furthermore, acute viral infections have been associated with SE[113] and the subsequent development of severe epilepsy in children,[114] including IS,[115] a particularly difficult-to-treat form of epilepsy. Thus, there is an emerging body of data that suggests that the limbic system is compromised in some forms of viral-induced encephalitis and that these patients are at increased risk for developing pharmacoresistent epilepsy.

Until recently, viral infections that lead to epilepsy have been difficult to address experimentally because, despite the fact that acute viral infection of rabbits, rats, and mice is associated with seizures,[116–119] infected animals either die from the acute viral encephalitis or, if they do not die, they do not go on to develop spontaneous recurrent seizures.

In contrast to herpes simplex virus encephalitis and Borna disease virus, mice inoculated with Daniels strain of Theiler's murine encephalomyelitis virus (TMEV) survive the initial infection and a high percentage ultimately develop recurrent seizure activity.[62] Theiler's murine encephalomyelitis virus is a positive-sense, single-stranded RNA picornavirus that naturally infects mice and can lead to chronic demyelinating disease in SJL mice[59,60,63] and acute behavioral seizures that resolve within a few days in C57BL/6 mice.[60] Mice that exhibit acute seizures following TMEV infection display substantially reduced thresholds to electrically evoked seizures months after the infection has cleared and after the acute seizures have resolved.[63] Of those mice that were observed to have acute symptomatic seizures postinoculation (p.i.) and were monitored continuously using video-EEG) monitoring for 1 week at 4 weeks p.i., 1 month at 16 weeks p.i., and 1 month again at 28 weeks p.i., 64%, 57%, and 40%, respectively, were observed to display spontaneous electrographic seizures that correlated with behavioral seizures lasting between 45 and 66 s[62] (Fig. 80–1). Moreover, 100% of these mice displayed abnormal brief epileptiform discharges that were associated with behavioral arrest.[62] Infected mice exhibiting acute seizures also exhibit profound temporal lobe sclerosis.[62]

Gene array experiments performed on tissue from TMEV-infected animals have shown dramatic increases in mRNA expression of TNF-α,[59] a cytokine recently linked to homeostatic synaptic scaling,[120–123] thus providing a putative direct link between a virus-initiated immune response and general neural circuit excitability.[59] Consistent with this observation, Fujinami and colleagues have shown that TNF-αR1 knockout mice infected with TMEV experienced fewer behavioral seizures than control C57BL/6 mice.[59] Collectively, these results suggest that TMEV infection in C57BL/6 mice represents a unique "hit-and-run" model of encephalitis-induced TLE that can be used to investigate the mechanisms underlying virus-induced acute symptomatic seizures, epileptogenesis, and epilepsy and serve as a novel model for therapy discovery.

Other Models of Acquired Epilepsy

As summarized in Table 80–1, a number of other models of acquired epilepsy have been proposed over the years. These include, but are not limited to, the tetanus toxin model of partial epilepsy, the neonatal hypoxia-ischemia

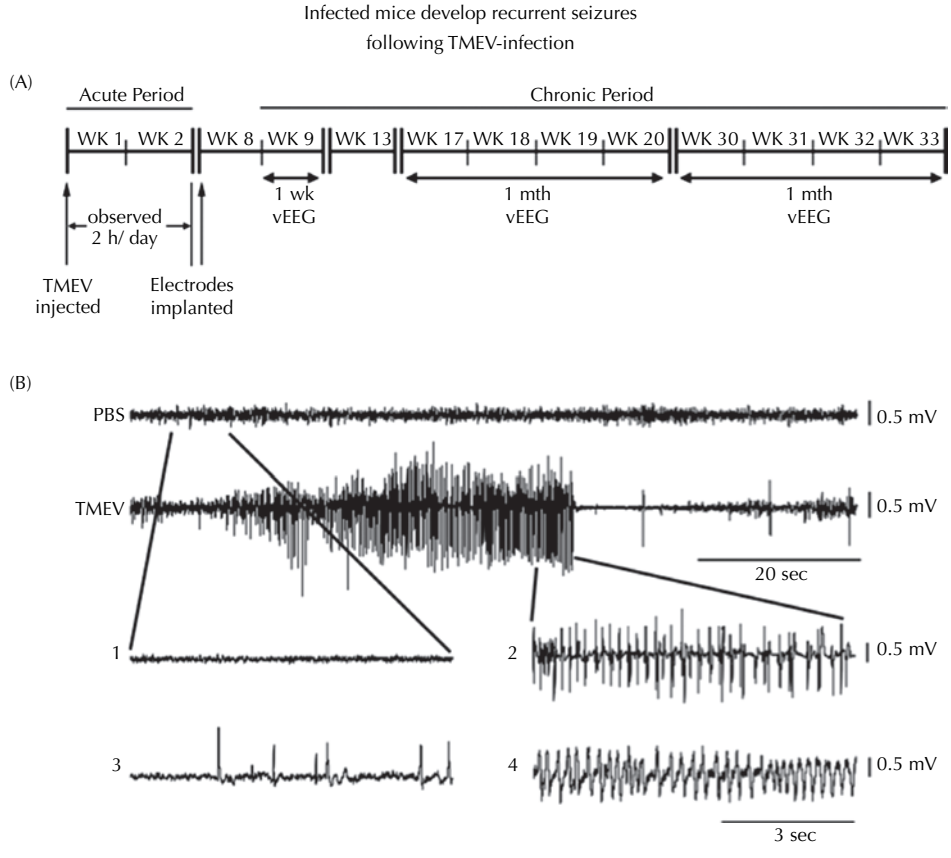

Figure 80–1. Chronology of procedures and representative seizures in epileptic TMEV-infected mice. **A.** Timeline of procedures. Animals were monitored with video-EEG at three different time points during the chronic period: 2 months p.i. for 1 week ($n = 14$), 4 months p.i. for 1 month ($n = 7$), and 7 months p.i. for 1 month ($n = 5$). **B.** Representative EEG traces showing baseline activity in a control (phosphate buffered saline [PBS]-injected] mouse (top trace) compared to high-frequency, high-amplitude, and rhythmic activity recorded during a Stage 5 seizure at 4 months p.i. Seizures were similar to those observed during the acute infection.[63] In the expanded traces (1–4), epileptiform activity associated with behavioral arrest (3) or no behavioral arrest (4) is present in TMEV mice but not in PBS mice (1). Activity observed during a Stage 5 seizure is expanded for comparison (2). From ref. 62.

model of hypoxic-ischemic injury, the neonatal hyperpyrexia model of febrile seizures, several models of IS, and models of cortical dysplasia. Each of these models display several phenotypic, histological, and electrophysiological similarities to their human counterpart and are likely to provide a framework for evaluating proposed antiepileptogenic and/or disease-modifying therapies.

Genetic Models: Unrealized Potential for Therapy Discovery

A number of genetic mouse models have been described in recent years that possess many of the features of human genetic epilepsy resulting from a specific defect in a particular receptor or voltage-gated ion channel. These have provided a wealth of information regarding the underpinnings of epileptogenesis and epilepsy at the molecular and genetic levels. For example, two excellent examples of animal models that have emerged from knowledge of human genetic mutations are the various mouse models that contain mutations in Nav1.1[124] and Kv7.2/7.3[125] genes. These mice recapitulate many of the phenotypic and pharmacological features of human severe myoclonic epilepsy of infancy (SMEI) and benign familial neonatal convulsions (BFNC), respectively. What is yet to be fully appreciated is the degree to which these and other emerging genetic mouse

models will aid in the identification and validation of novel targets for the treatment and prevention of epilepsy.

The Wistar Albino Glaxo Rat from Kijswijk (WAG/Rij), like the Genetic Absence Epileptic Rat from Strasbourg (GAERS), is an excellent model of human absence epilepsy.[126] In a novel experimental approach, Blumenfeld and colleagues demonstrated that early treatment with ethosuximide from postnatal day 21 through 5 months of age blocked changes in the expression of ion channels Nav1.1, Nav1.6, and (HCN1) normally associated with the onset and expression of spike-wave seizures in the WAG/Rij rat model of absence epilepsy.[127] Furthermore, they observed that early treatment was associated with prolonged suppression of seizures long after ethosuximide treatment was discontinued. This study does not show that ethosuximide prevented the development of epilepsy; however, it provides supportive information suggesting that early intervention with ethosuximide has the potential for disease modification and represents how innovative and strategic approaches can advance our efforts to identify novel therapies for the prevention of epilepsy.

CHALLENGES IN CHARACTERIZING THE ANTIEPILEPTOGENIC POTENTIAL OF AN INVESTIGATIONAL THERAPY

Conducting an intervention study with any one of the available models that display recurrent spontaneous seizures has its limitations.[128] Outcome measures include time to first seizure, seizure frequency, and severity of seizures at various time intervals postinsult and postintervention. As discussed above, there is substantial variability among individual animals in the latent period between their initial insult and the development of spontaneous seizures. Before declaring that a therapy is antiepileptogenic, the investigator is almost certainly obligated to conduct months of continuous video-EEG recording in order to ensure that a seizure was not missed due to a lapse in monitoring or due to the fact that seizures in animals, like those in humans, often cluster and could be easily missed by intermittent monitoring. Monitoring 24 h/day is clearly optimal, as intermittent sampling protocols are likely to introduce errors and underestimate the seizure frequency, particularly if seizures are clustered. Similarly, the optimal duration for monitoring may be 6 to12 months, but shorter periods are clearly more practical. It is important to note that it is always much easier to conclude that an animal has epilepsy than to conclude that an intervention prevented epileptogenesis. With that said, limited intermittent monitoring over the course of several weeks could serve to screen for potential antiepileptic drugs that could subsequently be submitted to more extensive and rigorous monitoring described above.

Another important issue that has to be considered when interpreting results from a positive intervention study is the possibility that the therapy itself decreased the insult-induced seizure severity and/or its duration.[128] This is not to imply that such a therapy would not be important; however, it becomes difficult under these conditions to separate the acute anticonvulsant effect of a therapy from its purported antiepileptogenic effects.

There have been a number of attempts to prevent the development of epilepsy in one or more of the available models (see ref. 3). Unfortunately, the results of these attempts have been somewhat disappointing and/or inconclusive given the caveats surrounding the study design and/or the seizure-monitoring protocol employed. Nonetheless, each of these studies has been extremely useful in setting the stage for further investigations and providing useful information regarding potential mechanisms underlying epileptogenesis for further hypothesis testing. The results obtained have been encouraging in that that they suggest that early treatment after an insult may offer some therapeutic benefit in terms of disease modification. These and other issues continue to be discussed, as the outcome of any intervention study is highly dependent on the question being asked and the extent to which a particular model mirrors the human condition being studied. Unfortunately, until the first antiepileptogenic or disease-modifying therapy is introduced, questions regarding the clinical validity of any animal model of epileptogenesis will remain.

The ability to prevent the development of epilepsy in the susceptible individual represents the holy grail of epilepsy research; however, preventing the cognitive decline and the

emergence of other debilitating comorbidities associated with epilepsy would represent a significant step forward for the patient with epilepsy. To this end, employing the currently available models to evaluate the cognition-sparing potential of a new therapy could provide the patient with an improved quality of life in the near term while the search for a cure continues.

CONCLUSIONS

The current approach to AED discovery is effective for identifying drugs that are useful for the symptomatic treatment of seizures. However, it would be naive to believe that therapies identified through this process would be useful for preventing or modifying the development of epilepsy in the susceptible person. A greater understanding of the pathophysiology of acquired epilepsy at the molecular and genetic levels will likely lead to the development of a new therapeutic approach that reaches beyond the symptomatic treatment of epilepsy to modify the progression or, dare we suggest, prevent the development of epilepsy in the susceptible patient. The realization of such a possibility will necessitate a change in our current AED discovery approach. Significant progress in our understanding of the factors that contribute to human epileptogenesis has been made in recent years. These advances have been made possible largely by the development and characterization of genetic-, insult-, and age-specific animal models of epileptogenesis and secondary neuronal hyperexcitability. The true validation of a given model of epileptogenesis requires the development of an effective therapy that prevents or delays the development of epilepsy or secondary hyperexcitability in the human condition and has activity that was predicted by preclinical testing. As we gain additional insight into the molecular biology and genetics of the acquired and genetic epilepsies, the day may come when new therapies that target the epileptogenic process will be developed.

DISCLOSURE STATEMENT

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REFERENCES

1. Mantegazza M, Rusconi R, Scalmani P, Avanzini G, Franceschetti S. Epileptogenic ion channel mutations: from bedside to bench and, hopefully, back again. *Epilepsy Res.* 2010;92:1–29.
2. Noebels JL, Treiman, D.M., Engel, J. Genetic models of epilepsy. In: Engel J, Pedley TA, Aicaridi J, Dichter MA, Moshe S, eds. *Epilepsy: A Comprehensive Textbook*. Philadephia: Lippincott Williams & Wilkins; 2008:445–455.
3. Loscher W, Brandt C. Prevention or modification of epileptogenesis after brain insults: experimental approaches and translational research. *Pharmacol Rev.* 2010;62:668–700.
4. Stables JP, Bertram EH, White HS, Coulter DA, Dichter MA, Jacobs MP, Loscher W, Lowenstein DH, Moshe SL, Noebels JL, Davis M. Models for epilepsy and epileptogenesis: report from the NIH workshop, Bethesda, Maryland. *Epilepsia*. 2002;43:1410–1420.
5. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol.* 1969;25:295–330.
6. Matagne A, Klitgaard H. Validation of corneally kindled mice: a sensitive screening model for partial epilepsy in man. *Epilepsy Res*. 1998;31:59–71.
7. Pitkanen A, Sutula TP. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. *Lancet Neurol*. 2002;1:173–181.
8. Stafstrom CE, Sutula TP. Models of epilepsy in the developing and adult brain: implications for neuroprotection. *Epilepsy Behav*. 2005;7(suppl 3):S18–S24.
9. Sutula T, Harrison C, Steward O. Chronic epileptogenesis induced by kindling of the entorhinal cortex: the role of the dentate gyrus. *Brain Res*. 1986;385: 291–299.
10. Sutula T, He XX, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science*. 1988;239:1147–1150.
11. Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience.* 1985;14: 375–403.
12. Ben-Ari Y, Lagowska J, Tremblay E, Le Gal La Salle G. A new model of focal status epilepticus: intra-amygdaloid application of kainic acid elicits repetitive secondarily generalized convulsive seizures. *Brain Res.* 1979;163:176–179.
13. Ben-Ari Y, Tremblay E, Ottersen OP. Injections of kainic acid into the amygdaloid complex of the rat: an electrographic, clinical and histological study in

relation to the pathology of epilepsy. *Neuroscience*. 1980;5:515–528.
14. Brandt C, Glien M, Potschka H, Volk H, Loscher W. Epileptogenesis and neuropathology after different types of status epilepticus induced by prolonged electrical stimulation of the basolateral amygdala in rats. *Epilepsy Res*. 2003;55:83–103.
15. Bumanglag AV, Sloviter RS. Minimal latency to hippocampal epileptogenesis and clinical epilepsy after perforant pathway stimulation-induced status epilepticus in awake rats. *J Comp Neurol.* 2008; 510:561–580.
16. Cavalheiro EA, Silva DF, Turski WA, Calderazzo-Filho LS, Bortolotto ZA, Turski L. The susceptibility of rats to pilocarpine-induced seizures is age-dependent. *Brain Res*. 1987;465:43–58.
17. Grabenstatter HL, Ferraro DJ, Williams PA, Chapman PL, Dudek FE. Use of chronic epilepsy models in antiepileptic drug discovery: the effect of topiramate on spontaneous motor seizures in rats with kainate-induced epilepsy. *Epilepsia.* 2005;46:8–14.
18. Hellier JL, Patrylo PR, Buckmaster PS, Dudek FE. Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: assessment of a rat model of temporal lobe epilepsy. *Epilepsy Res*. 1998;31:73–84.
19. Hoffmann AF, Zhao Q, Holmes GL. Cognitive impairment following status epilepticus and recurrent seizures during early development: support for the "two-hit hypothesis." *Epilepsy Behav*. 2004;5:873–877.
20. Holmes GL. The long-term effects of seizures on the developing brain: clinical and laboratory issues. *Brain Dev.* 1991;13:393–409.
21. Liu Z, Yang Y, Silveira DC, Sarkisian MR, Tandon P, Huang LT, Stafstrom CE, Holmes GL. Consequences of recurrent seizures during early brain development. *Neuroscience.* 1999;92:1443–1454.
22. Norwood BA, Bumanglag AV, Osculati F, Sbarbati A, Marzola P, Nicolato E, Fabene PF, Sloviter RS. Classic hippocampal sclerosis and hippocampal-onset epilepsy produced by a single "cryptic" episode of focal hippocampal excitation in awake rats. *J Comp Neurol*. 2010;518:3381–3407.
23. Stafstrom CE, Thompson JL, Holmes GL. Kainic acid seizures in the developing brain: status epilepticus and spontaneous recurrent seizures. *Brain Res Dev Brain Res*. 1992;65:227–236.
24. Turski L, Cavalheiro EA, Czuczwar SJ, Turski WA, Kleinrok Z. The seizures induced by pilocarpine: behavioral, electroencephalographic and neuropathological studies in rodents. *Pol J Pharmacol Pharm*. 1987;39:545–555.
25. Turski L, Ikonomidou C, Turski WA, Bortolotto ZA, Cavalheiro EA. Review: cholinergic mechanisms and epileptogenesis. The seizures induced by pilocarpine: a novel experimental model of intractable epilepsy. *Synapse.* 1989;3:154–171.
26. Williams PA, White AM, Clark S, Ferraro DJ, Swiercz W, Staley KJ, Dudek FE. Development of spontaneous recurrent seizures after kainate-induced status epilepticus. *J Neurosci*. 2009;29:2103–2112.
27. Jefferys JG. Mechanism of tetanus toxin in neuronal cell death. *Trends Pharmacol Sci*. 1992;13:13–14.
28. Jefferys JG. Chronic epileptic foci induced by intracranial tetanus toxin. *Epilepsy Res Suppl*. 1996;12: 111–117.
29. Jiang M, Lee CL, Smith KL, Swann JW. Spine loss and other persistent alterations of hippocampal pyramidal cell dendrites in a model of early-onset epilepsy. *J Neurosci*. 1998;18:8356–8368.
30. Avramescu S, Timofeev I. Synaptic strength modulation after cortical trauma: a role in epileptogenesis. *J Neurosci*. 2008;28:6760–6772.
31. Bush PC, Prince DA, Miller KD. Increased pyramidal excitability and NMDA conductance can explain posttraumatic epileptogenesis without disinhibition: a model. *J Neurophysiol*. 1999;82:1748–1758.
32. Nita DA, Cisse Y, Timofeev I, Steriade M. Increased propensity to seizures after chronic cortical deafferentation in vivo. *J Neurophysiol*. 2006;95:902–913.
33. Prince DA, Jacobs K. Inhibitory function in two models of chronic epileptogenesis. *Epilepsy Res*. 1998;32: 83–92.
34. Prince DA, Tseng GF. Epileptogenesis in chronically injured cortex: in vitro studies. *J Neurophysiol*. 1993;69:1276–1291.
35. Sharpless SK, Halpern LM. The electrical excitability of chronically isolated cortex studied by means of permanently implanted electrodes. *Electroencephalogr Clin Neurophysiol.* 1962;14:244–255.
36. Willmore LJ, Sypert GW, Munson JV, Hurd RW. Chronic focal epileptiform discharges induced by injection of iron into rat and cat cortex. *Science.* 1978;200:1501–1503.
37. D'Ambrosio R, Fairbanks JP, Fender JS, Born DE, Doyle DL, Miller JW. Post-traumatic epilepsy following fluid percussion injury in the rat. *Brain.* 2004;127: 304–314.
38. D'Ambrosio R, Fender JS, Fairbanks JP, Simon EA, Born DE, Doyle DL, Miller JW. Progression from frontal-parietal to mesial-temporal epilepsy after fluid percussion injury in the rat. *Brain*, 2005; 128:174–188.
39. D'Ambrosio R, Hakimian S, Stewart T, Verley DR, Fender JS, Eastman CL, Sheerin AH, Gupta P, Diaz-Arrastia R, Ojemann J, Miller JW. Functional definition of seizure provides new insight into post-traumatic epileptogenesis. *Brain.* 2009;132:2805–2821.
40. Eastman CL, Verley DR, Fender JS, Stewart TH, Nov E, Curia G, D'Ambrosio R. Antiepileptic and antiepileptogenic performance of carisbamate after head injury in the rat: blind and randomized studies. *J Pharmacol Exp Ther*. 2011;336:779–790.
41. Keck CA, Thompson HJ, Pitkanen A, LeBold DG, Morales DM, Plevy JB, Puri R, Zhao B, Dichter M, McIntosh TK. The novel antiepileptic agent RWJ-333369-A, but not its analog RWJ-333369, reduces regional cerebral edema without affecting neurobehavioral outcome or cell death following experimental traumatic brain injury. *Restor Neurol Neurosci*. 2007;25:77–90.
42. Pitkanen A, Immonen RJ, Grohn OH, Kharatishvili I. From traumatic brain injury to posttraumatic epilepsy: what animal models tell us about the process and treatment options. *Epilepsia*. 2009;50(suppl 2):21–29.
43. Pitkanen A, McIntosh TK. Animal models of post-traumatic epilepsy. *J Neurotrauma.* 2006;23:241–261.
44. Thompson HJ, Hoover RC, Tkacs NC, Saatman KE, McIntosh TK. Development of posttraumatic hyperthermia after traumatic brain injury in rats is associated with increased periventricular inflammation. *J Cereb Blood Flow Metab*. 2005;25:163–176.

45. Jensen FE. An animal model of hypoxia-induced perinatal seizures. *Ital J Neurol Sci.* 1995;16:59–68.
46. Jensen FE, Wang C, Stafstrom CE, Liu Z, Geary C, Stevens MC. Acute and chronic increases in excitability in rat hippocampal slices after perinatal hypoxia in vivo. *J Neurophysiol*. 1998;79:73–81.
47. Kadam SD, Dudek FE. Neuropathogical features of a rat model for perinatal hypoxic-ischemic encephalopathy with associated epilepsy. *J Comp Neurol.* 2007;505:716–737.
48. Kadam SD, White AM, Staley KJ, Dudek FE. Continuous electroencephalographic monitoring with radio-telemetry in a rat model of perinatal hypoxia-ischemia reveals progressive post-stroke epilepsy. *J Neurosci.* 2010;30:404–415.
49. Sanchez RM, Dai W, Levada RE, Lippman JJ, Jensen FE. AMPA/kainate receptor-mediated downregulation of GABAergic synaptic transmission by calcineurin after seizures in the developing rat brain. *J Neurosci*. 2005;25:3442–3451.
50. Williams PA, Dou P, Dudek FE. Epilepsy and synaptic reorganization in a perinatal rat model of hypoxia-ischemia. *Epilepsia.* 2004;45:1210–1218.
51. Williams PA, Dudek FE. A chronic histopathological and electrophysiological analysis of a rodent hypoxic-ischemic brain injury model and its use as a model of epilepsy. *Neuroscience*. 2007;149:943–961.
52. Baram TZ, Eghbal-Ahmadi M, Bender RA. Is neuronal death required for seizure-induced epileptogenesis in the immature brain? *Prog Brain Res.* 2002;135: 365–375.
53. Bender RA, Dube C, Baram TZ. Febrile seizures and mechanisms of epileptogenesis: insights from an animal model. *Adv Exp Med Biol.* 2004;548: 213–225.
54. Bender RA, Kirschstein T, Kretz O, Brewster AL, Richichi C, Ruschenschmidt C, Shigemoto R, Beck H, Frotscher M, Baram TZ. Localization of HCN1 channels to presynaptic compartments: novel plasticity that may contribute to hippocampal maturation. *J Neurosci*. 2007;27:4697–4706.
55. Avanzini GG, Treiman, D.M., Engel, J. Animal models of acquired epilepsies and status epilepticus. In: Engel J, Pedley TA, Aicaridi J, Dichter MA, Moshe S, eds. *Epilepsy: A Comprehensive Textbook*. Philadelphia: Lippincott, Williams & Wilkins; 2008:415–444.
56. Castro PA, Pleasure SJ, Baraban SC. Hippocampal heterotopia with molecular and electrophysiological properties of neocortical neurons. *Neuroscience*. 2002;114:961–972.
57. Chudomelova L, Scantlebury MH, Raffo E, Coppola A, Betancourth D, Galanopoulou AS. Modeling new therapies for infantile spasms. *Epilepsia*. 2010;51(suppl 3): 27–33.
58. Stafstrom CE. Infantile spasms: a critical review of emerging animal models. *Epilepsy Curr*. 2009;9: 75–81.
59. Kirkman NJ, Libbey JE, Wilcox KS, White HS, Fujinami RS. Innate but not adaptive immune responses contribute to behavioral seizures following viral infection. *Epilepsia.* 2010;51:454–464.
60. Libbey JE, Kirkman NJ, Smith MC, Tanaka T, Wilcox KS, White HS, Fujinami RS. Seizures following picornavirus infection. *Epilepsia*. 2008;49:1066–1074.
61. Libbey JE, Kirkman NJ, Wilcox KS, White HS, Fujinami RS. Role for complement in the development of seizures following acute viral infection. *J Virol*. 2010;84:6452–6460.
62. Stewart KA, Wilcox KS, Fujinami RS, White HS. Development of postinfection epilepsy after Theiler's virus infection of C57BL/6 mice. *J Neuropathol Exp Neurol*. 2010;69:1210–1219.
63. Stewart KA, Wilcox KS, Fujinami RS, White HS. Theiler's virus infection chronically alters seizure susceptibility. *Epilepsia*. 2010;51:1418–1428.
64. Sarkisian MR. Animal models for human seizure and epileptic activity. Reply. *Epilepsy Behav*. 2001;2: 506–507.
65. White HS. Epilepsy and disease modification: animal models for novel drug discovery. In: Rho JM, Stafstrom CE, eds. *Epilepsy: Mechanisms, Models, and Translational Perspectives.* Philadelphia: CRC Press; 2010:143–158.
66. Coulter DA, McIntyre DC, Loscher W. Animal models of limbic epilepsies: what can they tell us? *Brain Pathol.* 2002;12:240–256.
67. McIntyre DC, Poulter MO, Gilby K. Kindling: some old and some new. *Epilepsy Res*. 2002;50:79–92.
68. Smith M, Wilcox KS, White HS. Discovery of antiepileptic drugs. *Neurotherapeutics*. 2007;4:12–17.
69. Bialer M, White HS. Key factors in the discovery and development of new antiepileptic drugs. *Nat Rev Drug Discov*. 2010;9:68–82.
70. Lynch M, Sutula T. Recurrent excitatory connectivity in the dentate gyrus of kindled and kainic acid-treated rats. *J Neurophysiol.* 2000;83:693–704.
71. Dudek FE, Sutula TP. Epileptogenesis in the dentate gyrus: a critical perspective. *Prog Brain Res.* 2007;163: 755–773.
72. Sutula TP, Dudek FE. Unmasking recurrent excitation generated by mossy fiber sprouting in the epileptic dentate gyrus: an emergent property of a complex system. *Prog Brain Res*. 2007;163:541–563.
73. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol*. 1972;32:281–294.
74. Racine RJ. Modification of seizure activity by electrical stimulation. I. After-discharge threshold. *Electroencephalogr Clin Neurophysiol*. 1972;32: 269–279.
75. Loscher W, Honack D, Rundfeldt C. Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. *J Pharmacol Exp Ther.* 1998;284:474–479.
76. Stratton SC, Large CH, Cox B, Davies G, Hagan RM. Effects of lamotrigine and levetiracetam on seizure development in a rat amygdala kindling model. *Epilepsy Res.* 2003;53:95–106.
77. Silver JM, Shin C, McNamara JO. Antiepileptogenic effects of conventional anticonvulsants in the kindling model of epilespy. *Ann Neurol*. 1991;29:356–363.
78. Turner IM, Newman SM, Louis S, Kutt H. Pharmacological prophylaxis against the development of kindled amygdaloid seizures. *Ann Neurol*. 1977;2: 221–224.
79. Mazarati A, Lundstrom L, Sollenberg U, Shin D, Langel U, Sankar R. Regulation of kindling epileptogenesis by hippocampal galanin type 1 and type 2 receptors: The effects of subtype-selective agonists and the role of G-protein-mediated signaling. *J Pharmacol Exp Ther*. 2006;318:700–708.
80. Mazarati A, Shin D, Auvin S, Caplan R, Sankar R. Kindling epileptogenesis in immature rats leads

to persistent depressive behavior. *Epilepsy Behav.* 2007;10:377–383.
81. Mazarati A, Shin D, Auvin S, Sankar R. Age-dependent effects of topiramate on the acquisition and the retention of rapid kindling. *Epilepsia*. 2007;48:765–773.
82. Mazarati A, Shin D, Sankar R. Bumetanide inhibits rapid kindling in neonatal rats. *Epilepsia*. 2009;50: 2117–2122.
83. Mazarati A, Wu J, Shin D, Kwon YS, Sankar R. Antiepileptogenic and antiictogenic effects of retigabine under conditions of rapid kindling: an ontogenic study. *Epilepsia*. 2008;49:1777–1786.
84. Sankar R, Auvin S, Kwon YS, Pineda E, Shin D, Mazarati A. Evaluation of development-specific targets for antiepileptogenic therapy using rapid kindling. *Epilepsia*. 2010;51(suppl 3):39–42.
85. Lothman EW, Hatlelid JM, Zorumski CF, Conry JA, Moon PF, Perlin JB. Kindling with rapidly recurring hippocampal seizures. *Brain Res.* 1985;360:83–91.
86. Goodkin HR. Status epilepticus. In: Wyllie E, Gidal BE, Goodkin, HP, eds. *Wyllie's Treatment of Epilepsy*. 5th ed. Philadelphia: Lippincott, Williams & Wilkins; 2010:469–485.
87. Krumholz A. Epidemiology and evidence for morbidity of nonconvulsive status epilepticus. *J Clin Neurophysiol*. 1999;16:314–322; discussion 353.
88. Dudek FE, Hellier JL, Williams PA, Ferraro DJ, Staley KJ. The course of cellular alterations associated with the development of spontaneous seizures after status epilepticus. *Prog Brain Res.* 2002;135:53–65.
89. Sloviter RS. Hippocampal epileptogenesis in animal models of mesial temporal lobe epilepsy with hippocampal sclerosis: the importance of the "latent period" and other concepts. *Epilepsia*. 2008;49(suppl 9):85–92.
90. Sloviter RS, Zappone CA, Bumanglag AV, Norwood BA, Kudrimoti H. On the relevance of prolonged convulsive status epilepticus in animals to the etiology and neurobiology of human temporal lobe epilepsy. *Epilepsia*. 2007;48(suppl 8):6–10.
91. Annegers JF, Hauser WA, Coan SP, Rocca WA. A population-based study of seizures after traumatic brain injuries. *N Engl J Med*. 1998;338:20–24.
92. Lowenstein DH. Epilepsy after head injury: an overview. *Epilepsia.* 2009;50(suppl 2):4–9.
93. Hauser WA, Annegers JF, Kurland LT. Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935–1984. *Epilepsia.* 1993;34:453–468.
94. Giza CC. Posttraumatic seizures and epileptogenesis: good and bad plasticity. In: Rho JM, Stafstrom CE, eds. *Epilepsy: Mechanisms, Models, and Translational Perspectives*. Boca Raton, FL: CRC Press; 2010: 181–208.
95. Curia G, Levitt M, Fender JS, Miller JW, Ojemann J, D'Ambrosio R. Impact of injury location and severity on posttraumatic epilepsy in the rat: role of frontal neocortex. *Cereb Cortex*. 2010:21:1574–1592.
96. Eastman CL, Verley DR, Fender JS, Temkin NR, D'Ambrosio R. ECoG studies of valproate, carbamazepine and halothane in frontal-lobe epilepsy induced by head injury in the rat. *Exp Neurol.* 2010;224: 369–388.
97. Diaz-Arrastia R, Agostini MA, Madden CJ, Van Ness PC. Posttraumatic epilepsy: the endophenotypes of a human model of epileptogenesis. *Epilepsia*. 2009; 50(suppl 2):14–20.
98. Hudak AM, Trivedi K, Harper CR, Booker K, Caesar RR, Agostini M, Van Ness PC, Diaz-Arrastia R. Evaluation of seizure-like episodes in survivors of moderate and severe traumatic brain injury. *J Head Trauma Rehabil*. 2004;19:290–295.
99. D'Ambrosio R, Miller JW. Point. *Epilepsy Curr.* 2010;10(4):90.
100. Dudek FE, Bertram EH. Counterpoint to "what is an epileptic seizure?" by d'Ambrosio and Miller. *Epilepsy Curr*. 2010;10:91–94.
101. Dichter MA. Posttraumatic epilepsy: the challenge of translating discoveries in the laboratory to pathways to a cure. *Epilepsia*. 2009;50(suppl 2):41–45.
102. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol.* 2011;7: 31–40.
103. Vezzani A, Balosso S, Ravizza T. The role of cytokines in the pathophysiology of epilepsy. *Brain Behav Immun.* 2008;22:797–803.
104. Marcon J, Gagliardi B, Balosso S, Maroso M, Noe F, Morin M, Lerner-Natoli M, Vezzani A, Ravizza T. Age-dependent vascular changes induced by status epilepticus in rat forebrain: implications for epileptogenesis. *Neurobiol Dis.* 2009;34:121–132.
105. Ravizza T, Gagliardi B, Noe F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. *Neurobiol Dis*. 2008;29:142–160.
106. Zibell G, Unkruer B, Pekcec A, Hartz AM, Bauer B, Miller DS, Potschka H. Prevention of seizure-induced up-regulation of endothelial P-glycoprotein by COX-2 inhibition. *Neuropharmacology*. 2009;56:849–855.
107. Fabene PF, Navarro Mora G, Martinello M, Rossi B, Merigo F, Ottoboni L, Bach S, Angiari S, Benati D, Chakir A, Zanetti L, Schio F, Osculati A, Marzola P, Nicolato E, Homeister JW, Xia L, Lowe JB, McEver RP, Osculati F, Sbarbati A, Butcher EC, Constantin G. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. *Nat Med.* 2008;14:1377–1383.
108. Pitkanen A, Lukasiuk K. Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. *Epilepsy Behav.* 2009;14(suppl 1):16–25.
109. Balachandra K, Ayuthaya PI, Auwanit W, Jayavasu C, Okuno T, Yamanishi K, Takahashi M. Prevalence of antibody to human herpesvirus 6 in women and children. *Microbiol Immunol.* 1989;33:515–518.
110. Okuno T, Takahashi K, Balachandra K, Shiraki K, Yamanishi K, Takahashi M, Baba K. Seroepidemiology of human herpesvirus 6 infection in normal children and adults. *J Clin Microbiol.* 1989;27:651–653.
111. Donati D, Akhyani N, Fogdell-Hahn A, Cermelli C, Cassiani-Ingoni R, Vortmeyer A, Heiss JD, Cogen P, Gaillard WD, Sato S, Theodore WH, Jacobson S. Detection of human herpesvirus-6 in mesial temporal lobe epilepsy surgical brain resections. *Neurology.* 2003;61:1405–1411.
112. Fotheringham J, Donati D, Akhyani N, Fogdell-Hahn A, Vortmeyer A, Heiss JD, Williams E, Weinstein S, Bruce DA, Gaillard WD, Sato S, Theodore WH, Jacobson S. Association of human herpesvirus-6B with mesial temporal lobe epilepsy. *PLoS Med.* 2007;4:e180.
113. Singh RK, Stephens S, Berl MM, Chang T, Brown K, Vezina LG, Gaillard WD. Prospective study of new-onset seizures presenting as status epilepticus in childhood. *Neurology*. 2010;74:636–642.

114. Juntunen A, Herrgard E, Mannonen L, Korppi M, Linnavuori K, Vaheri A, Koskiniemi M. A major role of viruses in convulsive status epilepticus in children: a prospective study of 22 children. *Eur J Pediatr*. 2001;160:37–42.
115. Guggenheim MA, Frost JD Jr, Hrachovy RA. Time interval from a brain insult to the onset of infantile spasms. *Pediatr Neurol*. 2008;38:34–37.
116. Beers DR, Henkel JS, Schaefer DC, Rose JW, Stroop WG. Neuropathology of herpes simplex virus encephalitis in a rat seizure model. *J Neuropathol Exp Neurol*. 1993;52:241–252.
117. Griffith JF, Kibrick S, Dodge PR, Richardson EP. Experimental herpes simplex encephalitis. Electroencephalographic,clinical,virologic,andpathologic observations in the rabbit. *Electroencephalogr Clin Neurophysiol.* 1967;23:263–269.
118. Lehrmann E, Guidetti P, Love A, Williamson J, Bertram EH, Schwarcz R. Glial activation precedes seizures and hippocampal neurodegeneration in measles virus-infected mice. *Epilepsia*. 2008;49(suppl 2): 13–23.
119. Wu HM, Huang CC, Chen SH, Liang YC, Tsai JJ, Hsieh CL, Hsu KS. Herpes simplex virus type 1 inoculation enhances hippocampal excitability and seizure susceptibility in mice. *Eur J Neurosci*. 2003;18: 3294–3304.
120. Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC. Control of synaptic strength by glial TNFalpha. *Science*. 2002;295:2282–2285.
121. Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. *J Neurosci*. 2005;25:3219–3228.
122. Stellwagen D, Malenka RC. Synaptic scaling mediated by glial TNF-alpha. *Nature*. 2006;440:1054–1059.
123. Turrigiano GG. More than a sidekick: glia and homeostatic synaptic plasticity. *Trends Mol Med*. 2006;12: 458–460.
124. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci*. 2006;9: 1142–1149.
125. Singh NA, Otto JF, Dahle EJ, Pappas C, Leslie JD, Vilaythong A, Noebels JL, White HS, Wilcox KS, Leppert MF. Mouse models of human KCNQ2 and KCNQ3 mutations for benign familial neonatal convulsions show seizures and neuronal plasticity without synaptic reorganization. *J Physiol.* 2008;586: 3405–3423.
126. Coenen AM, Van Luijtelaar EL. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. *Behav Genet.* 2003;33:635–655.
127. Blumenfeld H, Klein JP, Schridde U, Vestal M, Rice T, Khera DS, Bashyal C, Giblin K, Paul-Laughinghouse C, Wang F, Phadke A, Mission J, Agarwal RK, Englot DJ, Motelow J, Nersesyan H, Waxman SG, Levin AR. Early treatment suppresses the development of spike-wave epilepsy in a rat model. *Epilepsia*. 2008;49:400–409.
128. Dudek FE. Commentary: a skeptical view of experimental gene therapy to block epileptogenesis. *Neurotherapeutics*. 2009;6:319–322.

Chapter 81

Strategies for Antiepileptogenesis

Antiepileptic Drugs versus Novel Approaches Evaluated in Post–Status Epilepticus Models of Temporal Lobe Epilepsy

Wolfgang Löscher

Epileptogenesis, that is, the process leading to epilepsy, is a common sequel of brain insults such as head trauma, cerebrovascular disease, brain tumors, neurosurgical procedures, neurodegenerative conditions, status epilepticus (SE), and complex febrile seizures.[1–3] Following such brain insults, there is a cascade of morphological and functional changes in the injured area over months to years before the occurrence of spontaneous recurrent seizures, that is, the hallmark of epilepsy (Fig. 81–1). This latent ("silent") period may offer a therapeutic window for the prevention of epileptogenesis and the subsequent development of unprovoked seizures and epilepsy.[2]

MOST ANTIEPILEPTIC DRUGS ARE NOT ANTIEPILEPTOGENIC

Based on the concept of a window of opportunity in which an appropriate treatment will stop the epileptogenic process induced by a brain insult, several clinical trials have been carried out to evaluate whether prolonged prophylactic administration of an antiepileptic (anticonvulsant, anti-ictal, anti-seizure) drug (AED) prevents the development of epilepsy after head trauma. However, in such clinical trials, administration of conventional AEDs such as phenytoin, phenobarbital, carbamazepine, or valproate following acute brain insults has thus far failed to prevent epileptogenesis.[4–6]

A similar lack of antiepileptogenic effects of clinically established AEDs has been found in most experimental studies, using post-SE rat models of temporal lobe epilepsy (TLE; Table 81–1). Post-SE models of TLE, in which SE is induced by sustained electrical stimulation of hippocampus or amygdala or by systemic administration of convulsants such as pilocarpine or kainate, display most of the major features of mesial TLE, that is, (1) neuropathological changes reminiscent of mesiotemporal sclerosis, (2) recurrent spontaneous seizures,

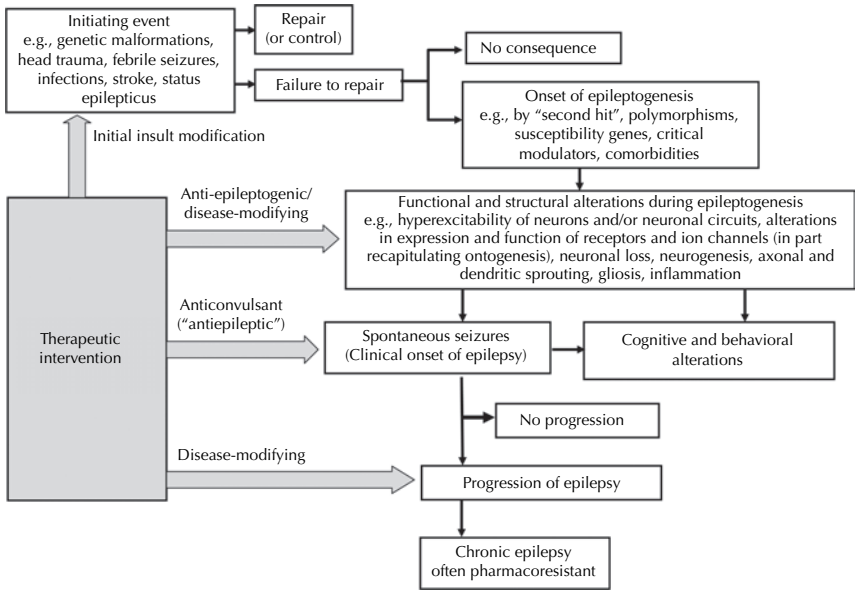

Figure 81–1. Steps in the development and progression of temporal lobe epilepsy and possible therapeutic interventions. The term *epileptogenesis* includes processes that take place before the first spontaneous seizure occurs to render the brain susceptible to spontaneous recurrent seizures and processes that intensify seizures and make them more refractory to therapy (progression). The concept illustrated in the figure is based on both experimental and clinical data. Adapted from Löscher[47] and Löscher et al.[48]

and (3) behavioral and cognitive changes associated with epilepsy.[7] Typically, depending on the severity and duration of SE, the spontaneous seizures first occur after a latent period following the SE of about 1–4 weeks, that is, the period of epileptogenesis (Figs. 81–1 and 81–2). In Table 81–1, only studies in which drugs were administered *after* onset of SE are shown. In various other studies, not included in this review, drugs were given *prior* to induction of SE, which may attenuate the severity or shorten the duration of SE and thereby reduce the long-term consequences of the brain insult. However, only a drug capable of preventing epilepsy *after* an initial insult such as SE would be clinically relevant.[8] A schematic illustration of drug testing in post-SE models of TLE, as used in our group, is shown in Fig. 81–2.

Antiepileptogenesis versus Disease Modification versus Initial Insult Modification

With respect to the lack of any antiepileptogenic or disease-modifying effect of most AEDs in most studies in post-SE models of TLE (Table 81–1), there are at least two critical issues. First, is it at all possible to prevent or modify epileptogenesis by a pharmacological treatment after SE? And, second, is there a critical time window in which to achieve such an effect after SE? When reviewing the literature in this respect, it is important to differentiate between drug effects resulting from *initial insult modification* and effects representing *true* antiepileptogenic or neuroprotective drug efficacy.[8,9] Initial insult modification means that the long-term consequences of the insult can be diminished by reducing the severity or duration of the initial brain insult, such as SE. This has, for instance, been demonstrated by reducing the duration of SE by phenobarbital, the *N*-methyl-D-aspartate (NMDA) antagonist MK-801 (dizocilpine), pregabalin, or diazepam in SE models in rats,[10–12] thus substantiating that early termination of SE is a powerful means for preventing or limiting its consequences.[13] In post-SE models of TLE with electrical SE induction, a SE duration of at least 3 h is needed to induce epileptogenesis in the majority of rats, so that any reduction of this duration by AEDs will result in a modification of

Table 81–1 **Prophylactic Effects of Treatment with Clinically Used Antiepileptic Drugs on the Long-Term Consequences of SE in Rats**

Drug	Model (Induction of SE)	Consequences of Drug Treatment				Reference
		Incidence of SRS	Frequency, Severity, or Duration of SRS	Neurodegeneration	Impairment of Learning and Memory	
Carbamazepine	Kainate	No effect	↓	↓ (hippocampus)	N.D.	Capella and Lemos[49]
Carbamazepine	Pilocarpine (in hippocampus)	N.D.	N.D.	↓ (CA1, CA3, hilus)	↓	Cunha et al.[41]
Carisbamate	Lithium-pilocarpine	N.D.	↓	↓ (CA1, PC, EC, amygdala, thalamus)	N.D.	Francois et al.[50] (A)
Diazepam*	Amygdala stimulation	↓	↓	↓ (hippocampus)	N.D.	Pitkänen et al.[12*]
Diazepam	Pilocarpine (in hippocampus)	N.D.	N.D.	↓ (CA1, CA3, hilus)	↓	Cunha et al.[41]
Fluorofelbamate*	Perforanth path stimulation	No effect	↓	N.D.	N.D.	Mazarati et al.[51*]
Gabapentin	Kainate	N.D.	N.D.	↓ (hippocampus)	No effect	Cilio et al.[52]
Lamotrigine	Perforanth path stimulation	N.D.	N.D.	↓ (CA3, hilus)	No effect	Halonen et al.[53]
Lamotrigine	Amygdala stimulation	No effect	No effect	No effect	N.D.	Nissinen et al.[54]
Levetiracetam	Pilocarpine	No effect	No effect	(↓) (hippocampus)	N.D.	Klitgaard et al.[55] (A)
Levetiracetam	Perforanth path stimulation	No effect	↓	N.D.	N.D.	Mazarati et al.[56] (A)
Levetiracetam	Amygdala stimulation	No effect	No effect	No effect	No effect	Brandt et al.[57]
Levetiracetam	Lithium-pilocarpine	No effect	N.D.	↓ (CA1, CA3, hilus)	No effect	Zhou et al.[58]
Phenobarbital	Kainate	No effect	No effect	No effect	Worsened	Mikati et al.[59]
Phenobarbital	Kainate	No effect	No effect	No effect	No effect	Bolanos et al.[60]
Phenobarbital*	Hippocampal stimulation	↓ (only for the 1 h after SE onset group)	N.D.	N.D.	N.D.	Prasad et al.[10*]
Phenobarbital	Lithium-Pilocarpine	No effect	↓	No effect (?)	N.D.	Brandt et al.[40]
Phenytoin	Hippocampal stimulation	No effect	N.D.	N.D.	N.D.	Prasad et al.[10]

(*continued*)

Table 81–1 **Prophylactic Effects of Treatment with Clinically Used Antiepileptic Drugs on the Long-Term Consequences of SE in Rats (Continued)**

Drug	Model (Induction of SE)	Consequences of Drug Treatment				Reference
		Incidence of SRS	Frequency, Severity, or Duration of SRS	Neurodegeneration	Impairment of Learning and Memory	
Phenytoin	Pilocarpine (in hippocampus)	N.D.	N.D.	↓ (CA1, CA3, hilus)	↓	Cunha et al.[41]
Pregabalin*	Lithium-pilocarpine	N.D.	N.D.	↓ (PC, EC)	N.D.	André et al.[11]*
Retigabine	Kainate	N.D	N.D	No effect	N.D	Ebert et al.[61]
Topiramate	Hippocampal stimulation	N.D.	N.D.	↓ (CA1, CA3, hilus)	N.D.	Niebauer and Gruenthal[62]
Topiramate	Pilocarpine	↓ (3–6 months after SE)	N.D.	↓ (CA1)	N.D.	DeLorenzo et al.[63] (A)
Topiramate	Lithium-pilocarpine	No effect	No effect	↓ (CA1, CA3)	N.D.	Rigoulot et al.[64]
Topiramate (plus diazepam)	Lithium-pilocarpine	No effect	No effect	↓ (CA1, hilus)	N.D.	Francois et al.[65]
Topiramate	Pilocarpine	N.D.	N.D.	↓ (CA1, CA3)	↓	Frisch et al.[66]
Topiramate	Lithium-pilocarpine	N.D.	N.D.	No effect (hippocampus)	(↓)	Shatskikh et al.[67]
Valproate	Kainate	↓ (during taper)	↓ (during taper)	↓ (CA1)	↓	Bolanos et al.[60]
Valproate	Amygdala stimulation	No effect	No effect	↓ (hippocampus and hilus)	No effect	Brandt et al.[15]
Valproate	Kainate	N.D.	N.D.	No effect (hippocampus and hilus)	↓	Jessberger et al.[68]
Vigabatrin*	Lithium-pilocarpine	No effect	No effect	↓ (CA1, CA3, and hilus)	N.D.	André et al.[69]*
Vigabatrin	Amygdala stimulation	No effect	No effect	No effect	No effect	Halonen et al.[70]

Notes: Only studies in which treatment started *after* onset of SE are included. A prophlylactic (beneficial) effect is indicated by "↓." Studies in which treatment effects were due to initial insult modification (i.e., reduction of SE duration or severity) rather than an antiepileptogenic effect are indicated by an asterisk (see text for discussion). Studies that are only available as abstracts are indicated by "A."

Abbreviations: EC, entorhinal cortex; N.D., not determined; PC, piriform cortex; SRS, spontaneous recurrent seizures.

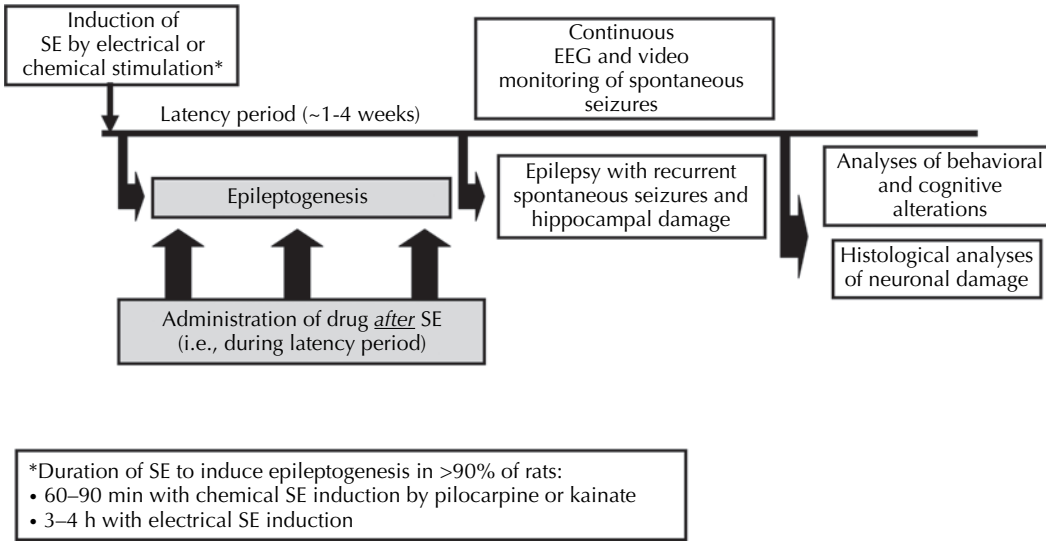

Figure 81–2. Schematic illustration of an experimental protocol to evaluate antiepileptogenic (or disease-modifying) drug effects by prophylactic drug treatment *after* SE.

the long-term consequences of the SE in such a way that fewer rats develop epilepsy or that the epilepsy that develops is milder.[2,8,9] Thus, in such SE models, the antiepileptogenic or neuroprotective potential of a drug should be tested by administering this drug *after* an SE of at least 3 h duration.[8] In chemical models of SE, such as the pilocarpine or kainate model, the critical duration of SE for induction of epileptogenesis and brain damage is considerably shorter, that is, about 60–90 min.[8] There are numerous studies that tested AEDs *after* such critical duration of SE for effects on epileptogenesis, brain damage, and/or behavioral and cognitive alterations in rats (Table 81–1). However, to our knowledge, there is no incontrovertible evidence supporting the idea that AEDs administered during the latent period following SE prevent the development of epilepsy, although some studies indicated that development of epilepsy may be delayed or the severity of spontaneous seizures may be reduced by such treatment (Table 81–1).

The ultimate goal of any prophylactic drug treatment after a brain insult such as SE is prevention of spontaneous recurrent seizures, that is, a true antiepileptogenic effect. However, alternative goals would be that the spontaneous seizures, if not prevented, are less frequent, less severe, and less resistant to AED treatment. Furthermore, any beneficial effect on the neuronal damage developing after brain insults or the cognitive and behavioral disturbances associated with epilepsy would be desirable. In this respect, it is important to note that several AEDs exerted neuroprotective effects when administered after SE (Table 81–1). At least in part, this neuroprotective effect was associated with positive effects on memory impairment or psychopathology. However, these data also indicate that protecting neurons from death is not sufficient for preventing the development of spontaneous seizures after brain insults, that is, for antiepileptogenic therapy.

NOVEL APPROACHES FOR ANTIEPILEPTOGENESIS

It is likely that antiepileptogenic drugs, if they exist, will have mechanisms of action distinct from those of traditional AEDs, as the molecular mechanisms underlying epileptogenesis and ictogenesis probably differ. Thus, a rational strategy for discovery of antiepileptogenic drugs would be testing of experimental compounds that interfere with one or several of the mechanisms underlying epileptogenesis (Fig. 81–1).

Neuroprotective Drugs

Because hippocampal damage has long been thought to be critically involved in the development of TLE, one potential strategy is administration of neuroprotective drugs after a brain insult (Table 81–2). For instance, we found that a single administration of a low dose (0.1 mg/kg) of the NMDA antagonist MK-801 after a kainate-induced SE of 90 min was capable of preventing most of the brain damage occurring in this model, but this treatment did not prevent the development of spontaneous seizures.[14] A similar finding was obtained more recently by starting prolonged treatment with valproate after 4 h of an electrically induced SE, which completely prevented any hippocampal damage, including cell loss in the hilus, but did not prevent development of spontaneous seizures.[15] These data thus substantiate the findings with MK-801 that an epileptogenic cascade, resulting in altered network excitability, may be triggered by SE, even in the absence of discernible neuronal injury in the hippocampal formation. Interestingly, although treatment with valproate after SE did not exert an antiepileptogenic effect, it did prevent most of the behavioral alterations developing after SE in rats.[15] We are currently evaluating the optimal therapeutic window and dosage protocol for these effects of valproate. Our data indicate that hippocampal damage is not critically involved in the development of spontaneous recurrent seizures but does play a major role in the psychopathology associated with epilepsy. We currently prove this hypothesis by experiments with the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) antagonist NS-1209, which has been shown to exert pronounced neuroprotective effects when administered after SE in rats (Table 81–2).

Anti-inflammatory Drugs

Another rational strategy for preventing or reducing the long-term consequences of brain insults is anti-inflammation. There is accumulating evidence that different types of brain insults, including SE, induce inflammatory processes in the brain that may critically contribute to epileptogenesis.[16] Thus, SE provoked experimentally in rodents triggers a prominent inflammatory response in brain areas recruited in the onset and propagation of epileptic activity.[16,17] This seizure-induced brain inflammation involves both the brain resident cells, such as glia and neurons, and cells of the peripheral innate immune systems such as granulocytes and macrophages, and shares molecules and pathways also activated by systemic infection, such as the Toll-like receptor signaling.[16] Various pro-inflammatory mediators are induced by SE in the brain, including cytokines such as interleukin (IL)-1ß, IL-6 or tumor necrosis factor-α (TNF-α), complement, and cyclooxygenase-2 (COX-2), which is responsible for generation of postaglandins from arachidonic acid.[16] Increase of these pro-inflammatory mediators is thought to be involved in impairment of blood-brain barrier function, neurodegeneration, and the neuronal hyperexcitability developing after SE.[16,17] Based on this hypothesis, Jung et al.[18] administered the COX-2 inhibitor, celecoxib, after a pilocarpine-induced SE in rats (Table 81–2). Compared to vehicle-treated controls, treatment with celecoxib prevented neuronal damage in the hippocampus and reduced the incidence and frequency of spontaneous recurrent seizures, that is, a true antiepileptogenic effect. This important finding prompted us to perform a similar study with the more selective COX-2 inhibitor, parecoxib. Parecoxib was administered twice daily at a rate of 10 mg/kg over 18 days, starting immediately after a pilocarpine-induced SE of 90 min duration. This treatment reduced hippocampal damage and the impairment of learning and memory in the Morris water maze test but did not prevent the development of spontaneous seizures, although seizures were less severe compared to those of controls.[19] Thus, anti-inflammation by COX-2 inhibition appears to constitute an interesting novel approach for disease modification after brain insults such as SE. However, a recent study with the COX-2 inhibitor SC58236 did not find any disease-modifying or neuroprotective effect when rats were treated after an electrically induced SE.[20] The most likely explanation for this different outcome of studies with prophylactic administration of COX-2 inhibitors after SE is the duration of the initial brain insult. Jung et al.[18] terminated SE after

Table 81–2 **Prophylactic Effects of Treatment with Various Drug Categories on the Long-Term Consequences of SE in Rats**

Drug	Model (Induction of SE)	Consequences of Drug Treatment				Reference
		Incidence of SRS	Frequency, Severity, or Duration of SRS	Neurodegeneration	Impairment of Learning and Memory	
Neuroprotective						
Ketamin* (NMDA antagonist)	Pilocarpine	↓ (K15)	N.D.	↓ (Ca1, Ca3) (K15 > K120)	↓ (K15 > K120)	Hort et al.[71]*
Ketamin	Pilocarpine (in hippocampus)	N.D.	N.D.	↓ (CA1, CA3, hilus)	↓	Cunha et al.[41]
MK-801* (NMDA antagonist)	Hippocampus stimulation	↓ (only for the 1 and 2 h after SE onset groups)	N.D.	N.D.	N.D.	Prasad et al.[10]*
MK-801	Kainate	No effect	No effect	↓ (CA1, CA3, PC, thalamus)	N.D.	Brandt et al.[14]
MK-801	Lithium-pilocarpine	N.D.	N.D.	↓ (CA1, CA3, PC, SN)	N.D.	Bankstahl et al.[72]
NS1209* (AMPA antagonist)	Amygdala stimulation	N.D.	N.D.	↓ (hippocampus)	N.D.	Pitkänen et al.[73]*
DEVD (caspase-3 inhibitor)	Kainate	N.D.	N.D.	No effect (hippocampus)	N.D.	Ebert et al.[61]
z-DEVD-fmk (caspase-3 inhibitor)	Amygdala stimulation	↓ (at 8–11 weeks post-SE)	No effect	↓ (CA3 and hilus)	No effect	Narkilahti et al.[74]
Anti-inflammatory						
Celecoxib (COX-2 inhibitor)	Lithium-pilocarpine	↓	↓	↓ (CA1, CA3, hilus)	N.D.	Jung et al.[18]
SC58236 (COX-2 inhibitor)	Hippocampus stimulation	No effect	No effect	No effect (hilus)	N.D.	Holtman et al.[20]
Parecoxib	Lithium-pilocarpine	No effect	↓	↓ (CA1, PC)	(↓)	Polascheck et al.[19]
Neuromodulators						
Atipamezole (α_2 antagonist)	Amygdala stimulation	No effect	↓	↓ (hilus)	No effect	Pitkänen et al.[22]
Rimonabant (CB1 antagonist)	Kainate	No effect	No effect	N.D.	N.D.	Pouliot et al.[25] (A)
Bumetanide	Lithium-Pilocarpine	No effect	No effect	No effect	N.D.	Brandt et al.[40]
Bumetanide + phenobarbital	Lithium-Pilocarpine	No effect	↓	No effect (?)	N.D.	Brandt et al.[40]

Notes: Only studies in which treatment started *after* onset of SE are included. A prophlylactic (beneficial) effect is indicated by "↓." Studies in which treatment effects were due to initial insult modification (i.e., reduction of SE duration or severity) rather than an anti-epileptogenic effect are indicated by an asterisk (see text for discussion). Studies that are only available as abstracts are indicated by "A."
Abbreviations: CB1, cannabinoid receptor 1; DEVD, a small peptide caspase-3 inhibitor with the amino acid sequence Asp-Glu-Val-Asp; N.D., not determined; PC, piriform cortex; SN, substantia nigra; SRS, spontaneous recurrent seizures.

60 min by diazepam and we did so after 90 min by diazepam, whereas Holtman et al.[20] terminated SE after 4 h by isoflurane anesthesia. However, SE was only transiently interrupted by anesthesia and continued for several more hours thereafter, resulting in a total SE duration of about 9–10 h.[20] Holtman et al.[20] suggested that the long duration of SE interfered with the outcome of COX-2 inhibition that started within this period. Thus, such technical details are very important when comparing studies on prophylactic drug treatment after SE.

Neuronal Modulators

A third rational strategy for preventing or modifying epileptogenesis and its consequences is to counteract the development of neuronal hyperexcitability after brain insults (Table 81–2). Interestingly, a number of studies have shown that different central nervous system (CNS)-stimulating drugs, including the adenosine antagonist caffeine, the α2 receptor antagonist atipamezole, and the cannabinoid (CB)-1 receptor antagonist rimobanant (SR141716A) exert neuromodulatory and/or antiepileptogenic and neuroprotective effects in epilepsy models[21–24] (but see Pouliot et al.[25]). Paradoxically, all of these compounds exert proconvulsant activity in normal animals, so that brain insults such as SE seem to change the pharmacology of these compounds. This is obviously a consequence of the molecular reorganization that develops after brain insults, resulting in alterations in the subunit composition and expression of receptors and ion channels and, thus, in their functions and pharmacology.[26–28] Furthermore, brain insults seem to induce a shift from adult to neonatal receptor and ion channel functions, indicating that epileptogenesis recapitulates ontogenesis.[29,30] Such a shift in GABAergic (gamma-aminobutyric acid) response polarity from hyperpolarizing to depolarizing has been described in human epileptic neurons recorded in the subiculum of hippocampal slices obtained from resections in patients suffering from mesial TLE.[31] This shift is thought to be a result of increased intraneuronal Cl^- levels caused by increased neuronal expression of the Na^+-K^+-$2Cl^-$ co-transporter NKCC1, an inwardly directed transporter that facilitates the accumulation of intracellular Cl^-, and downregulation of the K^+-Cl^- co-transporter KCC2, an outwardly directed transporter.[29,30,32,33] Upregulation of NKCC1 and downregulation of KCC2 in the hippocampus have been described both in patients with TLE and in the kindling and pilocarpine models of TLE.[33–39] This prompted us to evaluate whether inhibition of NKCC1 after SE affects the development of epilepsy in rats.[40] The diuretic bumetanide was used for these experiments, administered either alone or in combination with phenobarbital. Because bumetanide is very rapidly eliminated by rats and does not penetrate very well into the brain, various dosing protocols of bumetanide were evaluated in our experiments. Our data did not indicate any beneficial effects of bumetanide alone, but a combination of bumetanide and phenobarbital retarded development of epilepsy and reduced the frequency of spontaneous seizures (Table 81–2). However, this effect was not significantly different from that of treatment with phenobarbital alone. We are currently testing various pro-drugs of bumetanide to enhance its penetration into the brain of adult rats and mice. Furthermore, based on the observations with pro-convulsant drugs (Table 81–2), we have started experiments in which we administer the GABA receptor antagonist pentylenetetrazole at subconvulsant doses after SE to examine whether this treatment modifies epileptogenesis.

CONCLUSIONS

Novel drugs that interfere with epileptogenic processes may (1) prevent seizures (ultimate goal), (2) reduce the frequency, duration, or severity of seizures (disease modification), (3) prevent or reduce neurodegeneration, or (4) prevent or reduce the behavioral and cognitive alterations associated with epilepsy. New promising data with neuroprotective, anti-inflammatory, and neuromodulatory drugs seem to indicate that these goals are not unrealistic. However, because of the numerous pathological alterations that occur simultaneously during the epileptogenic cascade (Fig. 81–1), it will most certainly not be possible to halt epileptogenesis by targeting only one of these processes. Instead, cocktails of drugs that target different epileptogenic alterations should be

administered after brain insults, and we have started to explore this strategy. Furthermore, the models used to test potential antiepileptogenic agents should be modified to enhance the chance for identifying such agents. For instance, instead of inducing SE by systemic administration of kainate or pilocarpine, focal (e.g., intrahippocampal) injection of these convulsants may provide a more realistic scenario of TLE without the widespread brain damage and high mortality associated with conventional routes of administration. The recent study by Cunha et al.,[41] in which several drugs were tested for neuroprotective effects when given after SE induced by intrahippocampal pilocarpine, illustrates that the drug effects obtained in this model differ strikingly from the respective effects in models in which SE is induced by systemic injection of pilocarpine (Tables 81–1 and 81–2). Furthermore, the search for antiepileptogenic drugs should not rely solely on post-SE models of TLE; other approaches, including genetic animal models of epilepsy, should be included. In this respect, it is interesting to note that levetiracetam, which we reported to retard amygdala kindling,[42] failed to prevent development of spontaneous seizures after SE (Table 81–1) but more recently was found to exert antiepileptogenic or disease-modifying effects in spontaneously epileptic rats,[43,44] indicating that studies on kindling acquisition may be more predictive for such effects than data from SE models. The laboratory findings with levetiracetam prompted a clinical trial in which this AED will be examined for antiepileptogenic effects in patients with traumatic brain injury.[3] Based on experimental data indicating antiepileptogenic or disease-modifying effects of topiramate (Table 81–1), another clinical trial will determine whether topiramate decreases the risk of posttraumatic epilepsy.[3] Ultimately, only clinical trials can determine whether a drug possesses antiepileptogenic or disease-modifying potential. However, defining the clinical paradigm and selecting appropriate outcomes to detect such potential effects present challenges to clinicians studying the antiepileptogenic or neuroprotective properties of drugs.[45,46] Achieving a better understanding of the process of epileptogenesis, improved testing treatments that demonstrate antiepileptogenic effects in the laboratory, and performing thorough preclinical and clinical evaluations before attempting definitive trials should greatly improve the chance of identifying ways to prevent epilepsy after brain insults, providing the ultimate cure for this condition.[6]

DISCLOSURE STATEMENT

The author declares no conflict of interest.

REFERENCES

1. Herman ST. Epilepsy after brain insult: targeting epileptogenesis. *Neurology.* 2002;59(9 suppl 5):S21–S26.
2. Pitkänen A. New pharmacotherapy for epilepsy. *IDrugs.* 2004;7(5):471–477.
3. Dichter MA. Posttraumatic epilepsy: the challenge of translating discoveries in the laboratory to pathways to a cure. *Epilepsia.* 2009;50(suppl 2):41–45.
4. Temkin NR. Antiepileptogenesis and seizure prevention trials with antiepileptic drugs: meta-analysis of controlled trials. *Epilepsia.* 2001;42:515–524.
5. Temkin NR. Causes and prevention of symptomatic epilepsy. a clinical survey. In: Löscher W, Schmidt D, eds. New horizons in the development of antiepileptic drugs II: The search for new targets. *Epilepsy Res.* 2004; 60:80–83.
6. Temkin NR. Preventing and treating posttraumatic seizures: the human experience. *Epilepsia.* 2009; 50(suppl 2):10–13.
7. Morimoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog Neurobiol.* 2004;73(1):1–60.
8. Löscher W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Res.* 2002;50:105–123.
9. Pitkänen A. Drug-mediated neuroprotection and antiepileptogenesis: animal data. *Neurology.* 2002;59 (9 suppl 5):S27–S33.
10. Prasad A, Williamson JM, Bertram EH. Phenobarbital and MK-801, but not phenytoin, improve the long-term outcome of status epilepticus. *Ann Neurol.* 2002; 51(2):175–181.
11. André V, Rigoulot MA, Koning E, Ferrandon A, Nehlig A. Long-term pregabalin treatment protects basal cortices and delays the occurrence of spontaneous seizures in the lithium-pilocarpine model in the rat. *Epilepsia.* 2003;44(7):893–903.
12. Pitkänen A, Kharatishvili I, Narkilahti S, Lukasiuk K, Nissinen J. Administration of diazepam during status epilepticus reduces development and severity of epilepsy in rat. *Epilepsy Res.* 2005;63(1):27–42.
13. Lowenstein DH. The management of refractory status epilepticus: an update. *Epilepsia.* 2006;47(suppl 1): 35–40.
14. Brandt C, Potschka H, Löscher W, Ebert U. *N*-methyl-D-aspartate receptor blockade after status epilepticus protects against limbic brain damage but not against

epilepsy in the kainate model of temporal lobe epilepsy. *Neuroscience.* 2003;118:727–740.
15. Brandt C, Gastens AM, Sun MZ, Hausknecht M, Löscher W. Treatment with valproate after status epilepticus: effect on neuronal damage, epileptogenesis, and behavioral alterations in rats. *Neuropharmacology.* 2006;51:789–804.
16. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol.* 2011;7: 31–40.
17. Vezzani A, Baram TZ. New roles for interleukin-1 Beta in the mechanisms of epilepsy. *Epilepsy Curr.* 2007;7(2):45–50.
18. Jung KH, Chu K, Lee ST, Kim J, Sinn DI, Kim JM, et al. Cyclooxygenase-2 inhibitor, celecoxib, inhibits the altered hippocampal neurogenesis with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. *Neurobiol Dis.* 2006;23(2):237–246.
19. Polascheck N, Bankstahl M, Löscher W. The COX-2 inhibitor parecoxib is neuroprotective but not antiepileptogenic in the pilocarpine model of temporal lobe epilepsy. *Exp Neurol.* 2010; 224(1):219–233.
20. Holtman L, van Vliet EA, van Schaik R, Queiroz CM, Aronica E, Gorter JA. Effects of SC58236, a selective COX-2 inhibitor, on epileptogenesis and spontaneous seizures in a rat model for temporal lobe epilepsy. *Epilepsy Res.* 2009;84(1):56–66.
21. Rigoulot MA, Leroy C, Koning E, Ferrandon A, Nehlig A. Prolonged low-dose caffeine exposure protects against hippocampal damage but not against the occurrence of epilepsy in the lithium-pilocarpine model in the rat. *Epilepsia.* 2003;44(4):529–535.
22. Pitkänen A, Narkilahti S, Bezvenyuk Z, Haapalinna A, Nissinen J. Atipamezole, an alpha(2)-adrenoceptor antagonist, has disease modifying effects on epileptogenesis in rats. *Epilepsy Res.* 2004;61(1–3):119–140.
23. Chen K, Neu A, Howard AL, Foldy C, Echegoyen J, Hilgenberg L, et al. Prevention of plasticity of endocannabinoid signaling inhibits persistent limbic hyperexcitability caused by developmental seizures. *J Neurosci.* 2007;27(1):46–58.
24. Echegoyen J, Armstrong C, Morgan RJ, Soltesz I. Single application of a CB1 receptor antagonist rapidly following head injury prevents long-term hyperexcitability in a rat model. *Epilepsy Res.* 2009;85(1):123–127.
25. Dudek FE, Pouliot WA, Rossi CA, Staley KJ. The effect of the cannabinoid-receptor antagonist, SR141716, on the early stage of kainate-induced epileptogenesis in the adult rat. *Epilepsia*. 2010;51(suppl 3):126–130.
26. Coulter DA. Epilepsy-associated plasticity in gamma-aminobutyric acid receptor expression, function, and inhibitory synaptic properties. *Int Rev Neurobiol.* 2001;45:237–252.
27. Coulter DA, McIntyre DC, Löscher W. Animal models of limbic epilepsies: what can they tell us? *Brain Pathol.* 2002;12:240–256.
28. Stefan H, Lopes Da Silva FH, Löscher W, Schmidt D, Perucca E, Brodie MJ, et al. Epileptogenesis and rational therapeutic strategies. *Acta Neurol Scand.* 2006;113(3):139–155.
29. Köhling R. Neuroscience. GABA becomes exciting. *Science.* 2002;298(5597):1350–1351.
30. Ben-Ari Y, Holmes GL. The multiple facets of gamma-aminobutyric acid dysfunction in epilepsy. *Curr Opin Neurol.* 2005;18(2):141–145.
31. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science.* 2002;298(5597): 1418–1421.
32. Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, et al. BDNF-induced TrkB activation down-regulates the K^+-Cl^- cotransporter KCC2 and impairs neuronal Cl^- extrusion. *J Cell Biol.* 2002;159(5):747–752.
33. Palma E, Amici M, Sobrero F, Spinelli G, Di Angelantonio S, Ragozzino D, et al. Anomalous levels of Cl- transporters in the hippocampal subiculum from temporal lobe epilepsy patients make GABA excitatory. *Proc Natl Acad Sci USA.* 2006;103(22): 8465–8468.
34. Okabe A, Ohno K, Toyoda H, Yokokura M, Sato K, Fukuda A. Amygdala kindling induces upregulation of mRNA for NKCC1, a Na(+), K(+)-2Cl(−) cotransporter, in the rat piriform cortex. *Neurosci Res.* 2002; 44(2):225–229.
35. Rivera C, Voipio J, Kaila K. Two developmental switches in GABAergic signalling: the K^+-Cl^- cotransporter KCC2 and carbonic anhydrase CAVII. *J Physiol.* 2005;562(pt 1):27–36.
36. Okabe A, Yokokura M, Toyoda H, Shimizu-Okabe C, Ohno K, Sato K, et al. Changes in chloride homeostasis-regulating gene expressions in the rat hippocampus following amygdala kindling. *Brain Res.* 2003;990(1–2): 221–226.
37. Huberfeld G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, et al. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. *J Neurosci.* 2007;27(37):9866–9873.
38. Pathak HR, Weissinger F, Terunuma M, Carlson GC, Hsu FC, Moss SJ, et al. Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. *J Neurosci.* 2007;27(51):14012–14022.
39. Li X, Zhou J, Chen Z, Chen S, Zhu F, Zhou L. Long-term expressional changes of Na^+ -K^+ -Cl^- co-transporter 1 (NKCC1) and K^+ -Cl^- co-transporter 2 (KCC2) in CA1 region of hippocampus following lithium-pilocarpine induced status epilepticus (PISE). *Brain Res.* 2008;1221:141–146.
40. Brandt C, Nozadze M, Heuchert N, Rattka M, Löscher W. Disease-modifying effects of phenobarbital and the NKCC1 inhibitor bumetanide in the pilocarpine model of temporal lobe epilepsy. *J Neurosci.* 2010;30(25):8602–8612.
41. Cunha AO, Mortari MR, Liberato JL, dos Santos WF. Neuroprotective effects of diazepam, carbamazepine, phenytoin and ketamine after pilocarpine-induced status epilepticus. *Basic Clin Pharmacol Toxicol.* 2009;104(6):470–477.
42. Löscher W, Hönack D, Rundfeldt C. Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. *J Pharmacol Exp Ther.* 1998;284:474–479.
43. Yan HD, Ji-qun C, Ishihara K, Nagayama T, Serikawa T, Sasa M. Separation of antiepileptogenic and antiseizure effects of levetiracetam in the spontaneously epileptic rat (SER). *Epilepsia.* 2005;46(8):1170–1177.
44. Russo E, Citraro R, Scicchitano F, De Fazio S, Di Paola ED, Constanti A, et al. Comparison of the antiepileptogenic effects of an early long-term treatment with ethosuximide or levetiracetam in a genetic animal

model of absence epilepsy. *Epilepsia.* 2010;51(8): 1560–1569.
45. Sankar R. Neuroprotection in epilepsy: the Holy Grail of antiepileptogenic therapy. *Epilepsy Behav.* 2005;7(suppl 3):S1–S2.
46. Willmore LJ. Antiepileptic drugs and neuroprotection: current status and future roles. *Epilepsy Behav.* 2005;7(suppl 3):S25–S28.
47. Löscher W. Current status and future directions in the pharmacotherapy of epilepsy. *Trends Pharmacol Sci.* 2002;23:113–118.
48. Löscher W, Gernert M, Heinemann U. Cell and gene therapies in epilepsy—promising avenues or blind alleys? *Trends Neurosci.* 2008;31(2):62–73.
49. Capella HM, Lemos T. Effect on epileptogenesis of carbamazepine treatment during the silent period of the pilocarpine model of epilepsy. *Epilepsia.* 2002;43(suppl 5):110–111.
50. Francois J, Ferrandon A, Koning E, Nehlig A. Epileptic outcome is correlated with protection in temporal cortices: evidence from neuroprotection studies with a new drug, RWJ333369, in the lithium-pilocarpine model. *Epilepsia.* 2005;46(suppl 6):62–63.
51. Mazarati AM, Sofia RD, Wasterlain CG. Anticonvulsant and antiepileptogenic effects of fluorofelbamate in experimental status epilepticus. *Seizure.* 2002;11(7): 423–430.
52. Cilio MR, Bolanos AR, Liu Z, Schmid R, Yang Y, Stafstrom CE, et al. Anticonvulsant action and long-term effects of gabapentin in the immature brain. *Neuropharmacology.* 2001;40:139–147.
53. Halonen T, Nissinen J, Pitkänen A. Effect of lamotrigine treatment on status epilepticus-induced neuronal damage and memory impairment in rat. *Epilepsy Res.* 2001;46:205–223.
54. Nissinen J, Large CH, Stratton SC, Pitkänen A. Effect of lamotrigine treatment on epileptogenesis: an experimental study in rat. *Epilepsy Res.* 2004;58(2–3): 119–132.
55. Klitgaard HV, Matagne AC, Vanneste-Goemaere J, Margineanu DG. Effects of prolonged administration of levetiracetam on pilocarpine-induced epileptogenesis in rats. *Epilepsia.* 2001;42(suppl 7):114–115.
56. Mazarati AM, Baldwin RA, Klitgaard H, Matagne A, Wasterlain CG. Treatment with levetiracetam during the latent period after experimental status epilepticus reduces chronic spontaneous recurrent seizures. *Epilepsia.* 2003;44(suppl 9):223.
57. Brandt C, Glien M, Gastens AM, Fedrowitz M, Bethmann K, Volk HA, et al. Prophylactic treatment with levetiracetam after status epilepticus: lack of effect on epileptogenesis, neuronal damage, and behavioral alterations in rats. *Neuropharmacology.* 2007;53(2):207–221.
58. Zhou JL, Zhao Q, Holmes GL. Effect of levetiracetam on visual-spatial memory following status epilepticus. *Epilepsy Res.* 2007;73(1):65–74.
59. Mikati MA, Holmes GL, Chronopoulos A, Hyde P, Thurber S, Gatt A, et al. Phenobarbital modifies seizure-related brain injury in the developing brain. *Ann Neurol.* 1994;36:425–433.
60. Bolanos AR, Sarkisian M, Yang Y, Hori A, Helmers SL, Mikati M, et al. Comparison of valproate and phenobarbital treatment after status epilepticus in rats. *Neurology.* 1998;51:41–48.
61. Ebert U, Brandt C, Löscher W. Delayed sclerosis, neuroprotection, and limbic epileptogenesis after status epilepticus in the rat. *Epilepsia.* 2002;43(suppl 5): 86–95.
62. Niebauer M, Gruenthal M. Topiramate reduces neuronal injury after experimental status epilepticus. *Brain Res.* 1999;837:263–269.
63. DeLorenzo RJ, Morris A, Blair RE, Wallace M, Razvi B. Topiramate is both neuroprotective and antiepileptogenic in the pilocarpine model of status epilepticus. *Epilepsia.* 2002;43(suppl 7):15.
64. Rigoulot MA, Koning E, Ferrandon A, Nehlig A. Neuroprotective properties of topiramate in the lithium-pilocarpine model of epilepsy. *J Pharmacol Exp Ther.* 2004;308(2):787–795.
65. Francois J, Koning E, Ferrandon A, Nehlig A. The combination of topiramate and diazepam is partially neuroprotective in the hippocampus but not antiepileptogenic in the lithium-pilocarpine model of temporal lobe epilepsy. *Epilepsy Res.* 2006;72(2–3): 147–163.
66. Frisch C, Kudin AP, Elger CE, Kunz WS, Helmstaedter C. Amelioration of water maze performance deficits by topiramate applied during pilocarpine-induced status epilepticus is negatively dose-dependent. *Epilepsy Res.* 2007;73(2):173–180.
67. Shatskikh T, Zhao Q, Zhou JL, Holmes GL. Effect of topiramate on cognitive function and single units from hippocampal place cells following status epilepticus. *Epilepsy Behav.* 2009;14(1):40–47.
68. Jessberger S, Nakashima K, Clemenson GD Jr, Mejia E, Mathews E, Ure K, et al. Epigenetic modulation of seizure-induced neurogenesis and cognitive decline. *J Neurosci.* 2007;27(22):5967–5975.
69. André V, Ferrandon A, Marescaux C, Nehlig A. Vigabatrin protects against hippocampal damage but is not antiepileptogenic in the lithium-pilocarpine model of temporal lobe epilepsy. *Epilepsy Res.* 2001;47(1–2): 99–117.
70. Halonen T, Nissinen J, Pitkänen A. Chronic elevation of brain GABA levels beginning two days after status epilepticus does not prevent epileptogenesis in rats. *Neuropharmacology.* 2001;40:536–550.
71. Hort J, Brozek G, Mares P, Langmeier M, Komarek V. Cognitive functions after pilocarpine-induced status epilepticus: changes during silent period precede appearance of spontaneous recurrent seizures. *Epilepsia.* 1999;40:1177–1183.
72. Bankstahl JP, Hoffmann K, Bethmann K, Löscher W. Glutamate is critically involved in seizure-induced overexpression of P-glycoprotein in the brain. *Neuropharmacology.* 2008;54(6):1006–1016.
73. Pitkänen A, Mathiesen C, Ronn LC, Moller A, Nissinen J. Effect of novel AMPA antagonist, NS1209, on status epilepticus. An experimental study in rat. *Epilepsy Res.* 2007;74(1):45–54.
74. Narkilahti S, Nissinen J, Pitkänen A. Administration of caspase 3 inhibitor during and after status epilepticus in rat: effect on neuronal damage and epileptogenesis. *Neuropharmacology.* 2003;44(8):1068–1088.

Chapter 82

Neonatal Seizures and Neuronal Transmembrane Ion Transport

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Chloride is the primary permeant anion in the mammalian nervous system, and the regulation of its flux across cell membranes is important for a diversity of neurophysiological processes. Neurons carefully adjust the intracellular level of chloride to ensure the proper electrical response to the amino acid neurotransmitter gamma-aminobutyric acid (GABA), which binds ligand-gated $GABA_A$ receptors that operate chloride channels. In adult neurons, the level of intracellular chloride is low and the reversal potential for chloride currents is near the neuron's resting membrane potential. Minor changes in the intracellular chloride concentration can significantly affect the strength, and even polarity, of GABAergic neurotransmission.

In addition to setting the direction of $GABA_A$ receptor-mediated currents, the intracellular chloride concentration is an important osmotic determinant of cell volume. Neurons, glia, and most other cells in the brain alter their cellular chloride concentration to defend their cell volume against fluctuations of extracellular osmolality and/or intracellular solute content—perturbations that can imperil their structural integrity. Neuronal chloride homeostasis is thus a balancing act between volume regulation and GABA signaling.

In the nervous system, the intracellular chloride concentration is set, in part, by the chloride-importing Na-K-2Cl cotransporter NKCC1, in concert with several different chloride-extruding K-Cl cotransporters. These related cation-chloride cotransporters (CCCs) are all members of the *SLC12* gene family.

This book chapter will discuss recent work that has provided insight into the role of

NKCC1 in fostering excitatory GABAergic neurotransmission in the immature brain and how the pharmacological inhibition of NKCC1 might hold promise for the treatment of neonatal seizures.

THE EFFECTS OF GABA ARE LAREGLY DETERMINED BY THE INTRACELLULAR CONCENTRATION OF CHLORIDE

Gamma-aminobutyric acid is the main *inhibitory* neurotransmitter in the adult cortex, where its activity is essential for maintaining appropriate electrical activity by balancing inputs that are triggered by excitatory neurotransmitters like glutamate. In contrast, in the neonatal cortex, GABA is an *excitatory* neurotransmitter. There, it plays an important role in neuronal development and the activity-dependent wiring of circuits by triggering the depolarizing inputs that underlie large-scale spontaneous electrical activity.[1]

In neurons, GABA acts by binding to $GABA_A$ receptors, which function as ligand-gated chloride channels. Gama-aminobutyric acid triggers conformational changes in these receptors that facilitate the passive inflow or outflow of chloride ions, depending on the cell's equilibrium potential for chloride (E_{Cl}). $[Cl^-]_i$ is a key determinant of the neuronal response to GABA. When $[Cl^-]_i$ is high, such that E_{Cl} is positive relative to the neuron's membrane potential (V_m), the opening of chloride channels by $GABA_A$-receptor activation results in chloride efflux, which consequently depolarizes the neuron. When $[Cl^-]_i$ is low, such that E_{Cl} is negative relative to the neuron's V_m, activation of the $GABA_A$ receptor results in chloride influx, leading to hyperpolarization of the neuron. Depolarization increases the chance that the neuron will fire an action potential, whereas hyperpolarization is associated with a decreased chance of its firing an action potential. Therefore, high neuronal chloride concentrations are generally considered to subserve GABA-mediated excitation and low concentrations to facilitate GABA-mediated inhibition. There are, however, exceptions to these considerations (such as shunting inhibition mediated by depolarizing conductances and action potentials triggered by conductances that are activated by membrane hyperpolarization), so it is sometimes preferable to assay the net effect of endogenously released GABA based on the rate of action potentials in the neural network rather than extrapolate from the effect of GABA on membrane potential.[2]

The differential action (excitatory versus inhibitory) of GABA in the neonate and adult central nervous systems is likely due to a difference in the $[Cl^-]_i$ of immature versus mature neurons.[3] Compared to mature adult neurons, $[Cl^-]_i$ is about 20–40 mM *higher* in immature neonatal neurons, a difference that is sufficient to shift the action of GABA from inhibition to excitation, because the electrochemical driving force for chloride drives the flux of this anion out of the cell when GABA channels are opened. $[Cl^-]_i$ is determined in part by the Na-K-Cl cotransporter NKCC1, which mediates chloride transport into the cell,[4] and the K-Cl cotransporter KCC2,[5] which mediates chloride transport out of the cell. NKCC1 and KCC2 are related members of the *SLC12A* electroneutral cation-chloride cotransporter gene family.

NKCC1 AND KCC2 ARE ESSENTIAL CATION-CHLORIDE COTRANSPORTERS IN THE NERVOUS SYSTEM

The CCCs are targets of some of the most common drugs in medicine—the loop and thiazide diuretics—and are mutated in several inherited human diseases.[6] The CCCs are intrinsic membrane proteins that transport chloride ions, together with sodium and/or potassium ions, across the plasma membranes. The stoichiometric coupling and directionality of their translocated ions result in an electroneutral, secondarily active transport process driven energetically by transmembrane sodium and potassium gradients established by the Na^+-K^+-adenosine triphosphatase (ATPase).

Defined by their sensitivity to pharmacological inhibitors, stoichiometry of transported ions, and phylogeny, the CCCs are divided into two main branches. Given the large electrochemically favorable inward gradient for sodium, the sodium-coupled CCC branch that comprises the Na-(K)-Cl cotransporters (NCC, NKCC1, and NKCC2) loads chloride ions into the cell to raise $[Cl^-]_i$ above its electrochemical

equilibrium. Conversely, by coupling chloride transport to the outward gradient for potassium, the potassium-coupled CCC branch, comprised of four different K-Cl cotransporters (KCC1, KCC2, KCC3, and KCC4), primarily transports chloride ions out of the cell, reducing $[Cl^-]_i$ below its electrochemical equilibrium. The relative activities of these mediators of chloride influx and efflux determine the level of $[Cl^-]_i$ in numerous cell types, and consequently play key roles in the regulation of neuronal excitability, the homeostasis of cellular volume, and the transepithelial transport of salt, potassium, and water.

Consistent with the opposing directionality of the chloride they transport, the Na-K-Cl and K-Cl cotransporters exhibit reciprocal regulation by volume- and chloride-sensitive serine-threonine phosphorylation/dephosphorylation events.[7] Cell swelling (triggered by extracellular hypotonicity), high intracellular levels of chloride, and protein phosphatases (by promoting cotransporter dephosphorylation) stimulate the K-Cl cotransporters but inhibit NKCC1, thereby decreasing $[Cl^-]_i$. Conversely, cell shrinkage (triggered by extracellular hypertonicity), low intracellular chloride, and protein phosphatase inhibitors (by promoting cotransporter phosphorylation) activate NKCC1 and inhibit the K-Cl cotransporters, resulting in increased $[Cl^-]_i$.

NKCC1 is expressed early in development in neurons and glia in the cerebrum and cerebellum, as well as in the spinal cord, peripheral nervous system, and cerebral vascular endothelial cells in adults.[8] The expression of KCC2 is restricted to mature neurons in the retina, cortex, cerebellum, and the dorsal horn of the spinal cord.[9]

The WNK (With No K, i.e. lysine) kinases, members of which are mutated in a inherited syndrome of human hypertension,[10] and also a hereditary sensory and autonomic neuropathy,[11] along with the Ste20p-related Proline Alanine-rich Kinase (SPAK) and oxidative stress-responsive kinase-1 (OSR1),[12] are now thought to be the long-sought chloride/volume-sensitive regulatory kinases of the Na-K-Cl and K-Cl cotransporters.[13] Protein phosphorylation is likely the predominant mechanism by which acute, dynamic modulation of CCC function takes place; regulation of gene transcription and protein degradation are mechanisms by which the activity of the CCCs can be modulated over a less rapid time scale.

A DEVELOPMENTAL SWITCH IN NKCC1:KCC2 EXPRESSION RENDERS GABA HYPERPOLARIZING DURING NEURONAL DEVELOPMENT

A developmental switch in neuronal chloride gradients underlies the maturation of GABAergic signaling from excitatory to inhibitory.[14,15] Before synaptic transmission is established, GABA plays an important role in neuronal growth, development, and the activity-dependent wiring of circuits by triggering the excitatory inputs that underlie large-scale spontaneous electrical activity in the neonatal central and peripheral nervous systems.[1,16–18] In contrast to its action in the adult, during this early time period GABA is an excitatory neurotransmitter due to a relatively higher level of intraneuronal chloride, such that GABA triggers an outward flux of chloride that leads to membrane depolarization.[14,19] As discussed above, this depolarization can generate action potentials, as well as directly activate voltage-dependent calcium channels and indirectly activate *N*-methyl-D-aspartate (NMDA) receptors by removing the obstructive block of magnesium ions from the receptor pore. The subsequent GABA-induced elevations of intracellular calcium promote neuronal survival and differentiation, and are important for the genesis and maintenance of synaptic connections.[16] However, during later stages of development, excitatory GABAergic inputs are replaced by inhibitory GABAergic inputs.[19] A shift in the relative predominance of neuronal KCC2 to NKCC1 activity is now considered to be the mechanism that underlies the excitatory-to-inhibitory developmental transition of GABAergic signaling.

Influx of chloride into neurons is mediated largely by Na–K–2Cl cotransport via NKCC1. In embryonic and early postnatal life, neurons show robust expression of NKCC1 but minimal expression of KCC2.[20] This predominance of inward-directed chloride transport increases the $[Cl^-]_i$, resulting in a positive E_{Cl} relative to the V_m of the neuron, so that GABA stimulates an outward-directed chloride current that triggers neuronal depolarization and excitation. Sustained block of excitatory GABAergic signaling during development by persistent pharmacological inhibition or genetic disruption

of NKCC1 results in a lack of morphological maturation of cortical neurons, fewer mature spinal neurons, reduced dendritic arborization, disrupted motor activity, fewer motor neurons and interneurons, a reduction in the elaboration of axonal tracts, and smaller brains and spinal cords.[8,21]

Efflux of chloride out of neurons is mediated largely by the K–Cl cotransporter KCC2. In many species, this cotransporter is expressed at very low levels at birth.[19] In rat hippocampal and neocortical pyramidal neurons, a negative shift in the GABA reversal potential (E_{GABA}) is paralleled by a robust increase in KCC2 expression near the end of the second postnatal week.[14] Dzhala et al. demonstrated that KCC2 expression in the human neocortex begins to increase at 40 weeks after conception.[22] This increase in KCC2 expression, accompanied by a concurrent downregulation of NKCC1 expression, results in the dominance of chloride efflux over chloride influx, which decreases $[Cl^-]_i$ such that E_{Cl} is negative relative to the neuron's V_m, thereby rendering GABAergic signals hyperpolarizing.[14] *Kcc2*$^{-/-}$ mice have seizures and die at birth, in part owing to malfunction of the respiratory center.[23]

Gamma-aminobutyric acid itself, by acting as a self-limiting trophic factor, might promote this GABAergic switch by triggering specific intracellular cascades that upregulate KCC2 gene expression.[15] Recent work suggests that another regulatory factor might be cholinergic signaling. Spontaneous cholinergic activity, by triggering calcium-signaling cascades that upregulate KCC2 downstream of the acetylcholine nicotinic receptor, has been shown to be important for the GABAergic excitation-to-inhibition transition.[17] Synergistic with the newly established inhibitory GABAergic signaling, cholinergic signaling then triggers later events of neuronal development.

Both bumetanide and furosemide, well-known loop diuretics, are capable of inhibiting the cation-chloride cotransporters in vitro and in vivo.[24,25] Bumetanide has an approximately 500-fold greater affinity for NKCC1 (inhibition constant [K_i] of approximately 0.1 μM) than for KCC2 (K_i of approximately 25–50 μM). Furosemide inhibits NKCC1 and KCC2 with equal potency (K_i of approximately 25–50 μM). Therefore, at low at low doses (2–10 μM), bumetanide is a relatively specific inhibitor of NKCC1. The accumulation of bumetanide in the central nervous system after systemic administration has not been directly measured, but the drug's high lipid:water partition coefficient, its documented anticonvulsant effects in both animals and humans in vivo, and the developmental effects of chronic systemically administered bumetanide suggest that the drug is able to cross the blood-brain barrier.[8,26,27]

THE ROLE OF NKCC1-DEPENDENT CHLORIDE ACCUMULATION IN NEONATAL SEIZURES

Neonatal seizures, or epileptic episodes suffered by infants in the first 28 days of life, occur in 1% to 2% of patients in neonatal intensive care units and are the most common manifestation of an acute neurological disorder in newborn infants.[28] Most commonly caused by hypoxic-ischemic encephalopathy, hemorrhage, or cerebral infarction, the presence of neonatal seizures often portends severe neurological dysfunction later in life, with high rates of adult epilepsy and long-term cognitive and motor deficits in survivors. In animal models, neonatal seizures have been shown to be injurious to the development of the brain, inducing synaptic reorganization, altering synaptic plasticity, and priming cortical neurons to increased damage from seizures sustained later in life. Thus, the prompt diagnosis and successful treatment of neonatal seizures are important for improving the long-term neurological outcome.

While seizure activity in adults is usually clinically obvious and the electroencephalogram (EEG) reflects coordinated seizure activity, diagnosing seizures in neonates is difficult because seizures are often behaviorally subtle and the EEG typically demonstrates a multifocal process.[29,30] Nonetheless, seizures in neonates are currently diagnosed by EEG, with a discharge duration of 10 s (versus 3 s in older age groups) required to diagnose an electrographic seizure. However, because EEGs are not immediately available in many neonatal intensive care units, the initial diagnosis and treatment of seizures are often based on clinical assessment alone, and EEGs are performed *after* the administration of antiepileptic drugs. Unfortunately, it is all too common for electrographic seizures to persist in encephalopathic

neonates even when antiepileptic drug levels are "therapeutic."[31]

Conventional antiepileptic drugs have limited utility in treating neonatal seizures. Barbiturates and benzodiazepines, $GABA_A$ receptor agonists that are efficacious for treating adult seizures, are currently among the first-line drugs for neonatal seizures; however, they are often ineffective and have been shown to actually have the potential to increase seizure activity in the immature brain.[32] Phenytoin has been used with similarly limited success.[33] Barbiturates and benzodiazepines have also been known to produce a phenomenon termed *electroclinical dissociation* in neonates, whereby the overt clinical manifestations of seizures (e.g., convulsions) are inhibited but EEG-documented cortical seizure activity is either unaffected or exacerbated.[34] This insidious effect of the GABA agonists has the potential for great harm, as it provides physicians with a false impression that seizures are under control, while cortical seizure activity—and its potentially detrimental effects—rages on.

There have been few prospective studies or randomized, controlled trials of the antiepileptic drugs that are currently used to treat neonatal seizures.[32] To date, the only randomized trial of antiepileptic drugs for the treatment of neonatal seizures compared the current first-line drugs, phenobarbital to phenytoin.[33] In this study, the majority of neonates had asphyxia, infarction, or hemorrhage as the etiology of their seizures. Complete control of electrographic seizures was achieved with either drug in only ~25% of neonates whose seizure frequency was increasing. Seizure control was achieved in another 15% of newborns when both agents were used concurrently. Preliminary studies of antiepileptic drugs other than phenobarbital and phenytoin have shown only modest efficacy in smaller cohorts of neonates, though sufficiently powered randomized trials are needed to demonstrate conclusively whether any of these drugs are truly effective.[32] The lack of evidence-based treatment recommendations, coupled with the paucity of data regarding the underlying pathophysiology of neonatal seizures, has made their current management far from optimal.

Increasing synaptic excitation and/or decreasing synaptic inhibition can cause neurons to become hyperactive. Because neurons harbor a multiplicity of connections with other neurons, the electrical firing of even a small population of hyperexcitable neurons, when synchronized, can progressively entrain larger neural networks until seizures ensue. In neonates, while GABA-mediated excitation plays a role in neuronal development, it also renders the developing brain particularly susceptible to seizures. In the adult cortex, excitatory glutaminergic signaling is balanced by inhibitory GABAergic signaling. However, in the brain-injured neonate, the additional depolarization due to $GABA_A$ receptor activation likely adds to the excitation already initiated by glutamate neurotransmission to tip the balance of excitation/inhibition toward excessive excitation and a propensity to seizure activity. Experimentally, GABA-mediated excitation has been shown to support epileptogenesis in the developing hippocampus and also to decrease the seizure threshold of neonates.[22,35,36] The excitatory nature of GABA signaling in immature neurons also explains why GABA agonists like barbiturates and benzodiazepines are often ineffective in reducing neonatal seizures and can even exacerbate seizure activity. Clearly, new antiepileptic treatment strategies are needed for neonatal seizures.

Because the elevated $[Cl^-]_i$ of immature neurons is due to robust activity of NKCC1, this cotransporter is currently being explored as a target for novel anticonvulsant strategies for neonatal seizures. In theory, inhibition of NKCC1, by reducing $[Cl^-]_i$, could reduce the GABA-mediated excitation of immature neonatal neurons or even possibly convert the GABA response to inhibitory.

Dzhala et al. were the first to test this hypothesis in a recent seminal paper.[22] Previous groups had established that NKCC1 expression in rats is highest in cortical neurons during the first postnatal week, begins to decrease at postnatal day 14 (P14), and then drops to the low levels that are found in adults (reviewed in ref. 37). Conversely, the expression of KCC2 is minimal at birth in rat cortical neurons, is low during the first postnatal week, and then attains an expression level at P14 that is comparable to that of adults. Dzhala et al. demonstrated that a similar expression pattern is present in the human cortex, with high NKCC1 and low KCC2 expression during the neonatal time period and before the end of the first year of life—a time scale not different from that of the rat when adjusted for the greater length of time

required for the development of the human cortex relative to the rodent brain. These data supported the hypothesis that, similar to the situation in the rat, GABA is excitatory in immature human cortical neurons, and neonates may be susceptible to seizures due to the excitatory effects of GABA during development. Because NKCC1 is known to establish the elevated levels of $[Cl^-]_i$ that underlie excitatory GABAergic signaling in immature neurons, these data predicted that bumetanide, an inhibitor of NKCC1, might be an effective treatment for neonatal seizures.[22]

This hypothesis was tested in a series of elegant in vitro and in vivo physiological studies.[22] NKCC1 blockade by bumetanide inhibited cortical seizure activity in neonatal rats both in vitro and in vivo, and this inhibition was observed at doses that have already been extensively tested in human neonates in diuresis studies. Specifically, pharmacological inhibition of NKCC1 by bumetanide (1) produced a negative shift in E_{GABA}, (2) inhibited GABA-dependent synchronous excitatory activity in the immature hippocampus, (3) suppressed interictal and ictal-like activity in immature hippocampal slices in vitro, and (4) attenuated kainate-induced seizure activity in vivo in neonatal rats. These anticonvulsant effects of bumetanide were shown to be specific, as they (1) were achieved at low doses that selectively block NKCC1, (2) were blocked by antagonists of the $GABA_A$ receptor (indicating that bumetanide is acting through a $GABA_A$ receptor-associated signaling pathway), (3) did not affect epileptiform activity in brain slices from *NKCC1*$^{-/-}$ mice (indicating that inhibition of NKCC1 is the mechanism by which bumetanide exerts its GABA-dependent anticonvulsant effects), and (4) did not depress epileptiform activity in mature neurons (where the expression of NKCC1 is less than 10% of that in neonatal tissue).[22]

Given these promising findings, it seemed reasonable to combine bumetanide, which blocks the excitatory effect of GABA in immature neurons by decreasing $[Cl^-]_i$, with phenobarbital, a GABA agonist that opens $GABA_A$ receptor-associated chloride channels. Theoretically, such an increase in GABA-mediated conductance in neurons already targeted by bumetanide would serve to increase shunting inhibition and maximize the anticonvulsant power of the GABA system.

The efficacy of bumetanide, in combination with the GABA-enhancing anticonvulsant phenobarbital, was tested for the treatment of recurrent tonic-clonic epileptiform activity in the intact immature hippocampus in vitro.[35] In this study, a low-magnesium model of neonatal seizures in the intact immature hippocampal formation in vitro was employed. Such a model has the benefits of not altering the energy gradient for NKCC1-mediated cation-chloride transport, as well as preserving longitudinal intrahippocampal connections. Recurrent seizures were induced in the intact hippocampal preparation by a continuous 5 h exposure to low-magnesium solution, and the anticonvulsant efficacy of phenobarbital, bumetanide, and the combination of these drugs was then studied. While phenobarbital failed to abolish or depress recurrent seizures in 70% of immature hippocampi, phenobarbital *in combination with bumetanide* abolished seizures in 70% of immature hippocampi and significantly reduced the frequency, duration, and power of seizures in the remaining 30% of immature hippocampi. Taken together, the results of these in vitro and in vivo studies suggested that bumetanide, alone or in combination with other drugs such as phenobarbital, might be useful in the treatment of neonatal seizures in humans.[22,35]

Electrographic neonatal seizures frequently have no clinical manifestations, a phenomenon referred to as *electroclinical dissociation* or *uncoupling*.[29,30] Phenobarbital, an allosteric modulator of $GABA_A$ receptors, is the drug of choice for treating neonatal seizures. Video-EEG studies have demonstrated that phenobarbital inhibits electrographic seizure activity much less effectively than clinically apparent convulsive activity. This differential efficacy exacerbates electroclinical dissociation so that the incidence of electroclinical dissociation in neonates is 80% after anticonvulsive treatment.[29,38] The ontogeny of KCC2 mRNA expression follows a caudal-rostral pattern. Spinal cord and subcortical neurons begin to express KCC2 early during embryogenesis, while KCC2 expression in cortical neurons increases after birth.[8,9] These expression patterns of NKCC1 and KCC2 suggests that at birth, GABA should have a more inhibitory effect in spinal and subcortical neurons compared to cortical neurons, but there is no direct

evidence for a differential effect of GABA on cortical versus subcortical structures.

To gain insight into the mechanisms of electroclinical dissociation and its exacerbation by phenobarbital, Glykys et al.[39] tested the hypotheses that the neocortex and subcortical structures have different $[Cl^-]_i$ during postnatal development using the genetically expressed Cl-sensitive dual-wavelength fluorescent protein Clomeleon. They then tested whether these regions have oppositely directed responses to $GABA_A$ receptor agonists and phenobarbital, and whether bumetanide, an NKCC1 blocker, preferentially alters the responses to GABA in cortical areas expressing high levels of NKCC1. Experiments revealed that (1) $[Cl^-]_i$ varies substantially between neighboring neurons in both the developing thalamus and neocortex, but the average $[Cl^-]_i$ is significantly lower in thalamic versus cortical neurons; (2) phenobarbital is an effective anticonvulsant in the thalamus but not in the neocortex due to a net inhibitory effect of GABA in the thalamus but an excitatory effect in the neocortex; and (3) the combination of bumetanide and phenobarbital is effective in decreasing epileptiform activity in the neocortex, while it is not different from phenobarbital alone in the thalamus. These results supported the idea that caudal-rostral $[Cl^-]_i$ maturation determines neuronal responses to GABA, and therefore the effects of allosteric modulators of GABA receptor function, and supported the hypothesis that differences in $[Cl^-]_i$ comprise a candidate mechanism of electroclinical dissociation of neonatal seizures and the exacerbation of dissociation by GABAergic anticonvulsants.[39]

Dzhala et al. considered the possibility that *activity-dependent*, NKCC1-dependent changes in E_{GABA} help determine the time course of neonatal seizures, explaining the substantial variations in the efficacy of GABAergic anticonvulsants in different reported experimental settings.[36] This hypothesis is of considerable clinical importance because it provides an additional impetus for immediate and aggressive treatment of neonatal seizures: the more seizures that occur, the more E_{GABA} will shift, and the lower the probability that the seizures will respond to available anticonvulsants such as phenobarbital, particularly during the crescendo phase of neonatal seizures.[33] A corollary of this hypothesis is that, by blocking NKCC1, bumetanide should prevent or reduce the seizure-induced shift in E_{GABA}, potentially ameliorating the crescendo pattern of neonatal seizures. Finally, enhancement of the efficacy of GABAergic anticonvulsants by bumetanide should increase with the duration of the previous seizure activity.

These hypotheses were tested in whole hippocampal preparations from neonatal rats and CLM-1 mice expressing Clomeleon.[36] Recurrent seizures were shown to increase progressively the intracellular chloride concentration $[Cl^-]_i$ assayed by Clomeleon imaging and invert the net effect of $GABA_A$ receptor activation from inhibition to excitation assayed by the frequency of action potentials and intracellular Ca^{2+} transients. These changes correlated with increasing frequency of seizure-like events and reduction in phenobarbital efficacy. Bumetanide inhibited seizure-induced neuronal chloride accumulation and the consequent facilitation of recurrent seizures. Together, these results suggest that seizure activity leads to $[Cl^-]_i$ accumulation, thereby increasing the probability of subsequent seizures and providing a potential mechanism for the early crescendo phase of neonatal seizures.[36]

TARGETING NKCC1 AND OTHER CCCS AS AN ANTIEPILEPTIC STRATEGY

At low concentrations (2–10 μM), bumetanide is a specific inhibitor of NKCC1 and has well-established pharmacokinetic and pharmacodynamic properties in adult humans with few side effects (reviewed in ref. 40). Because the expression patterns of NKCC1 during development are similar in the human and rat cortex, bumetanide might be useful for the treatment of seizures in human neonates. Bumetanide has been extensively used in both healthy and critically ill human term and preterm infants to treat fluid volume overload due to cardiac and/or pulmonary disease, with few side effects other than minor electrolyte imbalances, so extrapolation from these studies might help guide the design of any potential pilot studies or clinical trials.[26] However, it will be important to investigate whether the pharmacokinetics of bumetanide are altered by any of the

underlying diseases that are responsible for triggering seizures in neonates, because many term newborns with refractory seizures have hypoxic-ischemic encephalopathy from perinatal asphyxia, which is often accompanied by multiorgan dysfunction (including hepatic and renal failure); such organ dysfunction can dramatically affect drug metabolism.

Perhaps the best new treatment strategy for neonatal seizures, and one that could be easily tested, is a combination regimen that would include bumetanide with a barbiturate like phenobarbital. In the neonatal rat brain, phenobarbital has been rendered a more effective anticonvulsant by coadministering it with bumetanide, which reverses the chloride gradient in immature neurons to a level such that $GABA_A$ receptor potentiation by phenobarbital results in synaptic inhibition. If such a trial were to take place, neonates with persistent seizures despite an initial loading dose of phenobarbital (the current standard of care) could be offered bumetanide along with the second dose of phenobarbital. Continuous EEG monitoring of patients could then be used to determine whether bumetanide reduces seizures compared with controls (i.e., those neonates treated with phenobarbital alone). Pilot studies of the efficacy of bumetanide, administered with phenobarbital, for the treatment of neonatal seizures, are now underway (FDA IND #101690; see http://www.cureepilepsy.org/research/current.asp).

The combination of bumetanide and a barbiturate should obviate the need to use the high doses of barbiturate or benzodiazepine that have been associated with significant side effects, such as apoptotic neurodegeneration in the developing brain and late cognitive/behavioral impairment.[4,5,22] Moreover, because of bumetanide's long-standing safe use in newborns as a diuretic, the low doses that are required to inhibit NKCC1 are not anticipated to produce short- or long-term side effects. However, caution must be exercised as work proceeds, and studies should be done to determine any potential side effects of inhibiting NKCC1 in the neonatal nervous system, since GABA-mediated excitation is important for neuronal development.[1] To date, bumetanide-mediated inhibition of NKCC1 in the brain, for periods of time that would far exceed the duration that would be used for the treatment of neonatal seizures, has been shown to have few developmental side effects. These modest but measurable side effects must be weighed against the well-known detrimental effects of persistent seizures in the immature brain on cortical development and the absence of knowledge regarding the developmental effects of NKCC1 inhibition in the setting of such seizures. Recently, bumetanide treatment was reported to decrease seizure duration and frequency in a 6-week-old baby girl with anticonvulsant-resistant status epilepticus secondary to meningitis.[41]

Brain-derived neurotrophic factor (BDNF), released upon seizure-like activity, causes downregulation of KCC2 that results in a decreased chloride extrusion capacity of neurons that propagates further seizure episodes.[42] Because inhibition of BDNF action via blockage of the TrkB receptor reverses depolarizing shifts in E_{GABA}, inhibitors of TrkB, by increasing the endogenous expression of KCC2, might help treat different seizure syndromes. Additionally, targeting the volume/chloride-sensitive regulatory kinases of the CCCs, the WNKs or SPAK/OSR1, might have utility. The role of these kinases in the mammalian nervous system is only beginning to be explored; however, in vitro experiments in mammalian cells and in vivo experiments in lower organisms suggest that these serine-threonine kinases—initially characterized in the kidney—might prove relevant to the physiology and pathophysiology of neuronal chloride transport, GABA signaling, and the origin and treatment of seizures.

SUMMARY

The incidence of seizures is higher in the immediate postnatal period than at any other age. The presence of seizures during this time period is a powerful predictor of long-term cognitive and developmental impairment. Electrographic neonatal seizures are notoriously resistant to phenobarbital and other allosteric modulators of ionotropic $GABA_A$ receptors, making their treatment difficult with the existing antiepileptic agents. Better drugs could be designed with an improved understanding of the pathophysiology underlying seizure activity in neonates and their resistance to GABAergic anticonvulsants (Fig. 82–1). The opening of $GABA_A$ receptors gates a transmembrane current that

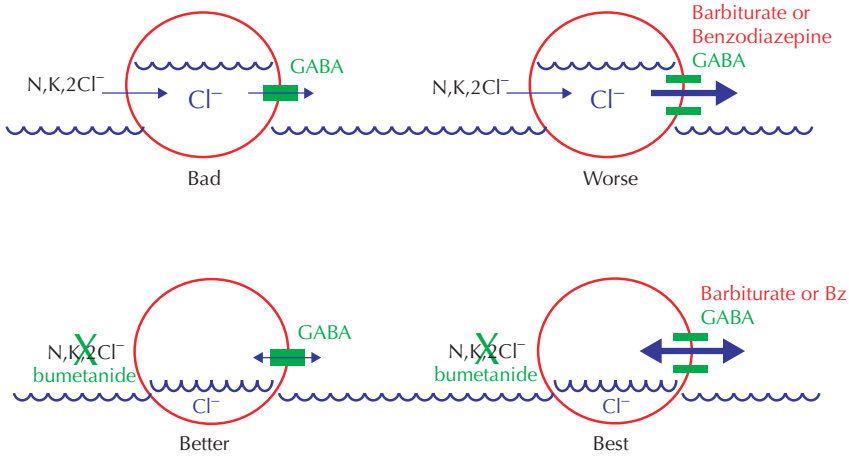

Figure 82–1. Anticonvulsant strategies for neonatal seizures. Top left: NKCC1 accumulates intracellular Cl^- in immature neurons. Neurons with high intracellular Cl^- levels will lose negatively charged chloride ions when GABA binds to $GABA_A$ receptors, opening a channel that is selectively permeable to anions. This loss of negative charge depolarizes neurons and may initiate excitatory processes such as action potentials and calcium waves. Top right: Allosteric modulators of the $GABA_A$ receptor such as anticonvulsant barbiturates and benzodiazepines increase the open probability of the anion channel, resulting in increased Cl^- efflux, increased depolarization, and increased probability of initiating frankly excitatory processes. Bottom left: Blocking NKCC1 is a better anticonvulsant strategy, because it blocks Cl^- accumulation and the net loss of Cl^- when the $GABA_A$ receptor channel opens. Although the neuron is not hyperpolarized by $GABA_A$ receptor activations, membrane conductance is increased, reducing the probability of initiating action potentials or calcium waves. Bottom right: the best anticonvulsant strategy may be to block NKCC1, thereby removing chloride accumulation and increasing the open probability of the $GABA_A$ receptor. This maximizes the increase in membrane conductance initiated by GABA binding and minimizes Cl^- loss that might otherwise initiate action potentials.

is carried by anions. The current's direction and effect (excitation or inhibition) depend on the neuron's resting membrane potential (RMP) and its relation to the reversal potential for GABA (E_{GABA}), which is primarily determined by the intracellular concentration of chloride $[Cl^-]_i$. If E_{GABA} is negative relative to the RMP, GABA will trigger Cl^- influx, membrane hyperpolarization, and neuronal inhibition; if E_{GABA} is positive relative to the RMP, GABA can trigger Cl^- efflux, membrane depolarization, and neuronal excitation. Relative to adult neurons, the $[Cl^-]_i$ of neurons in the cortex of seizing neonates is about 30 mM higher due to robust activity of the chloride-importing, bumetanide-sensitive Na-K-2Cl cotransporter NKCC1 and low activity of the chloride-exporting K-Cl cotransporter KCC2. An ontological switch from NKCC1 to KCC2 activity at around 2 weeks postnatally underlies the switch from excitatory to inhibitory GABA neurotransmission due to a reversal of Cl^- gradients in neocortical neurons. Though important for neuronal development and synaptogenesis, the excitatory actions of GABA in neonates lower the seizure threshold and might explain the poor EEG response to GABAergic anticonvulsants such as phenobarbital and benzodiazepines in this age group. Recent studies show that bumetanide, a diuretic that inhibits NKCC1, modulates Cl^- transport sufficiently to cause a more negative E_{GABA} in immature neurons, rendering the widely used antiepileptic drug phenobarbital effective in neonatal rats. This demonstration of rational anticonvulsant polypharmacy in animals has served as the basis for two double-blind, randomized, placebo-controlled multicenter trials testing the efficacy of phenobarbital and bumetanide in the treatment of neonatal seizures.

DISCLOSURE STATEMENT

The authors have no conflicts of interest.

REFERENCES

1. Ben-Ari Y. Excitatory actions of GABA during development: the nature of the nurture. *Nat Rev Neurosci*. 2002;3:728–739.

2. Staley KJ, Mody I. Shunting of excitatory input to dentate gyrus granule cells by a depolarizing $GABA_A$ receptor-mediated postsynaptic conductance. *J Neurophysiol*. 2003;68:197–212.
3. Delpire E. Cation-chloride cotransporters in neuronal communication. *News Physiol Sci*. 2002;15:309–312.
4. Yamada J, Okabe A, Toyoda H, Kilb W, Luhmann HJ, Fukuda A. Cl^- uptake promoting depolarizing GABA actions in immature rat neocortical neurones is mediated by NKCC1. *J Physiol* (*Lond*). 2004;557:829–841.
5. Zhu L, Lovinger D, Delpire E. Cortical neurons lacking KCC2 expression show impaired regulation of intracellular chloride. *J Neurophysiol*. 2005;93:1557–1568.
6. Gamba G. Molecular physiology and pathophysiology of electroneutral cation-chloride cotransporters. *Physiol Rev*. 2005;85:423–493.
7. Kahle KT, Rinehart J, Ring A, Gimenez I, Gamba G, Hebert SC, Lifton RP. WNK protein kinases modulate cellular Cl^- flux by altering the phosphorylation state of the Na-K-Cl and K-Cl cotransporters. *Physiology* (*Bethesda*) 2006;21:326–335.
8. Wang DD, Kriegstein AR. Blocking early GABA depolarization with bumetanide results in permanent alterations in cortical circuits and sensorimotor gating deficits. *Cereb Cortex*. 2011;21:574–587.
9. Stein V, Hermans-Borgmeyer I, Jentsch TJ, Hübner CA. Expression of the KCl cotransporter KCC2 parallels neuronal maturation and the emergence of low intracellular chloride. *J Comp Neurol*. 2004;468:57–64.
10. Wilson FH, Disse-Nicodème S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP. Human hypertension caused by mutations in WNK kinases. *Science*. 2001;293: 1107–1112.
11. Shekarabi M, Girard N, Rivière JB, Dion P, Houle M, Toulouse A, Lafrenière RG, Vercauteren F, Hince P, Laganiere J, Rochefort D, Faivre L, Samuels M, Rouleau GA. Mutations in the nervous system—specific HSN2 exon of WNK1 cause hereditary sensory neuropathy type II. *J Clin Invest*. 2008;118:2496–2505.
12. Delpire E, Gagnon KB. SPAK and OSR1: STE20 kinases involved in the regulation of ion homeostasis and volume control in mammalian cells. *Biochem J*. 2008;409:321–331.
13. Kahle KT, Ring AM, Lifton RP. Molecular physiology of the WNK kinases. *Annu Rev Physiol*. 2008;70:329–355.
14. Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*. 1999;397: 251–255.
15. Ganguly K, Schinder AF, Wong ST, Poo M. GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell*. 2001;105:521–532.
16. Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H. GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature*. 2006;439:589–593.
17. Liu Z, Neff RA, Berg DK. Sequential interplay of nicotinic and GABAergic signaling guides neuronal development. *Science*. 2006;314:1610–1613.
18. Cancedda L, Fiumelli H, Chen K, Poo MM. Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. *J Neurosci*. 2007;27: 5224–5235.
19. Lee H, Chen CX, Liu YJ, Aizenman E, Kandler K. KCC2 expression in immature rat cortical neurons is sufficient to switch the polarity of GABA responses. *Eur J Neurosci*. 2005;21:2593–2599.
20. Plotkin MD, Snyder EY, Hebert SC, Delpire E. Expression of the Na-K-2Cl cotransporter is developmentally regulated in postnatal rat brains: a possible mechanism underlying GABA's excitatory role in immature brain. *J Neurobiol*. 1997;33:781–795.
21. Reynolds A, Brustein E, Liao M, Mercado A, Babilonia E, Mount DB, Drapeau P. Neurogenic role of the depolarizing chloride gradient revealed by global overexpression of KCC2 from the onset of development. *J Neurosci*. 2008;28:1588–1597.
22. Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, Delpire E, Jensen FE, Staley KJ. NKCC1 transporter facilitates seizures in the developing brain. *Nat Med*. 2005;11:1205–1213.
23. Hübner CA, Stein V, Hermans-Borgmeyer I, Meyer T, Ballanyi K, Jentsch TJ. Disruption of KCC2 reveals an essential role of K-Cl cotransport already in early synaptic inhibition. *Neuron*. 2001;30:515–524.
24. Isenring P, Forbush B. Ion transport and ligand binding by the Na-K-Cl cotransporter, structure-function studies. *Comp Biochem Physiol A Mol Integr Physiol*. 2001;130:487–497.
25. Hannaert P, Alvarez-Guerra M, Pirot D, Nazaret C, Garay RP. Rat NKCC2/NKCC1 cotransporter selectivity for loop diuretic drugs. *Naunyn Schmiedebergs Arch Pharmacol*. 2002;365:193–199.
26. Sullivan JE, Witte MK, Yamashita TS, Myers CM, Blumer JL. Pharmacokinetics of bumetanide in critically ill infants. *Clin Pharmacol Ther*. 1996;60:405–413.
27. Lopez-Samblas AM, Adams JA, Goldberg RN, Modi MW. The pharmacokinetics of bumetanide in the newborn infant. *Biol Neonate*. 1997;72:265–272.
28. Jensen FE. Neonatal seizures: an update on mechanisms and management. *Clin Perinatol*. 2009;36: 881–900.
29. Connell J, Oozeer R, de Vries L, Dubowitz LM, Dubowitz V. Clinical and EEG response to anticonvulsants in neonatal seizures. *Arch Dis Child*. 1989;64: 459–464.
30. Boylan GB, Rennie JM, Pressler RM, Wilson G, Morton M, Binnie CD. Phenobarbitone, neonatal seizures, and video-EEG. *Arch Dis Child Fetal Neonat Ed*. 2002;86:F165–F170.
31. Murray DM, Boylan GB, Fitzgerald AP, Ryan CA, Murphy BP, Connolly S. Persistent lactic acidosis in neonatal hypoxic-ischaemic encephalopathy correlates with EEG grade and electrographic seizure burden. *Arch Dis Child Fetal Neonatal Ed*. 2008;93: F183–F186.
32. Booth D, Evans DJ. Anticonvulsants for neonates with seizures. *Cochrane Database Syst Rev*. 2004;18: CD004218.
33. Painter MJ, Scher MS, Stein AD, Armatti S, Wang Z, Gardiner JC, Paneth N, Minnigh B, Alvin J. Phenobarbital compared with phenytoin for the treatment of neonatal seizures. *N Engl J Med*. 1999; 341: 485–489.

34. Farwell JR, Lee YJ, Hirtz DG, Sulzbacher SI, Ellenberg JH, Nelson KB. Phenobarbital for febrile seizures—effects on intelligence and on seizure recurrence. *N Engl J Med*. 1990;322:364–369.
35. Dzhala VI, Brumback AC, Staley KJ. Bumetanide enhances phenobarbital efficacy in a neonatal seizure model. *Ann Neurol*. 2008;63:222–235.
36. Dzhala VI, Kuchibhotla KV, Glykys JC, Kahle KT, Swiercz WB, Feng G, Kuner T, Augustine GJ, Bacskai BJ, Staley KJ. Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. *J Neurosci*. 2011;30:11745–11761.
37. Payne JA, Rivera C, Voipio J, Kaila K. Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci*. 2003;26: 199–206.
38. Scher MS, Alvin J, Gaus L, Minnigh B, Painter MJ. Uncoupling of EEG-clinical neonatal seizures after antiepileptic drug use. *Pediatr Neurol.* 2003; 28: 277–280.
39. Glykys J, Dzhala VI, Kuchibhotla KV, Feng G, Kuner T, Augustine G, Bacskai BJ, Staley KJ. Differences in cortical versus subcortical GABAergic signaling: a candidate mechanism of electroclinical uncoupling of neonatal seizures. *Neuron.* 2009;63:657–672.
40. Kahle KT, Staley KJ. The bumetanide-sensitive Na-K-2Cl cotransporter NKCC1 as a potential target of a novel mechanism-based treatment strategy for neonatal seizures. *Neurosurg Focus.* 2008;25:E22–E27.
41. Kahle KT, Barnett SM, Sassower KC, Staley KJ. Decreased seizure activity in a human neonate treated with bumetanide, an inhibitor of the Na^+-K^+-$2Cl^-$ cotransporter NKCC1. *J Child Neurol*. 2009;24: 572–576.
42. Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, Kokaia Z, Airaksinen MS, Voipio J, Kaila K, Saarma M. BDNF-induced TrkB activation down-regulates the K^+-Cl^- cotransporter KCC2 and impairs neuronal Cl^- extrusion. *J Cell Biol*. 2002;159:747–752.

Chapter 83

Antiepileptogenesis, Plasticity of AED Targets, Drug Resistance, and Targeting the Immature Brain

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Yoel Yaari

INTRODUCTION

The cellular basis of epileptic seizures consists of high-frequency, synchronized discharges of neuronal ensembles. The ultimate goal of all antiepileptic therapies is to prevent the occurrence of such episodes or to substantially attenuate their severity. To this end, a multitude of antiepileptic compounds have been developed that are currently in clinical use. However, seizures remain uncontrolled by carefully monitored drug treatment in a substantial portion (~30%) of epilepsy patients. Therefore, a better understanding of the mode of action of different antiepileptic drugs is mandatory, along with an improved understanding of why these compounds fail in some epilepsy patients, with the ultimate goal of developing new therapeutic avenues.

So far, two hypotheses have been advanced to account for the cellular basis of pharmacoresistance in chronic epilepsy. The first hypothesis proposes that pharmacoresistance involves an upregulation of multidrug transporters at

the blood-brain barrier. This upregulation limits the access of antiepileptic drugs to the brain parenchyma and therefore leads to a reduced drug concentration at the respective drug target. Because multidrug transporter proteins are of central importance to this hypothesis, it has been termed the *transporter hypothesis*. The second hypothesis contends that the molecular targets of antiepileptic drugs are modified in chronic epilepsy. Consequently, they are less sensitive to these compounds. This hypothesis has been named the *target hypothesis*.[1,2] Clearly, these two hypotheses are not mutually exclusive. Rather, the underlying mechanisms may coexist and perhaps even act in synergy. The subject of this chapter is the target hypothesis. We describe the molecular mechanisms that alter the targets of antiepileptic drugs and how these mechanisms may interact with altered drug transporter function to cause pharmacoresistance.

DEFINING DRUG TARGETS

In general, antiepileptic drug targets are proteins involved in neuronal function that mediate a reduction in excitability after binding the antiepileptic drug at clinically relevant concentrations. A plethora of drug targets has been identified in central neurons (see Table 83–1). Most of these targets are neurotransmitter receptors or voltage-gated ion channels. A comprehensive review of the different ion channel types that are affected by antiepileptic drugs is beyond the scope of this review. However, a number of classes of ion channels are particularly prominent targets.

Sodium Channels

Voltage-gated sodium channels are implicated in the mode of action of a large number of common antiepileptic drugs, such as phenytoin, carbamazepine, valproate, lamotrigine, lacosamide, and eslicarbazepine.[3–5] These channels are found in all excitable cells and are capable of extremely rapid channel gating that mediates millisecond-scale physiological processes.[6] They open rapidly upon membrane depolarization, causing the upstroke of the action potential, and subsequently undergo fast inactivation, thus contributing to its downstroke. Upon repolarization of the cell membrane, sodium channels recover from inactivation with a complex time course. In many cells, however, a noninactivating, persistent component of the sodium current is also observed in addition to the rapidly inactivating component. This current component activates even during subthreshold depolarizations and therefore is important in controlling subthreshold activity. Both the transient and persistent components of the sodium current are potently inhibited by antiepileptic drugs, such as phenytoin, carbamazepine, and lamotrigine, in addition to other drugs. A characteristic effect of most of these sodium channel blockers is that they preferentially bind to channels that have entered an inactivated state following depolarization of the cell. In addition, a second major mechanism is that the recovery from depolarization-induced inactivation is prolonged by many antiepileptic drugs.[4,5] These two effects together explain why these drugs preferentially block repetitive high-frequency neuronal activity and long-lasting depolarizations, both of which typically occur during epileptic seizures.

Calcium Channels

Voltage-gated calcium channels are also targets for numerous antiepileptic drugs. Central nervous system (CNS) neurons express multiple types of calcium channels, which can be separated into two classes based on their biophysical properties, namely, high-threshold and low-threshold calcium channels.[7] A number of antiepileptic drugs have been shown to inhibit high-threshold calcium channels in native neurons at high therapeutic concentrations,[8–10] and this has been proposed to inhibit neurotransmitter release via a reduction of presynaptic action potential-induced calcium increases.[11] Some antiepileptic drugs potently inhibit low-threshold (also known as *T-type*) calcium channels, which are expressed in postsynaptic compartments,[12–14] and powerfully control postsynaptic excitability and the propensity to generate burst discharges.[15] Intriguingly, the antiepileptic drug gabapentin has been shown to exhibit strong and specific binding to the accessory calcium channel $\alpha 2\delta$ subunit,[16] but this binding seems to have no effect on the

Table 83–1 **Drug Targets for Common Antiepileptic Drugs**

	Voltage-Gated Ion Channels					Neurotransmission		
	I_{NaT}	I_{NaP}	I_{Ca}	I_K	I_H	GABA	Glu	Presynaptic.
Primarily targeting voltage-gated ion channels								
Phenytoin	+	+	+	+				
Carbamazepine	+		+					
Oxcarbazepine	+		+					
Lamotrigine	+	+	+	+	+			
Valproic acid	+	+/–	+			+		
Losigamone		+						
Retigabine				+				
Zonisamide	+		+					
Ethosuximide	–	+	+	+				
Mixed mechanism								
Felbamate	+		+			+	+	
Topiramate	+	+	+	+		+	+	
Primarily affecting neurotransmitter receptors, release or metabolism								
Levetiracetam	–	–	+	+				+ (SV2A)
Phenobarbital			+			+		
Benzodiazepine						+		
Vigabatrin						+		
Tiagabin						+		
Other								
Gabapentin, primary target alpha2delta-1 subunit	–	–	–	–	+	–	–	–

I_{NaP}, persistent sodium current; I_{NaT}, transient sodium current; I_{Ca}, calcium currents; I_K, voltage-gated potasium currents; I_H, H-current; GABA, activity on the GABAergic system; Glu, activity on the glutamatergic system.

functional properties of the channel complex, suggesting that gabapentin binding to these subunits exerts effects that are not dependent on calcium channel modulation.[17]

HCN Channels

Hyperpolarization-activated, cyclic nucleotide-gated (HCN) cation channels, responsible for generating the so called H-current, are cation-permeable channels that are activated by hyperpolarization and deactivate upon depolarization of the membrane potential.[18,19] H-currents modulate membrane resistance and resting potential, and can mediate pacemaker activity in some types of neurons due to their particular biophysical properties.[19] H-currents, and their corresponding HCN channel subunits, appear to be located mainly in dendrites.[20–22] Accordingly, they potently modify dendritic integration of excitatory input.[22,23] Interestingly, dendritic H-currents are potentiated by application of the antiepileptic drugs lamotrigine or gabapentin.[24,25] Thus, H-currents appear to be dendritic antiepileptic drug targets.

$GABA_A$ Receptors

A further group of antiepileptic drugs seems to exert its main effects via an increase in synaptic inhibition. Gamma-aminobutyric acid (GABA) is the predominant inhibitory neurotransmitter in the adult brain. The ionotropic $GABA_A$ receptor, which conducts chloride upon the binding of GABA, is an important target for antiepileptic drugs. Gamma-aminobutyric acid A receptor-active drugs include direct modulators such as benzodiazepines and barbiturates, which increase the $GABA_A$ receptor-mediated chloride currents via allosteric modulation.[26] A number of additional antiepileptic drugs indirectly enhance $GABA_A$ receptor-mediated action by inhibiting the reuptake of GABA or its catabolism. This class of compounds includes tiagabine and vigabatrin. Tiagabine inhibits the high-affinity GABA transporter

GAT1 that normally terminates synaptic action of GABA via rapid uptake into astrocytes, whereas vigabatrin is a GABA analog that inhibits GABA transaminase, one of the main GABA-degrading enzymes in the brain. Both compounds are capable of causing large elevations in brain GABA levels.

Glutamate Receptors

A number of newer antiepileptic drugs exert their action primarily or in part by reducing the activity of excitatory neurotransmitter receptors, namely, glutamate receptors. Felbamate exerts complex effects on the *N*-methyl-D-aspartate (NMDA) receptor, perhaps by interacting with the modulatory glycine-binding site of this receptor.[27–30] Some of the effects of felbamate have been shown to be dependent upon NMDA receptor subunit composition.[31,32] Topiramate,[33] as well as a number of compounds currently in clinical trials,[34] have been shown to reduce excitatory synaptic transmission via an inhibition of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors.

Presynaptic Proteins

Recently, the presynaptic vesicle protein SV2A has been identified as a target for the antiepileptic levetiracetam. More intriguingly, SV2A seems to be the only high-affinity receptor for levetiracetam in the rodent brain.[35] The SV2A protein is a vesicular component of the complex presynaptic release machinery and inhibits presynaptic neurotransmitter release in a use-dependent manner. Studies in knockout mice have suggested that SV2A is involved in regulating the release probability of quiescent neurons, either via protein interactions that facilitate vesicle priming or inhibit depriming or by transporting a protein involved in priming.[36–39] However, the precise effects of levetiracetam binding to SV2A, and how this in turn affects neurotransmitter release, are still unknown.

Unconventional Drug Targets

It should be emphasized that many of the antiepileptic drugs discussed so far show multiple modes of action. It is also very probable that the list of drug targets will have to be expanded. Research on drug targets has hitherto focused on voltage-gated or neurotransmitter-gated receptor ion channels, which are the most easily investigated drug targets in a quantitative manner. Studies of alternative drug targets that control processes on a slower time scale (e.g., neuroendocrine processes or neuronal structural and functional plasticity) have been conducted more rarely, likely because they are more technically demanding and labor intensive. One intriguing study has identified the calcium channel subunit α2δ-1 as a receptor for thrombospondin.[17] Thrombospondin is an astrocyte-secreted protein that promotes synaptogenesis via an α2δ-1 interaction. Intriguingly, gabapentin antagonizes thrombospondin binding to α2δ-1 and powerfully inhibits excitatory synapse formation in vitro and in vivo.[17] These findings were the first to identify a specific drug target that may act by inhibiting pathological structural plasticity. It should also be noted that studies concerning mechanisms of antiepileptic drug action and refractoriness have so far focused mostly on drug targets in neurons while neglecting many putative targets in glia. Glial function may also be affected by common antiepileptic drugs.[40]

ALTERED PHARMACOLOGY AND MOLECULAR PLASTICITY OF DRUG TARGETS IN CHRONIC EPILEPSY

In a number of cases, our knowledge of the cellular mechanisms of action of antiepileptic drugs has fueled studies aimed at finding whether these drug actions are altered in chronically epileptic tissue. Indeed, many drug targets undergo plastic changes on the molecular level in epilepsy models and probably also in human epilepsy. In this section, we will summarize what is known about epilepsy-associated modifications of drug targets.

Altered Pharmacology of Voltage-Gated Sodium Channels

Reduced activity of the antiepileptic drug carbamazepine on the transient sodium current in

dentate granule cells of the hippocampus has been shown in vitro, both in patients with pharmacoresistant epilepsy and in the pilocarpine model of temporal lobe epilepsy. This loss of activity was selective for the use-dependent or frequency-dependent sodium channel blocking effects of carbamazepine; other effects of this drug were not altered.[41] A similar but weaker effect was seen in pyramidal cells in the CA1 subregion of the hippocampus.[42] Intriguingly, in the pilocarpine model, phenytoin showed a similar but much less pronounced loss of its use- and voltage-dependent sodium channel blocking activity. In contrast, the use- and voltage-dependent blocking effects of lamotrigine and valproate were unchanged.[43] Likewise, data from both epilepsy patients and the kindling models of epilepsy suggest that valproate's effects on Na^+ channels are unaltered in epileptic tissue.[44,45]

These data indicate that the molecular mechanisms of pharmacoresistance likely are drug specific, so that changes in sodium channel properties affecting the efficacy of one antiepileptic drug, such as carbamazepine, are less relevant for other drugs interacting with the same channel.

The molecular mechanisms leading to the selective loss of use-dependent sodium channel block by carbamazepine, while other actions of carbamazepine on these channels remain untouched, are completely unclear. One potential molecular mechanism is downregulation of accessory β subunits shown to occur in epilepsy models.[46,47] However, this mechanism is unlikely to be relevant, because mice lacking either β^1 or β^2 subunits display undiminished use- and frequency-dependent block of sodium channels by carbamazepine.[48] A number of additional changes in the subunit composition of sodium channels have been discovered in epilepsy that may account for the reduced efficacy of carbamazepine.[49] Further possibilities are alternative splicing of sodium channels or the reexpression of neonatal isoforms of sodium channels.[50] In addition, posttranscriptional changes in sodium channel properties may alter the sensitivity of drug targets. For instance, there is evidence that phosphorylation of sodium channels alters the efficacy of some antiepileptic drugs in suppressing the persistent sodium current component.[51]

Loss of ion channel drug sensitivity occurs not only in the pilocarpine model of chronic epilepsy. A diminished effect of carbamazepine has also been observed on the steady-state inactivation properties of Na^+ channels in CA1 neurons from kindled animals.[45]

Plasticity of Calcium Channels: Emergence of a Novel Drug Target?

The altered expression of calcium channels and their functional consequences have been studied extensively in chronic epilepsy. So far, these studies have yielded no clear evidence of altered Ca^{2+} channel pharmacology in chronic epilepsy or of the formation of pharmacoinsensitive channels. However, in the pilocarpine model of epilepsy, the T-type calcium current was shown to be upregulated in CA1 pyramidal cells, at least during the early stage of epileptogenesis, causing the conversion of these ordinarily regularly firing neurons into aberrantly burst-firing neurons.[52,53] An upregulation of T-type calcium current was shown to result from a transcriptional upregulation of $Ca_v3.2$ calcium channel subunits and was critical for the development of the epileptic condition.[54] This finding suggests that $Ca_v3.2$ may constitute a novel drug target that emerges during epileptogenesis. However, studies so far have yielded no clear evidence of altered calcium channel pharmacology or of the formation of pharmacoresistant calcium channels in chronic epilepsy.

Plasticity of H-Current Expression: Loss of a Drug Target?

In chronic epilepsy, dramatic changes in the magnitude of dendritic H-currents, and expression of the underlying HCN subunits, particularly in pyramidal cells, have been reported in chronic epilepsy models.[55–57] Given that HCN channels are likely a major target in the antiepileptic action of lamotrigine and gabapentin, it is tempting to speculate that their downregulation causes pharmacoresistance to these drugs.

Altered Subunit Composition of $GABA_A$ Receptors

An enormous diversity of $GABA_A$ receptors has been reported in the CNS, reflecting the fact that in each receptor at least three different subunits are present, deriving from one of

eight structurally distinct and genetically distinct families.[58,59] An elegant study by Brooks-Kayal et al. has shown that $GABA_A$ receptor subunit composition is specifically altered in epilepsy models, and that this alteration is associated with reduced activity of $GABA_A$ receptor agonists.[60] Whether these changes are pertinent for pharmacoresistance to antiepileptic drugs acting on GABAergic inhibition remains to be explored. It is noteworthy that neither the efficacy of GABA uptake nor the sensitivity of the GABA transporter GAT-1 to tiagabine is altered in chronic experimental epilepsy.[61]

Increasing evidence suggests that the actions of GABA in epileptic brain tissue, as in immature brain, are not strictly inhibitory.[62] For instance, in the epileptic human subiculum, $GABA_A$ receptor activation in a subset of principal neurons causes depolarization rather than the ordinary hyperpolarization of the membrane potential.[63,64] This change in GABA action is caused by the altered expression of chloride transporters, leading to the intracellular accumulation of chloride.[65] Depolarizing GABAergic action in subsets of neurons would be expected to strongly reduce the antiepileptic activity of drugs acting on synaptic inhibition and thus may contribute to pharmacoresistance. This consideration illustrates a more general point: changes in drug targets have to be considered within the context of other epilepsy-related cellular changes.

RELATIONSHIP BETWEEN CHANGES IN ANTIEPILEPTIC DRUG TARGETS AND IN VIVO PHARMACORESISTANCE

What is the relationship of changes in specific drug targets to the pharmacoresistance observed either in epilepsy patients or in animal models of chronic epilepsy? Addressing this question requires investigators first to assess the antiepileptic efficacy of a drug in vivo in chronic epilepsy models and then to compare it to the drug's efficacy in modifying its putative target. This approach has been implemented in only a few studies. One study used kindled rats separated into two groups, one responsive and the other unresponsive to phenytoin.[66] In these groups, gross differences in the sensitivity of transient sodium currents to suppression by phenytoin were not found.[67] Further studies are needed to compare the two groups with respect to phenytoin's use-dependent sodium current block and its effect on the persistent sodium current. Nevertheless, this experimental approach, which involves comparing the pharmacosensitivity of putative antiepileptic drug targets in pharmacoresponsive versus pharmacoresistent epileptic animals, likely will lead to a better understanding of pharmacoresistance.[68]

A similar approach has been applied in studies of hippocampal tissue resected from carbamazepine-treated epileptic patients separated into pharmacoresistant and pharmacoresponsive groups. Interestingly, the use-dependent block of sodium currents by carbamazepine was evident in CA1 pyramidal cells in the responsive group but not in the resistant group.[41] Furthermore, epileptiform activity induced acutely was strongly inhibited by carbamazepine in hippocampal slices from the responsive group but not in slices from the resistant group.[41,69] Because changes in the blood-brain barrier cannot contribute to pharmacoresistance in these in vitro slice experiments, these findings support the presence of a target mechanism for pharmacoresistance.

RELATIONSHIP BETWEEN THE TARGET AND TRANSPORTER HYPOTHESES OF PHARMACORESISTANCE

Functionally relevant changes in antiepileptic drug targets, as well as in multidrug transporters, occur in chronic epilepsy.[2] To define the relative impact of these two mechanisms for each individual antiepileptic drug is of obvious therapeutic relevance. In the case of carbamazepine, for instance, a target mechanism is well established in human and experimental epilepsy.[41,69] On the other hand, recent studies have revealed that carbamazepine is not transported by numerous human multidrug transporters, including P-glycoprotein and multidrug resistance-associated proteins MRP1, MRP2, and MRP5.[70–72] These results suggest that a target mechanism of pharmacoresistance may be dominant in the case of carbamazepine. On the other hand, a number of other antiepileptic drugs are excellent substrates for the human multidrug transporter

Table 83–2 **Relative Importance of Target and Transporter Mechanisms**

	Transported by Human Multidrug Transporters				Target Mechanism: Reduction of Use-or Voltage-Dependent Block in Epilepsy Models
	MRP1	MRP2	MRP5	PGP	
Carbamazepine	–	–	–	–	Dentate gyrus: +++ [41,69] CA1: +, smaller effects[42]
Phenytoin	–	–	–	+	Dentate gyrus: +, small effects[43] CA1: +, small effects[42]
Lamotrigine	–	–	–	+	Dentate gyrus: no change in use-dependent block, only impaired tonic block[43]
Valproic acid	–	–	–	–	– [43,44,45]

Notes: MRP1, MRP2, MRP5, and PGP denote types of multidrug transporters. The data given for drug transporters (left side of the table) are based on results described for human multidrug transporters.[70–74]

P-glycoprotein 1 (PGP1).[71,73] In the case of phenytoin or lamotrigine, which are transported efficiently by PGP1,[71] target changes in drug sensitivity seem to be less pronounced than for carbamazepine.[42,43,67] These data support the notion that potentially pharmacoresistent mechanisms, and the predominance of either target or transporter mechanisms, have to be evaluated individually for each antiepileptic drug (see Table 83–2).

The therapeutic relevance of these considerations to pharmacoresistent epilepsy is underscored by the fact that a large number of increasingly selective inhibitors exist for multidrug transporters. Comedication of antiepileptic drugs with such compounds may overcome pharmacoresistance, provided that the antiepileptic drug is a substrate of the multidrug transporter and that upregulation of the transporter contributes importantly to pharmacoresistance to this drug. If a dominant target mechanism exists, the efforts to overcome pharmacoresistance should be aimed at designing novel compounds that act on the modified drug targets in the epileptic brain. Thus, a comprehensive understanding of the dominant mechanisms underlying pharmacoresistance to each antiepileptic drug will be required to develop strategies toward new therapeutic options.

DISCLOSURE STATEMENT

H.B. discloses that he has performed contract research for UCB and BIAL. Y.Y. has not disclosed any conflicts of interest.

REFERENCES

1. Heinemann U, Kann O, Remy S, Beck H. Novel mechanisms underlying drug resistance in temporal lobe epilepsy. *Adv Neurol.* 2006;97:85–95.
2. Remy S, Beck H. Molecular and cellular mechanisms of pharmacoresistance in epilepsy. *Brain.* 2006;129: 18–35.
3. Köhling R. Voltage-gated sodium channels in epilepsy. *Epilepsia*. 2002;43:1278–1295.
4. Catterall WA. Molecular properties of brain sodium channels: an important target for anticonvulsant drugs. *Adv Neurol*. 1999;79:441–456.
5. Ragsdale DS, Avoli M. Sodium channels as molecular targets for antiepileptic drugs. *Brain Res Brain Res Rev*. 1998;26:16–28.
6. Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Netter YB, Noda M, Tamkun MM, Waxman SG, Wood JN, Catterall WA. Nomenclature of voltage-gated sodium channels. *Neuron*. 2002;28:365–368.
7. Ertel EA, Campbell KP, Harpold MM, Hofmann F, Mori Y, Perez-Reyes E, Schwartz A, Snutch TP, Tanabe T, Birnbaumer L, Tsien RW, Catterall WA. Nomenclature of voltage-gated calcium channels. *Neuron*. 2000;25:533–535.
8. Schumacher TB, Beck H, Steinhaeuser C, Schramm J, Elger CE. Effects of phenytoin, carbamazepine, and gabapentin on calcium channels in hippocampal granule cells from patients with temporal lobe epilepsy. *Epilepsia*. 1998;39:355–363.
9. Stefani A, Spadoni F, Bernardi G. Gabapentin inhibits calcium currents in isolated rat brain neurons. *Neuropharmacology*. 1998;37:83–91.
10. Stefani A, Spadoni F, Bernardi G. Voltage-activated calcium channels: targets of antiepileptic drug therapy? *Epilepsia*. 1997;38:959–965.
11. Fink K, Meder W, Dooley DJ, Göthert M. Inhibition of neuronal Ca^{2+} influx by gabapentin and subsequent reduction of neurotransmitter release from rat neocortical slices. *Br J Pharmacol*. 2000;130:900–906.
12. Yaari Y, Hamon B, Lux HD. Development of two types of calcium channels in cultured mammalian hippocampal neurons. *Science*. 1987;235:680–682.

13. Gomora JC, Daud AN, Weiergraber M, Perez-Reyes E. Block of cloned human T-type calcium channels by succinimide antiepileptic drugs. *Mol Pharmacol*. 2001;60:1121–1132.
14. Coulter DA, Huguenard JR, Prince DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann Neurol*. 1989;25:582–593.
15. Huguenard JR. Low-threshold calcium currents in central nervous system neurons. *Annu Rev Physiol*. 1996;58:329–348.
16. Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. *J Biol Chem*. 1996;271:5768–5776.
17. Eroglu C, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, Chakraborty C, Mulinyawe SB, Annis DS, Huberman AD, Green EM, Lawler J, Dolmetsch R, Garcia KC, Smith SJ, Luo ZD, Rosenthal A, Mosher DF, Barres BA. Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell*. 2009;139: 380–392.
18. Ludwig A, Zong X, Jeglitsch M, Hofmann F, Biel M. A family of hyperpolarization-activated mammalian cation channels. *Nature*. 1998;393:587–591.
19. Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: from molecules to physiological function. *Annu Rev Physiol*. 2003;65:453–480.
20. Lorincz A, Notomi T, Tamas G, Shigemoto R, Nusser Z. Polarized and compartment-dependent distribution of HCN1 in pyramidal cell dendrites. *Nat Neurosci*. 2002;5:1185–1193.
21. Stuart G, Spruston N. Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. *J Neurosci*. 1998;18:3501–3510.
22. Magee JC. Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *J Neurosci*. 1998;18: 7613–7624.
23. Magee JC. Dendritic lh normalizes temporal summation in hippocampal CA1 neurons. *Nat Neurosci*. 1999;2:508–514.
24. Surges R, Freiman TM, Feuerstein TJ. Gabapentin increases the hyperpolarization-activated cation current Ih in rat CA1 pyramidal cells. *Epilepsia*. 2003;44: 150–156.
25. Poolos NP, Migliore M, Johnston D. Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. *Nat Neurosci*. 2002;5: 767–774.
26. Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, Mohler H. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. *Nature*. 1999;401:796–800.
27. Rho JM, Donevan SD, Rogawski MA. Mechanism of action of the anticonvulsant felbamate: opposing effects on *N*-methyl-D-aspartate and gamma-aminobutyric acid A receptors. *Ann Neurol*. 1994;35:229–234.
28. Subramaniam S, Rho JM, Penix L, Donevan SD, Fielding RP, Rogawski MA. Felbamate block of the *N*-methyl-D-aspartate receptor. *J Pharmacol Exp Ther*. 1995;273:878–886.
29. Kuo CC, Lin BJ, Chang HR, Hsieh CP. Use-dependent inhibition of the *N*-methyl-D-aspartate currents by felbamate: a gating modifier with selective binding to the desensitized channels. *Mol Pharmacol*. 2004;65:370–380.
30. White HS, Harmsworth WL, Sofia RD, Wolf HH. Felbamate modulates the strychnine-insensitive glycine receptor. *Epilepsy Res*. 1995;20:41–48.
31. Kleckner NW, Glazewski JC, Chen CC, Moscrip TD. Subtype-selective antagonism of *N*-methyl-D-aspartate receptors by felbamate: insights into the mechanism of action. J *Pharmacol Exp Ther*. 1999;289:886–894.
32. Harty TP, Rogawski MA. Felbamate block of recombinant *N*-methyl-D-aspartate receptors: selectivity for the NR2B subunit. *Epilepsy Res*. 2000;39:47–55.
33. Qian J, Noebels JL. Topiramate alters excitatory synaptic transmission in mouse hippocampus. *Epilepsy Res*. 2003;55:225–233.
34. Chappell AS, Sander JW, Brodie MJ, Chadwick D, Lledo A, Zhang D, Bjerke J, Kiesler GM, Arroyo S. A crossover, add-on trial of talampanel in patients with refractory partial seizures. *Neurology*. 2002;58: 1680–1682.
35. Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, Fuks B. The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. *Proc Natl Acad Sci USA*. 2004;101: 9861–9866.
36. Janz R, Goda Y, Geppert M, Missler M, Sudhof TC. SV2A and SV2B function as redundant Ca^{2+} regulators in neurotransmitter release. *Neuron*. 1999;24: 1003–1016.
37. Crowder KM, Gunther JM, Jones TA, Hale BD, Zhang HZ, Peterson MR, Scheller RH, Chavkin C, Bajjalieh SM. Abnormal neurotransmission in mice lacking synaptic vesicle protein 2A (SV2A). *Proc Natl Acad Sci USA*. 1999;96:15268–15273.
38. Chang WP, Sudhof TC. SV2 renders primed synaptic vesicles competent for Ca^{2+} -induced exocytosis. *J Neurosci*. 2009;29:883–897.
39. Yao J, Nowack A, Kensel-Hammes P, Gardner RG, Bajjalieh SM. Cotrafficking of SV2 and synaptotagmin at the synapse. *J Neurosci*. 2010;30:5569–5578.
40. Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M. An astrocytic basis of epilepsy. *Nat Med*. 2005;11:973–981.
41. Remy S, Gabriel S, Urban BW, Dietrich D, Lehmann TN, Elger CE, Heinemann U, Beck H. A novel mechanism underlying drug-resistance in chronic epilepsy. *Ann Neurol*. 2003;53:469–479.
42. Schaub C, Uebachs M, Beck H. Diminished response of CA1 neurons to antiepileptic drugs in chronic epilepsy. *Epilepsia*. 2007;48:1339–1350.
43. Remy S, Urban BW, Elger CE, Beck H. Anticonvulsant pharmacology of voltage-gated Na^+ channels in hippocampal neurons of control and chronically epileptic rats. *Eur J Neurosci*. 2003;17:2648–2658.
44. Vreugdenhil M, Van Veelen CWM, Van Rijen PC, Da Silva FHL, Wadman WJ. Effect of valproic acid on sodium currents in cortical neurons from patients with pharmaco-resistant temporal lobe epilepsy. *Epilepsy Res*. 1998;32:309–320.
45. Vreugdenhil M, Wadman WJ. Modulation of sodium currents in rat CA1 neurons by carbamazepine and valproate after kindling epileptogenesis. *Epilepsia*. 1999;40:1512–1522.
46. Ellerkmann RK, Remy S, Chen J, Sochivko D, Elger CE, Urban BW, Becker A, Beck H. Molecular and

functional changes in voltage-dependent Na^+ channels following pilocarpine-induced status epilepticus in rat dentate granule cells. *Neuroscience*. 2003;119: 323–333.
47. Gastaldi M, Robaglia-Schlupp A, Massacrier A, Planells R, Cau P. mRNA coding for voltage-gated sodium channel beta2 subunit in rat central nervous system: cellular distribution and changes following kainate-induced seizures. *Neurosci Lett*. 1998;249: 53–56.
48. Uebachs M, Opitz T, Royeck M, Dickhof G, Horstmann MT, Isom LL, Beck H. Efficacy loss of the anticonvulsant carbamazepine in mice lacking sodium channel beta subunits via paradoxical effects on persistent sodium currents. *J Neurosci.* 2010;30:8489–8501.
49. Bartolomei F, Gastaldi M, Massacrier A, Planells R, Nicolas S, Cau P. Changes in the mRNAs encoding subtypes I, II and III sodium channel alpha subunits following kainate-induced seizures in rat brain. *J Neurocytol*. 1997;26:667–678.
50. Gastaldi M, Bartolomei F, Massacrier A, Planells R, Robaglia-Schlupp A, Cau P. Increase in mRNAs encoding neonatal II and III sodium channel alpha- isoforms during kainate-induced seizures in adult rat hippocampus. *Brain Res Mol Brain Res.* 1997;44:179–190.
51. Curia G, Aracri P, Sancini G, Mantegazza M, Avanzini G, Franceschetti S. Protein-kinase C-dependent phosphorylation inhibits the effect of the antiepileptic drug topiramate on the persistent fraction of sodium currents. *Neuroscience*. 2004;127:63–68.
52. Sanabria ER, Su H, Yaari Y. Initiation of network bursts by Ca^{2+}-dependent intrinsic bursting in the rat pilocarpine model of temporal lobe epilepsy. *J Physiol*. 2001;532:205–216.
53. Su H, Sochivko D, Becker A, Chen J, Jiang Y, Yaari Y, Beck H. Upregulation of a T-type Ca^{2+} channel causes a long-lasting modification of neuronal firing mode after status epilepticus. *J Neurosci*. 2002;22:3645–3655.
54. Becker AJ, Pitsch J, Sochivko D, Opitz T, Staniek M, Chen CC, Campbell K, Schoch S, Yaari Y, Beck H. Transcriptional upregulation of $Ca_v3.2$ mediates epileptogenesis in the pilocarpine model of epilepsy. *J Neurosci*. 2008;28:13341–13353.
55. Shah MM, Anderson AE, Leung V, Lin X, Johnston D. Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. *Neuron*. 2004;44:495–508.
56. Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ. Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. *J Neurosci.* 2003;23: 6826–6836.
57. Jung S, Jones TD, Lugo JN Jr, Sheerin AH, Miller JW, D'Ambrosio R, Anderson AE, Poolos NP. Progressive dendritic HCN channelopathy during epileptogenesis in the rat pilocarpine model of epilepsy. *J Neurosci*. 2007;27:13012–13021.
58. Costa E. From $GABA_A$ receptor diversity emerges a unified vision of GABAergic inhibition. *Annu Rev Pharmacol Toxicol*. 1998;38:321–350.
59. Sperk G, Furtinger S, Schwarzer C, Pirker S. GABA and its receptors in epilepsy. *Adv Exp Med Biol*. 2004;548:92–103.
60. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat Med*. 1998;4:1166–1172.
61. Frahm C, Stief F, Zuschratter W, Draguhn A. Unaltered control of extracellular GABA-concentration through GAT-1 in the hippocampus of rats after pilocarpine-induced status epilepticus. *Epilepsy Res*. 2003;52: 243–252.
62. Cossart R, Bernard C, Ben-Ari Y. Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signalling in epilepsies. *Trends Neurosci.* 2005;28:108–115.
63. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science*. 2002;298:1418–1421.
64. Wozny C, Kivi A, Lehmann TN, Dehnicke C, Heinemann U, Behr J. Comment on "On the origin of interictal activity in human temporal lobe epilepsy in vitro." *Science*. 2003;301:463–46I.
65. Huberfeld G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, Rivera C. Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. *J Neurosci*. 2007;27:9866–9873.
66. Loscher W, Cramer S, Ebert U. Selection of phenytoin responders and nonresponders in male and female amygdala-kindled Sprague-Dawley rats. *Epilepsia*. 1998;39:1138–1147.
67. Jeub M, Beck H, Siep E, Ruschenschmidt C, Speckmann EJ, Ebert U, Potschka H, Freichel C, Reissmuller E, Loscher W. Effect of phenytoin on sodium and calcium currents in hippocampal CA1 neurons of phenytoin-resistant kindled rats. *Neuropharmacology.* 2002;42:107–116.
68. Nissinen JPT, Pitkänen A. An animal model with spontaneous seizures: a new tool for testing the effects of antiepileptic compounds. *Epilepsia*. 2000;41:136–136.
69. Jandova K, Pasler D, Antonio LL, Raue C, Ji S, Njunting M, Kann O, Kovacs R, Meencke HJ, Cavalheiro EA, Heinemann U, Gabriel S, Lehmann TN. Carbamazepine-resistance in the epileptic dentate gyrus of human hippocampal slices. *Brain*. 2006;129: 3290–3306.
70. Baltes S, Gastens AM, Fedrowitz M, Potschka H, Kaever V, Loscher W. Differences in the transport of the antiepileptic drugs phenytoin, levetiracetam and carbamazepine by human and mouse P-glycoprotein. *Neuropharmacology*. 2007;52:333–346.
71. Luna-Tortos C, Fedrowitz M, Loscher W. Several major antiepileptic drugs are substrates for human P-glycoprotein. *Neuropharmacology*. 2008;55: 1364–1375.
72. Luna-Tortos C, Fedrowitz M, Loscher W. Evaluation of transport of common antiepileptic drugs by human multidrug resistance-associated proteins (MRP1, 2 and 5) that are overexpressed in pharmacoresistant epilepsy. *Neuropharmacology.* 2010;58:1019–1032.
73. Luna-Tortos C, Rambeck B, Jurgens UH, Loscher W. The antiepileptic drug topiramate is a substrate for human P-glycoprotein but not multidrug resistance proteins. *Pharm Res*. 2009;26:2464–2470.
74. Baltes S, Fedrowitz M, Tortos CL, Potschka H, Loscher W. Valproic acid is not a substrate for P-glycoprotein or multidrug resistance proteins 1 and 2 in a number of in vitro and in vivo transport assays. *J Pharmacol Exp Ther*. 2007;320:331–343.

Chapter 84

Drug Resistance

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INTRODUCTION

Drug resistance poses a major challenge to the therapeutic management of epilepsy patients. Multiple studies have explored the epidemiological factors and mechanisms of drug resistance in order to increase knowledge and provide a basis for optimized therapeutic protocols and strategies. However, the lack of a common definition of clinical drug resistance hampers the comparison of data. Therefore, it is a major step forward that a task force of the International League against Epilepsy has recently proposed a definition.[1] According to this task force, drug resistance may be defined as failure of adequate drug trials of two tolerated and appropriately chosen and used antiepileptic drug (AED) schedules (whether as monotherapy or in combination) to achieve freedom from seizures. The proposal is consistent with the clinical observation that therapeutic success is unlikely when these conditions are met.[2] The task force emphasized that the definition should be considered as work in progress; therefore, clinicians are encouraged to test and apply the definition and provide feedback.

In individual patients, the time course of drug resistance can differ significantly. Whereas seizure control might never be achieved in some patients, others might initially respond and then develop drug resistance, or might exhibit alternate phases with seizures being controlled and uncontrolled.[3] Moreover, seizure activity can be uncontrolled in the initial phase but become controlled with ongoing treatment. Long-term studies indicate that many patients with intractable seizures exhibit drug resistance from the onset of treatment.

A large number of seizures before treatment onset are generally considered a major predictor of a poor outcome of drug therapy. Mohanraj and Brodie[2] also explored seizure density as a parameter and found that the greater the number of seizures before treatment, the higher the risk of drug resistance. Based on these data, seizure density seems to be an even more significant predictive factor than the overall number of pretreatment seizures. Considering the epidemiological data, Rogawski and Johnson[4] proposed the *intrinsic severity hypothesis*, which suggests that common neurobiological factors contribute to both

epilepsy severity and drug refractoriness (see below). Further factors that are discussed as clinical predictors of drug resistance include a family history of epilepsy, prior febrile convulsions, seizure clusters, traumatic brain injury, and psychiatric comorbidities, particularly depression.[5]

Therapeutic management of patients with drug-resistant epilepsy can include nonpharmacological approaches such as epilepsy surgery, vagal nerve stimulation, or a ketogenic diet. Recent data suggest that although the number of responders might be low, it is also worthwhile to test further AEDs in patients who have been classified as drug resistant based on two or more drug trials.[6] The authors emphasize that no matter how many AED therapies have failed, there is always hope for meaningful seizure remission in this population.

Research efforts have been made to elucidate specific mechanisms of drug resistance. The identification of these mechanisms might provide a basis for the future design and development of novel pharmacological approaches to overcome resistance.

MECHANISMS OF DRUG RESISTANCE

The two main hypotheses concerning a mechanism for pharmacoresistant epilepsy, which have attracted the most attention over the past 15 years, are the *target hypothesis* and the *drug transporter hypothesis* (Fig. 84–1). The target hypothesis posits that the AED fails to act because of a molecular alteration of its target. The transporter hypothesis posits that the AED fails to reach its target in sufficient concentration because of overexpressed drug transporters at the blood-brain barrier. As mentioned above, another hypothesis that has been put forward recently is the *intrinsic severity hypothesis*.[4]

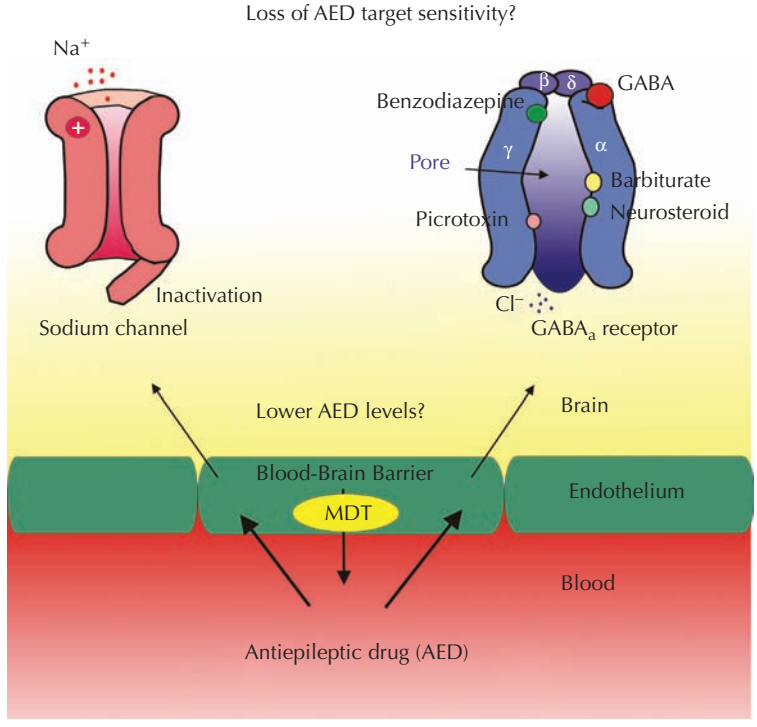

Figure 84–1. Schematic representation of the drug transporter hypothesis and the target hypothesis. MDT illustrates the multidrug transporter proteins on the luminal site of the endothelial cells. Overexpression of MDT in epilepsy leads to reduced AED levels at the targets. The $GABA_A$ receptor, with its multiple binding sites, and the sodium channels are examples of two important targets for AEDs. Changes of AED binding sites can alter its sensitivity. Both lack of AED availability at the target and altered target sensitivity to the AED may play a role in drug resistance. Adapted from ref. 59.

Although the neurobiological mechanisms are still elusive, the authors propose that some of the factors related to the severity of the disease also cause drug resistance. Below, we discuss these hypotheses separately in more detail.

Target Hypothesis

The main targets of the current AEDs are voltage-gated ion channels and neurotransmitter receptors.[7] Carbamazepine, phenytoin, valproate, oxcarbazepine, lamotrigine, rufinamide, zonisamide, lacosamide, and topiramate act mainly on voltage-gated sodium channels, although some of these drugs also act partially on other channels. Other AEDs act mainly on voltage-gated calcium channels (e.g., gabapentin, pregabalin, ethosuximide). Barbiturates, benzodiazepines, valproate, tiagabine, and vigabatrin are AEDs that amplify inhibitory gamma-aminobutyric acid (GABA) action. Specific potassium channel subunits and glutamate receptor (subunits) are also potential targets, and AEDs for some of these targets are just recently marketed (e.g., retigabine) or cautiously used in more severe syndromes (e.g., felbamate). More unconventional targets are enzymes (e.g., GABA transaminase, inhibited by vigabatrin, and carbonic anhydrase, inhibited by acetazolamide) and the synaptic vesicle protein SV2A (the binding site for levetiracetam and related compounds). The target hypothesis implies that molecular changes in AED targets, whether intrinsic (e.g., polymorphisms) or acquired (e.g., by the disease process, seizures, drugs, etc.), may lead to reduced sensitivity to AEDs.

The voltage-dependent sodium channels are the most intensively studied targets. The first studies that addressed changes in sodium channel function and reduced AED sensitivity in the remaining CA1 neurons of epileptic rats and of epilepsy patients were reported by Wadman and colleagues.[8,9] Beck and colleagues extended these studies and reported reduced carbamazepine and phenytoin sensitivity to sodium channels in hippocampal dentate neurons in the pilocarpine model for temporal lobe epilepsy (TLE) as well as in hippocampal neurons of tissue from TLE patients obtained after surgical resection.[10,11] Antiepileptic drugs like carbamazepine act by shifting the voltage dependency of the steady-state inactivation in a more hyperpolarized direction and by producing a use-dependent block. In tissue of epileptic rats as well as in tissue of carbamazepine-resistant patients, carbamazepine loses its ability to shift this voltage dependency in a more hyperpolarized direction; the use-dependent block by carbamazepine is also diminished. Lack of a cellular response to carbamazepine was particularly evident in a group of patients who were also unresponsive to carbamazepine, while a small group of patients who still responded to carbamazepine also still showed a cellular response to carbamazepine in vitro.[10]

Most of the changes reported above may have developed over time as part of the epileptogenic process and may have involved changes in transcription or RNA processing (e.g., alternative splicing). A change in subunit gene expression can be obtained rapidly. For instance, increased expression of a neonatal splice variant of rNav1.2/rNav1.3 was detected within 4 h after electrically induced status epilepticus (SE) in rats.[12] However, although a change in sodium channel properties has been detected after electrically induced SE,[13] it has not been determined whether this change is related to this neonatal channel function with altered sensitivity to AEDs. Phosphorylation can also alter the AED sensitivity of targets. Changes in the topiramate sensitivity of sodium channels have been detected after protein kinase C activation in rat cortical neurons, suggesting that phosphorylation may limit the effect of this AED on the persistent fraction of the sodium current.[14]

There are significant cell-specific changes in $GABA_A$ receptor subunit expression after epileptic insults induced by kindling stimulations, by electrically or pharmacologically induced SE, and in TLE patients with hippocampal sclerosis. A clear example of the changes in subunit expression and their effects on drug sensitivity has been shown in dentate granule cells after pilocarpine-induced SE; decreased sensitivity to benzodiazepines was detected after $GABA_A$ receptor activation,[15] which appeared to be related to alterations in subunit expression.[16] Reduced benzodiazepine sensitivity was detected within hours after electrically induced SE in rats. However, this was not believed to be caused by transcriptional changes but was attributed to GABA receptor subunit trafficking away from the membrane into the cytoplasm.[17]

The aforementioned studies are examples of changes in target function due to modification of the target itself. However, target function could also be altered by changes in the ion environment caused by ion transporters. For instance, a number of studies have shown that expression of the sodium-potassium chloride cotransporter (NKCC1) is increased in a subset of neurons obtained from epileptic patients and epileptic animals.[18,19] This transporter, whose expression is also increased during early development, is responsible for an increase in intracellular chloride, which leads to a depolarizing effect of GABA (or GABA agonists). These insights may lead to clinical trials using drugs that inhibit the function of NKCC1, so that the hyperpolarizing action of GABA will be obtained or restored.[20]

SV2A, the binding site for levetiracetam, is significantly changed after SE and in tissue of patients with hippocampal sclerosis. Cell-specific downregulation of SV2A expression has been observed, with significant permanent downregulation in the mossy fibers of the dentate gyrus of post-SE rats and of patients with hippocampal sclerosis.[21] Whether this has consequences for the action or the efficacy of levetiracetam needs to be determined.

Most of the experimental studies mentioned above have described AED effects in cells measured in in vitro slice preparations and obtained from epileptic or control animals. Whether AEDs were effective in the same animals was not tested. Only one study related to the target hypothesis has been performed in responder versus nonresponder rats with spontaneous seizures.[22] Therefore, our insights would benefit if, in future studies, more experiments are designed in which the effects of the AED are tested on cellular responses in epileptic animals in which it is known whether or not seizures can be controlled by AEDs. Also, most studies have mainly investigated the effects of a few first-generation AEDs on cellular responses in patients who suffer from TLE (or in TLE animal models). Future studies should also test cellular responses of newer AEDs in animal models that mimic not only TLE but also other epilepsy syndromes (e.g., the rat methylazoxymethanol model of cortical dysplasia, the 6 Hz psychomotor model of partial epilepsies, or traumatic brain injury epilepsy models[23]) and take advantage of resected tissue that becomes available from pharmacoresistant epilepsy syndromes other than TLE.

Although the data above provide support for the target hypothesis, some issues remain unresolved. For instance, although carbamazepine sensitivity is altered in epileptic tissue compared to control tissue (which in human studies is often obtained from cortex), sensitivity to phenytoin is less affected, while sensitivity to lamotrigine is hardly changed.[11] This is surprising, considering the fact the voltage-gated sodium channels are the main, although not the exclusive, target of these AEDs. This also suggests that the target hypothesis cannot describe the only mechanism explaining drug resistance; other mechanisms also play a role. One such alternative mechanism is proposed by the drug transporter hypothesis.

Drug Transporter Hypothesis

The fact that pharmacoresistant epilepsy patients are resistant to a broad range of AEDs with different mechanisms of action suggests that nonspecific mechanisms contribute to drug resistance. By analogy with cancer research, where drug resistance to chemotherapy is also a huge problem, the drug transporter hypothesis has been proposed for pharmacoresistant epilepsy. This hypothesis states that drug resistance is caused by overexpression of multidrug transporters that prevent therapeutic drugs from entering the brain in sufficient concentrations.[24]

The best-known drug transporters at the blood-brain barrier are members of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter superfamily. The multidrug transporters (MDTs) that are present at the blood-brain barrier normally prevent the entrance of many different lipophilic toxic compounds or xenobiotics from blood to brain by an active (ATP-driven) efflux mechanism. Their presence is important and necessary in order to protect the brain, but it is not very useful when one needs to get drugs into the brain for therapeutic purposes. Of the many different known drug transporters, P-glycoprotein (P-gp; ABCB1) is the most intensively studied, followed by multidrug resistance-related proteins

(MRPs, ABCC1–5) and breast cancer-related protein (BCRP; ABCG2). P-glycoprotein, MRP1, MRP2, MRP5, and BCRP are reported to be present at the luminal site of endothelial cells and are therefore in a position to extrude their substrates back into the bloodstream.

The study of Tishler et al.[25] led to the formulation of the drug transporter hypothesis for epilepsy. These authors reported increased expression of P-glycoprotein on brain capillaries and perivascular astrocytes in epileptic tissue. Numerous studies have confirmed this finding not only in tissue of patients with hippocampal sclerosis but also in epilepsies associated with cortical malformations. There is now general agreement that drug transporters such as P-gp, MRP1, MRP2, MRP5, and BCRP are overexpressed in brain tissue of patients with a pharmacoresistant epilepsy syndrome as well as in different epilepsy animal models (reviewed in ref. 24). P-glycoprotein overexpression can be detected within 1 day after SE, but it does not appear to play a role in reduced AED efficacy during SE.[26]

In comparison with anticancer drugs, AEDs are much weaker substrates for the drug transporters. Therefore, AED transport may be detected only when the transporters are overexpressed. There is ample evidence that increased expression of drug transporters correlates negatively with brain levels of several major AEDs in epileptic animals. One study in epileptic patients reports a negative correlation between P-gp expression and the brain concentration of an active metabolite of oxcarbamazepine.[27]

Pharmacological P-gp inhibition in rats or absence of P-gp by genetic deletion of *mdr1a/b* in mice led to increased brain levels of phenytoin, carbamazepine, phenobarbital, lamotrigine, and felbamate.[28,29] A study with MRP2-deficient rats indicates that phenytoin, but not carbamazepine, lamotrigine, and phenobarbital, is a substrate for MRP2.[30] Great skepticism concerning the relevance of drug transporters for pharmacoresistant epilepsy in humans arose, however, when it was reported that human P-gp did not transport major AEDs such as phenytoin, carbamazepine, or levetiracetam.[31] However, this doubt was refuted by subsequent studies by the same authors and others who tested AED transport in more sensitive screening assays. Using these transport assays, it has been shown that major AEDs, including phenytoin, lamotrigine, levetiracetam, and phenobarbital (but not carbamazepine), can be transported by human P-gp transfected in kidney cell lines.[32] This was confirmed for phenytoin transport in brain capillary endothelial cells from AED-resistant patients and controls.[33] Transport measurements in cell lines transfected with human MRP1, MRP2, or MRP5 revealed that none of these drugs were transported by these MRPs.[34] The authors acknowledge, however, that in vitro assays may produce false-negative results and suggest that the potential role of MRPs should be further investigated in vivo, including positron emission tomography (PET) studies with labeled MRP and P-gp substrates.

More support for a role of multidrug transporters in pharmacoresistant epilepsy came from two proof-of-principle studies in chronic epileptic rats in which brain AED concentrations were increased and seizure control was obtained when P-gp was inhibited with the selective P-gp inhibitor tariquidar.[35,36] In the study of van Vliet et al.,[36] therapeutic doses of phenytoin were used for 7 days in rats that had developed epilepsy with frequent daily seizures after electrically induced SE. Phenytoin treatment alone produced only a slight reduction in daily seizure frequency. However, almost complete seizure control was observed when phenytoin was administered together with tariquidar. Unfortunately, this control was present for only 3–4 days, after which seizure frequency increased again to pretreatment levels, despite the fact that brain phenytoin levels were higher than the levels in control rats. Loscher and colleagues performed similar proof-of-principle experiments in a rat post-SE model.[35] They used phenobarbital as the AED and discriminated between rats that responded to phenobarbital and those that did not. In previous experiments, these rats were characterized by low and high endothelial P-gp expression, respectively. [37] The nonresponders became responsive to phenobarbital when tariquidar was coadministered. Thus, although these findings clearly showed that inhibition of P-gp improves AED action and produces complete seizure control (which is not obtained with the AED alone), the temporary effects that were observed with phenytoin in the study of van Vliet et al.[36] may indicate that other factors can come into play, reducing the efficacy of phenytoin once again. One such mechanism might

be related to the development of tolerance to the AED.[38]

Despite quite convincing indications from animal studies that drug transporters play a role in pharmacoresistant epilepsy, clinical proof is still lacking. In this respect, PET studies in pharmacoresistant patients using specific transporter inhibitors are needed to provide more definite evidence of the clinical relevance of this putative mechanism.

Drug transporters are regulated in a complex manner and by different processes, including oxidative stress and inflammation.[39] Ligand-activated nuclear receptors, β-amyloid, glutamate, and elements of the innate immune response can regulate drug transporter expression. In relation to epilepsy, the immune response and glutamate are particularly relevant since they both play a crucial role in the epileptogenic process. The role of inflammation as a regulator of drug transporters is very complex and may be dose-, model-, and time-dependent. Both increases and decreases in drug transporter expression on endothelial cells have been reported, depending on the timing of the activation of specific inflammatory pathways. While increased expression of P-gp is evident after an ischemic insult and in a variety of epilepsy syndromes (that are associated with inflammation), decreased P-gp expression has been observed in endothelial cells of multiple sclerosis patients and in the animal model for this disease.[40] It has been shown that glutamate increases P-gp expression in endothelial cells via *N*-methyl-D-aspartate (NMDA) receptor activation and cyclo-oxygenase-2 (COX-2) activation.[41] This opens the possibility of targeting COX-2 in order to regulate P-gp expression in epileptic patients.

There are several other proposed mechanisms of drug resistance that have been less intensively investigated or that may be more closely related to the specific properties of the AED. Like overexpression of drug transporters, interaction between the AED and serum proteins can lead to reduced AED levels near the target (Table 84–1). This can occur during brain edema and was recently put forward as the *protein-binding hypothesis* of drug resistance.[42] The idea behind this hypothesis is that plasma proteins penetrate into the brain because of disturbance of blood-brain barrier integrity. As a consequence, enhanced protein binding in the brain extracellular space might decrease free concentrations of AEDs with a high protein-binding rate. Disease-related structural alterations, such as tumors and scars, can also form physical barriers that may prevent the AED from reaching its target and contribute to drug resistance. Other important factors are metabolic or pharmacokinetic tolerance induced by AED-metabolizing enzymes. This is mainly a problem for the older AEDs. The development of pharmacodynamic or functional tolerance that is due to adaptation of AED targets (e.g., by loss of receptor sensitivity) may be another reason for failure of drug treatment. This has been shown experimentally for all AEDs that lose activity during prolonged treatment.[38]

Intrinsic Severity Hypothesis

As mentioned above, Rogawski and Johnson have recently proposed the intrinsic severity hypothesis,[4] which incorporates the intriguing epidemiological finding that a higher seizure frequency in the early phase of epilepsy before treatment increases the risk of drug resistance. An essential point of this hypothesis is that common neurobiological factors may underlie both epilepsy severity and drug resistance. The general concept of this hypothesis is that seizures that are easily triggered can result in frequent seizures that may be difficult to suppress. The neurobiological or molecular factors are still unknown, but one could speculate that they include changes of targets and alterations of network properties (e.g., via elimination of cellular elements and/or abnormal network plasticity) that lead to unstable neuronal networks, making it impossible to control seizures with the currently available AEDs. Although this is an attractive hypothesis, Schmidt and Loscher[42] have pointed out that it also has weak points: despite the fact that high seizure frequency in patients is often associated with drug resistance, a subgroup of patients can become seizure-free after a change in medication. Moreover, there are patients who start with only few seizures and become pharmacoresistant.[43] As a consequence, future research needs to determine the neurobiological factors that critically affect the severity of the disease. The studies should be based on the definition of common measures that provide a basis for scoring epilepsy severity.

Table 84–1 **Overview of Mechanistic Hypotheses, Their Limitations, and Putative Implications**

	Hypotheses		
	Target	**Transporter**	**Protein Binding**
Experimental evidence from rodent epilepsy models	Loss or reduction of drug resistance	Expression correlates with drug sensitivity Modulation of transporters improves AED efficacy	Relevance not yet demonstrated in an epilepsy model
Clinical evidence	Limited evidence from a small number of patients	Expression levels ↑ but functional relevance not proven	Protein extravasation demonstrated, but impact not proven
Major limitations	Alterations in one target do not explain broad drug resistance	Controversial data on transport of AEDs	Only of relevance for older AEDs with high rates of protein binding
Implications for therapy or drug development	Develop AEDs with affinity to altered target sites or to novel targets	Select/develop AEDs that are not transporter substrates Modulate transporter function Prevent transporter induction By-pass transporters.	As already done for other reasons: select AEDs with low protein binding Prevent disturbance of blood-brain barrier function
Identification in patients	Development of radiolabeled ligands with affinity sensitive to molecular alterations	Analyze brain kinetics of radiolabeled transporter substrates	Image protein extravasation

NOVEL APPROACHES TO OVERCOME DRUG RESISTANCE

Ongoing efforts to elucidate the mechanisms of drug resistance raise the question of whether increasing knowledge might provide a basis for future drug development and novel therapeutic strategies (Figure 84–2). The target hypothesis implicates that drug development should consider the molecular and functional alterations occurring in promising target sites. Based on precise identification of these changes, it should be possible to develop in vitro screening tests for an affinity of developmental compounds to altered target sites. This would allow the selection of advantageous compounds at early stages of drug development. Moreover, the target hypothesis points to the fact that the use of chronic epilepsy models during in vivo testing is of particular importance. In acute models with seizure elicitation, naive mice do not exhibit alterations in target sites. A major problem is that chronic epilepsy models characterized by disease-associated molecular and cellular alterations are generally elaborate and time-consuming to use. Thus, recommendations based on the target hypothesis pose a major challenge to the choice of initial in vivo screening models used as gatekeepers in the selection of promising compounds.[23]

The implications of the protein-binding hypothesis for drug development have been considered during the last decades. In order to avoid drug interactions, compounds have been selected that exhibit a low protein-binding rate. This is reflected by the pharmacokinetic characteristics of the majority of second- and third-generation AEDs. Therefore, protein binding following protein extravasation into the brain extracellular space is an issue for older AEDs. Since further support for the relevance of protein binding in the extracellular space of the epileptic brain has been obtained, future strategies might aim to maintain the integrity of the blood-brain barrier despite the occurrence of seizure activity. Anti-inflammatory drugs such

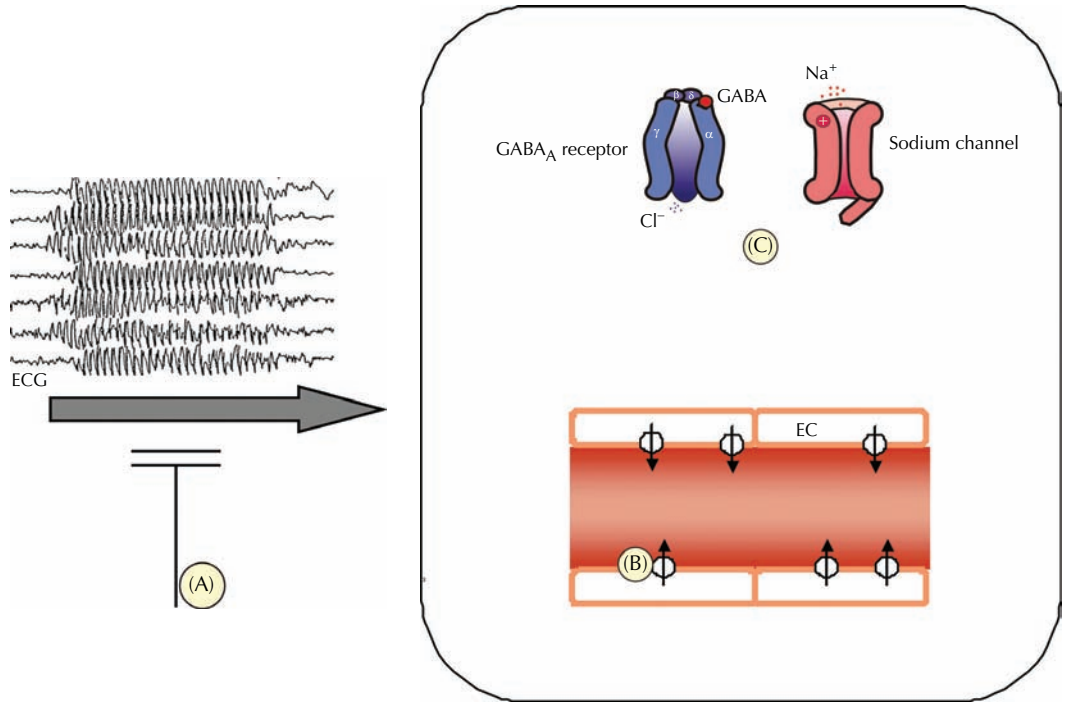

Figure 84–2. Implications of mechanistic hypotheses for drug development or strategies to overcome resistance. **A.** Mechanisms of resistance might be acquired as a consequence of seizure activity (indicated by the arrow and the EEG high-amplitude spiking pattern). As a novel approach, acquired alterations such as target alterations, transporter overexpression, and blood-brain barrier disruption can be prevented by interference with involved molecular and signaling events. **B.** Transporter-mediated resistance might be targeted by inhibiting or bypassing transporters. **C.** The target hypothesis suggests development of AEDs tailored for altered target sites or novel targets. EC, endothelial cells.

as steroids have been suggested as therapeutic add-on options with the ability to repair or protect the blood-brain barrier.[44]

The transporter hypothesis has different implications for drug development and therapy. In general, the developmental process of central nervous system drugs already includes testing whether compounds are efflux transporter substrates. However, a series of in vitro analyses suggests that determining the substrate specificities of highly lipophilic compounds such as AEDs involves particular requirements for the sensitivity of the transport assay used. Thus, care should be taken to integrate sensitive assays into the screening process. Add-on strategies might aim to modulate transporter function or to prevent induction of transporter expression. As already described, experimental proof of principle has been obtained in acute and chronic epilepsy models demonstrating that the addition of P-gp modulators enhances the anticonvulsant efficacy of AEDs and can even help to overcome drug resistance.[35,36] The use of transporter modulators that reduce the transport capacity in a competitive or noncompetitive manner requires careful safety considerations. Efflux transporters such as P-gp serve important protective functions. Through their activity in biological barriers, excretory organs, and hematopoietic cells, they significantly limit the exposure of sensitive tissues and cells to harmful xenobiotics. For instance, evidence exists that low expression of P-gp predisposes to Alzheimer's disease, Parkinson's disease, and inflammatory bowel disease. Long-term pharmacological inhibition of P-gp might be associated with comparable risks. Thus, translational development should consider transient phases of add-on therapy.

Progress has been made in the identification of key signaling factors upregulating P-gp in the epileptic brain.[45] Seizure-associated induction of P-gp has been prevented by targeting these key factors, including COX-2 inhibitors and

prostaglandin E2 (PGE_2) receptor type 1 (EP1) antagonists.[46–48] More importantly, COX-2 inhibition increased the brain penetration rate of phenytoin and restored pharmacosensitivity to phenobarbital in chronic epileptic rats.[49] As these preventive strategies have been proven to keep P-gp expression at control levels, targeting P-gp upregulation will not interfere with basal transport function. However, the specific side effects of compounds targeting arachidonic acid signaling need to be taken into consideration.

Bypassing overexpressed efflux transporters might be another option. Approaches might include nanoparticle encapsulation of AEDs and invasive strategies involving intracerebral drug administration.

Any strategy that aims to overcome one of the suggested mechanisms of resistance has several challenges. First, the paucity or lack of convincing clinical support demands further efforts to explore the clinical relevance of specific resistance mechanisms. Moreover, drug resistance has generally been considered a multifactorial problem. Therefore, it remains questionable whether overcoming one selected mechanism is sufficient to restore pharmacosensitivity in patients. In this context, clinical studies also need to explore whether specific mechanisms might predominate in a subgroup of patients.

APPROACHES TO IDENTIFY RESISTANCE MECHANISMS IN PATIENTS

Evaluation of the clinical relevance of a suggested mechanism of drug resistance would benefit significantly from the availability of tools to study the alterations in individual patients. Pharmacogenetic analyses might be helpful in the identification of intrinsic mechanisms of drug resistance.[50] The presence of genetic differences in drug targets might affect the affinity and efficacy of AEDs. Considering voltage-gated sodium channels as one of the major targets of AEDs, it is of specific interest that a single nucleotide polymorphism (SNP) has been identified in the *SCN1A* gene that, by affecting the splicing of the gene, might alter the functional properties of the Na^+ channel. In an initial analysis of a large cohort of patients from the United Kingdom, the SNP proved to be associated with the maximal prescribed dose of the AEDs carbamazepine and phenytoin.[51] However, studies in a Chinese patient population, as well as a replication study in a European cohort, failed to detect a respective association.[52,53] Thus, the clinical relevance of this SNP remains questionable.

An association between the assembly of the pentameric $GABA_A$ receptor and its sensitivity to the agonist zolpidem was reported several years ago. Based on these data, it has been suggested that functional polymorphisms at sites that regulate the expression of $GABA_A$ receptor subunits might affect drug responsiveness.[54] Further research is necessary to explore the pharmacogenetic relevance of the described polymorphisms.

Many AEDs act by multiple mechanisms, representing one of the major challenges in efforts to identify a link between a selected polymorphism in one drug target and drug responsiveness. This problem is considered a major cause of the failure to reproduce an association in replication studies.

Considering the putative differences in brain penetration of AEDs, several groups have performed association studies analyzing a link between polymorphisms in the P-gp-encoding gene *ABCB1* and resistance to treatment.[54,55] Evidence exists that the most intensely analyzed SNP located in exon 27 (C3435T) is associated with altered protein expression rates, although this SNP does not affect the amino acid sequence. Single nucleotide polymorphisms that are in linkage disequilibrium with the C3435T SNP have been suggested to mediate the functional consequences. An initial study indicated an association between the C3435T polymorphism and resistance to multiple AEDs. Since then, a series of studies has supported this link, but a number of other studies failed to confirm the association.[54,56]

Controversial data might be related to differences in inclusion criteria. Among other factors, the failure to focus on AEDs that are transported by P-gp should be considered as a major limitation in some of the studies. Inconsistencies in the outcome might also reflect the complexity of drug resistance mechanisms. Even the focus on P-gp as one transporter might require much more complex considerations. Experimental data indicate that overexpression of this transporter in the epileptic brain is an acquired

phenomenon occurring as a consequence of seizure activity. Therefore, differences between responders and nonresponders might lie in regulatory mechanisms. Thus, genetic analyses need to include regulatory sequences that control *ABCB1* expression as well as the different signaling factors driving P-gp expression during seizures. Given these issues, investigators are unlikely to obtain explicit and reproducible results from analyses of SNPs in the *ABCB1* coding sequence.

Imaging techniques might allow a more direct evaluation of intrinsic or acquired mechanisms of resistance. Although a series of PET tracers proved to be affected by P-gp, the vast majority of studies so far have used the [^{11}C]-labeled (R)-enantiomer of verapamil. Using this radiotracer, a pilot PET study in epileptic patients revealed evidence of asymmetries in R-[^{11}C] verapamil distribution in homologous brain regions located ipsilateral and contralateral to the seizure focus.[57] The failure to reach significance was attributed to the small sample size of the study. Explicit conclusions obviously need to await the completion of more comprehensive studies.

Progress has been made in the optimization of sensitive techniques in that the reversal of P-gp function can also be monitored when a P-gp substrate radiotracer is combined with a P-gp modulator such as tariquidar.[58] Subsequent PET scanning using a radiotracer with and without pretreatment with a P-gp inhibitor will probably allow an even more precise determination of P-gp function when analyzing the difference in tracer kinetics between both scans.

Evaluation of the transport function of further efflux transporters at the blood-brain barrier is currently hampered by the lack of excellent tracer candidates. With regard to the target hypothesis, it might be worthwhile to develop radioligands with an affinity sensitive to target variations. Such tracers might allow evaluation of the predominant variant in individual patients.

SUMMARY

Despite the ongoing development of novel AEDs, drug resistance remains a major problem in the clinical management of epilepsy patients. Thus, strategies to break new grounds in the development of novel AEDs or alternative therapeutic approaches are urgently needed. Based on epidemiological data as well as experimental studies, several hypotheses have been proposed to explain the phenomenon of drug resistance. These hypotheses suggest specific implications for future drug development and clinical therapeutic management. However, clinical evidence for the drug resistance mechanisms is still limited. Therefore, novel tools to study putative resistance mechanisms in individual patients are needed. New techniques might also guide individualized therapeutic decisions in the future.

DISCLOSURE STATEMENT

J.A.G. has no conflicts of interest to disclose. H.P. acts as a consultant, speaker, and collaborator for different pharmaceutical companies.

REFERENCES

1. Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, Moshe SL, Perucca E, Wiebe S, French J. Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia*. 2010;51:1069–1077.
2. Mohanraj R, Brodie MJ. Diagnosing refractory epilepsy: response to sequential treatment schedules. *Eur J Neurol*. 2006;13:277–282.
3. Schmidt D, Loscher W. Drug resistance in epilepsy: putative neurobiologic and clinical mechanisms. *Epilepsia*. 2005;46:858–877.
4. Rogawski MA, Johnson MR. Intrinsic severity as a determinant of antiepileptic drug refractoriness. *Epilepsy Curr*. 2008;8:127–130.
5. Hitiris N, Mohanraj R, Norrie J, Sills GJ, Brodie MJ. Predictors of pharmacoresistant epilepsy. *Epilepsy Res*. 2007;75:192–196.
6. Callaghan BC, Anand K, Hesdorffer D, Hauser WA, French JA. Likelihood of seizure remission in an adult population with refractory epilepsy. *Ann Neurol*. 2007;62:382–389.
7. Meldrum BS, Rogawski MA. Molecular targets for antiepileptic drug development. *Neurotherapeutics*. 2007;4:18–61.
8. Vreugdenhil M, van Veelen CW, van Rijen PC, Lopes da Silva FH, Wadman WJ. Effect of valproic acid on sodium currents in cortical neurons from patients with pharmaco-resistant temporal lobe epilepsy. *Epilepsy Res*. 1998;32:309–320.
9. Vreugdenhil M, Wadman WJ. Modulation of sodium currents in rat CA1 neurons by carbamazepine and valproate after kindling epileptogenesis. *Epilepsia*. 1999;40:1512–1522.

10. Remy S, Gabriel S, Urban BW, Dietrich D, Lehmann TN, Elger CE, Heinemann U, Beck H. A novel mechanism underlying drug resistance in chronic epilepsy. *Ann Neurol*. 2003;53:469–479.
11. Remy S, Urban BW, Elger CE, Beck H. Anticonvulsant pharmacology of voltage-gated Na^+ channels in hippocampal neurons of control and chronically epileptic rats. *Eur J Neurosci*. 2003;17:2648–2658.
12. Aronica E, Yankaya B, Troost D, van Vliet EA, Lopes da Silva FH, Gorter JA. Induction of neonatal sodium channel II and III alpha isoform mRNAs in neurons and microglia after status epilepticus in the rat hippocampus. *Eur J Neurosci*. 2001;13:1261–1266.
13. Ketelaars SO, Gorter JA, van Vliet EA, Lopes da Silva FH, Wadman WJ. Sodium currents in isolated rat CA1 pyramidal and dentate granule neurones in the post-status epilepticus model of epilepsy. *Neuroscience*. 2001;105:109–120.
14. Curia G, Aracri P, Colombo E, Scalmani P, Mantegazza M, Avanzini G, Franceschetti S. Phosphorylation of sodium channels mediated by protein kinase-C modulates inhibition by topiramate of tetrodotoxin-sensitive transient sodium current. *Br J Pharmacol*. 2007;150:792–797.
15. Gibbs JW 3rd, Shumate MD, Coulter DA. Differential epilepsy-associated alterations in postsynaptic $GABA_A$ receptor function in dentate granule and CA1 neurons. *J Neurophysiol*. 1997;77:1924–1938.
16. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Kelly ME, Coulter DA. γ-Aminobutyric $acid_A$ receptor subunit expression predicts functional changes in hippocampal dentate granule cells during postnatal development. *J Neurochem*. 2001;77:1266–1278.
17. Naylor DE, Liu H, Wasterlain CG. Trafficking of $GABA_A$ receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci.* 2005;25:7724–7733.
18. Bragin DE, Sanderson JL, Peterson S, Connor JA, Muller WS. Development of epileptiform excitability in the deep entorhinal cortex after status epilepticus. *Eur J Neurosci*. 2009;30:611–624.
19. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science.* 2002;298:1418–1421.
20. Kahle KT, Barnett SM, Sassower KC, Staley KJ. Decreased seizure activity in a human neonate treated with bumetanide, an inhibitor of the Na^+-K^+-$2Cl^-$ cotransporter NKCC1. *J Child Neurol*. 2009;24:572–576.
21. van Vliet EA, Aronica E, Redeker S, Boer K, Gorter JA. Decreased expression of synaptic vesicle protein 2A, the binding site for levetiracetam, during epileptogenesis and chronic epilepsy. *Epilepsia*. 2009;50:422–433.
22. Bethmann K, Fritschy JM, Brandt C, Loscher W. Antiepileptic drug resistant rats differ from drug responsive rats in $GABA_A$ receptor subunit expression in a model of temporal lobe epilepsy. *Neurobiol Dis*. 2008;31:169–187.
23. Bialer M, White HS. Key factors in the discovery and development of new antiepileptic drugs. *Nat Rev Drug Discov*. 2010;9:68–82.
24. Loscher W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. *Nat Rev Neurosci*. 2005;6:591–602.
25. Tishler DM, Weinberg KI, Hinton DR, Barbaro N, Annett GM, Raffel C. MDR1 gene expression in brain of patients with medically intractable epilepsy. *Epilepsia.* 1995;36:1–6.
26. Loscher W. Mechanisms of drug resistance in status epilepticus. *Epilepsia*. 2007;48(suppl 8):74–77.
27. Marchi N, Guiso G, Rizzi M, Pirker S, Novak K, Czech T, Baumgartner C, Janigro D, Caccia S, Vezzani A. A pilot study on brain-to-plasma partition of 10,11-dyhydro-10-hydroxy-5H-dibenzo(b,f)azepine-5-carboxamide and MDR1 brain expression in epilepsy patients not responding to oxcarbazepine. *Epilepsia*. 2005;46:1613–1619.
28. Rizzi M, Caccia S, Guiso G, Richichi C, Gorter JA, Aronica E, Aliprandi M, Bagnati R, Fanelli R, D'Incalci M, Samanin R, Vezzani A. Limbic seizures induce P-glycoprotein in rodent brain: functional implications for pharmacoresistance. *J Neurosci.* 2002;22:5833–5839.
29. Potschka H, Fedrowitz M, Loscher W. P-glycoprotein-mediated efflux of phenobarbital, lamotrigine, and felbamate at the blood-brain barrier: evidence from microdialysis experiments in rats. *Neurosci Lett*. 2002;327:173–176.
30. Potschka H, Fedrowitz M, Loscher W. Multidrug resistance protein MRP2 contributes to blood-brain barrier function and restricts antiepileptic drug activity. *J Pharmacol*. Exp Ther 2003;306:124–131.
31. Baltes S, Gastens AM, Fedrowitz M, Potschka H, Kaever V, Loscher W. Differences in the transport of the antiepileptic drugs phenytoin, levetiracetam and carbamazepine by human and mouse P-glycoprotein. *Neuropharmacology*. 2007;52:333–346.
32. Luna-Tortos C, Fedrowitz M, Loscher W. Several major antiepileptic drugs are substrates for human P-glycoprotein. *Neuropharmacology*. 2008;55:1364–1375.
33. Cucullo L, Hossain M, Rapp E, Manders T, Marchi N, Janigro D. Development of a humanized in vitro blood-brain barrier model to screen for brain penetration of antiepileptic drugs. *Epilepsia*. 2007;48:505–516.
34. Luna-Tortos C, Fedrowitz M, Loscher W. Evaluation of transport of common antiepileptic drugs by human multidrug resistance-associated proteins (MRP1, 2 and 5) that are overexpressed in pharmacoresistant epilepsy. *Neuropharmacology*. 2010;58:1019–1032.
35. Brandt C, Bethmann K, Gastens AM, Loscher W. The multidrug transporter hypothesis of drug resistance in epilepsy: proof-of-principle in a rat model of temporal lobe epilepsy. *Neurobiol Dis*. 2006;24:202–211.
36. van Vliet EA, van Schaik R, Edelbroek PM, Redeker S, Aronica E, Wadman WJ, Marchi N, Vezzani A, Gorter JA. Inhibition of the multidrug transporter P-glycoprotein improves seizure control in phenytoin-treated chronic epileptic rats. *Epilepsia*. 2006;47:672–680.
37. Volk HA, Loscher W. Multidrug resistance in epilepsy: rats with drug-resistant seizures exhibit enhanced brain expression of P-glycoprotein compared with rats with drug-responsive seizures. *Brain*. 2005;128:1358–1368.
38. Loscher W, Schmidt D. Experimental and clinical evidence for loss of effect (tolerance) during prolonged treatment with antiepileptic drugs. *Epilepsia*. 2006;47:1253–1284.
39. Potschka H. Targeting regulation of ABC efflux transporters in brain diseases: a novel therapeutic approach. *Pharmacol Ther*. 2010;125:118–127.
40. Kooij G, van Horssen J, de Lange EC, Reijerkerk A, van der Pol SM, van Het Hof B, Drexhage J,

Vennegoor A, Killestein J, Scheffer G, Oerlemans R, Scheper R, van der Valk P, Dijkstra CD, de Vries HE. T lymphocytes impair P-glycoprotein function during neuroinflammation. *J Autoimmun*. 2010;34:416–425.
41. Bauer B, Hartz AM, Pekcec A, Toellner K, Miller DS, Potschka H. Seizure-induced up-regulation of P-glycoprotein at the blood-brain barrier through glutamate and cyclooxygenase-2 signaling. *Mol Pharmacol*. 2008;73:1444–1453.
42. Marchi N, Betto G, Fazio V, Fan Q, Ghosh C, Machado A, Janigro D. Blood-brain barrier damage and brain penetration of antiepileptic drugs: role of serum proteins and brain edema. *Epilepsia*. 2009;50:664–677.
43. Schmidt D, Loscher W. New developments in antiepileptic drug resistance: an integrative view. *Epilepsy Curr*. 2009;9:47–52.
44. Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. *Epilepsia*. 2005;46: 1724–1743.
45. Potschka H. Modulating P-glycoprotein regulation: future perspectives for pharmacoresistant epilepsies? *Epilepsia*. 2010;51:1333–1347.
46. van Vliet EA, Zibell G, Pekcec A, Schlichtiger J, Edelbroek PM, Holtman L, Aronica E, Gorter JA, Potschka H. COX-2 inhibition controls P-glycoprotein expression and promotes brain delivery of phenytoin in chronic epileptic rats. *Neuropharmacology.* 2010;58: 404–412.
47. Pekcec A, Unkruer B, Schlichtiger J, Soerensen J, Hartz AM, Bauer B, van Vliet EA, Gorter JA, Potschka H. Targeting prostaglandin E2 EP1 receptors prevents seizure-associated P-glycoprotein up-regulation. *J Pharmacol Exp Ther*. 2009;330:939–947.
48. Zibell G, Unkruer B, Pekcec A, Hartz AM, Bauer B, Miller DS, Potschka H. Prevention of seizure-induced up-regulation of endothelial P-glycoprotein by COX-2 inhibition. *Neuropharmacology.* 2009;56:849–855.
49. Schlichtiger J, Pekcec A, Bartmann H, Winter P, Fuest C, Soerensen J, Potschka H. Celecoxib treatment restores pharmacosensitivity in a rat model of pharmacoresistant epilepsy. *Br J Pharmacol.* 2010;160: 1062–1071.
50. Kasperaviciute D, Sisodiya SM. Epilepsy pharmacogenetics. *Pharmacogenomics.* 2009;10:817–836.
51. Tate SK, Depondt C, Sisodiya SM, Cavalleri GL, Schorge S, Soranzo N, Thom M, Sen A, Shorvon SD, Sander JW, Wood NW, Goldstein DB. Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. *Proc Natl Acad Sci USA*. 2005;102: 5507–5512.
52. Tate SK, Singh R, Hung CC, Tai JJ, Depondt C, Cavalleri GL, Sisodiya SM, Goldstein DB, Liou HH. A common polymorphism in the SCN1A gene associates with phenytoin serum levels at maintenance dose. *Pharmacogenet Genomics*. 2006;16:721–726.
53. Zimprich F, Stogmann E, Bonelli S, Baumgartner C, Mueller JC, Meitinger T, Zimprich A, Strom TM. A functional polymorphism in the SCN1A gene is not associated with carbamazepine dosages in Austrian patients with epilepsy. *Epilepsia*. 2008;49:1108–1109.
54. Loscher W, Klotz U, Zimprich F, Schmidt D. The clinical impact of pharmacogenetics on the treatment of epilepsy. *Epilepsia*. 2009;50:1–23.
55. Kasperaviciute D, Catarino CB, Heinzen EL, Depondt C, Cavalleri GL, Caboclo LO, Tate SK, Jamnadas-Khoda J, Chinthapalli K, Clayton LM, Shianna KV, Radtke RA, Mikati MA, Gallentine WB, Husain AM, Alhusaini S, Leppert D, Middleton LT, Gibson RA, Johnson MR, Matthews PM, Hosford D, Heuser K, Amos L, Ortega M, Zumsteg D, Wieser HG, Steinhoff BJ, Kramer G, Hansen J, Dorn T, Kantanen AM, Gjerstad L, Peuralinna T, Hernandez DG, Eriksson KJ, Kalviainen RK, Doherty CP, Wood NW, Pandolfo M, Duncan JS, Sander JW, Delanty N, Goldstein DB, Sisodiya SM. Common genetic variation and susceptibility to partial epilepsies: a genome-wide association study. *Brain*. 2010;133(pt 7):2136–2147.
56. Bournissen FG, Moretti ME, Juurlink DN, Koren G, Walker M, Finkelstein Y. Polymorphism of the MDR1/ABCB1 C3435T drug-transporter and resistance to anticonvulsant drugs: a meta-analysis. *Epilepsia*. 2009;50:898–903.
57. Langer O, Bauer M, Hammers A, Karch R, Pataraia E, Koepp MJ, Abrahim A, Luurtsema G, Brunner M, Sunder-Plassmann R, Zimprich F, Joukhadar C, Gentzsch S, Dudczak R, Kletter K, Muller M, Baumgartner C. Pharmacoresistance in epilepsy: a pilot PET study with the P-glycoprotein substrate R-[^{11}C]verapamil. *Epilepsia.* 2007;48:1774–1784.
58. Bankstahl JP, Kuntner C, Abrahim A, Karch R, Stanek J, Wanek T, Wadsak W, Kletter K, Muller M, Loscher W, Langer O. Tariquidar-induced P-glycoprotein inhibition at the rat blood-brain barrier studied with (R)-^{11}C-verapamil and PET. *J Nucl Med.* 2008;49: 1328–1335.
59. Loscher W. Mechanisms of drug resistance. *Epileptic Disord*. 2005;7(suppl 1):S3–S9.

Chapter 85

Neural Stem Cell Therapy for Temporal Lobe Epilepsy

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Temporal lobe epilepsy (TLE), one of the common types of epilepsy, is characterized by multiple hippocampal abnormalities. These include a substantial reduction in the numbers of different subclasses of inhibitory gamma-amino butyric acid-positive (GABAergic) interneurons, aberrant synaptic reorganization, hippocampal hyperexcitability due to an increase in the overall excitatory tone vis-à-vis the inhibitory function, spontaneous recurrent seizures [SRS]) originating mostly from the hippocampus, and impairments in hippocampal-dependent cognitive function and mood.[1–10] Although regular treatment with the antiepileptic drugs (AEDs) has been found to be useful for controlling seizures in many patients, ~35% of people with TLE have chronic seizures that are resistant to AEDs.[2,11,12] Furthermore, AED therapy is associated with side effects, and most TLE patients have learning and memory impairments and depression that are not alleviated with AED therapy.[4,5,13–18] From the above perspectives, alternative therapies that have the potential for easing the frequency and intensity of SRS, learning and memory impairments, and depression in TLE are needed.

Cell transplantation strategies have received significant attention as an alternative therapy for TLE in preclinical studies.[19–26] This is because these approaches have promise for restraining epileptogenesis as well as preventing TLE development and cognitive and mood dysfunction when applied shortly after an initial precipitating hippocampal injury resulting from status epilepticus (SE), head injury, or stroke. Cell therapy may also be useful for restraining seizures and reversing cognitive and mood dysfunction when applied after the onset of TLE. Neural stem cells (NSCs) are one of the donor cell candidates considered for grafting in the domain of cell-based therapy for TLE. Neural stem cells are attractive as donor cells

for grafting in TLE because these cells can be expanded in culture for extended periods from diverse sources such as the fetal, postnatal, and adult brain, human embryonic stem (ES) cells, and human induced pluripotent stem (iPS) cells.[27–36] The promise of NSC grafting therapy for restraining SRS in TLE stems particularly from their ability to migrate extensively into different regions of the hippocampus and produce significant numbers of neurons synthesizing the inhibitory neurotransmitter GABA and astrocytes secreting the anticonvulsant factors such as the glial cell-derived neurotrophic factor (GDNF).[37–39] Moreover, the NSC grafting approach is also expected to ease the cognitive and mood dysfunction observed in TLE, as these cells can secrete a multitude of neurotrophic factors that are known to enhance both endogenous NSC proliferation and overall hippocampal neurogenesis,[40] one of the substrates important for maintaining the hippocampal-dependent cognitive functions and mood.[41–45]

The major objective of this chapter is to evaluate the contemporary knowledge and to put forward perspectives concerning the NSC grafting therapy for TLE. Since the efficiency of grafting of neural progenitors obtained from the fetal brain or ES cells are covered in other chapters in this book, this chapter is deliberately confined to studies on the efficacy of NSC grafts in TLE prototypes. The first section will focus on the prospects for preventing or minimizing SRS using NSC grafting procedures that are performed shortly after the initial precipitating injury (IPI), such as SE or a direct excitotoxic lesion. The second section will consider the promise of NSC grafting therapy for restraining seizures and easing cognitive dysfunction when applied shortly after or at prolonged periods after the onset of SE- or kindling- induced TLE. Several critical issues that need to be resolved before initiating the clinical application of NSC grafting therapy for TLE are also discussed.

EFFICACY OF THE NSC GRAFTING STRATEGY FOR PREVENTING EPILEPTOGENESIS AND/OR CHRONIC EPILEPSY AFTER HIPPOCAMPAL INJURY

Several studies have investigated the efficacy of NSC grafts for restraining seizures in different models of TLE through quantification of SRS or spontaneous spike activity via intermittent behavioral observations or/and electroencephalographic (EEG) recordings over a period of time after NSC grafting. In these studies, the effects of NSC grafts placed into hippocampi at early time points after the injury mediated by an excitotoxic lesion or SE were examined.

Effects of Adult Subventricular Zone NSC Grafts in a Unilateral Hippocampal Injury Model

Jing and colleagues[46] expanded NSCs obtained from the adult subventricular zone (SVZ) in culture as neurospheres and transplanted a suspension of neurosphere cells into the rat hippocampus 1 week after a hippocampal injury inflicted through a unilateral intracerebroventricular (ICV) administration of a small dose of kainic acid (KA). They examined the effects of NSC grafts on spontaneous spike activity using EEG recordings for 3 weeks after grafting.

DONOR CELLS AND THE TLE PROTOTYPE

The SVZ is one of the neurogenic regions in the adult brain where stem cells persist throughout life and produce neuroblasts that migrate normally into the olfactory bulb and differentiate mostly into interneurons.[31] Neural stem cells from both postnatal and adult SVZ can be expanded in culture for prolonged periods without losing their self-renewal and multipotent properties.[34,47–49] Upon differentiation, these NSCs have the ability to give rise to substantial numbers of GABAergic neurons, astrocytes, and oligodendrocytes. The prototype induced through a unilateral ICV KA administration is considered a relatively milder model of TLE, as neurodegeneration (characterized by a substantial loss of hippocampal CA3 pyramidal neurons and dentate hilar neurons), inflammation, aberrant neurogenesis, and hyperexcitability are mostly restricted to the hippocampus ipsilateral to the area of KA administration.[50–57] Additionally, the overall SRS after a unilateral ICV KA-induced hippocampal injury are much fewer and occur much later after the injury in this model in comparison to SE models of TLE.[55]

EFFECTS OF SVZ-NSC GRAFTING ON ABNORMAL SPIKE ACTIVITY AND HOST HIPPOCAMPAL INTERNEURONS

The abnormal spike frequencies in the injured hippocampus obtained through EEG recordings did not differ between the KA-treated rats that received NSC grafts and the KA-treated rats that received nonspecific grafts such as fibroblasts in the first week after grafting. However, in the second and third weeks after grafting, the KA-treated rats receiving NSC grafts displayed reduced frequencies of abnormal spikes in comparison to the KA-treated rats receiving nonspecific grafts, suggesting that NSC grafts are capable of decelerating the process of epileptogenesis. However, the major limitation of this study is that the effects of NSC grafts on SRS were not determined. Histological analyses revealed the survival of graft-derived cells and also their differentiation into glial fibrillary acidic protein positive (GFAP+) astrocytes and neuron-specific nuclear antigen positive (NeuN+) mature neurons. Interestingly, the frequency of abnormal spikes was found to be negatively correlated with the number of surviving graft-derived cells, suggesting that a reduced frequency of abnormal spikes in grafted animals is linked to the presence of graft-derived cells. The mechanisms underlying this phenomenon are yet to be determined. Additionally, the differentiation of graft-derived cells into GABAergic interneurons was not evaluated in this study. Analyses of the injured host hippocampi that received NSC grafts revealed improved survival of subclasses of host GABAergic interneurons and reduced aberrant sprouting of mossy fibers into the dentate molecular layer. As reductions in the number of GABAergic interneurons and aberrant mossy fiber sprouting are both hallmarks of TLE and are believed to contribute to the enhanced excitatory tone in the chronically epileptic hippocampus, the above results suggested that NSC grafting shortly after injury exerts an antiepileptogenic effect on the injured hippocampus.

Effects of Intravenous Administration of Human NSCs in a Pilocarpine Prototype of TLE

A study by Chu et al.[58] examined the efficacy of intravenous administration of beta galactosidase-encoded human NSCs on SRS in a pilocarpine model of TLE in rats. Administration of NSCs was performed just 1 day after the induction of SE. At 28–35 days after SE, 87% of the rats that did not receive NSCs (epilepsy-only group) exhibited SRS. In contrast, only 13% of the rats that received NSCs after SE displayed SRS. The severity of SRS was also diminished in rats receiving NSCs in comparison to the epilepsy-only group. Analyses of the field excitatory postsynaptic potentials (fEPSPs) in the CA1 region through stimulation of the CA3 Schaffer collaterals revealed that, in animals that received NSC grafts, fEPSPs were smaller than their counterparts in the epilepsy-only group. Histological analyses of the brains of rats that received intravenous administration of β gal$^+$ NSCs 6 weeks postadministration revealed the presence of β gal$^+$ cells in multiple regions of the brain, which included the hippocampal CA1 and CA3 subfields, the dentate hilus, the subiculum, amygdala, and piriform cortex. Investigation of the phenotypes of β gal$^+$ cells demonstrated that only a few cells derived from NSCs expressed markers of mature neurons. A fraction of β gal$^+$ cells coexpressed markers of interneurons such as GABA and parvalbumin (PV) in the hippocampus and the piriform cortex, suggesting that the cells derived from NSCs differentiate into GABA-synthesizing cells. A few cells derived from NSCs also expressed GFAP (presumably astrocytes or undifferentiated NSCs). These results suggested that NSCs have the ability to differentiate into GABA-synthesizing cells following engrafting into the injured hippocampus. It is possible that introduction of these new GABA-synthesizing cells into the inhibitory circuitry of the injured hippocampus decreased overall neuronal excitability and resulted in considerable suppression of SRS after SE. However, this mechanism needs to be confirmed in future studies.

Effects of Hippocampal NSC Grafts in an SE Model

A recent study investigated the efficacy of grafting of NSCs expanded from the fetal hippocampi into hippocampi of adult rats 1 week after the induction of SE for preventing or minimizing the SRS that typically occur several months after SE.[37]

DONOR CELLS FOR GRAFTING AND THE TLE PROTOTYPE

Neural stem cells were expanded from the embryonic day 19 (E19) hippocampi in vitro as neurospheres,[30] and the neurosphere cells were labeled via the addition of chlorodeoxyuridine (CldU; a thymidine analog and cell birth-dating marker) to the stem cell proliferation medium. The hippocampus is another region in the developing brain and the adult brain rich in NSCs. Neural stem cells from the fetal, postnatal, and adult hippocampi can be expanded in culture for prolonged periods.[28,30,59–61] Upon differentiation, these NSCs have the ability to give rise to all three central nervous system (CNS) phenotypes and GABAergic neurons. For grafting, neurospheres were triturated gently, and the resulting suspension of neurosphere cells was treated with differentiation factors such as fibroblast growth factor-2 (FGF-2) and brain-derived neurotrophic factor (BDNF) and transplanted into hippocampi of adult rats 7 days after the induction of SE. Status epilepticus was induced via graded injections of the KA,[62,63] and the KA-induced continuous acute seizure activity (i.e., SE) was terminated 3 h after the onset through an injection of diazepam (5 mg/kg body weight). Since virtually all rats treated with KA as described above typically develop SRS (or chronic TLE) 3–4 months after SE,[62] this is an excellent model to test the efficacy of NSC grafts for seizure suppression.

EFFECTS OF HIPPOCAMPAL NSC GRAFTS ON SRS

Graft-mediated beneficial effects on the frequency and intensity of SRS were quantified at 4–6 months postgrafting through intermittent behavioral observations (4 h per session, two sessions per week for 3 months). To authenticate the direct effects of hippocampal NSC grafts on SRS, seizure data were compared with those from another group of rats that underwent SE and sham surgery (involving injections of the culture medium instead of the hippocampal NSC grafts) 1 week post-SE. This comparison clearly revealed considerably diminished frequency and intensity of SRS in the rats that received hippocampal NSC grafts after SE in comparison to those that received sham-grafting surgery after SE (82%–90% reduction in all seizures and 89%–93% reduction in stage 5 seizures; $p < .01$). Thus, the beneficial effects in terms of the frequency and intensity of SRS observed with hippocampal NSC grafting in this SE model appeared specific to the presence of NSC-derived cells, as the animals that received sham-grafting surgery did not exhibit these beneficial changes.

Additional analyses of the SRS and high-amplitude spikes were done at 6 months postgrafting using continuous EEG recordings for 4 days in comparison to two age-matched control groups (i.e., rats receiving dead NSC grafts after SE or rats receiving no grafts after SE).[64] These analyses also demonstrated that grafting of hippocampal NSCs into hippocampi of rats 1 week post-SE considerably diminishes the frequency of SRS (53%–63% reduction; $p < 0.001$) and the total numbers of high-amplitude spikes (40%–55% reduction; $p < 0.05$) in the chronic phase after SE in comparison to control groups.[64] Histological analyses revealed an extensive migration of NSC graft-derived cells into different regions of the hippocampus. The overall yield of graft-derived cells was equivalent to ~50% of the injected cells. Phenotypic analyses of graft-derived cells revealed the ability of hippocampal NSCs to give rise to mature neurons (including the differentiation of ~15% of cells into GABAergic neurons) and mature astrocytes following grafting into the epileptic hippocampus.[64] Hippocampal NSC grafting also appeared to have a neuroprotective effect on the survival of host interneurons positive for neuropeptide Y (NPY) and PV. This neuroprotective effect, as well as the addition of new GABAergic neurons, could be the mechanism underlying the suppression of SRS in this grafting model. Overall, this study, using both behavioral observations and EEG recordings, indicated that grafting of hippocampal NSCs into adult hippocampi shortly after SE is an excellent strategy for greatly restraining SE-induced chronic epilepsy for prolonged periods. The seizure-suppressing effect demonstrated with grafting of hippocampal NSCs in this study is relevant for the future clinical application of NSC grafting for restraining TLE development after a hippocampal injury due to acute seizures, SE, stroke, or head injury.

EFFICACY OF NSC GRAFTING FOR EASING CHRONIC TLE

Application of NSC grafts at early time points after the injury or insult, using TLE models as described above, is useful for unraveling the antiepileptogenic effects of NSC grafts and for developing a pretreatment strategy that prevents or diminishes the development of chronic epilepsy after an injury to the hippocampus. However, such studies are not relevant for treating patients who are already suffering from chronic TLE. As patients with intractable epilepsy are the most likely candidates for NSC grafting therapy among the epilepsy patient population as an alternative to hippocampal resection, it is vital that studies are performed in prototypes where animals are epileptic at the time of grafting. As seizures in TLE originate mainly from the hippocampus and are associated with cognitive impairments and reduced hippocampal neurogenesis, it is also important to examine the effects of grafting NSCs into hippocampi of animals that are not only chronically epileptic but also have cognitive impairments at the time of grafting. The following section discusses findings from NSC grafting studies in rats that exhibited SRS and/or cognitive impairments at the time of grafting.

Effects of NSC Grafts in an SE-Induced Chronic Epilepsy Model

A recent study in our laboratory ascertained whether grafting of NSCs that are capable of adding new GABAergic interneurons and GDNF-expressing astrocytes to the epileptic hippocampus restrains SRS in chronic TLE.[39] In this study, NSCs expanded in vitro from the embryonic medial ganglionic eminence (MGE) were grafted bilaterally into hippocampi of adult rats exhibiting chronic TLE with cognitive impairments for prolonged periods to ascertain the efficacy of such grafts for treating chronic TLE.

PREPARATION AND PROPERTIES OF DONOR NSCS

For preparation of donor NSCs, MGEs of E14 fetuses were dissected, dissociated, and expanded as neurospheres in an NSC proliferation medium containing the birth-dating marker CldU. The reasons for choosing MGE-NSCs for grafting in this study are based on the fact that the MGE is the source of most hippocampal GABAergic interneurons in the developing brain and the observation that GABAergic interneurons derived from primary MGE cells display the ability for both functional integration and increasing the extent of inhibition when grafted into the normal postnatal brain.[65] For grafting, the MGE-NSC-derived neurospheres were dissociated and a suspension of neurosphere cells was prepared. Parallel differentiation cultures of neurosphere cells were also performed, which revealed the ability of MGE-derived neurosphere cells to differentiate into all three CNS phenotypes. Additionally, 15% of NSC-derived cells differentiated into GABAergic neurons in culture.[39] Figure 85–1 illustrates examples of the beta-III tubulin positive (TuJ-1+) and GABAergic neurons derived from the MGE-NSCs.

CHARACTERISTICS OF CHRONICALLY EPILEPTIC RATS USED FOR THE GRAFTING STUDY

Chronically epileptic rats used in this study were generated using a KA-induced SE.[63] Since the extent of SRS varies among animals, a group of age-matched chronically epileptic rats exhibiting a similar extent of SRS were chosen from a larger pool of animals that were chronically epileptic for ~12 months. In addition, all chronically epileptic animals chosen for grafting were also examined with a water maze test to confirm the spatial learning and memory impairments in these rats at the time of grafting. These rats were grafted with MGE-NSCs, with each rat receiving four grafts per hippocampus (80,000 live cells per graft). In order to exclude the effects of grafting surgery on SRS, sham-grafting surgery was performed in an additional group of rats exhibiting a similar extent of SRS.

EFFICACY OF MGE-DERIVED NSC GRAFTS FOR EASING SRS

Grafting of MGE-NSCs greatly reduced not only the frequency but also the duration and severity of SRS[39] (Fig. 85–2). At 3 months

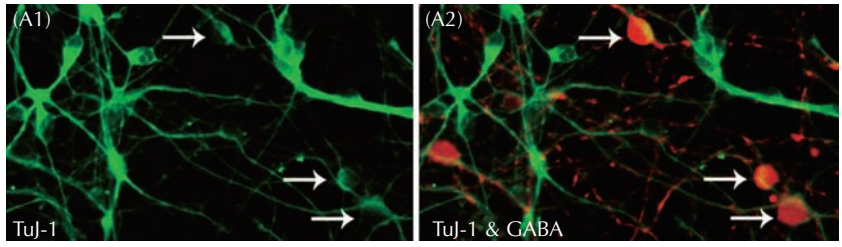

Figure 85–1. A significant fraction of the MGE-NSCs differentiate into TujJ-1+ neurons and GABA+ neurons in culture. The figure shows examples of TujJ-1+ neurons and GABA+ neurons from an MGE-NSC culture after 8 days of incubation in a differentiation medium. Arrows denote the TuJ-1+ neurons that express GABA. From ref. 39.

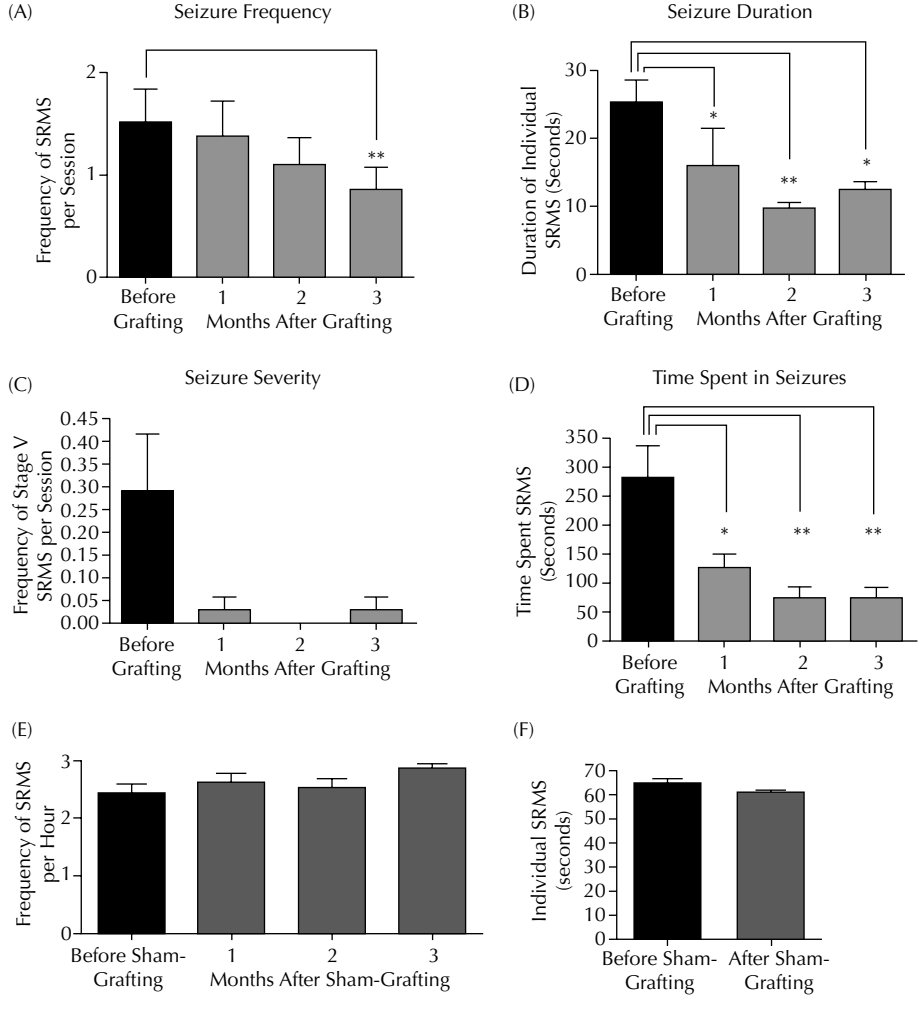

Figure 85–2. The MGE-NSC grafting procedure considerably eases spontaneous recurrent motor seizures (SRMS) in chronically epileptic rats. The *y*-axis in bar charts **A** and **C** denotes the average number of seizures per session (4 h block) of observation. Note that MGE-NSC grafting considerably decreases the seizure frequency (**A**), the duration of individual seizures (**B**), the severity of seizures (**C**), and the total time spent in seizures (**D**). $^{*}p < .05$; $^{**}p < .01$. The bar charts in **E** and **F** show the average seizure frequency and the average seizure duration in chronically epileptic animals that received the sham-grafting surgery. The sham-grafting surgery did not alter the seizure frequency or the seizure duration in chronically epileptic rats. From ref. 39.

postgrafting, the overall decreases were 43% for the frequency of SRS, 51% for the duration of individual SRS, and 90% for the frequency of stage 5 seizures (the most severe form of SRS). In addition, there was a 74% decrease in the total time in seizures during the third month after grafting. Sham-grafting surgery had no effect on chronic epilepsy, however, as the animals in this group displayed no decrease in either seizure frequency or duration of individual SRS.[39]

SURVIVAL AND DIFFERENTIATION OF MGE-NSC GRAFTS IN THE CHRONICALLY EPILEPTIC HIPPOCAMPUS

Analyses of brain tissues with immunostaining for CldU (the marker used for identifying graft-derived cells in this study) revealed the presence of graft-derived cells in hippocampi after 3 months of grafting. Graft-derived cells migrated extensively into different layers of the CA3 subfield and the lateral regions of the CA1 and dentate gyrus subfields throughout the antero-posterior axis of the hippocampus[39] (Fig. 85–3). However, graft-derived cells were only occasionally observed in the dentate subgranular zone and the granule cell layer. Quantification of the total number of graft-derived cells demonstrated a yield that was equivalent to 28% of injected cells or ~81,536 cells per hippocampus. Furthermore, the grafted MGE-NSCs differentiated into the NeuN+ neurons, the GABA+ interneurons (Fig. 85–4), the (S-100β+) mature astrocytes, and the neuron-glia chondritin sulfate proteoglycan 2 positive (NG2+) oligodendrocyte progenitors. Extrapolation of the yield of graft-derived cells with percentages of different cell types suggested that MGE-NSC grafting resulted in the addition of over 10,000 new neurons, 46,000 new astrocytes, 2000 new oligodendrocyte progenitors, and 8000 new GABAergic neurons into each hippocampus of chronically epileptic rats. A substantial fraction (~50%) of cells derived from the MGE-NSC grafts also expressed GDNF, which morphologically appeared to be astrocytes. Considering the overall yield of graft-derived cells, this amounted to the addition of over 40,000 new GDNF+ cells into the each hippocampus of epileptic rats.[39]

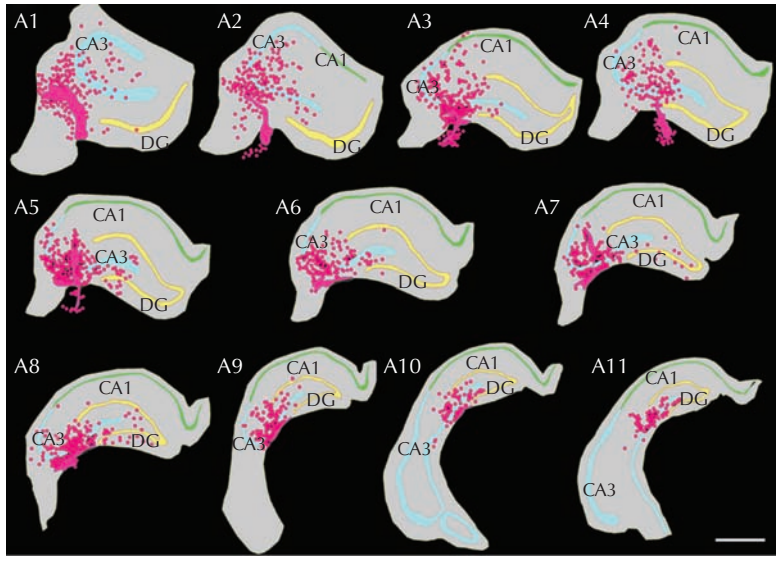

Figure 85–3. Cells derived from the MGE-NSC grafts migrate extensively in the chronically epileptic hippocampus. The figures illustrate the location of transplants and the transplant-derived cells (shown in pink based on the donor cell marker CldU+ immunoreactivity) with respect to the hippocampal cell layers and the subfields in a chronically epileptic rat after 3 months of grafting. These tracings, performed using Neurolucida software (Microbrightfield Inc.), represent every tenth 30 μm thick section through a chronically epileptic hippocampus that received four MGE-NSC grafts. Note that the grafts and graft-derived cells were located mostly in the CA3 subfield and the lateral ends of the CA1 subfield and the dentate gyrus. The core of transplants partially projected ventrally into the thalamus at certain levels. Scale bar, A1–A11, 1000 μm. From ref. 39.

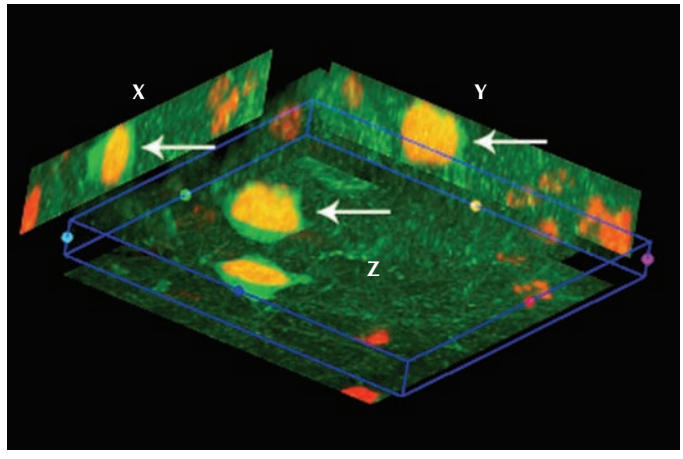

Figure 85–4. A fraction of the cells derived from the MGE-NSC grafts differentiate into GABA-positive neurons in the chronically epileptic hippocampus. The figure illustrates a three-dimensional view of (in X, Y, Z planes) a GABAergic neuron (indicated by arrows) derived from an MGE-NSC graft. This neuron was visualized through dual immunofluorescence for CldU (a birth-dating marker used for the donor MGE-NSCs) and GABA (a marker of inhibitory neurons) followed by Z-section and three-dimensional analyses in a confocal microscope.

EFFECTS OF MGE-NSC GRAFTING ON HOST HIPPOCAMPAL GDNF EXPRESSION

This study demonstrated that the vast majority of astrocytes (≥86%) in all subfields of the intact hippocampus of naive rats express the anticonvulsant protein GDNF but a substantially smaller percentage of astrocytes (≤42%) express GDNF in the hippocampus of chronically epileptic rats. Interestingly, MGE-NSC grafting into the hippocampus restored GDNF expression (Fig. 85–5), as ≥76% of S-100β+ astrocytes in the hippocampus of rats that received MGE-NSC grafts expressed GDNF.

POTENTIAL MECHANISMS OF NSC GRAFT-MEDIATED SUPPRESSION OF SRS

While this study did not investigate the precise mechanisms underlying the NSC graft- mediated suppression of SRS in chronically epileptic rats, correlative observations suggested two potential mechanisms. One pertains to the addition of over 8000 new GABAergic neurons per hippocampus through NSC grafting. This addition is clearly significant, considering the extent of GABAergic interneuron loss and loss of the functional inhibition observed in TLE,[1,6,7,9,50,51] as well as the observation that grafting of cells that release only GABA can also facilitate transient antiseizure effects.[23,25,66–68] Based on these observations, it is possible that the antiseizure effect is a consequence of an increase in the GABA concentration mediated by the GABAergic neurons derived from the NSC grafts. It is also possible that the inhibitory synaptic integration of graft-derived GABAergic neurons on hippocampal principal excitatory neurons underlies the seizure suppression demonstrated in this study. This is because the overall suppression of SRS was improved over the 3-month postgrafting period, a sizable number of graft-derived GABAergic neurons survived for 3 months, and previous studies have shown that axons of GABAergic neurons derived from the MGE precursor cell grafts integrate with the host brain and increase the level of inhibition.[65,69] Nevertheless, electrophysiological and electron microscopic studies are needed to confirm the synaptic integration of NSC graft-derived GABAergic neurons. The second potential mechanism is related to the addition of over 40,000 GDNF+ cells per hippocampus and restoration of GDNF expression in the host hippocampal astrocytes with MGE-NSC grafting because of the anticonvulsant property of GDNF noted in models of TLE.[70,71] Thus, suppression of SRS by NSC grafts in this study appeared to be due to both addition of GABAergic neurons and GDNF-secreting

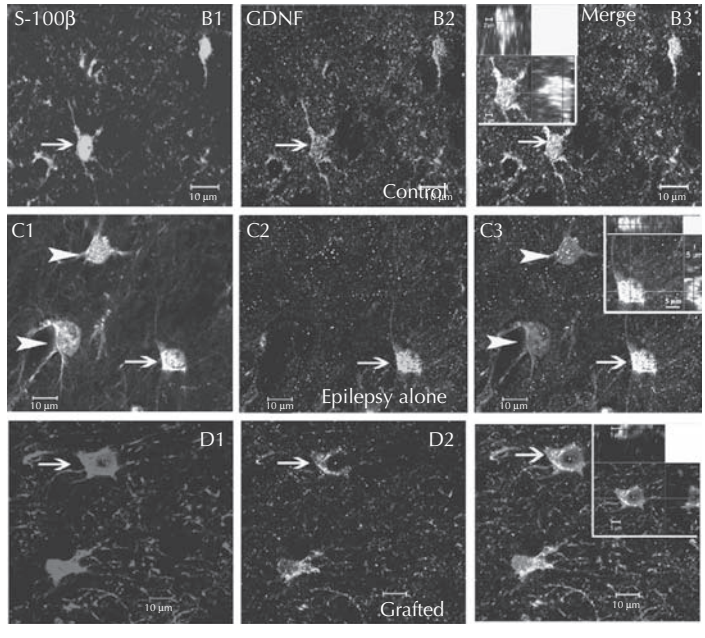

Figure 85–5. The MGE-NSC grafting into the chronically epileptic hippocampus restores the expression of GDNF in the host hippocampal astrocytes. Figures **B1–D3** show confocal microscopic images of S-100β+ hippocampal astrocytes that exhibit GDNF expression in an age-matched control rat (**B1–B3**), a rat with chronic epilepsy alone (**C1–C3**), and a chronically epileptic rat that received MGE-NSC grafts (**D1–D3**). The insets in **B3**, **C3**, and **D3** show orthogonal views of cells that are indicated by arrows in **B1** and **B2**, **C1** and **C2**, and **D1** and **D2**, respectively.. Note that these cells are positive for both S-100β and GDNF. Arrowheads in **C1** and **C3** denote S-100β+ astrocytes that lack GDNF expression in the epilepsy-alone group. Scale bar, 10 μm. From ref. 39.

cells and restoration of GDNF expression in the host hippocampal astrocytes.

EFFICACY OF MGE-NSC GRAFTS FOR EASING LEARNING AND MEMORY DYSFUNCTION IN CHRONICALLY EPILEPTIC RATS

Evaluation of chronically epileptic rats prior to grafting with a water maze test had revealed the existence of impairments in learning and memory function in comparison to age-matched control rats. In the second water maze test performed 2 months postgrafting, their posttransplantation learning and memory scores were not improved in comparison to their pretransplantation scores. Thus, transplantation of the MGE-NSC grafts did not reverse the hippocampal-dependent spatial learning and memory deficits in chronically epileptic rats. Considering the importance of neurogenesis for hippocampal-dependent learning and memory function,[42–45] the failure to reverse the cognitive dysfunction likely reflects the inability of the grafts to enhance the hippocampal neurogenesis that almost shuts down in chronic epilepsy.[55,72] Indeed, no effects of MGE-NSC grafting on neurogenesis were observed when the chronically epileptic rats were examined after 3 months of grafting. Lack of graft-mediated effects on neurogenesis might be attributable to insufficient engrafting of the graft-derived NSCs into the neurogenic niche of the dentate gyrus. Another reason for the lack of improvement in cognitive function is that the MGE-NSC grafts did not give rise to CA1 or CA3 pyramidal neurons to restore the neuron loss in CA1 and CA3 cell layers. Restoration of the cognitive pathways might require grafting of NSCs that are capable of engrafting substantially into the neurogenic region of the dentate gyrus[40] and/or cells that have the ability to differentiate into hippocampal pyramidal neurons and restore the damaged CA1-CA3 circuitry.[6,8,19,20,21]

Overall, this study provided novel evidence that MGE-NSC grafting into the hippocampus is an effective approach for suppressing SRS

in chronic TLE. The limitation of this study is that continuous EEG recordings were not performed to ascertain all of the SRS occurring in the postgrafting observation period, which is necessary in future NSC grafting studies using chronic epilepsy models to advance this therapy for TLE patients.

Effects of NSC Grafts in Suppressing SRS in a Kindling Model

Shindo and colleagues examined the efficacy of transplantation of NSCs generated from mouse ES cells into the hippocampus of kindled epileptic mice for suppressing SRS.[38]

DONOR CELLS FOR GRAFTING AND THE ANIMAL MODEL

Pluripotent mouse ES cells were first grown into aggregates of cells called *embryoid bodies* (EBs). Following this, NSCs derived from EBs were selectively expanded in serum-free defined media containing FGF-2 to generate neurospheres. The neurospheres were then dissociated into single cells and used for grafting. The host epileptic rats were prepared using the amygdala kindling paradigm.[38] This comprised implantation of electrodes into the left amygdala followed by electrical stimulation of the left amygdala (twice daily) with a biphasic square wave pulse and evaluation of the induced seizures using a modified Racine scale.[38] The stimulation was continued until animals displayed stage V seizures. The animals exhibiting stage V seizures were then used for intrahippocampal grafting of the NSCs (one transplant to the left hippocampus comprising 400,000 live cells).

EFFECTS OF ES CELL-DERIVED NSC GRAFTS ON SRS

Evaluation of SRS 6 weeks after grafting through behavioral observations revealed partial recovery from seizures in all animals that received NSC grafts.[38] Recovery was, however, manifested only in terms of reduction in the intensity of seizures (i.e., from stage V to stage III/IV seizures) but was significant in comparison to recovery in the sham-operated (i.e., culture media- injected) rats exhibiting stage V seizures during the same time period. Qualitative immunohistochemical analyses of the transplanted NSCs 6 weeks postgrafting revealed some differentiation of the graft-derived cells into neurons, including neurons that were positive for the GABA-synthesizing enzyme glutamic acid decarboxylase-67 (GAD-67).[38] Overall, this study suggested that NSCs derived from ES cells are also efficacious for restraining SRS in a TLE model. However, the limitations of this study include the brief intermittent analyses of SRS (i.e., only at 2, 4, and 6 weeks after grafting), the lack of EEG recordings to document all (electrographic as well as behavioral) SRS occurring during the entire postgrafting observation period, and the lack of quantitative correlation between the phenotype of the NSC graft-derived cells (such as cells positive for GAD-67) and SRS.

CONCLUSIONS AND FUTURE DIRECTIONS

Neural stem cell grafting into the hippocampus shortly after the initial precipitating injury has shown considerable beneficial effects in terms of restraining injury- or SE-induced epileptogenesis and SRS in animal models of TLE.[37,46,58,64] However, the beneficial effects of such early grafting strategies for preventing the injury-induced cognitive dysfunction and depression have not been ascertained so far. Grafting of NSCs has also shown considerable efficacy for restraining SRS when applied shortly or at prolonged periods after the onset of TLE.[38,39] While the precise mechanisms underlying NSC graft-mediated seizure suppression are yet to be determined, correlative analyses point to the addition of substantial numbers of GABAergic neurons and GDNF-secreting cells into the epileptic host hippocampus and restoration of GDNF in host hippocampal astrocytes by NSC grafts.[39]

Nonetheless, additional rigorous studies are needed prior to the clinical application of NSC grafts for treating TLE. First, it is vital to study human NSCs such as those derived from fetal, postnatal, and adult brain tissues, human ES cells, or human iPS cells for their ability to survive for a long time and their ability to give rise to substantial numbers of GABAergic neurons and/or GDNF-secreting astrocytes after being

grafted into the hippocampus in animal models of chronic TLE. Second, it is important to rigorously assess the ability of NSC grafts to give rise to subclasses of interneurons that exhibit substantial depletions in number in the chronically epileptic hippocampus (such as those positive for NPY, somatostatin, and PV). Third, it will be useful to determine whether or not the appropriate synaptic integration of NSC graft-derived GABAergic neurons underlies seizure suppression in chronically epileptic prototypes. Fourth, as strategies that suppress SRS (e.g., increased inhibition) may not necessarily improve cognitive function and mood in chronic epilepsy,[39] it will be important to examine the efficacy of combination strategies such as grafting of NSCs into the hippocampi with systemic administration of neurogenesis-enhancing factors such as antidepressants, neurotrophic factors, antioxidants, or dietary supplements. In addition, studies will be needed for developing strategies that both increase the overall yield of NSC graft-derived GABAergic neurons and GDNF-secreting cells and also promote the engraftment of graft-derived NSCs into the dentate subgranular zone to restore the greatly attenuated hippocampal neurogenesis observed in chronic epilepsy.

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DISCLOSURE STATEMENT

The author has no conflicts of interest to disclose.

REFERENCES

1. de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res.* 1989;495:387–395.
2. Engel J Jr. Mesial temporal lobe epilepsy: what have we learned? *Neuroscientist*. 2001;7:340–352.
3. Kobayashi M, Buckmaster PS. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. *J Neurosci*. 2003;23:2440–2452.
4. Devinsky O. Therapy for neurobehavioral disorders in epilepsy. *Epilepsia.* 2004;45(S2)2:34–40.
5. Detour J, Schroeder H, Desor D, Nehlig A. A 5-month period of epilepsy impairs spatial memory, decreases anxiety, but spares object recognition in the lithium-pilocarpine model in adult rats. *Epilepsia*. 2005;46: 499–508.
6. Shetty AK, Turner DA. Fetal hippocampal CA3 cell transplants restore host hippocampal GAD-positive interneuron numbers in a rat model of TLE. *J Neurosci*. 2000;20:8788–8801.
7. Shetty AK, Turner DA. Glutamic acid decarboxylase positive hippocampal interneurons undergo a permanent reduction in number following kainic acid induced destruction of CA3 pyramidal neurons. *Exp Neurol*. 2001;176:276–297.
8. Shetty AK, Zaman V, Hattiangady B. Repair of the injured adult hippocampus through graft-mediated modulation of the plasticity of the dentate gyrus in a rat model of temporal lobe epilepsy. *J Neurosci.* 2005;25:8391–8401.
9. Shetty AK, Hattiangady B, Rao MS. Vulnerability of hippocampal GABA-ergic interneurons to kainate induced excitotoxic injury during old age. *J Cell Mol Med*. 2009;13(8B):2408–2423.
10. Dudek FE, Sutula TP. Epileptogenesis in the dentate gyrus: a critical perspective. *Prog Brain Res*. 2007;163:755–773.
11. Litt B, Esteller R, Echauz J, D'Alessandro M, Shor R, Henry T, Pennell P, Epstein C, Bakay R, Dichter M, Vachtsevanos G. Epileptic seizures may begin hours in advance of clinical onset: a report of five patients. *Neuron*. 2001;30:51–64.
12. Spencer SS. When should temporal-lobe epilepsy be treated surgically? *Lancet Neurol*. 2002;1:375–382.
13. Alessio A, Damasceno BP, Camargo CH, Kobayashi E, Guerreiro CA, Cendes F. Differences in memory performance and other clinical characteristics in patients with mesial temporal lobe epilepsy with and without hippocampal atrophy. *Epilepsy Behav*. 2004;5: 22–27.
14. Jokeit H, Krämer G, Ebner A. Do antiepileptic drugs accelerate forgetting? *Epilepsy Behav*. 2005;6: 430–432.
15. Strine TW, Kobau R, Chapman DP, Thurman DJ, Price P, Balluz LS. Psychological distress, comorbidities, and health behaviors among U.S. adults with seizures: results from the 2002 National Health Interview Survey. *Epilepsia.* 2005;46:1133–1139.
16. Mainio A, Alamäki K, Karvonen K, Hakko H, Särkioja T, Räsänen P. Depression and suicide in epileptic victims: a population-based study of suicide victims during the years 1988–2002 in northern Finland. *Epilepsy Behav*. 2007;11:389–393.
17. Neville BG, Scott RC. Re: severe memory impairment in a child with bi-hippocampal injury after status epilepticus. *Dev Med Child Neurol*. 2007;49:398–399.
18. Chen J, Quan QY, Yang F, Wang Y, Wang JC, Zhao G, Jiang W. Effects of lamotrigine and topiramate on hippocampal neurogenesis in experimental temporal-lobe epilepsy. *Brain Res*. 2010;1313:270–282.

19. Shetty AK, Turner DA. Development of fetal hippocampal grafts in intact and lesioned hippocampus. *Prog Neurobiol*. 1996;50:597–653.
20. Turner DA, Shetty AK. Clinical prospects for neural grafting therapy for hippocampal lesions and epilepsy. *Neurosurgery*. 2003;52:632–644.
21. Rao MS, Hattiangady B, Rai KS, Shetty AK. Strategies for promoting anti-seizure effects of hippocampal fetal cells grafted into the hippocampus of rats exhibiting chronic temporal lobe epilepsy. *Neurobiol Dis*. 2007;27:117–132.
22. Shetty AK, Hattiangady B. Concise review: prospects of stem cell therapy for temporal lobe epilepsy. *Stem Cells*. 2007;25:2396–2407.
23. Löscher W, Gernert M, Heinemann U. Cell and gene therapies in epilepsy—promising avenues or blind alleys? *Trends Neurosci*. 2008;31:62–73.
24. Richardson RM, Barbaro NM, Alvarez-Buylla A Baraban SC. Developing cell transplantation for temporal lobe epilepsy. *Neurosurg Focus.* 2008;24 (3–4):E17..
25. Thompson K. Transplantation of GABA-producing cells for seizure control in models of temporal lobe epilepsy. *Neurotherapeutics.* 2009;6:284–294.
26. Naegele JR, Maisano X, Yang J, Royston S, Ribeiro E. Recent advancements in stem cell and gene therapies for neurological disorders and intractable epilepsy. *Neuropharmacology.* 2010;58:855–864.
27. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science.* 1992;255:1707–1710.
28. Shetty AK, Turner DA. In vitro survival and differentiation of neurons derived from epidermal growth factor-responsive postnatal hippocampal stem cells: inducing effects of brain-derived neurotrophic factor. *J Neurobiol*. 1998;35:395–425.
29. Palmer TD, Markakis EA, Willhoite AR, Safar F, Gage FH. Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. *J Neurosci*. 1999;19:8487–8497.
30. Shetty AK. Progenitor cells from the CA3 region of the embryonic day 19 rat hippocampus generate region-specific neuronal phenotypes in vitro. *Hippocampus.* 2004;14:595–614.
31. Alvarez-Buylla A, Kohwi M, Nguyen TM, Merkle FT. The heterogeneity of adult neural stem cells and the emerging complexity of their niche. *Cold Spring Harb Symp Quant Biol.* 2008;73:357–365.
32. Elkabetz Y, Studer L. Human ESC-derived neural rosettes and neural stem cell progression. *Cold Spring Harb Symp Quant Biol*. 2008;73:377–387.
33. Zhang SC, Li XJ, Johnson MA, Pankratz MT. Human embryonic stem cells for brain repair? *Philos Trans R Soc Lond B Biol Sci.* 2008;363:87–99.
34. Leonard BW, Mastroeni D, Grover A, Liu Q, Yang K, Gao M, Wu J, Pootrakul D, van den Berge SA, Hol EM, Rogers J. Subventricular zone neural progenitors from rapid brain autopsies of elderly subjects with and without neurodegenerative disease. *J Comp Neurol*. 2009;515:269–294.
35. Saha K, Jaenisch R. Technical challenges in using human induced pluripotent stem cells to model disease. *Cell Stem Cell*. 2009;5:584–595.
36. Yamanaka S. Patient-specific pluripotent stem cells become even more accessible. *Cell Stem Cell*. 2010; 7:1–2.
37. Kuruba R, Hattiangady B, Shuai B, Shetty AK. Effects of grafting of hippocampal stem/progenitor cells shortly after status epilepticus on the development of chronic epilepsy. *Cell Transplant*. 2009;18:221–221.
38. Shindo A, Nakamura T, Matsumoto Y, Kawai N, Okano H, Nagao S, Itano T, Tamiya T. Seizure suppression in amygdala-kindled mice by transplantation of neural stem/progenitor cells derived from mouse embryonic stem cells. *Neurol Med Chir*. 2010;50: 98–105.
39. Waldau B, Hattiangady B, Kuruba R, Shetty AK. Medial ganglionic eminence-derived neural stem cell grafts ease spontaneous seizures and restore GDNF expression in a rat model of chronic temporal lobe epilepsy. *Stem Cells*. 2010;8:1153–1164.
40. Hattiangady B, Shuai B, Cai J, Coksaygan T, Rao MS, Shetty AK. Increased dentate neurogenesis after grafting of glial restricted progenitors or neural stem cells in the aging hippocampus. *Stem Cells*. 2007;25: 2104–2117.
41. Sahay A, Hen R. Adult hippocampal neurogenesis in depression. *Nat Neurosci*. 2007;10:1110–1115.
42. Dupret D, Revest JM, Koehl M, Ichas F, De Giorgi F, Costet P, Abrous DN, Piazza PV. Spatial relational memory requires hippocampal adult neurogenesis. *PLoS One*. 2008;3:e1959.
43. Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T, Yamaguchi M, Mori K, Ikeda T, Itohara S, Kageyama R. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci*. 2008;11:1153–1161.
44. Clelland CD, Choi M, Romberg C, Clemenson GD Jr, Fragniere A, Tyers P, Jessberger S, Saksida LM, Barker RA, Gage FH, Bussey TJ. A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science.* 2009;325:210–213.
45. Jessberger S, Clark RE, Broadbent NJ, Clemenson GD Jr, Consiglio A, Lie DC, Squire LR, Gage FH. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn Mem.* 2009;16:147–154.
46. Jing M, Shingo T, Yasuhara T, Kondo A, Morimoto T, Wang F, Baba T, Yuan WJ, Tajiri N, Uozumi T, Murakami M, Tanabe M, Miyoshi Y, Zhao S, Date I. The combined therapy of intrahippocampal transplantation of adult neural stem cells and intraventricular erythropoietin-infusion ameliorates spontaneous recurrent seizures by suppression of abnormal mossy fiber sprouting. *Brain Res*. 2009;295:203–217.
47. Lois C, Alvarez-Buylla A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc Natl Acad Sci USA.* 1993;90:2074–2077.
48. Ayuso-Sacido A, Roy NS, Schwartz TH, Greenfield JP, Boockvar JA. Long-term expansion of adult human brain subventricular zone precursors. *Neurosurgery*. 2008;62:223–229.
49. Ahlenius H, Kokaia Z. Isolation and generation of neurosphere cultures from embryonic and adult mouse brain. *Methods Mol Biol.* 2010;633:241–252.
50. Cornish SM, Wheal HV. Long-term loss of paired pulse inhibition in the kainic acid-lesioned hippocampus of the rat. *Neuroscience*. 1989;28:563–571.
51. Turner DA, Wheal HV. Excitatory synaptic potentials in kainic acid-denervated rat CA1 pyramidal neurons. *J Neurosci*. 1991;11:2786–2794.

52. Shetty AK, Turner DA. Intracerebroventricular kainic acid administration in adult rat alters hippocampal calbindin and non-phosphorylated neurofilament expression. *J Comp Neurol*. 1995;363:581–599.
53. Shetty AK, Turner DA. Vulnerability of the dentate gyrus to aging and intracerebroventricular administration of kainic acid. *Exp Neurol*. 1999;158:491–503.
54. Shetty AK, Turner DA. Aging impairs axonal sprouting response of dentate granule cells following target loss and partial deafferentation. *J Comp Neurol*. 1999;414: 238–254.
55. Hattiangady B, Rao MS, Shetty AK. Chronic temporal lobe epilepsy is associated with severely diminished dentate neurogenesis in the adult hippocampus. *Neurobiol Dis*. 2004;17:473–490.
56. Hattiangady B, Rao MS, Shetty AK. Plasticity of hippocampal stem/progenitor cells to increase neurogenesis in response to injury is lost by middle age. *Aging Cell*. 2008;7:207–224.
57. Shetty AK, Hattiangady B, Rao MS, Shuai B. Deafferentation enhances neurogenesis in the young and middle aged hippocampus but not in the aged hippocampus. *Hippocampus*. 2011; 21:631–646
58. Chu K, Kim M, Jung KH, Jeon D, Lee ST, Kim J, Jeong SW, Kim SU, Lee SK, Shin HS, Roh JK. Human neural stem cell transplantation reduces spontaneous recurrent seizures following pilocarpine-induced status epilepticus in adult rats. *Brain Res*. 2004;1023:213–221.
59. Ray J, Peterson DA, Schinstine M, Gage FH. Proliferation, differentiation, and long-term culture of primary hippocampal neurons. *Proc Natl Acad Sci USA*. 1993;90:3602–3606.
60. Gage FH, Coates PW, Palmer TD, Kuhn HG, Fisher LJ, Suhonen JO, Peterson DA, Suhr ST, Ray J. Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. *Proc Natl Acad Sci USA*. 1995;92:11879–11883.
61. Shetty AK, Turner DA. Neurite outgrowth from progeny of epidermal growth factor-responsive hippocampal stem cells is significantly less robust than from fetal hippocampal cells following grafting onto organotypic hippocampal slice cultures: effect of brain-derived neurotrophic factor. *J Neurobiol*. 1999;38: 391–413.
62. Rao MS, Hattiangady B, Reddy DS, Shetty AK. Hippocampal neurodegeneration, spontaneous seizures, and mossy fiber sprouting in the F344 rat model of temporal lobe epilepsy. *J Neurosci Res*. 2006;83: 1088–1105.
63. Rao MS, Hattiangady B, Shetty AK. Status epilepticus during old age is not associated with enhanced hippocampal neurogenesis. *Hippocampus*. 2008;18: 931–944.
64. Hattiangady B, Kuruba R, Parihar VK, Shetty AK. Intrahippocampal grafting of NSCs after status epilepticus eases both spontaneous seizures and cognitive dysfunction in a rat model of temporal lobe epilepsy. *Soc Neurosci Abstr*. 2010; 29.30.
65. Alvarez-Dolado M, Calcagnotto ME, Karkar KM, Southwell DG, Jones-Davis DM, Estrada RC, Rubenstein JL, Alvarez-Buylla A, Baraban SC. Cortical inhibition modified by embryonic neural precursors grafted into the postnatal brain. *J Neurosci*. 2006;26: 7380–7389.
66. Gernert M, Thompson KW, Löscher W, Tobin AJ. Genetically engineered GABA-producing cells demonstrate anticonvulsant effects and long-term transgene expression when transplanted into the central piriform cortex of rats. *Exp Neurol*. 2002;176:183–192.
67. Thompson KW. Genetically engineered cells with regulatable GABA production can affect afterdischarges and behavioral seizures after transplantation into the dentate gyrus. *Neuroscience*. 2005;133:1029–1037.
68. Castillo CG, Mendoza-Trejo S, Aguilar MB, Freed WJ, Giordano M. Intranigral transplants of a GABAergic cell line produce long-term alleviation of established motor seizures. *Behav Brain Res*. 2008;193:17–27.
69. Baraban SC, Southwell DG, Estrada RC, Jones DL, Sebe JY, Alfaro-Cervello C, García-Verdugo JM, Rubenstein JL, Alvarez-Buylla A. Reduction of seizures by transplantation of cortical GABAergic interneuron precursors into Kv1.1 mutant mice. *Proc Natl Acad Sci USA*. 2009;106:15472–15477.
70. Kanter-Schlifke I, Georgievska B, Kirik D, Kokaia M. Seizure suppression by GDNF gene therapy in animal models of epilepsy. *Mol Ther*. 2007;15:1106–1113.
71. Kanter-Schlifke I, Fjord-Larsen L, Kusk P, Angehagen M, Wahlberg L, Kokaia M. GDNF released from encapsulated cells suppresses seizure activity in the epileptic hippocampus. *Exp Neurol*. 2009;216:413–419.
72. Hattiangady B, Shetty AK. Decreased neuronal differentiation of newly generated cells underlies reduced hippocampal neurogenesis in chronic temporal lobe epilepsy. *Hippocampus*. 2010;20:97–112.

Chapter 86

Embryonic Stem Cell Therapy for Intractable Epilepsy

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Envisioning a world in which humans are able to regenerate severed limbs, rewire neural pathways, and enhance sensory perception has been a mainstay of science fiction novels and movies for decades. While human limb regeneration is still not possible, remarkable developments in the fields of stem cell biology and neuroscience are leading the way for stem cell-based therapies to amend brain and spinal cord damage and repair sensory organs. In this chapter, we discuss recent efforts to derive neural stem cells from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) and their applications to treating conditions such as temporal lobe epilepsy (TLE) and neurodegenerative diseases.

Information about the spatiotemporal patterns of transcription factor expression in the developing nervous system suggests that the major classes of neurons in the brain and spinal cord become specified by combinatorial codes of transcription factors. Studies aimed at directing pluripotent stem cells toward neural fates have utilized this information to monitor the differentiation of ESCs and iPSCs into neural progenitors. There are several examples in which sufficient advances in production of clinical-grade cells have led to clinical trials for neurological disorders. Geron initiated the first clinical trial with transplanted human ESC-derived glial progenitors for repairing spinal cord injuries in paraplegic patients (http://www.geron.com/). Due

to financial constraints however, the company halted the trial and withdrew from the field of stem cell therapy. A second clinical trial with retinal grafts of human ESC (hESC)-derived retinal pigment epithelial cells will establish whether blindness caused by macular degeneration is treatable by this approach. In both cases, the cell-based therapies were extensively validated in animal models of the disorders. Systematic studies of the fates and functional properties of neurons and glia derived from ESCs and iPSCs in experimental models of epilepsy are still in their infancy. Relatively few studies have evaluated the efficacy of transplanting different cell types for seizure control in epilepsy.

MIGHT STEM CELL THERAPIES BE EFFECTIVE FOR CONTROLLING SEIZURES IN EPILEPSY?

Spontaneous seizures in patients may induce changes in gene expression and trigger widespread inflammation. It is not well understood how these and other seizure-induced changes in the brain will influence the survival, differentiation, or integration of transplanted neural precursors. Therefore, stem cell cures for complex and heterogeneous neurological disorders such as TLE may be years in the future.

Mesial temporal lobe epilepsy (MTLE) is often acquired after prolonged status epilepticus caused by prolonged high fever, a brain tumor, stroke, or traumatic brain injury, but it is also found in patients diagnosed with Alzheimer's disease. Following the initial brain injury, neural plasticity is thought to lead to an imbalance between synaptic excitation and inhibition in the dentate gyrus, abetting epileptogenesis.[1] Magnetic resonance imaging studies show that hippocampal atrophy is common in patients with childhood-onset MTLE,[2] and seizures in childhood are associated with cognitive decline.[3,4] Partial complex epilepsy involving the mesial temporal lobes can be difficult to control with anticonvulsant medications, and the high doses that are often required can cause debilitating cognitive side effects and toxicity. Surgical resection of hippocampus or severing the corpus callosum are alternative therapeutic interventions that may be beneficial for eliminating the seizure focus or reducing the spread of seizures,[5] but hippocampal resection can only be used in MTLE patients with a well-defined and unilateral seizure focus. When pharmacological and surgical approaches are not feasible, stem cell therapies might be an alternative; these therapies also offer the additional potential for cell replacement and neural circuit repair. The availability of hESCs and strategies for directing their differentiation toward specific types of forebrain neurons and glia is beginning to offer hope that a stem cell cure may be on the horizon for treating hippocampal sclerosis in some forms of acquired epilepsy.[6,7]

REPAIRING DYSFUNCTIONAL NEURAL CIRCUITRY IN TLE

Epileptogenesis refers to cellular and molecular changes occurring during the latent period after an initial insult or seizure, when the brain rewires and becomes prone to spontaneous seizures. Epileptogenesis is hypothesized to be caused by disruption of the normal balance between inhibitory and excitatory connections within limbic circuits.[8] One hypothetical mechanism in epileptogenesis is the loss of GABAergic (gamma-aminobutyric acid) interneurons and inhibitory synapses with granule cells after an initial precipitating injury. Gamma-aminobutyric acid is the principal inhibitory neurotransmitter in the adult neocortex and hippocampus, where it constrains the spread of neuronal excitation. The GABA-producing interneurons also modulate and integrate information in the cortex and hippocampus by synchronizing cortical oscillations underlying brain function and preventing the development of hyperexcitability and epileptiform activity. Two of the hallmark neuropathological changes in patients with MTLE resulting from traumatic brain injury or prolonged febrile seizures are reduced numbers of hilar interneurons and mossy cells in the dentate gyrus.[2,9,10]

In experimental MTLE in adult rodents, prolonged seizures or head injury can lead to hyperexcitability of granule neurons[11] and deficits in functional subclasses of GABAergic interneurons that coexpress neuropeptide Y or somatostatin.[12–14] These observations have spurred efforts to repair damaged neural circuits with GABAergic neuron precursors generated from ESCs or fetal neural progenitor cells (NPCs), as discussed below.

In both the neocortex and hippocampus, inhibitory cells also regulate networks of synaptically interconnected excitatory pyramidal neurons. Due to recurrent networks of excitatory connections, "runaway excitation" and prolonged burst firing of pyramidal neurons can result when synaptic inhibition is reduced. It has been proposed that prolonged status epilepticus impairs the efficacy of inhibitory GABAergic synaptic transmission onto granule neurons,[15] caused by dysregulation of $GABA_A$ receptors.[16,17] In addition, recent studies in the pilocarpine model suggest that despite a significant loss of hilar GABAergic interneurons, a compensatory response in the residual hippocampal interneurons causes them to hypertrophy, resulting in a net increase in GABAergic synapses in the inner molecular layer of the dentate gyrus.[18] Failure of these new connections to regulate dentate granule neuron hyperexcitability further supports the hypothesis that inhibitory synaptic transmission becomes dysfunctional in some forms of acquired MTLE.[18,19] Developmental disorders affecting the specification and migration of GABAergic interneurons in neocortical and hippocampal regions have also been shown to cause abnormal neuronal firing properties and spontaneous seizures.[20–24]

Taken together, the evidence from human neuroimaging data, postmortem histological studies of tissue from MTLE patients, and experimental models in rodents supports the idea that defective inhibitory neurotransmission in the hippocampus is a key factor in epileptogenesis and recurrent spontaneous seizures. As the above examples show, however, effective cell replacement in acquired forms of MTLE is likely to require extensive integration of specific functional types of GABAergic neurons into the dentate gyrus and correction of some of the other deficits in limbic circuits that underlie epileptogenesis.

In addition to requiring selective cell replacement in MTLE, effective therapies may need to address activity-dependent changes in gene expression induced by seizure activity that alter patterns of granule cell neurogenesis in the dentate gyrus.[25] Spontaneous recurrent seizures in MTLE are also associated with sprouting of mossy fibers from granule neurons, creating new excitatory synaptic connections in the inner molecular layer of the dentate gyrus.[26–28] While the exact mechanisms remain unclear, aberrant migration of the new neurons and abnormal axonal and dendritic growth of granule neurons contribute to network dysfunction in MTLE.[25, 29–31]

Glial cell involvement in chronic epilepsy is another feature adding to the complexity of the cellular and molecular changes. Glial cells are an important source of the anticonvulsant molecule adenosine, and augmenting adenosine levels has a powerful anticonvulsant effect.[32–35] However, glial cells also produce proinflammatory molecules that contribute to hyperexcitability. Taken together, these studies implicate multiple cell types and pathophysiological processes in acquired focal TLE. Even with the advent of new methods for replacing specific types of neurons and glia in the brain by stem cell therapy, treatment strategies may need to be devised that incorporate multiple approaches to correct the defects, including gene therapy, neuroprotection, and dietary modifications such as the ketogenic diet.

STEM CELLS FOR NEURODEGENERATION AND EPILEPSY

Fetal neural precursor cells (NPCs), adult neural stem cells, mesenchymal stem cells (MSCs), cord blood cells, ESCs, and iPSCs are all being tested for therapeutic effects in experimental models of neurodegenerative diseases and epilepsy. By definition, neural stem cells are self-renewing and can generate neurons or glial cells through asymmetric cell division.

Recent studies demonstrate that stem cells exist not only in the developing embryo, but also in the adult body and brain. The discovery and isolation of neural stem cells from the fetal and adult nervous system has shown that they retain the potential to generate the three major cell types in the central nervous system, namely, neurons, astrocytes, and oligodendrocytes. Once the molecules that govern stem cell self-renewal were identified, it became possible to harvest neural stem cells from the fetal or adult nervous system, expand their populations as neurospheres in vitro, or transplant them directly after removal from fetal brains. To date, significantly more progress has been made in examining the efficacy of grafts derived from fetal progenitors or

genetically modified cell lines, compared to neural stem cells derived from ESCs or iPSCs, for suppressing seizures in experimental models of TLE, ion channel mutations, or developmental disorders of interneurons associated with spontaneous seizures.[36–41]

Classification systems for the different brain-specific stem cells are still evolving.[42] Populations of neural stem cells in the adult nervous system reside within a specialized stem cell niche surrounding the lateral ventricles called the *subependymal zone*. Another specialized niche is the *subgranular zone* of the dentate gyrus, but here the stem cells are defined as progenitors, because separate populations appear to give rise to neurons or glial cells and have very limited self-renewal capacity.

In tissues of the adult body, stem cells are hard to find, but they do exist. An advantage of patient-derived stem cells is that they may be used for autologous transplants, reducing complications caused by immune incompatibility and transplant rejection.[43,44] Increasingly, neural stem cells derived from patients with genetic disorders of the nervous system are being studied in vitro to gain insights into basic disease mechanisms and for drug discovery. However, limited availability of human fetal tissue precludes widespread use of fetal neural stem cells for most clinical or biotechnology applications. To circumvent this problem, protocols have been developed to improve the yield of fetal or adult neural stem cells from the brain by propagating them as neurosphere-forming cells. While rarely tumorigenic when transplanted into the brain, propagation of human NPCs (hNPCs) in culture may select for chromosomal aneuploidy; therefore, cytogenetic screening of hNPCs is necessary prior to their use in clinical applications.[45]

PLURIPOTENT STEM CELLS

The pluripotent stem cells derived from the inner cell mass of human or mouse blastocyst have the capacity to generate all tissue types of the embryo and these cells have been used to generate ESCs. Protocols for differentiating ESCs into neural stem cells in vitro have been developed for adherent ESC lines, as well as those that require an initial stage of growth as embryoid bodies. They were shown to undergo extensive migration and integration after transplantation into the developing rodent brain.[46–48] The three main in vitro approaches for generating neural stem cells from ESCs include the formation of embryoid bodies, growth of neural stem cells in monolayer cultures on feeder layers in the presence of growth factors such as fibroblast growth factor-2 (FGF-2), or neurosphere cultures grown in the presence of defined growth factors. The challenge has been to obtain neural stem cells that can be patterned with regional identities upon differentiation into specific neural and glial types. Rapid progress toward this goal has been aided by identification of the transcriptional codes that specify functionally distinct subsets of neurons at different levels of the developing nervous system.

Although deriving neural stem cells from human ESCs (hESCs) requires more specialized conditions than fresh dissection of fetal neural stem cells from the embryo, the difficulty of obtaining fetal tissue presents an even larger obstacle for clinical applications. One of the biggest challenges in hESC research has been to obtain consistent batches of stem cells with regional and laminar identities. Recent modifications to culture conditions resulted in the growth of mESC- and hESC-derived neural stem cells into neural tube-like rosette structures.[49] As these neural rosettes grow, they recapitulate some notable features of the growing neural tube. Symmetrical mitotic divisions characterize the early-stage rosettes, without forming postmitotic neurons. There is a close temporal link between the neural rosette stage and its potential for neural patterning, similar to that of the neural tube. As neural rosettes develop into late-stage rosettes, they divide asymmetrically, producing neurons and glia that migrate away and differentiate. At this stage, radial glial-like progenitors appear in the rosettes and show interkinetic nuclear migration, similar to that of radial glial cells of the neural tube. They also have apical end feet that form a structure similar to the center of the neural tube. Human ESC-derived neural stem cells have been found to develop laminated structures with distinctive cell populations in the different layers reminiscent of the cerebral cortex. As shown in Fig. 86–1, human H9 pluripotent stem cells can be differentiated into neural stem cells in adherent cultures. These cells are beyond the neural rosette stage and

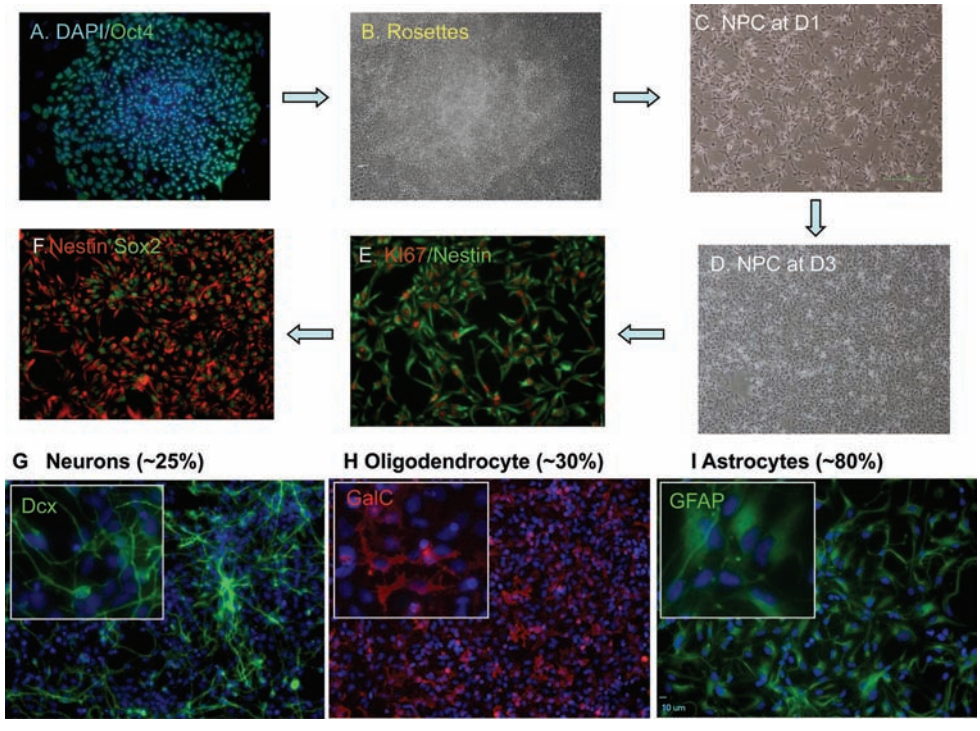

Figure 86–1. Neural induction of hESCs to neural stem cells followed by differentiation into neuronal and glial lineages. Human ESCs (H9) are pluripotent and able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. Pluripotency is typically indicated by the expression of the transcription factor Oct-4 (octamer-binding transcription factor **4**). **A.** Embryoid body formation and subsequent adherent culture of embryoid bodies to generate neural rosettes. **B.** Phase contrast images (10x) of GIBCO® hNSCs cultured in StemPro® NSC SFM (Serum-free media) at day 1 (**C**) and at day 3 (**D**) after thawing. Fluorescence images (20x) of GIBCO® hNSCs that were cultured in StemPro® NSC SFM for three passages and then allowed to differentiate into neurons, oligodendrocytes, or astrocytes. The cells were stained with antibodies recognizing a marker of cellular proliferation: Ki67 (red) in combination with the neural stem cell markers nestin (green, **E**) or with nestin (red) and SRY (sex determining region Y)-box 2 (SOX2) green, **F**). Upon directed differentiation, NSC types may be further distinguished based on their expression of the immature neuronal marker Dcx (doublecortin; green, **G**), the oligodendrocyte marker GalC (galactosylceramidase; red, **H**), or the astrocyte intermediate filament protein GFAP (glial fibrillary acidic protein: green, **I**). Nuclei are identified by staining with the dye DAPI (4′,6-diamidino-2-phenylindole; blue).

can be readily patterned toward a wide range of neural and glial fates. Studies have also shown that transplanted ESC-derived human or mouse NPCs migrate in the rodent brain after transplantation into the cerebral cortex or hippocampus and differentiate in a region-specific manner.[41,46,50] It is not known whether these cells can provide long-term seizure suppression or neuroprotection in TLE.

INDUCED PLURIPOTENT STEM CELLS

Induced pluripotent stem cells (iPSCs) are genetically reprogrammed adult cells that exhibit a pluripotent stem cell-like state like that of hESCs. Induced pluripotent stem cells may be derived by several different methods that induce select gene expression to confer pluripotency.[51–53] Functionally distinct types of neurons have been generated from iPSCs, and studies are now examining their ability to migrate and successfully modify symptoms of neurological disease.[54–56] One of the advantages of studying iPSC-derived neurons from patients with epilepsy is the possibility of gaining insights at the cellular level into how a particular genetic mutation affects neuronal excitability, migration, dendritic or axonal differentiation, or synapse formation. This approach is now being used to study iPSCs from patients with inherited forms of epilepsy. These cells may also be

quite useful for screening anticonvulsant drugs. Based on their cellular responses to different drug compounds, more effective anticonvulsant drugs can be selected and then administered to individual patients.

DIRECTED DIFFERENTIATION OF ESCS AND IPSCS

Recent advances in directing the fates of hESCs and iPSCs toward neural cell types that have relevance to TLE have utilized defined media conditions, reporter genes, or bacterial artificial chromosomes to select for neural stem cells with GABAergic fates.[50,57] For example, transcriptional codes for specifying GABAergic interneuron subtypes have been identified,[58,59] and this information has been used to direct ESC-derived NPCs to differentiate into GABAergic interneurons.[60] In one study, floating stem cell aggregates were first generated to obtain neural progenitors with a transgene, *Lhx6-GFP* (*Lim homeobox6-green fluorescent protein*). These were then grown on adherent substrates in the presence of growth factors and the signaling molecule sonic hedgehog to obtain ventral telencephalon neural progenitors. By harvesting the neural precursors at this stage, they were able to use the fluorescent transgene in fluorescence-activated cell sorting (FACS) to identify and prospectively isolate the *Lhx6*-expressing GABAergic progenitors prior to transplantation.[60] When the isolated interneuron precursors were then transplanted into the developing mouse brain, they differentiated into functionally defined types of interneurons and exhibited mature electrophysiological properties. Studies are now beginning to test systematically whether transplants of interneuron progenitors obtained with similar protocols have disease-modifying effects in experimental models of temporal lobe epilepsy.

BACTERIAL ARTIFICIAL CHROMOSOME TRANSGENESIS

Another powerful approach is to force the differentiation of hESC-derived neural stem cells toward particular neural fates by introducing bacterial artificial chromosomes (BAC) into the cells. This approach, called *BAC transgenesis*, is a novel tool to define and isolate different neural and glial lineages from ESCs.[61–63] This approach has used the GENSAT library of BACs, which have been engineered to express GFP under the control of key genes related to neural development. To obtain stable BAC transgenesis in hESCs, the cells had to be dissociated into single-cell suspensions using enzymatic digestion and an inhibitor of the Rho-associated kinase. This relatively simple step provided a substantial improvement in survival and cloning efficiency. The BACS were then nucleofected into the dissociated hESCs and selected for antibiotic resistance to obtain hESCs with different GFP transgenes that reported differentiation into motor neuron or dopamine neuron fates.

CLINICAL GRADE PLURIPOTENT STEM CELLS IN XENO- AND FEEDER-FREE CULTURE SYSTEMS

Successful stem cell therapies for central nervous system (CNS) diseases require large-scale production of neural stem cells from hESCs or iPSCs. In addition, such neural stem cells should be nontransformed and well characterized, with adequate bioprocess controls such as safety, sterility, and traceability. These cells, upon transplantation, should differentiate into appropriate target cell types, survive in diseased CNS tissue, and integrate with the existing neural network to improve the functional efficacy and clinical outcome.

A variety of protocols and cell culture conditions have been reported for the generation, expansion, and differentiation of neural stem cells from hESCs. A typical induction protocol from hESCs to human neural stem cells is shown in a schematic diagram (Fig. 86–1), and this can be achieved either by spontaneous or directed differentiation methods.[64–66] Some of the earliest protocols used serum-free culture conditions with either N2 or B27 neural supplements, but the process was not robust enough for reproducibility and scalability and neural induction efficiency was low (0.2% hESC to hNPC) with spontaneous differentiation.[67] Higher efficiency for neural induction was achieved by adding retinoic acid,[68] medium conditioned with stromal cells,[69] or bone morphogenetic protein (BMP) pathway inhibitors.[70]

For human cell therapy, however, using animal serum in the media, stromal feeder cells derived from animals, cocultures, or preconditioned media is incompatible because animal cell- and serum-free conditions are necessary. Guidelines for the manufacture of biologics have been developed and codified under chemistry and manufacturing controls (CMC) and good manufacturing practices (GMP). Deriving the first hESC line with these properties for clinical applications was an important advance.[71] It was necessary to demonstrate the ability to obtain pure populations of long-term self-renewing rosette-type hESC-derived neural stem cells that exhibited extensive self-renewal, clonogenicity, and stable neurogenesis upon transplantation.[72] Recently, an hESC line was generated that has the capacity to produce dopaminergic precursors in completely xeno-free cultures using four steps for producing functional dopaminergic neurons for clinical purposes.[73] Neurons generated by this process were shown to survive in experimental animal models of Parkinson's disease. A third important advance was the development of an efficient method to generate hNSCs from hESC or induced pluripotent stem cells by a combined blockade of SMAD (the SMAD proteins are homologs of two proteins, the drosophila protein, mothers against decapentaplegic (MAD) and the *Caenorhabditis elegans* protein SMA and the name is a combination of these two homologs) signaling with Noggin and a small molecule, SB431542, to achieve full neural conversion. This method is regulatory compliant, as it obviates the need for intermediary steps of generating embryoid bodies, the use of stromal feeders, and even the isolation of rosette structures.[74] The commercial availability of expandable populations of neural stem cells with stable phenotype marker expression will facilitate efforts to produce neural stem cells under xeno-free culture conditions for epilepsy research and clinical applications.

TRANSPLANT STUDIES OF FETAL NEURAL STEM CELLS FOR SEIZURE SUPPRESSION IN EXPERIMENTAL MODELS

Neural stem cells and fetal NPCs have been transplanted into a variety of experimental models of neurological disorders including ischemic stroke, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, spinal cord injury, and epilepsy.[40,41,50,75–83]

Evidence from experimental studies in several models of TLE in rodents has provided proof of concept that fetal neural stem cell transplants or cell lines genetically engineered to release GABA at multiple sites in the brain reduce seizure severity and/or frequency.[36,84–92] Studies with fetal cell transplants showed reduction of abnormal electrical discharges in the hippocampus or substantia nigra. Moreover, when GABAergic NPCs from the embryonic medial ganglionic eminence were transplanted into the forebrains of young mice, they showed widespread migration across the hippocampus and neocortex. The grafted cells differentiated into distinct types of GABAergic interneurons, functionally integrated, and increased the inhibitory tone in the host cortex and hippocampus.[37] These studies serve as proof of concept that increasing the number of forebrain GABAergic neurons in several different models of epilepsy can not only raise seizure thresholds in genetic models but also suppress recurrent spontaneous seizures in acquired MTLE. Studies employing fetal neural stem cells are described in detail in additional chapters in this volume (Chapters 85 and 87).

CHALLENGES IN CELL THERAPY APPROACHES TO TREAT EPILEPSY

While there are several advantages of stem cell-based therapies over gene therapy and other traditional approaches for treating epilepsy, significant technical hurdles still impede cell therapy for treating epilepsy. Unlike other tissues of the body, the nervous system has a limited capacity for self-repair because mature neurons cannot regenerate, and despite the presence of neural stem cells in the adult brain, their ability to respond to injury is limited. Improving the efficacy of stem cell therapies for replacing neurons or glial cell types destroyed by damage or disease is an extremely active area of investigation. To be successful, grafts of stem cells and their differentiated derivatives in the epileptic brain must not only survive for long periods of time, they must also migrate to the appropriate sites, integrate, and

establish the correct types of synaptic connections with the host brain. The importance of this last point is underscored by studies showing that seizures induce the genesis of ectopically positioned neurons from endogenous neural stem populations, and these ectopic neurons can contribute to increased excitability and epileptogenesis.[29,93]

Immune incompatibility between the donor and host is one of the more formidable problems in the field of cell replacement therapy. Currently, cell therapies based on fetal cell transplants require that patients receiving fetal stem cells also take immunosuppressive drugs to prevent rejection of fetal cell grafts.[40] Autologous stem cell grafts, in which the patient is also the stem cell donor, may help overcome the problem of graft rejection. Another major hurdle for ESC-based therapy is that the risk of tumor formation is high because these cells are pluripotent and mitotically active. To address this problem, hESCs cells have been engineered with suicide genes to allow elimination if the transplanted cells proliferate excessively or form tumors.[94]

CONCLUSIONS AND THE PATH FORWARD

Testing the feasibility of ESC-derived neurons for seizure suppression and hippocampal sclerosis in epilepsy will require a better understanding of how seizures alter the environment of the brain. Activity-dependent changes in the expression of a large number of genes have been found in experimental models of epilepsy. Additionally, even brief seizures may cause epigenetic changes that alter gene expression. Evidence for host brain influences on transplanted neural stem cells suggests that these local changes in the brain's milieu may be powerful influences on stem cell survival and migration after transplantation.

To achieve further advances in stem cell therapies for epilepsy, it will be necessary to improve the ability to track the transplanted cells after transplantation in the human brain. It will also be necessary to achieve long-term survival of transplants, circumvent the immunological problem of graft rejection, and tailor therapies for individual patients. The development of protocols for directing iPSCs into particular cell fates is one way to remove immunological barriers, since these cells can be generated from the patient's skin and offer the possibility of being transplanted back into the same patient. Lastly, before moving into the clinic, it will be necessary to produce clinical-grade human pluripotent cell-derived neural stem cells that differentiate into particular classes of GABAergic interneurons or other cell types that are injured in epilepsy.

With further new technological advances in the field of stem cell biology, cell therapies to treat neurological disorders such as epilepsy may soon become feasible. Stem cell transplants that employ NPCs for subclasses of GABAergic interneurons show great promise for controlling "runaway" excitation in the brain and spontaneous seizures in acquired forms of TLE. However, further studies are needed in a range of translational models of TLE to determine whether hESC- or iPSC-derived neurons transplanted into the hippocampus have the capacity to survive over the long term, form synapses with their appropriate synaptic targets, and regulate pyramidal and granule neuron hyperexcitability without causing adverse neurological side effects.

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DISCLOSURE STATEMENT

M.C.V. is currently a full time director for the division of Primary and Stem Cell Culture Systems at Life Technologies (LIFE), a publicly traded global company. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in the subject matter or materials discussed in the manuscript apart from those disclosed. J.R.N. and L.S. have no financial interests to disclose.

REFERENCES

1. Chang BS, Lowenstein DH. Epilepsy. *N Engl J Med*. 2003;349:1257–1266.
2. Mathern GW, Pretorius JK, Babb TL. Influence of the type of initial precipitating injury and at what age it occurs on course and outcome in patients with temporal lobe seizures. *J Neurosurg*. 1995;82:220–227.
3. Bjornaes H, Stabell K, Henriksen O, Loyning Y. The effects of refractory epilepsy on intellectual functioning in children and adults. A longitudinal study. *Seizure*. 2001;10:250–259.
4. Kolk A, Talvik T. Cognitive outcome of children with early-onset hemiparesis. *J Child Neurol*. 2000;15: 581–587.
5. Engel J Jr, Wiebe S, French J, Sperling M, Williamson P, Spencer D, Gumnit R, Zahn C, Westbrook E, Enos B. Practice parameter: temporal lobe and localized neocortical resections for epilepsy: report of the Quality Standards Subcommittee of the American Academy of Neurology, in association with the American Epilepsy Society and the American Association of Neurological Surgeons. *Neurology*. 2003;60:538–547.
6. Naegele JR, Maisano X. Gene and stem cell therapies for treating epilepsy. In: Rho JM, Sankar R, Stafstrom CE, es. *Epilepsy: Mechanisms, Models, and Translational Perspectives*. Boca Raton, FL: CRC Press; 2010:583–601.
7. Naegele JR, Maisano X, Yang J, Royston S, Ribeiro E. Recent advancements in stem cell and gene therapies for neurological disorders and intractable epilepsy. *Neuropharmacology.* 2010;58:855–864.
8. Cossart R, Bernard C, Ben-Ari Y. Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signalling in epilepsies. *Trends Neurosci*. 2005;28:108–115.
9. Swartz BE, Houser CR, Tomiyasu U, Walsh GO, DeSalles A, Rich JR, Delgado-Escueta A. Hippocampal cell loss in posttraumatic human epilepsy. *Epilepsia*. 2006;47:1373–1382.
10. de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res*. 1989;495: 387–395.
11. Golarai G, Greenwood AC, Feeney DM, Connor JA. Physiological and structural evidence for hippocampal involvement in persistent seizure susceptibility after traumatic brain injury. *J Neurosci*. 2001;21: 8523–8537.
12. McDonald AJ, Mascagni F. Immunohistochemical characterization of somatostatin containing interneurons in the rat basolateral amygdala. *Brain Res.* 2002;943:237–244.
13. Tuunanen J, Halonen T, Pitkanen A. Decrease in somatostatin-immunoreactive neurons in the rat amygdaloid complex in a kindling model of temporal lobe epilepsy. *Epilepsy Res.* 1997;26:315–327.
14. Choi YS, Lin SL, Lee B, Kurup P, Cho HY, Naegele JR, Lombroso PJ, Obrietan K. Status epilepticus-induced somatostatinergic hilar interneuron degeneration is regulated by striatal enriched protein tyrosine phosphatase. *J Neurosci*. 2007;27:2999–3009.
15. Bonislawski DP, Schwarzbach EP, Cohen AS. Brain injury impairs dentate gyrus inhibitory efficacy. *Neurobiol Dis*. 2007;25:163–169.
16. Coulter DA, Carlson GC. Functional regulation of the dentate gyrus by GABA-mediated inhibition. *Prog Brain Res*. 2007;163:235–243.
17. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat Med*. 1998;4:1166–1172.
18. Zhang W, Yamawaki R, Wen X, Uhl J, Diaz J, Prince DA, Buckmaster PS. Surviving hilar somatostatin interneurons enlarge, sprout axons, and form new synapses with granule cells in a mouse model of temporal lobe epilepsy. *J Neurosci*. 2009;29:14247–14256.
19. Kobayashi M, Buckmaster PS. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. *J Neurosci*. 2003;23:2440–2452.
20. Cobos I, Broccoli V, Rubenstein JL. The vertebrate ortholog of Aristaless is regulated by Dlx genes in the developing forebrain. *J Comp Neurol*. 2005;483: 292–303.
21. Price MG, Yoo JW, Burgess DL, Deng F, Hrachovy RA, Frost JD Jr, Noebels JL. A triplet repeat expansion genetic mouse model of infantile spasms syndrome, Arx(GCG)10+7, with interneuronopathy, spasms in infancy, persistent seizures, and adult cognitive and behavioral impairment. *J Neurosci.* 2009;29: 8752–8763.
22. Marsh E, Fulp C, Gomez E, Nasrallah I, Minarcik J, Sudi J, Christian SL, Mancini G, Labosky P, Dobyns W, Brooks-Kayal A, Golden JA. Targeted loss of Arx results in a developmental epilepsy mouse model and recapitulates the human phenotype in heterozygous females. *Brain*. 2009;132(pt 6):1563–1576.
23. Martins GJ, Plachez C, Powell EM. Loss of embryonic MET signaling alters profiles of hippocampal interneurons. *Dev Neurosci.* 2007;29:143–158.
24. Powell EM, Campbell DB, Stanwood GD, Davis C, Noebels JL, Levitt P. Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy, and behavioral dysfunction. *J Neurosci*. 2003;23:622–631.
25. Ma DK, Jang MH, Guo JU, Kitabatake Y, Chang ML, Pow-Anpongkul N, Flavell RA, Lu B, Ming GL, Song H. Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science.* 2009;323:1074–1077.
26. Tauck DL, Nadler JV. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. *J Neurosci*. 1985;5:1016–1022.
27. Sloviter RS, Zappone CA, Harvey BD, Frotscher M. Kainic acid-induced recurrent mossy fiber innervation of dentate gyrus inhibitory interneurons: possible anatomical substrate of granule cell hyperinhibition in chronically epileptic rats. *J Comp Neurol*. 2006;494:944–960.
28. Shibley H, Smith BN. Pilocarpine-induced status epilepticus results in mossy fiber sprouting and spontaneous seizures in C57BL/6 and CD-1 mice. *Epilepsy Res*. 2002;49:109–120.
29. Parent JM. Adult neurogenesis in the intact and epileptic dentate gyrus. *Prog Brain Res*. 2007;163: 529–540.
30. Scharfman HE, Gray WP. Relevance of seizure-induced neurogenesis in animal models of epilepsy to the etiology of temporal lobe epilepsy. *Epilepsia*. 2007; 48(suppl 2):33–41.
31. Ribak CE, Tran PH, Spigelman I, Okazaki MM, Nadler JV. Status epilepticus-induced hilar basal dendrites on

rodent granule cells contribute to recurrent excitatory circuitry. *J Comp Neurol*. 2000;428:240–253.
32. Boison D. Adenosine kinase, epilepsy and stroke: mechanisms and therapies. *Trends Pharmacol Sci*. 2006;27:652–658.
33. Boison D. The adenosine kinase hypothesis of epileptogenesis. *Prog Neurobiol*. 2008;84:249–262.
34. Boison D. Adenosine augmentation therapies (AATs) for epilepsy: prospect of cell and gene therapies. *Epilepsy Res*. 2009;85:131–141.
35. Masino SA, Kawamura M, Wasser CA, Pomeroy LT, Ruskin DN. Adenosine, ketogenic diet and epilepsy: the emerging therapeutic relationship between metabolism and brain activity. *Curr Neuropharmacol*. 2009;7:257–268.
36. Castillo CG, Mendoza S, Freed WJ, Giordano M. Intranigral transplants of immortalized GABAergic cells decrease the expression of kainic acid-induced seizures in the rat. *Behav Brain Res*. 2006;171:109–115.
37. Alvarez-Dolado M, Calcagnotto ME, Karkar KM, Southwell DG, Jones-Davis DM, Estrada RC, Rubenstein JL, Alvarez-Buylla A, Baraban SC. Cortical inhibition modified by embryonic neural precursors grafted into the postnatal brain. *J Neurosci*. 2006;26: 7380–7389.
38. Baraban SC, Southwell DG, Estrada RC, Jones DL, Sebe JY, Alfaro-Cervello C, Garcia-Verdugo JM, Rubenstein JL, Alvarez-Buylla A. Reduction of seizures by transplantation of cortical GABAergic interneuron precursors into Kv1.1 mutant mice. *Proc Natl Acad Sci USA*. 2009;106:15472–15477.
39. Bengzon J, Kokaia Z, Lindvall O. Specific functions of grafted locus coeruleus neurons in the kindling model of epilepsy. *Exp Neurol*. 1993;122:143–154.
40. Bjorklund A, Lindvall O. Cell replacement therapies for central nervous system disorders. *Nat Neurosci*. 2000;3:537–544.
41. Carpentino JE, Hartman NW, Grabel LB, Naegele JR. Region-specific differentiation of embryonic stem cell-derived neural progenitor transplants into the adult mouse hippocampus following seizures. *J Neurosci Res*. 2008;86:512–524.
42. Seaberg RM, van der Kooy D. Stem and progenitor cells: the premature desertion of rigorous definitions. *Trends Neurosci*. 2003;26:125–131.
43. Gage FH. Mammalian neural stem cells. *Science*. 2000;287:1433–1438.
44. Kokovay E, Shen Q, Temple S. The incredible elastic brain: how neural stem cells expand our minds. *Neuron*. 2008;60:420–429.
45. Sareen D, McMillan E, Ebert AD, Shelley BC, Johnson JA, Meisner LF, Svendsen CN. Chromosome 7 and 19 trisomy in cultured human neural progenitor cells. *PLoS One*. 2009;4(10):e7630.
46. Tabar V, Panagiotakos G, Greenberg ED, Chan BK, Sadelain M, Gutin PH, Studer L. Migration and differentiation of neural precursors derived from human embryonic stem cells in the rat brain. *Nat Biotechnol*. 2005;23:601–606.
47. Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol*. 2001;19:1129–1133.
48. Reubinoff BE, Itsykson P, Turetsky T, Pera MF, Reinhartz E, Itzik A, Ben-Hur T. Neural progenitors from human embryonic stem cells. *Nat Biotechnol*. 2001;19:1134–1140.
49. Elkabetz Y, Panagiotakos G, Al Shamy G, Socci ND, Tabar V, Studer L. Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. *Genes Dev*. 2008;22:152–165.
50. Maisano X, Carpentino J, Becker S, Lanza R, Aaron G, Grabel L, Naegele JR. Embryonic stem cell-derived neural precursor grafts for treatment of temporal lobe epilepsy. *Neurotherapeutics*. 2009;6:263–277.
51. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861–872.
52. Takahashi K, Okita K, Nakagawa M, Yamanaka S. Induction of pluripotent stem cells from fibroblast cultures. *Nat Protoc*. 2007;2:3081–3089.
53. Kim SU, de Vellis J. Stem cell-based cell therapy in neurological diseases: a review. *J Neurosci Res*. 2009;87:2183–2200.
54. Karumbayaram S, Novitch BG, Patterson M, Umbach JA, Richter L, Lindgren A, Conway AE, Clark AT, Goldman SA, Plath K, Wiedau-Pazos M, Kornblum HI, Lowry WE. Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. *Stem Cells*. 2009;27:806–811.
55. Dimos JT, Rodolfa KT, Niakan KK,Weisenthal LM, Mitsumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R, Wichterle H, Henderson CE, Eggan K. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*. 2008;321:1218–1221.
56. Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, Soldner F, Broccoli V, Constantine-Paton M, Isacson O, Jaenisch R. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc Natl Acad Sci USA*. 2008;105:5856–5861.
57. Cai C, Grabel L. Directing the differentiation of embryonic stem cells to neural stem cells. *Dev Dyn*. 2007;236:3255–3266.
58. Butt SJ, Cobos I, Golden J, Kessaris N, Pachnis V, Anderson S. Transcriptional regulation of cortical interneuron development. *J Neurosci*. 2007;27: 11847–11850.
59. Butt SJ, Fuccillo M, Nery S, Noctor S, Kriegstein A, Corbin JG, Fishell G. The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron*. 2005;48:591–604.
60. Maroof AM, Brown K, Shi SH, Studer L, Anderson SA. Prospective isolation of cortical interneuron precursors from mouse embryonic stem cells. *J Neurosci*. 2010;30:4667–4675.
61. Placantonakis DG, Tomishima MJ, Lafaille F, Desbordes SC, Jia F, Socci ND, Niale A, Lee H, Harrison N, Studer L, Tabar VS. Enriched motor neuron populations derived from bacterial artificial chromosome-transgenic human embryonic stem cells. *Clin Neurosurg*. 2009;56:125–132.
62. Placantonakis DG, Tomishima MJ, Lafaille F, Desbordes SC, Jia F, Socci ND, Viale A, Lee H, Harrison N, Tabar V, Studer L. BAC transgenesis in human embryonic stem cells as a novel tool to define the human neural lineage. *Stem Cells*. 2009;27: 521–532.
63. Tomishima MJ, Hadjantonakis AK, Gong S, Studer L. Production of green fluorescent protein transgenic embryonic stem cells using the GENSAT bacterial

artificial chromosome library. *Stem Cells*. 2007;25: 39–45.
64. Schulz TC, Palmarini GM, Noggle SA, Weiler DA, Mitalipova MM, Condie BG. Directed neuronal differentiation of human embryonic stem cells. *BMC Neurosci*. 2003;4:27.
65. Gerrard L, Rodgers L, Cui W. Differentiation of human embryonic stem cells to neural lineages in adherent culture by blocking bone morphogenetic protein signaling. *Stem Cells*. 2005;23:1234–1241.
66. Shin S, Mitalipova M, Noggle S, Tibbitts D, Venable A, Rao R, Stice SL. Long-term proliferation of human embryonic stem cell-derived neuroepithelial cells using defined adherent culture conditions. *Stem Cells*. 2006;24:125–138.
67. Tropepe V, Hitoshi S, Sirard C, Mak TW, Rossant J, van der Kooy D. Direct neural fate specification from embryonic stem cells: a primitive mammalian neural stem cell stage acquired through a default mechanism. *Neuron*. 2001;30:65–78.
68. Eiges R, Schuldiner M, Drukker M, Yanuka O, Itskovitz-Eldor J, Benvenisty N. Establishment of human embryonic stem cell-transfected clones carrying a marker for undifferentiated cells. *Curr Biol*. 2001;11:514–518.
69. Perrier AL, Tabar V, Barberi T, Rubio ME, Bruses J, Topf N, Harrison NL, Studer L. Derivation of midbrain dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci USA*. 2004;101(34):12543–12548.
70. Itsykson P, Ilouz N, Turetsky T, Goldstein RS, Pera MF, Fishbein I, Segal M, Reubinoff BE. Derivation of neural precursors from human embryonic stem cells in the presence of noggin. *Mol Cell Neurosci*. 2005;30: 24–36.
71. Klimanskaya I, Chung Y, Meisner L, Johnson J, West MD, Lanza R. Human embryonic stem cells derived without feeder cells. *Lancet*. 2005;365(9471): 1636–1641.
72. Koch P, Opitz T, Steinbeck JA, Ladewig J, Brustle O. A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. *Proc Natl Acad Sci USA*. 2009;106:3225–3230.
73. Swistowski A, Peng J, Liu Q, Mali P, Rao MS, Cheng L, Zeng X. Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions. Stem Cells. 2010;28: 1893–1904.
74. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol*. 2009;27:275–280.
75. Lindvall O. Clinical application of neuronal grafts in Parkinson's disease. *J Neurol.* 1994;242(1 suppl 1): S54–S56.
76. Zaman V, Shetty AK. Fetal hippocampal CA3 cell grafts transplanted to lesioned CA3 region of the adult hippocampus exhibit long-term survival in a rat model of temporal lobe epilepsy. *Neurobiol Dis.* 2001;8:942–952.
77. Turner DA, Shetty AK. Clinical prospects for neural grafting therapy for hippocampal lesions and epilepsy. *Neurosurgery.* 2003;52:632–644; discussion 641–644.
78. Ruschenschmidt C, Koch PG, Brustle O, Beck H. Functional properties of ES cell-derived neurons engrafted into the hippocampus of adult normal and chronically epileptic rats. *Epilepsia*. 2005;46(suppl 5): 174–183.
79. Rao MS, Hattiangady B, Rai KS, Shetty AK. Strategies for promoting anti-seizure effects of hippocampal fetal cells grafted into the hippocampus of rats exhibiting chronic temporal lobe epilepsy. *Neurobiol Dis*. 2007;27:117–132.
80. Hattiangady B, Rao MS, Shetty AK. Grafting of striatal precursor cells into hippocampus shortly after status epilepticus restrains chronic temporal lobe epilepsy. *Exp Neurol*. 2008;212:468–481.
81. Aubry L, Bugi A, Lefort N, Rousseau F, Peschanski M, Perrier AL. Striatal progenitors derived from human ES cells mature into DARPP32 neurons in vitro and in quinolinic acid-lesioned rats. *Proc Natl Acad Sci USA*. 2008;105:16707–16712.
82. Bacigaluppi M, Pluchino S, Martino G, Kilic E, Hermann DM. Neural stem/precursor cells for the treatment of ischemic stroke. *J Neurol Sci*. 2008;265:73–77.
83. Raedt R, Van Dycke A, Vonck K, Boon P. Cell therapy in models for temporal lobe epilepsy. *Seizure*. 2007;16:565–578.
84. Chu K, Kim M, Jung KH, Jeon D, Lee ST, Kim J, Jeong SW, Kim SU, Lee SK, Shin HS, Roh JK. Human neural stem cell transplantation reduces spontaneous recurrent seizures following pilocarpine-induced status epilepticus in adult rats. *Brain Res*. 2004;1023:213–221.
85. Clough R, Statnick M, Maring-Smith M, Wang C, Eells J, Browning R, Dailey J, Jobe P. Fetal raphe transplants reduce seizure severity in serotonin-depleted GEPRs. *Neuroreport*. 1996;8:341–346.
86. Gernert M, Thompson KW, Loscher W, Tobin AJ. Genetically engineered GABA-producing cells demonstrate anticonvulsant effects and long-term transgene expression when transplanted into the central piriform cortex of rats. *Exp Neurol*. 2002;176:183–192.
87. Kokaia M, Aebischer P, Elmer E, Bengzon J, Kalen P, Kokaia Z, Lindvall O. Seizure suppression in kindling epilepsy by intracerebral implants of GABA- but not by noradrenaline-releasing polymer matrices. *Exp Brain Res*. 1994;100:385–394.
88. Loscher W, Ebert U, Lehmann H, Rosenthal C, Nikkhah G. Seizure suppression in kindling epilepsy by grafts of fetal GABAergic neurons in rat substantia nigra. *J Neurosci Res*. 1998;51:196–209.
89. Loscher W, Gernert M, Heinemann U. Cell and gene therapies in epilepsy—promising avenues or blind alleys? *Trends Neurosci*. 2008;31:62–73.
90. Thompson KW. Genetically engineered cells with regulatable GABA production can affect afterdischarges and behavioral seizures after transplantation into the dentate gyrus. *Neuroscience*. 2005;133:1029–1037.
91. Thompson KW, Suchomelova LM. Transplants of cells engineered to produce GABA suppress spontaneous seizures. *Epilepsia.* 2004;45:4–12.
92. Waldau B, Hattiangady B, Kuruba R, Shetty AK. Medial ganglionic eminence-derived neural stem cell grafts ease spontaneous seizures and restore GDNF expression in a rat model of chronic temporal lobe epilepsy. *Stem Cells.* 2010;28:1153–1164.
93. Scharfman HE. Functional implications of seizure-induced neurogenesis. *Adv Exp Med Biol*. 2004;548: 192–212.
94. Schuldiner M, Itskovitz-Eldor J, Benvenisty N. Selective ablation of human embryonic stem cells expressing a "suicide" gene. *Stem Cells*. 2003;21:257–265.

Chapter 87

Cell Therapy Using GABAergic Neural Progenitors

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EMBRYONIC ORIGIN OF CORTICAL INTERNEURONS

Pioneering work in the late 1990s confirmed the concept that neurons arising in the ganglionic eminences of the ventral subpallium of the telencephalon migrate in a tangential manner to developing neocortex, hippocampus, and olfactory bulb, where they generate gamma-aminobutyric acid (GABA)-containing interneurons.[1–3] These cells are thought to arise from two primary, and transient, embryonic structures known as the *medial* and *caudal ganglionic eminences* (MGE and CGE, respectively).[4,5] Seminal studies of interneuron migration in explants[6,7] and in vivo fate mapping[8,9] revealed distinct subtypes of cortical interneurons that arise from each of these two locations. The MGE mainly gives rise to two neurochemically defined subgroups, those that express the calcium binding protein parvalbumin (PV) and those that express the neuropeptide somastostatin[10,11]; the CGE gives rise to vertically oriented calretinin- and vasoactive intestinal peptide (VIP)-expressing interneurons,[10,11] as well as neuropeptide Y (NPY)- and reelin-expressing neurogliaform subclasses.[12,13] Within these neurochemically defined subgroups, there are numerous interneuron subtypes defined by combinations of neurochemical, physiological, and axon-targeting criteria.[14]

Genetically engineered mice lacking transcription factors expressed in these embryonic regions (e.g., *Dlx1* or *Mash1*) lack interneurons confirming a ventral telencephalic origin for these cells and, relevant to this volume, demonstrate that the consequence of interneuron deficiency is often epilepsy. For example, inactivation of a homeobox transcription factor, *Dlx1*, resulted in mutant mice featuring an age-dependent loss of somatostatin$^+$, NPY$^+$, and calretinin$^+$ interneurons (identified through immunohistochemical and in situ hybridization studies), with a subsequent reduction in cortical/hippocampal inhibition (evaluated in acute slice experiments) and spontaneous electrographic

seizures (assessed in video-electroencephalographic [EEG] recordings).[15] Late-onset epilepsy in these *Dlx1* mutants, and another mouse with subtype-specific reductions in cortical interneuron density, that is, the *uPAR*$^{-/-}$ mouse[16] provide strong support for the hypothesis that correct interneuron number and function plays a key antiepileptic role in the adult central nervous system (CNS). Decreased numbers of PV^{+} and NPY^{+} interneurons also correlate with increased seizure susceptibility in a recently described neuropilin-2 knockout mouse,[17] and genetic manipulation of the Aristaless-related homeobox gene (*ARX*) in mice results in a reduction of cortical calbindin-positive interneurons and a variety of seizure phenotypes.[18,19] Taken together, these discoveries and the identification of an epileptic phenotype in children with *ARX* mutations (and reduced cortical interneuron function) have prompted a new *interneuronopathy* epilepsy designation.[20]

TRANSPLANTATION OF MGE PRECURSOR CELLS

Grafted neural progenitors can produce functionally integrated neurons, even after host neurogenesis is complete, and in brain areas outside traditional neurogenic regions. For example, progenitors derived from fetal midbrain integrate into host striatum as dopaminergic neurons, and immortalized neural progenitors (RN33B) transplanted into cortex or hippocampus of neonatal rats differentiate into cells with the morphological features of pyramidal neurons.[21] In electrophysiological studies, these cells can generate action potentials and receive excitatory or inhibitory input from neighboring cells. Using grafted embryonic stem (ES) cells expressing green fluorescent protein (GFP) and voltage-clamp analysis of postsynaptic currents, Wernig et al.[22] confirmed synapse formation between host and donor neurons. These studies, though elegant in design and buttressed by anatomical data indicating synapse formation onto GFP cells, failed to examine synaptic integration in the opposite direction—for example, from donor neuron to host brain. Additionally, large tumors were noted in most animals receiving ES-GFP cell grafts. Embryonic stem cell-derived neural progenitor cells transplanted into hippocampus also display a "marked tendency" to form tumors[23] or appear as "clumps" or "clusters" of cells with mixed lineage near the transplant site.[24,25] Although a promising alternative to currently available drug therapies, functional integration of these ES-derived cells within the host brain is probably quite limited.

Immature neurons arising from the embryonic MGE, in stark contrast to ES-derived cell lines, exhibit a unique ability to migrate widely in host brain following early postnatal transplantation.[7] Medial ganglionic eminence-derived cells express neuronal markers such as NeuN and Hu[24] but exhibit only limited, or no, expression of nonneuronal markers such as glial fibrillary acidic protein, vimentin, or Olig-2.[7,26–29] Transplanted MGE cells are also immune-negative for tyrosine hydroxylase, choline acetyltransferase, calcium/calmodulin-dependent (CaM) kinase IIα or the neuronal glutamate transporter excitatory amino acid carrier-1. Consistent with lineage tracing and fate-mapping studies, nearly all MGE-derived neurons in the host brain are GABAergic and stain with antibodies to GABA or GAD67. Subpopulations of MGE-derived interneurons can be double-labeled with antibodies to PV, somatostatin, NPY, and calretinin. Most importantly, MGE-derived neurons can migrate up to 5 mm from the injection site and show signs of synapse formation in electron micrographic studies.[7,29,30] These anatomical findings suggest that MGE-derived cells could be a source of new, and functionally integrated, interneurons in the host brain. In vitro electrophysiology studies using GFP-labeled, MGE-derived interneurons confirmed that these cells exhibit intrinsic membrane and active firing properties that would classify them as mature interneurons following transplantation.[26,27,30] Moreover, as direct evidence that these cells can integrate functionally and influence GABA-mediated inhibition in the host brain, slice electrophysiology studies consistently show an increase in synaptic[26,28,30] and extrasynaptic inhibition[30] in regions of the host brain containing MGE-derived GFP^{+} interneurons.

CELL THERAPY USING TRANSPLANTED MGE PRECURSOR CELLS

Epilepsy can be a devastating neurological condition characterized by unpredictable

abnormal electrical discharges (seizures) that can result in various combinations of uncontrolled motor output, loss of consciousness, and sometimes death. Nearly 3 million Americans suffer from some form of epilepsy, it has long been recognized that loss (or reduction) of GABA-mediated inhibition can be a contributing factor, and available antiepileptic drugs (including those that target GABAergic signaling pathways) are not effective in approximately one-third of these patients. Cell therapy could offer an alternative treatment option. One critical advantage of a transplantation strategy over conventional antiepileptic drug therapy is that cell treatments can be locally restricted, whereas drugs have widespread and systemic adverse effects. What will be critical to the success of this therapy is the type of neuron that is generated. Early attempts at establishing a cell-based therapy for epilepsy used fetal noradrenergic neurons that were grafted bilaterally to the hippocampus of adult rats following chemical lesioning of the central catecholamine pathway.[31,32] However, the adrenergic cell grafts did not suppress kindling-induced seizure discharge in nonlesioned animals, nor did they suppress seizures when grafted postkindling. Fetal catecholamine-releasing cell grafts were shown to be only moderately effective at suppressing seizure-like activity in a variety of animal models.[33,34] Grafting fetal serotonergic or cholinergic neurons was shown to suppress seizures in epilepsy-prone animals in which these signaling pathways were lesioned.[35,36] Because inhibition is a key deficit in epilepsy, several early attempts to enhance GABA-mediated inhibition have been tried: (1) grafting fetal "GABAergic" neurons putatively derived from the rat embryonic ganglionic eminence (no immunohistochemical confirmation of the cell type was performed) only produced modest effects that were similar to those observed with control cell grafts from the sciatic nerve[37]; (2) grafts of fetal GABA-rich cells into substantia nigra produced transient effects on afterdischarge activity in kindled rats[38,39]; and (3) grafting of an immortalized cortical cell line engineered to produce GABA also produced only a modest suppression of afterdischarge activity.[39,40] Though encouraging, none of these studies demonstrated the ability to selectively generate interneurons that migrate, function, and integrate into host brain in a manner similar to that of the endogenous interneuron cell population.

Although interneuron dysfunction can be a feature of the epileptic brain, and although GABA-enhancing or GABA-mimetic antiepileptic drugs are already in widespread clinical use, a strategy to modify host brain circuitry by exploiting the embryonic source of these cells (i.e., MGE) has only recently been explored. Using rat embryonic day 14 (E14) fetal tissue to generate putative MGE neural stem cells (NSCs) for in vivo grafting into the hippocampus of rats made chronically epileptic by the intraperitoneal injection of kainic acid, Shetty and coworkers reported a reduction in behaviorally monitored seizure frequency and duration.[41] Although it was suggested that increased numbers of new GABAergic interneurons were responsible for the observed suppression of seizure behavior, only 10% of graft-derived cells were immunoreactive for GABA in these studies and whether subdissection of embryonic MGE was performed prior to the derivation of NSC neurospheres was not explicitly described. A more promising study by Zipancic and coworkers[28] described transplantation of E12.5 embryonic MGE progenitor cells into the hippocampus of adult mice 1 week after ablation of a subpopulation of GABAergic interneurons using a neurotoxic saporin conjugated to substance P (SSP-Sap). Gamma-aminobutyric acid-mediated inhibition, shown to be decreased in voltage-clamp recordings of miniature and spontaneous inhibitory postsynaptic currents from hippocampal slices prepared from SSP-Sap mice, was restored to normal levels following MGE transplantation. In additional studies, MGE-grafted SSP-Sap mice were found to be less sensitive to pentylenetetrazole (PTZ)-induced seizures than age-matched SSP-Sap control mice. We also used mouse embryonic MGE progenitor cell transplantations in our attempts to develop an interneuron-based cell therapy for epilepsy. First, we examined the thresholds for induction of seizure activity in wild-type CD1 mice. To induce seizures, we chose the pilocarpine model, as activity is triggered by cholinergic mechanisms and is sensitive to endogenous GABA tone.[42] Acute pilocarpine administration induces ictal and interictal discharge activity in electrographic recordings (Fig. 87–1A), which is correlated with a sequence of behavioral alterations that include akinesia, ataxic lurching, and facial automatisms (Stage II), progressing to tonic-clonic motor seizures (Stage III). Following brief concentration-response

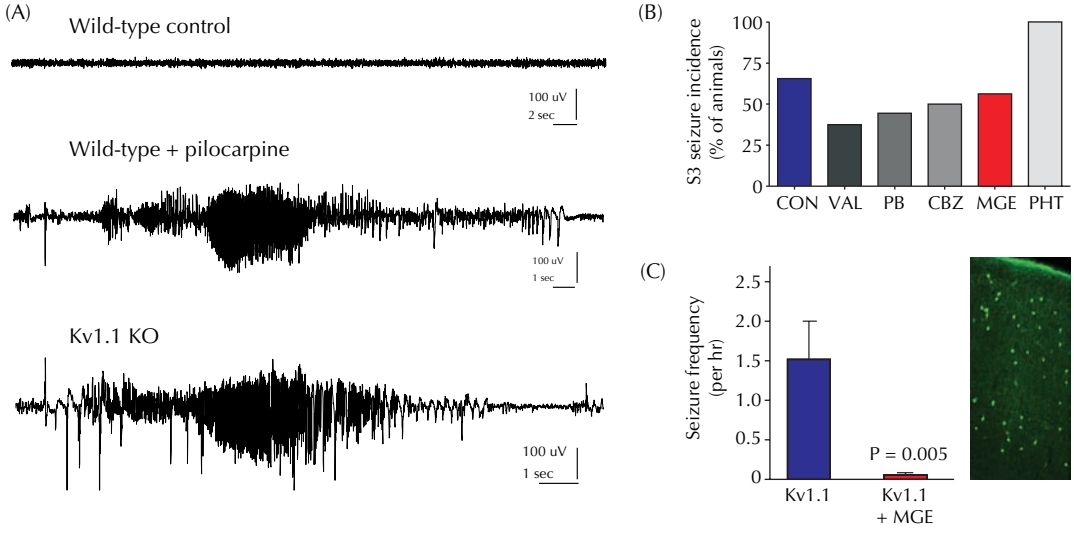

Figure 87–1. Medial ganglionic eminence cell therapy for epilepsy. **A.** Sample EEG traces from a wild-type CD1 mouse (top), a CD1 mouse injected with 300 mg/kg pilocarpine (middle), and a Kv1.1$^{-/-}$ mouse (bottom); seizures are observed as high-frequency, large-amplitude events with durations greater than 45 s. **B.** Plot of the percentage of mice scored as reaching a Stage III seizure in response to pilocarpine. CON, control ($n = 18$); VAL, valproate ($n = 8$); PB, phenobarbital ($n = 9$); CBZ, carbamazepine ($n = 8$); MGE, medial ganglionic eminence ($n = 16$; red bars); PHT, phenytoin ($n = 8$). **C.** Plot of the seizure frequency (measured as EEG-verified seizures per hour) for Kv1.1$^{-/-}$ mice receiving sham surgery or dead cells ($n = 8$) and Kv1.1$^{-/-}$ mice receiving MGE grafts at P2 ($n = 8$). Coronal section of neocortex showing the distribution and spread of MGE-derived GFP-positive neurons in the host brain (inset at the right); the section is approximately 900 mm caudal to the injection site.

studies, a pilocarpine concentration (300 mg/kg, intraperitonal) was chosen that elicits Stage III seizures in approximately 70% of control mice. As an initial demonstration of "seizure protection" conferred by MGE cell grafts, only 55% of grafted mice were observed to exhibit pilocarpine-induced Stage III seizures at 45 days after transplantation; in all grafted animals post hoc immunohistochemistry was used to confirm the presence of at least 40,000 MGE-GFP cells in cortex. To place the MGE graft "protection" data in a therapeutic context, we also preadministered three conventional antiepileptic drugs (AEDs) and measured pilocarpine-induced Stage III seizure incidence. Interestingly, MGE grafts conferred protection that was comparable to that of available AEDs and was clearly superior to at least one, phenytoin (Fig. 87–1B). Next, we tested our early MGE transplantation strategy in Kv.1.1 null mice that mimic a human form of epilepsy associated with mutation of the *Kv1.1/Kcna1* channel. These mice exhibited a spontaneous seizure frequency of at least one tonic-clonic seizure per hour and were shown to be predisposed to sudden unexplained death in epilepsy (SUDEP).[43–45] Medial ganglionic eminence grafts dramatically reduced the frequency of electrographic seizures (Fig. 87–1C) in Kv1.1-deficient mice and when rare electrographic seizure events did occur in grafted animals, they were reduced in duration by over 50%.[30]

POTENTIAL SOURCES OF MGE-LIKE INTERNEURON PRECURSORS

The studies described above provide exciting evidence that interneuron transplantation could become a new therapy for medication-resistant seizures. However, this point begs the obvious question: where would "MGE cells" for such therapy come from? Recently, mouse and human ES cells have been differentiated into ventral telencephalic progenitors that give rise to GABA+ cells.[46–48] One approach is to initially use signaling pathway inhibitors to allow ES cells to take what appears to be, at least for human cells, their default fate of telencephalic, multipotent progenitors.[47,49,50] The progenitors are then ventralized to subcortical fates with

the morphogen Sonic Hedgehog.[48,51] Induced pluripotent stem cells (iPSCs), which are generated by the dedifferentiation of somatic, mitotic cells such as fibroblasts, also default to a telencephalic-like progenitor stage[52] and thus should also be amenable to ventralization into MGE-like progenitors of GABA-producing neurons. Conceivably, the latter approach would permit the generation of interneurons from a patient's own somatic cells.

CONCLUSION

While the above studies demonstrate the feasibility of generating progenitors of GABA+ telencephalic neurons from mouse and human stem cells, none of them determined whether putative MGE-derived cortical interneurons were being generated. This issue is critical since the vast majority of subcortical telencephalic neurons are GABAergic, including several large classes of projection neurons. To achieve this goal, promoter elements from the *Lhx6* gene have been used to generate a mouse ES cell line that, following differentiation to ventral telencephalic progenitors, expresses GFP in putative postmitotic cortical interneuron precursors.[53] Green fluorescent protein-positive cells from this line migrate extensively after transplantation into neocortex, express markers such as PV or somatostatin that define cortical interneuron subgroups, and express intrinsic firing properties typical of these subgroups—for example, critical features of MGE-derived progenitors harvested from the mouse embryo. Promoter elements that impart MGE expression of *Lhx6* downstream of the interneuron fate-determining gene *Nkx2.1* are highly conserved between mice and humans, increasing the likelihood that the same or similar reporter constructs could also be used to isolate cortical interneuron precursors from telencephalon-directed cultures of human ES cells or IPSCs. However, even if this particular approach is successful in generating putative human interneurons, a major challenge will be proving this point, since either very long-term cultures or xenographic transplants with long-term survival will be needed due to the extended period of maturation of cortical interneurons in humans. Of course, it will then be necessary to demonstrate that these integrated cell lines are capable of suppressing spontaneous seizure activity. These caveats aside, the prospect of developing a new cell-based therapy based on transplantation of GABA progenitor cells is an exciting one and clearly merits further study.

DISCLOSURE STATEMENT

S.C.B. is a cofounder of, and has a financial interest in, Neurona Therapeutics.

REFERENCES

1. De Carlos JA, López-Mascaraque L, Valverde F. Dynamics of cell migration from the lateral ganglionic eminence in the rat. *J Neurosci*. 1996;16:6146–6156.
2. Tamamaki N, Fujimori KE, Takauji R. Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. *J Neurosci*. 1997;17:8313–8323.
3. Anderson SA, Eisenstat DD, Shi L, Rubenstein JL. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science*. 1997;278:474–476.
4. Anderson SA, Marín O, Horn C, Jennings K, Rubenstein JL. Distinct migrations from the medial and lateral ganglionic eminences. *Development*. 2001;128:353–363.
5. Nery S, Fishell G, Corbin JG. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. *Nat Neurosci*. 2002;5:1279–1287.
6. Lavdas AA, Grigoriou M, Pachnis V, Paravelas JG. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. *J Neurosci*. 1999;19:7881–7888.
7. Wichterle H, Garcia-Verdugo JM, Herrera DG, Alvarez-Buylla A. Young neurons from medial ganglionic eminence disperse in adult and embryonic brain. *Nat Neurosci*. 1999;2:461–466.
8. Wichterle H, Turnbull DH, Nery S, Fishell G, Alvarez-Buylla A. In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. *Development*. 2001;128:3759–3771.
9. Anderson SA, Kaznowski CE, Horn C, Rubenstein JL, McConell SK. Distinct origins of neocortical projection neurons and interneurons in vivo. *Cereb Cortex*. 2002;12:702–709.
10. Xu Q, Cobos I, De La Cruz E, Rubenstein JL, Anderson SA. Origins of cortical interneuron subtypes. *J Neurosci*. 2004;24:2612–2622.
11. Butt SJ, Fuccillo M, Nery S, Noctor S, Kriegstein A, Corbin JG, Fishell G. The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron*. 2005;48:591–604.
12. Miyoshi G, Hjerling-Leffler J, Karayannis T, Sousa VH, Butt SJ, Battiste J, Johnson JE, Machold RP, Fishell G. Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and

diverse population of superficial cortical interneurons. *J Neurosci*. 2010;30:1582–1594.
13. Tricoire L, Pelkey KA, Daw MI, Sousa VH, Miyoshi G, Jeffries B, Cauli B, Fishell G, McBain CJ. Common origins of hippocampal Ivy and nitric oxide synthase expressing neurogliaform cells. *J Neurosci*. 2010;30: 2165–2176.
14. Ascoli GA, Alonso-Nanclares L, Anderson SA, Barrionuevo G, Benavides-Piccione R, Burkhalter A, Buzsaki G, Cauli B, Defelipe J, Fairen A, Feldmeyer D, Fishell G, Fregnac Y, Freund TF, Gardner D, Gardner EP, Goldberg JH, Helmstaedter M, Hestrin S, Karube F, Kisvarday ZF, Lambolez B, Lewis DA, Marin O, Markram H, Munoz A, Packer A, Petersen CC, Rockland KS, Rossier J, Rudy B, Somogyi P, Staiger JF, Tamas G, Thomson AM, Toledo-Rodriguez M, Wang Y, West DC, Yuste R. Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nat Rev Neurosci*. 2008; 9:557–568.
15. Cobos I, Calcagnotto ME, Vilaythong AJ, Thwin MT, Noebels JL, Baraban SC, Rubenstein JL. Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. *Nat Neurosci*. 2005;8:1059–1068.
16. Powell EM, Campbell DB, Stanwood GD, Davis C, Noebels JL, Levitt P. Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy, and behavioral dysfunction. *J Neurosci*. 2003;23:622–631.
17. Gant JC, Thibault O, Blalock EM, Yang J, Bachstetter A, Kotick J, Schauwecker PE, Hauser KF, Smith GM, Mervis R, Li Y, Barnes GN. Decreased number of interneurons and increased seizures in neuropilin 2 deficient mice: implications for autism and epilepsy. *Epilepsia*. 2009;50:629–645.
18. Price MG, Yoo JW, Burgess DL, Deng F, Hrachovy RA, Frost JD Jr, Noebels JL. A triplet repeat expansion genetic mouse model of infantile spasms syndrome, Arx(GCG)10+7, with interneuronopathy, spasms in infancy, persistent seizures, and adult cognitive and behavioral impairment. *J Neurosci*. 2009;29: 8752–8763.
19. Marsh E, Fulp C, Gomez E, Nasrallah I, Minarcik J, Sudi J, Christian SL, Mancini G, Labosky P, Dobyns W, Brooks-Kayal A, Golden JA. Targeted loss of Arx results in a developmental epilepsy mouse model and recapitulates the human phenotype in heterozygous females. *Brain*. 2009;132:1563–1576.
20. Kato M, Dobyns WB. X-linked lissencephaly with abnormal genitalia as a tangential migration disorder causing intractable epilepsy: proposal for a new term, "interneuronopathy." *J Child Neurol*. 2005;20: 392–397.
21. Kim JH, Auerbach JM, Rodriguez-Gomez JA, Velasco I, Gavin D, Lumelsky N, Lee SH, Nguyen J, Sanchez-Pernaute R, Bankiewicz K, McKay R. Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature*. 2002;418: 50–56.
22. Wernig M, Benninger F, Schmandt T, Rade M, Tucker KL, Bussow H, Beck H, Brustle O. Functional integration of embryonic stem cell-derived neurons in vivo. *J Neurosci.* 2004;24:5258–5268.
23. Carpentino JE, Hartman NW, Grabel LB, Naegele JR. Region-specific differentiation of embryonic stem cell-derived neural progenitor transplants into the adult mouse hippocampus following seizures. *J Neurosci Res*. 2008;86:512–524.
24. Ruschenschmidt C, Koch PG, Brustle O, Beck H. Functional properties of ES cell-derived neurons engrafted into the hippocampus of adult normal and chronically epileptic rats. *Epilepsia*. 2005;46(suppl 5): 174–183.
25. Shetty AK, Zaman V, Hattiangady B. Repair of the injured adult hippocampus through graft-mediated modulation of the plasticity of the dentate gyrus in a rat model of temporal lobe epilepsy. *J Neurosci*. 2005;25:8391–8401.
26. Alvarez-Dolado M, Calcagnotto ME, Karkar KM, Southwell DG, Jones-Davis DM, Estrada RC, Rubenstein JLR, Alvarez-Buylla A, Baraban SC. Cortical inhibition modified by embryonic neural precursors grafted into the postnatal brain. *J Neurosci*. 2006;26:7380–7389.
27. Martínez-Cerdeño V, Noctor SC, Espinosa A, Ariza J, Parker P, Orasji S, Daadi MM, Bankiewicz K, Alvarez-Buylla A, Kriegstein AR. Embryonic MGE precursor cells grafted into adult rat striatum integrate and ameliorate motor symptoms in 6-OHDA-lesioned rats. *Cell Stem Cell*. 2010;6:238–250.
28. Zipancic I, Calcagnotto ME, Piquer-Gil M, Mello LE, Alvarez-Dolado M. Transplant of GABAergic precursors restores hippocampal inhibitory function in a mouse model of seizure susceptibility. *Cell Transplant*. 2010;19:549–564.
29. Daadi MM, Lee SH, Arac A, Grueter BA, Bhatnagar R, Maag AL, Schaar B, Malenka RC, Palmer TD, Steinberg GK. Functional engraftment of the medial ganglionic eminence cells in experimental stroke model. *Cell Tranpslant*. 2009;18:815–826.
30. Baraban SC, Southwell DG, Estrada RC, Jones DL, Sebe JY, Alfaro-Cervello C, García-Verdugo JM, Rubenstein JLR, Alvarez-Buylla A. Reduction of seizures by transplantation of cortical GABAergic interneuron precursors into Kv1.1 mutant mice. *Proc Natl Acad Sci USA*. 2009;106:15472–15477.
31. Barry DI, Kikvadze I, Brundin P, Bolwig TG, Bjorklund A, Lindvall O. Grafted noradrenergic neurons suppress seizure development in kindling-induced epilepsy. *Proc Natl Acad Sci USA*. 1987;84:8712–8715.
32. Barry DI, Wanscher B, Kragh J, Bolwig TG, Kokaia M, Brundin P, Bjorklund A, Lindvall O. Grafts of fetal locus coeruleus neurons in rat amygdala-piriform cortex suppress seizure development in hippocampal kindling. *Exp Neurol*. 1989;106:125–132.
33. Holmes GL, Thompson JL, Huh K, Holmes C, Carl GF. Effect of neural transplants on seizure frequency and kindling in immature rats following kainic acid. *Brain Res Dev Brain Res*. 1991;64:47–56.
34. Holmes GL, Thompson JL, Huh K, Stuart JD, Carl GF. Effects of neural transplantation on seizures in the immature genetically epilepsy-prone rat. *Exp Neurol.* 1992;116:52–63.
35. Clough RW, Browning RA, Maring ML, Statnick MA, Wang C, Jobe PC. Effects of intraventricular locus coeruleus transplants on seizure severity in genetically epilepsy-prone rats following depletion of brain norepinephrine. *J Neural Transplant Plast*. 1994;5: 65–79.
36. Ferencz I, Kokaia M, Elmer E, Keep M, Kokaia Z, Lindvall O. Suppression of kindling epileptogenesis

in rats by intrahippocampal cholinergic grafts. *Eur J Neurosci*. 1998;10:213–220.
37. Fine A, Meldrum BS, Patel S. Modulation of experimentally induced epilepsy by intracerebral grafts of fetal GABAergic neurons. *Neuropsychologia*. 1990;28:627–634.
38. Loscher W, Ebert U, Lehmann H, Rosenthal C, Nikkhah G. Seizure suppression in kindling epilepsy by grafts of fetal GABAergic neurons in rat substantia nigra. *J Neurosci Res*. 1998;51:196–209.
39. Thompson K, Anantharam V, Behrstock S, Bongarzone E, Campagnoni A, Tobin AJ. Conditionally immortalized cell lines, engineered to produce and release GABA, modulate the development of behavioral seizures. *Exp Neurol*. 2000;161:481–489.
40. Gernert M, Thompson KW, Loscher W, Tobin AJ. Genetically engineered GABA-producing cells demonstrate anticonvulsant effects and long-term transgene expression when transplanted into the central piriform cortex of rats. *Exp Neurol*. 2002;176:183–192.
41. Waldau B, Hattiangady B, Kuruba R, Shetty AK. Medial ganglionic eminence-derived neural stem cell grafts ease spontaneous seizures and restore GDNF expression in a rat model of chronic temporal lobe epilepsy. *Stem Cells*. 2010;28:1153–1164.
42. Turski L, Cavalheiro EA, Sieklucka-Dziuba M, Ikonomidou-Turski C, Czucwar SJ, Turski WA. Seizures produced by pilocarpine: neuropathological sequelae and activity of glutamate decarboxylase in the rat forebrain. *Brain Res*. 1986;398:37–48.
43. Smart SL, Lopantsev V, Zhang CL, Robbins CA, Wang H, Chiu SY, Schwartzkroin PA, Messing A, Tempel BL. Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. *Neuron*. 1998;20:809–819.
44. Wenzel HJ, Vacher H, Clark E, Trimmer JS, Lee AL, Sapolsky RM, Tempel BL, Schwartzkroin PA. Structural consequences of Kcna1 gene deletion and transfer in the mouse hippocampus. *Epilepsia*. 2007;48:2023–2046.
45. Glasscock E, Yoo JW, Chen TT, Klassen TL, Noebels JL. Kv1.1 potassium channel deficiency reveals brain-drive cardiac dysfunction as a candidate mechanism for sudden unexplained death in epilepsy. *J Neurosci*. 2010;30:5167–5175.
46. Barberi T, Klivenyi P, Calingasan NY, Lee H, Kawamata H, Loonam K, Perrier AL, Bruses J, Rubio ME, Topf N, Tabar V, Harrison NL, Beal MF, Moore MA, Studer L. Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. *Nat Biotechnol*. 2003;21:1200–1207.
47. Eiraku M, Watanabe K, Matsuo-Takasaki M, Kawada M, Yonemura S, Matsumura M, Wataya T, Nishiyama A, Muguruma K, Sasai Y. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell*. 2008;3:519–532.
48. Watanabe K, Kamiya D, Nishiyama A, Katayama T, Nozaki S, Kawasaki H, Watanabe Y, Mizuseki K, Sasai Y. Directed differentiation of telencephalic precursors from embryonic stem cells. *Nat Neurosci*. 2005;8:288–296.
49. Pankratz MT, Li XJ, Lavaute TM, Lyons EA, Chen X, Zhang SC. Directed neural differentiation of human embryonic stem cells via an obligated primitive anterior stage. *Stem Cells*. 2007;25:1511–1520.
50. Elkabetz Y, Panagiotakos G, Al Shamy G, Socci ND, Tabar V, Studer L. Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. *Genes Dev*. 2008;22:152–165.
51. Li XJ, Zhang X, Johnson MA, Wang ZB, Lavaute T, Zhang SC. Coordination of sonic hedgehog and Wnt signaling determines ventral and dorsal telencephalic neuron types from human embryonic stem cells. *Development*. 2009;136:4055–4063.
52. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol*. 2009;27:275–280.
53. Maroof AM, Brown K, Shi SH, Studer L, Anderson SA. Prospective isolation of cortical interneuron precursors from mouse embryonic stem cells. *J Neurosci*. 2010;30:4667–4675.

Chapter 88

Reversing Disorders of Neuronal Migration and Differentiation in Animal Models

Jean-Bernard Manent
Joseph J. LoTurco

The developing brain has a remarkable capacity for plasticity at both the structural and functional levels. For example, lesions and traumatic injury in the developing rodent neocortex can often be difficult to detect later in life[1,2] and can even trigger new neurogenesis.[3] Similarly, activity-dependent synaptic plasticity is greatest early in development.[4–7] The high degree of plasticity in the developing brain suggests the possibility that after an initially detected disrupted developmental pattern, one that could predispose to seizures, it may be possible to restore a normal developmental state by reactivating appropriate developmental processes to reduce or even prevent the development of seizures.

In this chapter, we review results from animal models that begin to provide evidence that genetically based developmental abnormalities leading to epilepsy can be reversed by reactivating developmental programs. The abnormalities reversed include disruptions in neuronal migration and differentiation. Moreover, molecular genetic and pharmacological interventions in animal models have been shown to reduce morphological disruptions, seizures, and associated behavioral impairments. Significant challenges remain, particularly with respect to translating the approaches used in animal models into viable human therapies. However, the diversity of both the methods used and the disruptions successfully targeted to date should encourage future research and therapy development in this area.

REVERSAL OF A MODEL OF SUBCORTICAL BAND HETEROTOPIA

Disorders in neuronal migration cause aberrations in the normal patterns of cellular

architecture, including alterations in lamination of the neocortex.[8,9] The term *neuronal migration disorder* (NMD) has come to encompass a number of syndromes, including lissencepahly and subcortical band heterotopia. Alterations that occur in cytoarchitecture and structure in NMD are diverse in terms of type and severity.[10] Disruptions range from subtle, only seen with microscopic analysis in isolated regions, to major disruptions covering large regions of the brain and easily apparent with magnetic resonance imaging (MRI). Genetic studies have now revealed many gene mutations that cause syndromes associated with disorders in neuronal migration and abnormal development of the human neocortex.[9,11] Studies focusing on the functions of the products of these genes have, in turn, revealed proteins and pathways required for distinct stages in neocortical development.[10]

Neuronal migration disorder has come to include a variety of early developmental disruptions that may cause displacement of neurons from the typical pattern. It is, however, important to appreciate that the actual underlying disruption that causes NMD may not be a direct deficit in the process of neuronal motility or migration during development. For example, changes in cell proliferation, cell death, cellular differentiation, or cellular adhesion can all cause disruptions in normal cellular patterns of the neocortex. Changes in any of these may alter the movements and ultimate positioning of neurons by blocking or changing physical pathways, or by altering a pattern of cellular growth, without causing any direct change in an immature neuron's intrinsic ability to move or migrate. Nevertheless, mispositioned neurons, independent of how they became mispositioned, could theoretically be repositioned by reactivating a neuronal migration program in those neurons.

Subcortical band heterotopia (SBH) and lissencephaly are both caused by defective neuronal migration during fetal brain development. Subcortical band heteropia is associated with mild to moderate mental retardation and intractable epilepsy in most patients.[12,13] The majority of individuals with SBH are females with mutations in the X-linked gene doublecortin (*DCX*) that encodes a microtubule-binding protein essential to neuronal migration.[14,15] *DCX* mutations in males usually cause anterior lissencephaly, but SBH associated with *DCX* mutations has also been described rarely in males.[16]

Subcortical band heterotopia is the type of malformation that is most clearly a direct consequence of stalled migration in the developing neocortex [17]. In its extreme form, a large band of cells can form a separate mass of gray matter throughout cortex, and the presence of such a large SBH has been referred to as *double cortex syndrome*.[17] During the formation of SBH malformations, many neurons fail to migrate through the intermediate zone and into normally forming cortical lamina, becoming embedded within what will become the white matter of neocortex. With enhanced MRI imaging methods the identification of small, potentially benign areas of SBH has increased, and small SBH-like aggegates of cells are often a feature of type I focal cortical dysplasias.[18–20]

Heterotopia of any type in which subsets of neurons and glia occupy abnormal positions, while others migrate and pattern normally within the same area, suggests a degree of somatic mosaicism in neuronal migration disorders. How such mosaicism occurs is clear for two X-linked genetic causes of heterotopia: periventricular heterotopia and SBH. Heterozygous mutation in the *DCX* gene in females is the most common cause of SBH.[21,22] Random X-inactivation in somatic cells naturally creates a cellular mosaic. Heterozygous *DCX* mutations combined with X-inactivation result in some cells with an active copy of the mutant gene, and these cells fail to migrate into normotopic cortex [14,23]. Somatic mosaicism may also occur from somatic mutations in early stages of embryonic development, creating a mixture of cells with functional and nonfunctional copies of genes essential to migration. Evidence for this type of mosaic pattern has been found by genotyping hair follicles in some SBH cases and finding a mosaic pattern of *DCX* or *LIS1* mutation[24,25]. Such somatic mosaicism in *DCX* mutations can be a cause of SBH in males.[26] Because many, and often most, neurons attain a normal position in mosaic disruptions of neuronal migration, the general substrates necessary for neuronal migration, radial glia and neurons, may be sufficiently intact to guide additional migration if stalled cells could be reactivated to migrate.

The clinical features of SBH show a wide range of severity, but typical deficits are mental retardation with a high incidence of seizures.[27–29]

Importantly for the prospects of reversing the neurological effects of heterotopia, the size of SBH is correlated with the severity.[27,28] The primary reasons for a range in the size of SBH in different cases may be due to the natural variation of random X-inactivation or the specific causative *DCX* mutation, with some mutations causing larger malformations than others. Some milder mutations can even cause SBH in males in which lissencepahly is the typical result. Some migrating cortical neurons may be able to migrate normally even with a defective copy of the *DCX* gene.

Although SBH malformations do not form in mouse genetic models of *Dcx* loss of function,[30,31] Bai and colleagues showed that a rat model for SBH can be generated efficiently by knocking down *Dcx* expression with in utero RNA interference (RNAi). RNA interference targeting of *Dcx* expression in a subset of migrating neurons caused a mosaic of migratory arrest, and *Dcx*-deficient neurons formed the core of an SBH.[32] This model accurately reproduces the main genetic and anatomical features of the human disease, and is also associated with aberrant network activities[33] and spontaneous seizures in the animal model.[34]

Subcortical band heterotopia formation in the RNAi model was shown to be prevented with concurrent expression of *Dcx* in the rat fetus.[35] Manent and colleagues further tested whether delayed reexpression of *Dcx* in already formed SBH after birth could restimulate a developmental program that would lead to SBH regression.[36] Temporal control of *Dcx* reexpression was obtained using a system of drug-inducible DNA plasmid expression vectors that allowed conditional expression of *Dcx* upon injection of 4-hydroxy-tamoxifen (4-OHT). Strikingly, reexpressing *Dcx* stimulated neuronal migration in aberrantly positioned neurons stalled in SBH, leading to both SBH regression and relocation of neurons to their correct laminar destination in the cortex (Fig. 88–1A–D). Moreover, whereas animals with SBH were more sensitive to the induction of seizures by convulsants (Fig. 88–1E,F), animals with regressed SBH showed levels of sensitivity to convulsants no different from those seen in malformation-free controls (Fig. 88–1E,F). Morphological rescue in terms of reduction of the SBH had a critical period and was restricted to early postnatal ages. Restarting migration was no longer possible by the middle of the second postnatal week in the rat model. These observations presented the first proof of concept that neuronal migration disorders may eventually be treatable by molecular or pharmacological interventions aimed at restarting migration to reduce malformations and the neuronal hyperexcitability associated with their presence. The Manent et al. study also suggested that such interventions, at least for migration disorders, may have a critical period to be most effective.

Significant challenges remain for restarting migration caused by deficiencies in *Dcx*, including extending the window for reinitiating migration to later developmental periods and applying gene delivery approaches that do not require fetal surgery. The Manent et al. study examined a relatively narrow period of reactivation and did not extend observations after reexpression of *DCX* to periods longer than 3 weeks. In addition, several *DCX*-interacting proteins, including *LIS1*, are required for migration, and if these decrease in expression or function as well, then they may also need to be reexpressed to restart migration in stalled cells.

PHARMACOLOGICAL RESCUE IN A MODEL OF LISSENCEPHALY

Classical lissencephaly in human patients is caused primarily by mutations in either the *DCX* or *LIS1* genes.[21,37–41] Miller-Dieker syndrome is a severe neurodevelopmental disorder that involves lissencephaly as well as other disruptions in central nervous system (CNS) structure. The syndrome is caused by deletions or translocations involving the short arm of chromosome 17 and heterozygous mutations of at least the two genes *LIS1* and *YWHAE*. Isolated lissencepahly (iLIS), without all the defining features of Miller-Dieker syndrome, can be caused by mutation in *LIS1* alone. Miller-Dieker syndrome is characterized by pachygyria[42] and, compared to X-linked lissencepahly (XLIS), caused typically by mutations in *DCX*, has a greater degree of agyria in posterior and occipital regions of the cortex.[43,44] Miller-Dieker syndrome is also associated with enlarged ventricles, hypoplasia of the corpus callosum, and hypoplasia of the cerebral peduncles and cerebral pyramids, further adding to the severity of the neural disruption. Miller-Dieker syndrome and iLIS

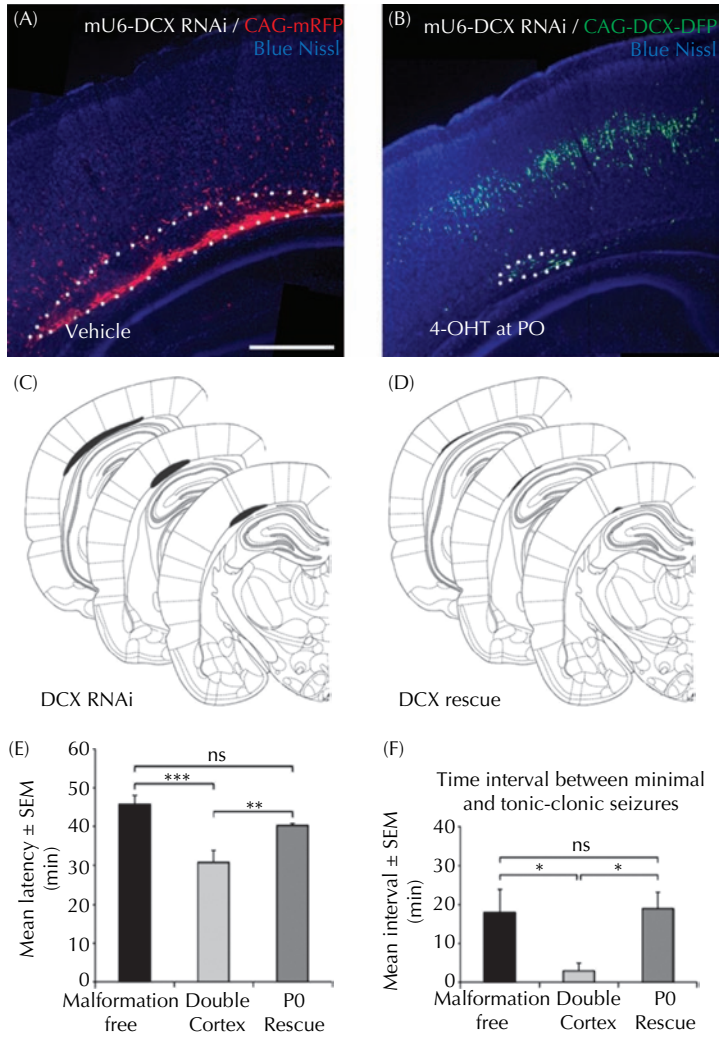

Figure 88–1. Rescue by reexpression of *Dcx* of SBH in a rat model of NMD. **A.** Subcortical band heterotopia is indicated by the dotted line and aggregates of monomeric red fluorescent protein (mRFP)-labeled (red) neurons just below the normatopic neocortex. The SBH was created by RNAi against *Dcx*. **B.** Greatly reduced SBH in a brain in which *Dcx* was reexpressed. Green fluorescent protein was used to mark the cells manipulated in **B**, and red fluorescent protein was used to mark the cells in **A. C,D.** Serial section reconstruction of SBH remaining in a control brain, an RNAi- alone brain (**C**), and a *Dcx* rescue brain in which *Dcx* was reexpressed after birth (**D**). **E,F.** *Dcx* reexpression at P0 (P0 rescue) restored the response to a ramped dosage of the convulsant PTZ to levels similar to those of malformation-free animals. This was seen both in the latency to the first induced seizure (**E**) and in the time interval between minimal seizure signs and the first generalized tonic-clonic seizure episode. Adapted from ref. 36.

patients show severe to profound mental retardation and early-onset seizures that may lead to intractable epilepsy. Seizures start early in life, near birth to 4 months of age,[45] and there is a significantly shortened lifespan.[46] The clinical severity of lissencepahly, like that of SBH, correlates with anatomical measures of morphological and neurodevelopmental disruption and genetic mutation.[47]

The *LIS1* gene, also known as *PAFAH1B*, codes for a protein initially described as a subunit in the enzyme for processing platelet activating factor (PAF). It was the first gene to be shown to be involved in lissencephaly when mutated.[38] Its role in neuronal migration has not been found to be linked to PAF activity; instead, it functions primarily in neurodevelopment through interactions with

dynein, microtubules, 14–3-3 epsilon protein, and nudel protein. The genetic evidence for Miller-Dieker syndrome and the protein interaction evidence for *LIS1* and *YWHAE* are consistent.[48] In addition, *LIS1-NDEL1* interaction plays a well-conserved role throughout biology in the movement of nuclei within cells.[49]

Heterozygous loss of *Lis1* function in mutant mice, as in humans, causes migration disruptions that result in disorganized lamination of the neocortex, hippocampus, and olfactory bulb[41] and epilepsy. Inspired by the finding that *LIS1* is degraded by calpain-dependent proteolysis, Yamada and colleagues[50] tested whether inhibition of calpains could increase *LIS1* protein abundance and effectively bring the expression levels of the heterozygous mutant back to wild-type levels, and if so, reverse the developmental disruptions. Yamada et al. showed that either treatment with calpain inhibitors or knockdown of calpain expression by small interfering RNA (siRNA) improved the abnormal cellular phenotype caused by heterozygous loss of *Lis1* in cell culture. They also showed that treatment with calpain inhibitors rescued migration of $Lis1^{+/-}$ cerebellar granule neurons from heterozygous mutant mice in an in vitro migration assay. These in vitro observations were followed by experiments in which calpain inhibitors were administered to pregnant $Lis1^{+/-}$ mice during embryonic corticogenesis. Remarkably, both pharmacological inhibition and knockdown of calpain in vivo rescued defective neuronal migration and abnormal cortical and hippocampal layering in heterozygous mutant pups ($Lis1^{+/-}$) born to treated mothers. Therefore, pharmacologically increasing Lis1 protein abundance normalized neurogenesis, and neuronal migration by blocking Lis1 protein degradation was sufficient to partially reverse the neuro-morphological deficit.[50,51] Pharmacological restoration of Lis1 protein levels by inhibition of calpain also ameliorated behavioral symptoms in $Lis1^{+/-}$ mice.[50,51] Whereas untreated $Lis1^{+/-}$ mice displayed abnormal motor behavior, $Lis1^{+/-}$ mice embryonicaly treated with calpain inhibitors showed significant levels of improvement in motor function. Considering the potential to reverse developmental insults even after birth, as revealed in the *DCX* reexpression experiments, it would be interesting to test whether calpain inhibition after the onset of early migration disruption phenotypes in neonatal animals can rescue the migration alterations in $Lis^{+/-}$ mutants. In addition, normalization of neural excitatability and the seizure risk in this model has not yet been fully characterized, but considering the nearly complete rescue of motor behaviors by calpain inhibition, seizures are also likely to be ameliorated.

REVERSAL OF NEURODEVELOPMENTAL DISRUPTION BEYOND NEURONAL MIGRATION DISORDERS

Molecular rescue of neurodevelopmental disorders after the onset of neurological and neuromorphological changes is not restricted to the two examples of NMD described above. Several experiments involving rodent models of Rett syndrome, caused by mutations in the gene *MECP2* and models of tuberous sclerosis complex (TSC) have been used to test both molecular genetic and pharmacological rescue of neurodevelopmental disorders. The late rescue of genetically based neurodevelopmental disorders may therefore be a general phenomenon, not restricted to disorders of neuronal migration.

Genetic Rescue of Rett Syndrome

Luikenhuis and colleagues[52] engineered a transgenic mouse strain (*Tau-Mecp2* knock-in) such that *Mecp2* expression was placed under the control of the Tau promoter to drive neuron-specific embryonic expression of *Mecp2* in mutant animals. Unlike *Mecp2-null* mutant mice that displayed reduced weight and brain size and exhibited impaired locomotor and exploratory activity, offspring of *Mecp2-null* mice mated to *Tau-Mecp2* mice were indistinguishable from wild-type animals and displayed no Rett syndrome-like symptoms. These observations indicated that reexpressing *Mecp2* in *Mecp2*-deficient neurons was sufficient to reverse Rett syndrome-associated developmental impairments.

Reexpression of *Mecp2* later in development as a means to prevent the Rett phenotype was tested in a study by Giacometti and colleagues.[53] A mouse was produced carrying

a conditional "*Mecp2* rescue transgene" engineered so that the *Mecp2* coding sequence was placed downstream of a lox-Stop-lox (LSL) cassette. These *LSL Mecp2* mice were then crossed with *Mecp2-null* mutants to generate animals carrying the *Mecp2-null* allele as well as the conditional "rescue" construct. In order to test whether *Mecp2* reexpression at late embryonic and postnatal ages could ameliorate the Rett syndrome-like phenotype, the investigators crossed *LSL Mecp2;Mecp2-null* double mutants with Cre-expressing transgenic mouse lines to induce reexpression of *Mecp2* in the brain at either early embryonic or postnatal periods in development. Whereas *Mecp2-null* mice developed Rett syndrome-associated symptoms and died early, mice in which *Mecp2* expression was reintroduced at embryonic ages displayed significantly delayed behavioral alterations and an increased lifespan. Similarly, mice in which *Mecp2* expression was reintroduced at early or even late postnatal ages displayed significant improvements including an increased lifespan. Reexpressing *Mecp2* at presymptomatic postnatal ages positively affected the course of the disease and significantly ameliorated Rett syndrome-like symptoms. Guy and colleagues[54] used a slightly different strategy. They created a mouse in which the endogenous *Mecp2* gene was silenced by insertion of a LSL cassette that could be conditionally deleted using Cre-mediated excision to activate the gene later in development. This mouse was crossed with a mouse carrying a tamoxifen-activatable Cre transgene so that the endogenous *Mecp2* gene in the offspring could be reactivated upon tamoxifen-induced deletion of the LSL cassette. In this model, when an active copy of *Mecp2* was restored in males at an age after which they exhibited advanced symptoms, the animals significantly improved their behavioral scores and had an increased lifespan.[54] Similarly, in postnatal female mice with advanced behavioral symptoms, a significant reversal of both behavioral and electrophysiological deficits associated with the Rett syndrome-like phenotype was observed when an active copy of the *Mecp2* gene was activated. Together, these reexpression studies in different Rett syndrome models indicate that the neurological deficits associated with *Mecp2* deficiency can be reversed by reintroduction of the gene even after the appearance of the first developmental deficits.

Pharmacological Rescue of TSC

Mutations in the *Tsc1* or *Tsc2* genes cause TSC and result in hyperactivatation of the mammalian target of rapamycin (mTOR) pathway.[55,56] Several research groups hypothesized that treating TSC model animals with the mTOR inhibitor rapamycin or its derivates could lead to phenotypic ameliorations.[57] Zeng et al. first investigated this issue in the astrocyte-specific TSC mouse model by administering rapamycin at presymptomatic or symptomatic stages of the disease in mice with mutated conditional alleles of *Tsc1*.[57] Rapamycin treatment prevented the development of seizures and significantly increased the lifespan of conditional *Tsc1* mutant mice. Remarkably, late rapamycin treatment in already symptomatic animals also decreased seizures and improved survival. Moreover, both early and late treatment resulted in reduced morphological signs of decreased *Tsc* function, including astrogliosis and disorganization in hippocampal lamination. In similar experiments, Meikle and colleagues carried out experiments in the neuron-specific TSC mouse model[58] and administered rapamycin or 40-O-(2-hydroxyethyl)-rapamycin (RAD001, also called *SDZ RAD* or *everolimus*) to postnatal day (7–9 (P7–9) animals for up to 100 days. Whereas *Tsc1*$^{\text{null-neuron}}$ mice displayed hyperactivity, postural and behavioral abnormalities, poor weight gain, and development of seizures and died at P33, both rapamycin- and RAD001-treated mice showed clinical improvement. Postural and behavioral abnormalities, weight gain, and survival were significantly improved in long-term treated animals, as well as in animals in which treatment was stopped after 30 days. Moreover, some histopathological improvement was obtained, including reduced neuronal hypertrophy and increased myelination.[58] Disorganized cortical lamination, abnormal cortical neurons, dendritic orientation, and reduced spine density remained unchanged. Interestingly, some histopathological amelioration was transient and progressively reversed after cessation of drug treatment, while major clinical benefits persisted for several weeks after the treatment was stopped.

Models of *Tsc2* loss can also be rescued with rapamycin. Ehninger and colleagues administered rapamycin to adult *Tsc2*$^{+/-}$ mice and observed a remarkable restoration of impaired spatial learning deficits, context discrimination,

and an abnormal late-phase long-term potentiation (LTP) threshold.[59] Importantly, learning and memory deficits were ameliorated even after brief rapamycin treatment periods. In the same study, the authors treated heterozygous neuron-specific *Tsc1*^{null} mice with a similar rapamycin regimen. Whereas *Tsc1*$^{null\text{-}neuron}$ mice displayed severe brain abnormalities such as macrocephaly, neuronal hypertrophy, and astrogliosis and exhibited neurological impairments, after rapamycin treatment mice showed significantly improved motor behavior, an increased lifespan, and decreased brain size.[59] This suggests that rescue for some phenotypes in TSC models does not depend upon reversal of morphological developmental defects.

The tumor suppressor gene *PTEN* (phosphatase and tensin homolog on chromosome 10) is a negative regulator of the mTOR signaling pathway, and *PTEN* mutations have been identified in several clinical disorders grouped as *PTEN*-hamartoma tumor syndromes.[60] Ljungberg and colleagues analyzed *Pten*^{loxP}; *GFAP-Cre* mice (referred to as *NS-PTEN* mice in their study) and observed that these animals display widespread neurodevelopmental abnormalities.[61] In addition to the presence of enlarged cortical, hippocampal, and cerebellar structures, detailed analysis of Pten immunolabeling revealed the presence of significant numbers of Pten-negative glutamatergic neurons with abnormal hypermorphic morphology in the neocortex. Moreover, the mTOR pathway was found to be hyperactive in these neocortical neurons, consistent with the previously described role of Pten in this pathway. As previously shown by Kwon et al., *NS-PTEN* mice after a few weeks of age exhibited mainly subclinical spontaneous epileptiform activity that often evolved to tonic-clonic seizures.[62] Altogether, these observations led Ljungberg and colleagues to propose that *NS-PTEN* mice could be considered as a model of focal cortical dysplasia (FCD), recapitulating some physiopathological aspects of the human disease, namely, hypermorphic cortical neurons and spontaneous epileptic manifestations. Similar to a previous study, the authors hypothesized that treating animals with the mTOR inhibitor rapamycin could restore mTOR activity. Indeed, after 2 weeks of treatment with rapamycin, they found a significant decrease in mTOR activity and reduced hypertrophy of neurons. As dysregulated mTOR activity was found in patients with FCD, hemimegalencephaly, and TSC, the authors concluded that modulating mTOR activity in such patients may help to control seizures resistant to AED therapy and/or epilepsy surgery.[61] The results also suggest that FCDs that result from developmental disruption in an earlier developmental period can be ameliorated in terms of both neuroanatomical abnormality and seizures.[61] This serves as another dramatic example of an intervention after a malformation or developmental disruption reversing in part the developmental abnormality.

SUMMARY AND FUTURE CHALLENGES

Reactivation of migration in molecularly and genetically defined models of NMDs, SBH, and lissencepahly can reduce the morphological and behavioral impairments associated with these NMDs in animal models. Future studies must now extend these approaches to other neurodevelopmental disorders, including NMD caused by mutations in genes other than *Lis1* and *Dcx,* as well as migration disruption caused by environmental insults or trauma. In addition, alternative pharmacological and molecular approaches that may be more amenable to human therapy than the ones used so far need to be tested in animal models.

Gene therapy approaches, both viral and virus-free, have advanced significantly in the past several years.[63–66] For example, adeno-associated virus technologies have approached a level where genes can be reintroduced safely into primates.[63,64] Different serotypes effectively create stable transgenesis with little or no toxicity in neurons and glia. Similarly, nonviral liposomal-mediated transgenesis has progressed rapidly and has been shown to be successful in treating animal models of neurological disease.[63,64] These two technologies should now be tested in neurodevelopmental models to attempt to genetically reactivate developmental pathways and genes in a way that does not require the use of specialized genetic models that allow for conditional gene expression. Alternatives to gene therapy should also be tested in neurodevelopmental disorders. Indeed, as already shown for inhibition of calpain in *Lis1* mutants and rapamycin

treatment in TSC models, it is possible to overcome impaired developmental pathways either directly or indirectly. Specific small molecule screens might be successful in identifying novel agents to enhance pathways impaired by loss of specific genes.

DISCLOSURE STATEMENT

The authors have no conflicts of interest.

REFERENCES

1. Kolb B, Nonneman AJ. Functional development of prefrontal cortex in rats continues into adolescence. *Science*. 1976;193:335–336.
2. Kolb B, Petrie B, Cioe J. Recovery from early cortical damage in rats, VII. Comparison of the behavioural and anatomical effects of medial prefrontal lesions at different ages of neural maturation. *Behav Brain Res*. 1996;79:1–14.
3. Covey MV, Jiang Y, Alli VV, Yang Z, Levison SW. Defining the critical period for neocortical neurogenesis after pediatric brain injury. *Dev Neurosci*. 2010;32: 488–498.
4. Maffei A, Lambo ME, Turrigiano GG. Critical period for inhibitory plasticity in rodent binocular V1. *J Neurosci*. 2010;30:3304–3309.
5. Maffei A, Turrigiano G. The age of plasticity: developmental regulation of synaptic plasticity in neocortical microcircuits. *Prog Brain Res.* 2008;169:211–223.
6. Hubel DH, Wiesel TN. The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J Physiol*. 1970;206:419–436.
7. Wiesel TN, Hubel DH. Effects of visual deprivation on morphology and physiology of cells in the cat's lateral geniculate body. *J Neurophysiol*. 1963;26:978–993.
8. Dobyns WB, Andermann E, Andermann F, Czapansky-Beilman D, Dubeau F, Dulac O, Guerrini R, Hirsch B, Ledbetter DH, Lee NS, Motte J, Pinard JM, Radtke RA, Ross ME, Tampieri D, Walsh CA, Truwit CL. X-linked malformations of neuronal migration. *Neurology*. 1996;47:331–339.
9. Guerrini R, Dobyns WB, Barkovich AJ. Abnormal development of the human cerebral cortex: genetics, functional consequences and treatment options. *Trends Neurosci.* 2008;31:154–162.
10. Gleeson JG, Walsh CA. Neuronal migration disorders: from genetic diseases to developmental mechanisms. *Trends Neurosci*. 2000;23:352–359.
11. Jaglin XH, Chelly J. Tubulin-related cortical dysgeneses: microtubule dysfunction underlying neuronal migration defects. *Trends Genet*. 2009;25:555–566.
12. Barkovich AJ, Jackson DE Jr, Boyer RS. Band heterotopias: a newly recognized neuronal migration anomaly. *Radiology*. 1989;171:455–458.
13. Tanaka T, Gleeson JG. Genetics of brain development and malformation syndromes. *Curr Opin Pediatr*. 2000;12:523–528.
14. Gleeson JG, Allen KM, Fox JW, Lamperti ED, Berkovic S, Scheffer I, Cooper EC, Dobyns WB, Minnerath SR, Ross ME, Walsh CA. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. *Cell*. 1998;92:63–72.
15. des Portes V, Pinard JM, Billuart P, Vinet MC, Koulakoff A, Carrié A, Gelot A, Dupuis E, Motte J, Berwald-Netter Y, Catala M, Kahn A, Beldjord C, Chelly J. A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. *Cell*. 1998;92:51–61.
16. D'Agostino MD, Bernasconi A, Das S, Bastos A, Valerio RM, Palmini A, Costa da Costa J, Scheffer IE, Berkovic S, Guerrini R, Dravet C, Ono J, Gigli G, Federico A, Booth F, Bernardi B, Volpi L, Tassinari CA, Guggenheim MA, Ledbetter DH, Gleeson JG, Lopes-Cendes I, Vossler DG, Malaspina E, Franzoni E, Sartori RJ, Mitchell MH, Mercho S, Dubeau F, Andermann F, Dobyns WB, Andermann E. Subcortical band heterotopia (SBH) in males: clinical, imaging and genetic findings in comparison with females. *Brain.* 2002;125(pt 11):2507–2522.
17. Palmini A, Andermann F, Aicardi J, Dulac O, Chaves F, Ponsot G, Pinard JM, Goutières F, Livingston J, Tampieri D. Diffuse cortical dysplasia, or the "double cortex" syndrome: the clinical and epileptic spectrum in 10 patients. *Neurology.* 1991;41:1656–1662.
18. Palmini A, Luders HO. Classification issues in malformations caused by abnormalities of cortical development. *Neurosurg Clin North Am–*. 2002;13(1)1–16.
19. Palmini A, Najm I, Avanzini G, Babb T, Guerrini R, Foldvary-Schaefer N, Jackson G, Luders HO, Prayson R, Spreafico R, Vinters HV. Terminology and classification of the cortical dysplasias. *Neurology*. 2004; 62(6 suppl 3):S2–S8.
20. Fauser S, Huppertz HJ, Bast T, Strobl K, Pantazis G, Altenmueller DM, Feil B, Rona S, Kurth C, Rating D, Korinthenberg R, Steinhoff BJ, Volk B, Schulze-Bonhage A. Clinical characteristics in focal cortical dysplasia: a retrospective evaluation in a series of 120 patients. *Brain.* 2006;129(pt 7):1907–1916.
21. Pilz DT, Matsumoto N, Minnerath S, Mills P, Gleeson JG, Allen KM, Walsh CA, Barkovich AJ, Dobyns WB, Ledbetter DH, Ross ME. LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. *Hum Mol Genet*. 1998;7:2029–2037.
22. Gleeson JG, Luo RF, Grant PE, Guerrini R, Huttenlocher PR, Berg MJ, Ricci S, Cusmai R, Wheless JW, Berkovic S, Scheffer I, Dobyns WB, Walsh CA. Genetic and neuroradiological heterogeneity of double cortex syndrome. *Ann Neurol*. 2000;47: 265–269.
23. des Portes V, Francis F, Pinard JM, Desguerre I, Moutard ML, Snoeck I, Meiners LC, Capron F, Cusmai R, Ricci S, Motte J, Echenne B, Ponsot G, Dulac O, Chelly J, Beldjord C. Doublecortin is the major gene causing X-linked subcortical laminar heterotopia (SCLH). *Hum Mol Genet*. 1998;7:1063–1070.
24. Gleeson JG, Minnerath S, Kuzniecky RI, Dobyns WB, Young ID, Ross ME, Walsh CA. Somatic and germline mosaic mutations in the doublecortin gene are associated with variable phenotypes. *Am J Hum Genet*. 2000;67:574–581.

25. Sicca F, Kelemen A, Genton P, Das S, Mei D, Moro F, Dobyns WB, Guerrini R. Mosaic mutations of the LIS1 gene cause subcortical band heterotopia. *Neurology*. 2003;61:1042–1046.
26. Poolos NP, Das S, Clark GD, Lardizabal D, Noebels JL, Wyllie E, Dobyns WB. Males with epilepsy, complete subcortical band heterotopia, and somatic mosaicism for DCX. *Neurology*. 2002;58:1559–1562.
27. Guerrini R, Carrozzo R. Epilepsy and genetic malformations of the cerebral cortex. *Am J Med Genet*. 2001;106:160–173.
28. Guerrini R, Carrozzo R. Epileptogenic brain malformations: clinical presentation, malformative patterns and indications for genetic testing. *Seizure*. 2002;11(suppl A):532–543; quiz 544–537.
29. Barkovich AJ, Guerrini R, Battaglia G, Kalifa G, N'Guyen T, Parmeggiani A, Santucci M, Giovanardi-Rossi P, Granata T, D'Incerti L. Band heterotopia: correlation of outcome with magnetic resonance imaging parameters. *Ann Neurol*. 1994;36:609–617.
30. Deuel TA, Liu JS, Corbo JC, Yoo SY, Rorke-Adams LB, Walsh CA. Genetic interactions between doublecortin and doublecortin-like kinase in neuronal migration and axon outgrowth. *Neuron*. 2006;49:41–53.
31. Corbo JC, Deuel TA, Long JM, LaPorte P, Tsai E, Wynshaw-Boris A, Walsh CA. Doublecortin is required in mice for lamination of the hippocampus but not the neocortex. *J Neurosci*. 2002;22:7548–7557.
32. Bai J, Ramos RL, Ackman JB, Thomas AM, Lee RV, LoTurco JJ. RNAi reveals doublecortin is required for radial migration in rat neocortex. *Nat Neurosci*. 2003;6:1277–1283.
33. Ackman JB, Aniksztejn L, Crépel V, Becq H, Pellegrino C, Cardoso C, Ben-Ari Y, Represa A. Abnormal network activity in a targeted genetic model of human double cortex. *J Neurosci*. 2009;29:313–327.
34. Lapray D, Popova IY, Kindler J, Jorquera I, Becq H, Manent JB, Luhmann HJ, Represa A. Spontaneous epileptic manifestations in a DCX knockdown model of human double cortex. *Cereb Cortex*. 2010;20: 2694–2701.
35. Ramos RL, Bai J, LoTurco JJ. Heterotopia formation in rat but not mouse neocortex after RNA interference knockdown of DCX. *Cereb Cortex*. 2006;16: 1323–1331.
36. Manent J-B, Wang Y, Chang Y, Paramasivam M, LoTurco JJ. Dcx reexpression reduces subcortical band heterotopia and seizure threshold in an animal model of neuronal migration disorder. *Nat Med*. 2009;15: 84–90.
37. Dobyns WB, Truwit CL. Lissencephaly and other malformations of cortical development: 1995 update. *Neuropediatrics*. 1995;26:132–147.
38. Hattori M, Adachi H, Tsujimoto M, Arai H, Inoue K. Miller-Dieker lissencephaly gene encodes a subunit of brain platelet-activating factor acetylhydrolase [corrected]. *Nature*. 1994;370:216–218.
39. Lo Nigro C, Chong CS, Smith AC, Dobyns WB, Carrozzo R, Ledbetter DH. Point mutations and an intragenic deletion in LIS1, the lissencephaly causative gene in isolated lissencephaly sequence and Miller-Dieker syndrome. *Hum Mol Genet*. 1997;6: 157–164.
40. Reiner O, Carrozzo R, Shen Y, Wehnert M, Faustinella F, Dobyns WB, Caskey CT, Ledbetter DH. Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats. *Nature*. 1993;364:717–721.
41. Hirotsune S, Fleck MW, Gambello MJ, Bix GJ, Chen A, Clark GD, Ledbetter DH, McBain CJ, Wynshaw-Boris A. Graded reduction of Pafah1b1 (Lis1) activity results in neuronal migration defects and early embryonic lethality. *Nat Genet*. 1998;19:333–339.
42. Dobyns WB, Reiner O, Carrozzo R, Ledbetter DH. Lissencephaly. A human brain malformation associated with deletion of the LIS1 gene located at chromosome 17p13. *JAMA*. 1993;270:2838–2842.
43. Dobyns WB, Stratton RF, Greenberg F. Syndromes with lissencephaly. I: Miller-Dieker and Norman-Roberts syndromes and isolated lissencephaly. *Am J Med Genet*. 1984;18:509–526.
44. Dobyns WB, Truwit CL, Ross ME, Matsumoto N, Pilz DT, Ledbetter DH, Gleeson JG, Walsh CA, Barkovich AJ. Differences in the gyral pattern distinguish chromosome 17-linked and X-linked lissencephaly. *Neurology*. 1999;53:270–277.
45. Dobyns WB, Stratton RF, Parke JT, Greenberg F, Nussbaum RL, Ledbetter DH. Miller-Dieker syndrome: lissencephaly and monosomy 17p. *J Pediatr*. 1983;102:552–558.
46. Dobyns WB, Elias ER, Newlin AC, Pagon RA, Ledbetter DH. Causal heterogeneity in isolated lissencephaly. *Neurology*. 1992;42:1375–1388.
47. Cardoso C, Leventer RJ, Dowling JJ, Ward HL, Chung J, Petras KS, Roseberry JA, Weiss AM, Das S, Martin CL, Pilz DT, Dobyns WB, Ledbetter DH. Clinical and molecular basis of classical lissencephaly: Mutations in the LIS1 gene (PAFAH1B1). *Hum Mutat*. 2002;19:4–15.
48. Wynshaw-Boris A. Lissencephaly and LIS1: insights into the molecular mechanisms of neuronal migration and development. *Clin Genet*. 2007;72:296–304.
49. Morris NR. Nuclear migration. From fungi to the mammalian brain. *J Cell Biol*. 2000;148:1097–1101.
50. Yamada M, Yoshida Y, Mori D, Takitoh T, Kengaku M, Umeshima H, Takao K, Miyakawa T, Sato M, Sorimachi H, Wynshaw-Boris A, Hirotsune S. Inhibition of calpain increases LIS1 expression and partially rescues in vivo phenotypes in a mouse model of lissencephaly. *Nat Med*. 2009;15:1202–1207.
51. Yamada M, Hirotsune S, Wynshaw-Boris A. A novel strategy for therapeutic intervention for the genetic disease: preventing proteolytic cleavage using small chemical compound. *Int J Biochem Cell Biol*. 2010;42: 1401–1407.
52. Luikenhuis S, Giacometti E, Beard CF, Jaenisch R. Expression of MeCP2 in postmitotic neurons rescues Rett syndrome in mice. *Proc Natl Acad Sci USA*. 2004;101:6033–6038.
53. Giacometti E, Luikenhuis S, Beard C, Jaenisch R. Partial rescue of MeCP2 deficiency by postnatal activation of MeCP2. *Proc Natl Acad Sci USA*. 2007;104: 1931–1936.
54. Guy J, Gan J, Selfridge J, Cobb S, Bird A. Reversal of neurological defects in a mouse model of Rett syndrome. *Science*. 2007;315:1143–1147.
55. Crino PB. Focal brain malformations: a spectrum of disorders along the mTOR cascade. *Novartis Found Symp*. 2007;288:260–272; discussion 272–281.
56. Ljungberg MC, Bhattacharjee MB, Lu Y, Armstrong DL, Yoshor D, Swann JW, Sheldon M, D'Arcangelo G. Activation of mammalian target of rapamycin in cytomegalic neurons of human cortical dysplasia. *Ann Neurol*. 2006;60:420–429.

57. Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol*. 2008;63:444–453.
58. Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, Kwiatkowski DJ. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci*. 2008;28:5422–5432.
59. Ehninger D, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ, Ramesh V, Silva AJ. Reversal of learning deficits in a $Tsc2^{+/-}$ mouse model of tuberous sclerosis. *Nat Med*. 2008;14:843–848.
60. Hobert JA, Eng C. PTEN hamartoma tumor syndrome: an overview. *Genet Med*. 2009;11:687–694.
61. Ljungberg MC, Sunnen CN, Lugo JN, Anderson AE, D'Arcangelo G. Rapamycin suppresses seizures and neuronal hypertrophy in a mouse model of cortical dysplasia. *Dis Model Mech*. 2009;2:389–398.
62. Kwon CH, Zhu X, Zhang J, Baker SJ. mTor is required for hypertrophy of Pten-deficient neuronal soma in vivo. *Proc Natl Acad Sci USA*. 2003;100:12923–12928.
63. McCown TJ. The future of epilepsy treatment: focus on adeno-associated virus vector gene therapy. *Drug News Perspect*. 2010;23:281–286.
64. McCown TJ. Adeno-associated virus (AAV) vectors in the CNS. *Curr Gene Ther*. 2005;5:333–338.
65. Hester ME, Foust KD, Kaspar RW, Kaspar BK. AAV as a gene transfer vector for the treatment of neurological disorders: novel treatment thoughts for ALS. *Curr Gene Ther*. 2009;9:428–433.
66. Dodge JC, Haidet AM, Yang W, Passini MA, Hester M, Clarke J, Roskelley EM, Treleaven CM, Rizo L, Martin H, Kim SH, Kaspar R, Taksir TV, Griffiths DA, Cheng SH, Shihabuddin LS, Kaspar BK. Delivery of AAV-IGF-1 to the CNS extends survival in ALS mice through modification of aberrant glial cell activity. *Mol Ther*. 2008;16:1056–1064.

Chapter 89

Gene Therapy of Focal-Onset Epilepsy Using Adeno-Associated Virus Vector-Mediated Overexpression of Neuropeptide Y

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Although various new antiepileptic drugs (AEDs) with diverse mechanisms of action have been developed in the last 15 years with improved tolerability and pharmacokinetic properties,[1,2] there has been relatively little improvement in their ability to control pharmacoresistant epilepsies compared to traditional AEDs. Thus, about 30% of patients with epilepsy still have seizures that are resistant to available AEDs. Drug-resistant epileptic patients are considered for surgical resection of the epileptic focus: this invasive procedure, however, is suitable only for a minority of them and results in complete control of epilepsy (seizure freedom without AEDs) in about 30% to 50% of cases, according to a 5- to 10-year follow-up study.[3] Additionally, most AEDs provide symptomatic suppression of seizures without apparently interfering with the mechanisms involved in the epileptic process.[4,5] Currently, AEDs mainly target neurotransmitter receptors, mechanisms of neurotransmitter release or reuptake, or ion channels.[1] There is therefore an urgent need to find novel treatment strategies, particularly for the most refractory forms, such as temporal lobe epilepsy (TLE).[4,6]

As an alternative approach to pharmacotherapy, preclinical studies in models of seizures

and epilepsy are addressing the possibility of suppressing seizures by inducing the overexpression of neuromodulatory molecules in the area(s) of seizure origin or propagation. This may be achieved using gene therapy that has been developed, and clinically applied, in other neurological disorders.[7–12] The intent of experimental studies is to establish the proof-of-concept evidence that gene therapy may be envisioned as a novel therapeutic approach to control drug-resistant seizures in focal-onset epilepsies.

GENE THERAPY IN THE CENTRAL NERVOUS SYSTEM AND THE CHOICE OF TOOLS FOR GENE DELIVERY

Gene manipulation in the central nervous system (CNS) encounters specific problems related to the presence of the blood-brain barrier, which is impermeable to many xenobiotics (including carriers that may be used to deliver the therapeutic gene into the brain). Furthermore, gene therapy must target cells in a defined brain area with a specific function relevant to epilepsy. Finally, most cells in the CNS are postmitotic and therefore inadequate for gene transduction with vectors requiring DNA integration.

Neurotropic adeno-associated viral (AAV) vectors are the tool of choice for gene delivery into brain tissue and consequent neuron transfection. These vectors can efficiently express single or multiple transgenes together with a wide range of regulatory elements; they are nonpathogenic and appear to be innocuous for normal brain physiology.[13,14] For these reasons, AAV is considered a safe vector for gene transfer and has been used in various clinical trials (see http://www.clinicaltrials.gov).

For gene therapy purpose, recombinant (r)AAV vectors are used[15,16] in which all viral genes encoding wild-type viral proteins are removed to avoid viral replication and reduce toxicity and immunogenicity while permitting the insertion of therapeutic genes with a length of up to 4.7 kb.

There are several serotypes of rAAV that can be used for gene transfer in the CNS, each of them possessing a distinct cellular tropism that depends on the expression of cell membrane receptors that can mediate vector transport inside the cells: heparan sulfate proteoglycans are the main receptors for AAV serotype 2; AAV serotype 1 binds to α2,3 and α2,6 sialic acids, which are present on N-linked glycoproteins. This evidence suggests that it is possible to use different serotypes, or even modify the capsid composition of the viral vector, for targeting specific cell populations. Moreover, it is possible to use specific promoters or other regulatory elements[17] to generate cell- and tissue-specific gene expression.

Gene therapy techniques in epilepsy are designed to successflully transfer a putative therapeutic gene, and its related protein, into the ictogenic brain area(s) and express them, thus permitting long-term CNS expression of neuromodulatory molecules with potential anticonvulsive, neuroprotective, and antiepileptogenic properties. Two main approaches have been developed for the transfer of a specific gene into the CNS: the ex vivo approach, consisting of in vitro genetic modifications of cells that are then transferred into the brain to provide a source of release of the therapeutic molecule, and the in vivo approach that allows direct transgene transfection in the brain using viral vectors or systemic delivery of the genes using strategies that favor their brain penetration, such as immunoliposomes.[18,19] Up to now, in vivo direct brain gene transfer has been used more frequently for introducing genes into postmitotic cells such as neurons and to promote long-term expression of the related proteins.[20]

There is a wide range of potential therapeutic targets that could modulate neuronal activity in vivo and inhibit hyperexcitability. The selection of the appropriate therapeutic gene is one crucial aspect of gene therapy for epilepsy.

GENE THERAPY: THE CHOICE OF THE THERAPEUTIC GENE

So far, therapeutic genes have been used to decrease the effect of excitatory neurotransmitters (e.g., using antisense sequences against *N*-methyl-D-aspartate [NMDA] receptor subunit 1),[21] to increase inhibitory neurotransmission (e.g., gamma-aminobutyric acid A [$GABA_A$] receptor subunit α-1, glutamic acid decarboxylase 65 [GAD65], adenosine kinase),[22–24] and to

enhance the release of neurotrophic factors, such as glial cell-derived neurotrophic factor (GDNF),[25] brain-derived neurotrophic factor (BDNF), and fibroblast growth factor (FGF),[26] or neuropeptides with anticonvulsant properties such as galanin[27,28] and neuropeptide Y (NPY).[29,30]

Recent studies have focused on endogenous neuropeptides with neuroprotective and neuromodulatory functions. Indeed, in contrast to classical neurotransmitters, neuropeptides are preferentially released by neurons during high-frequency neuronal activity, such as during seizures, while they do not appear to contribute significantly to synaptic transmission in physiological conditions,[31–33] thus reducing the chances of ensuing side effects by targeting these systems.

THE FOCUS ON NPY

Both galanin and NPY are peptides with powerful anticonvulsant properties in various experimental models of seizures.[34,35] Both peptides inhibit glutamate release, via different mechanisms, and this effect appears to be mainly responsible for their anticonvulsant actions.[29,36] Gene therapy approaches using either galanin or NPY as transgenes have been developed to reduce seizures. In this chapter, we will focus on preclinical studies related to NPY overexpression mediated by AAV vectors.

Changes in the Endogenous NPY System during Seizures

Overexpression of NPY in the hippocampus and temporal cortex has been described in several models of seizures and in human brain tissue from TLE patients. [29,37,38]
In the hippocampus, seizure-induced NPY overexpression occurs both in interneurons, where NPY is physiologically colocalized mainly with GABA and/or somatostatin, and ectopically in granule cells of the dentate gyrus and in CA1 pyramidal neurons.[35] The changes in peptide expression are concomitant with modifications in NPY receptor subtypes (i.e., Y2 receptors are increased on presynaptic glutamatergic terminals, whereas Y1 receptors are decreased on granule cell dendrites) and are accompanied by increased NPY release,[29,35,39,40] thus suggesting that NPY-mediated neurotransmission is altered in the epileptic tissue. A similar pattern of NPY changes was reported in patients with intractable TLE,[38] except for the long-lasting ectopic expression of NPY in granule cells and their mossy fibers, typically observed in seizure models but lacking in human epileptic tissue.[29]

The anticonvulsant effect of NPY is mainly ascribed to its release from hippocampal GABAergic interneurons, as well as from glutamatergic mossy fibers terminals. Acting on presynaptic Y2 receptors localized on glutamatergic terminals, NPY inhibits glutamate release,[41,42] thus reducing the excitability of the trisynaptic pathway of the hippocampal formation.[42,43] One main action of NPY is to block the synchronization of granule cell discharge, thus inhibiting epileptiform activity dependent on the recurrent collaterals of mossy fibers.[40,44] Y5 receptors in granule cells of the dentate gyrus and in pyramidal cell layers may also contribute to NPY inhibitory actions,[45,46] although this possibility has been challenged in in vitro and in vivo studies.[47,48]

In experimental models of seizures, intracerebral administration of NPY[29] or Y2/Y5 receptor agonists[49–51] resulted in strong anticonvulsant effects[37] (Table 89–1). Accordingly, the susceptibility to seizure was decreased in transgenic rats overexpressing NPY in CA1 pyramidal neurons,[52] whereas mice with NPY or Y2 gene deletion were more susceptible to seizures.[48,53] Moreover, NPY suppressed epileptiform activity and attenuated excitatory responses in dentate granule cells evoked by perforant path stimulation in brain tissue surgically resected from medically intractable TLE patients.[54]

These findings suggest that increased NPY-mediated neurotrasmission during seizures represents a homeostatic mechanism to counteract hyperexcitability. Moreover, anticonvulsant effects can be achieved by increasing endogenous NPY actions by pharmacological or genetic manipulation.

GENE THERAPY WITH NPY IN SEIZURE MODELS

AAV-Mediated NPY Overexpression

To study the expression of the NPY transgene in the CNS using gene therapy approaches, the

Table 89–1 **Anticonvulsant Effects of NPY Injected in Rodent Seizure Models**

Dose (nmol)	Route of administration	Model	Effects	References
6	Intracerebroventricular	Kainic acid (intraperitoneal)	↑ Onset ↓ Time in seizures ↓ Seizures severity	49
12	Intracerebroventricular	Electrical stimulation	↓ Afterdischarge duration ↓ Wet dog shakes	50,55,56
12–24	Intracerebroventricular	Pentylenetetrazol	↑ Onset ↓ Seizures severity	57
3–12	Intracerebroventricular	GAERS	↓ SWD frequency ↓ SWD duration	58,59
0.08	Intrahippocampal	Electrical stimulation	No secondary AD No WDS	56
1	Intrahippocampal	Electrically-induced SE	↓ SE severity Spike suppression	60

AD, afterdischarge; GAERS: Genetic Absence Epilepsy Rat from Strasbourg; SE, status epilepticus; SWD, spike-and-wave discharge; WDS wet dog shakes

human pre-pro-NPY cDNA sequence has been cloned into a highly purified recombinant rAAV vector. Rat and human NPY cDNAs have a remarkable degree of homology (about 75% in the translated region) and are identical in their amino acid sequence in the mature form.[61] Recombinant rAAV vectors with different serotypes have been tested in rats in order to develop the most suitable vector for clinical application: a nonimmunogenic and nontoxic vector able to transfect hippocampal neurons and to express NPY long-term at sufficiently high levels and in a releasable form.

Serotype 2 (rAAV2), chimeric serotype 1/2 (rAAV1/2), and serotype 1 (rAAV1) vectors have been used in different sets of experiments. The rAAV2-NPY vector increased peptide expression in hilar interneurons only,[62] while chimeric rAAV1/2 (expressing capsid surface proteins of both serotype 1 and serotype 2) and rAAV1-NPY vectors mediated more widespread expression, including granule cells and their mossy fibers, and pyramidal cells in the hippocampus and the subiculum.[62,63]

Two different promoters have been tested on the backbone of serotype 2 plasmid, namely, neuron-specific enolase (NSE) and cytomegalovirus (CMV)-chicken β-actin (CBA) promoters. The neuron-specific enolase promoter leads primarily to expression of gene products in neurons, providing a good level of specificity; CBA is a very powerful promoter that provides higher levels of protein expression in neurons for at least 18 months postinjection.[64]

Using the NSE promoter, the hippocampal injection of rAAV2-NSE-NPY vector induced transgene expression for about 1.5 mm around the injection site. Using the chimeric rAAV1/2-NSE-NPY vector, or the CBA promoter coupled to chimeric or to AAV1 serotypes (rAAV1/2-CBA and rAAV1-CBA-NPY vectors), a larger spread of the transgene was obtained (i.e., about 2.5 mm around the injection site).[62,63] Moreover, NPY immunoreactivity was increased to the greatest extent in neurons for at least 6 months using the CBA promoter (unpublished data).

High-performance liquid chromatography (HPLC) analysis demonstrated that NPY encoded by transduced neurons elutes with the synthetic rat peptide.[30] In situ hybridization analysis of mRNA sequence specific for the vector genome, as well as peptide immunohistochemistry, showed that NPY expression was confined to neurons within the hippocampal formation and did not spread outside the injected hippocampus, except for its axonal transport to synaptic terminals in the contralateral hemisphere.[62,63] Neurochemical experiments in hippocampal slices from rAAV-NPY-injected rats showed that NPY is released by neurons only under high potassium chloride (KCl) depolarization (about 10-fold more than control slices), and in vivo electrophysiology confirmed that transduced NPY is released by high-frequency stimulation.[30,33] Overexpression of NPY was associated with decreased levels of Y1 receptors likely due to peptide-induced

internalization,[65] while the levels of Y2 receptors were unchanged.[30]

Anticonvulsant Effects Mediated by Transgene NPY

Hippocampal rAAV-mediated NPY overexpression in rats was associated with powerful anticonvulsant effects in various rat models of seizures and epilepsy (Table 89–2). The first study was carried out in a rat model of acute focal seizures induced by intrahippocampal administration of kainic acid: the overexpression of NPY was driven by the NSE promoter, resulting in 50% or 75% seizure reduction when the serotype 2 or chimeric serotype 1/2 vector, respectively, was used.[62] Thus, the greater levels of NPY produced with the chimeric vector provided more effective seizure control. When the CBA promoter and the chimeric rAAV1/2 or rAAV1 vector were used, the number of focal seizures was also significantly reduced.[63] Total focal seizure duration was decreased in all experimental settings, up to 70% in rats injected with the serotype 1 vector. Moreover, status epilepticus induced by intraventricular injection of kainic acid in rats could not be evoked in rats injected with the rAAV1/2-NSE-NPY vector. Thus, continuous epileptic activity recorded electroencephalographically in the hippocampus and in the overlying cortex was precluded in NPY-overexpressing rats, as well as clonic motor seizures that were instead observed in control animals.[62] These findings showed that NPY overexpression results in reduction of focal seizure activity and anticipates a role for this peptide in reducing seizure generalization as well, as subsequently demonstrated in kindling and using systemic administration of chemoconvulsants.[33,62,66,67]

To address the antiepileptogenic potential of this gene therapy approach, we used the rapid hippocampal kindling model; rats injected with the rAAV1/2-NSE-NPY vector showed a significant delay in acquisition of generalized seizures, a significant increase in the threshold current for inducing a local afterdischarge, and a decrease in afterdischarge duration.[33,62] A subsequent study supported and extended these findings[66] by demonstrating that rAAV-mediated overexpression of NPY13–36, a peptide fragment that activates Y2 receptors, in the rat piriform cortex attenuates generalized limbic seizures induced by systemic kainate. In this set of experiments, the authors used a rAAV vector construct that included insertion of the secretion signal sequence of fibronectin, which promotes the *constitutive release* of the encoded peptide, as opposed to the *regulated release* mediated by the above-described vectors.

The inhibitory effects mediated by NPY overexpression on status epilepticus and the associated motor seizures, as well as on kindling progression, demonstrate a prominent reduction of seizure generalization.

Importantly, rAAV-NPY-based gene therapy was reported to be effective in reducing spontaneous recurrent limbic seizures when injected in the hippocampus of chronic epileptic rats mimicking TLE.[30] The increased NPY levels resulted in a significant decrease in spontaneous seizures frequency compared to preinjection baseline levels. Moreover, rAAV-NPY-injected rats showed a reduction in the symptomatic progression of the disease (i.e., no increase in seizure frequency and reduced behavioral seizure severity over time) compared to control epileptic rats, thus suggesting a disease-modifying effect. Disease progression is typical of this epilepsy model,[68] and is also described in TLE patients who do not respond adequately to AEDs and are eventually evaluated for surgical resection of the seizure focus.[69] Since the NPY transgene could not be switched off in these sets of experiments, it remains unresolved if NPY overexpression results in genuine disease-modifying effects or if the suppression of seizure progression was due mainly to the peptide's anticonvulsant properties.[70]

As noted in acute seizure models, the extent of seizure reduction provided by the rAAV-NPY vector in chronic epileptic rats correlated positively with the extent of NPY overexpression in the hippocampus.[30] A significant inverse correlation was found between hippocampal NPY levels and seizure progression, indicating that the NPY level within the epileptic focus is a key factor determining the antiepileptic efficacy of this approach.

GENE THERAPY: ANY ADVERSE EFFECTS?

Gene therapy approaches may encounter great difficulties related both to the invasiveness of

Table 89–2 **Effects of rAAV-NPY Vectors Injection in Rat Models of Seizures and Epilepsy**

Vector	Injection site	Seizure Model	Effects	References
rAAV2-NSE-NPY	Intrahippocampal	Kainic acid (intrahippocampal)	↑ Onset ↓ 50% number of seizures ↓ 50% time in seizures	62
rAAV1/2-NSE-NPY	Intrahippocampal	Kainic acid (intrahippocampal)	↑ Onset ↓ 75% number of seizures ↓ 75% time in seizures	62
		Kainic acid (intracerebroventricular)	↑ Onset No SE	62
		Electrical kindling	↑ 40% treshold current ↑ number of stimuli to generalized seizures ↓ number of generalized seizures ↓ Afterdischarge duration	33,62,67
rAAV1/2-CBA-NPY	Intrahippocampal	Kainic acid (intrahippocampal)	↓ 35% number of seizures ↓ 40% time in seizures	63
		Epilepsy	↓ Spontaneous seizures frequency ↓ number of generalized seizures ↓ Seizure progression	30
rAAV1-CBA-NPY	Intrahippocampal	Kainic acid Intrahippocampal	↓ 55% number of seizures ↓ 70% time in seizures	63
rAAV1/2-CBA-(FIB)NPY	Piriform Cortex	Kainic acid intraperitoneal	No generalized seizures	66

the intervention and to the specific targeting of the brain region; moreover, toxic effects may arise due to the injection of the vector that might induce an inflammatory response in brain tissue or trigger immune host reactions to capsid proteins or to the gene product. The possible occurrence of these effects should be carefully considered since brain inflammatory molecules may exacerbate seizures[71] and the presence of antibodies against the vector could affect its ability to transfect neurons, resulting in decreased or impaired efficacy of gene therapy.[72–74] These considerations have been recently addressed in studies in which normal rats were injected in the hippocampus with the rAAV1-CBA-NPY vector,[63] which appears to be suitable for clinical application.[75–77] Light microscopy showed lack of a local reaction to the vector injection except for nonspecific damage due to needle insertion, which was similar in vehicle-injected rats. Neutralizing serum antibodies against the serotype 1 AAV vector were not detected except in one rat, which, however, showed increased transgene expression and was significantly protected from seizures.

Another factor that should be considered is the pleiotropic effects of NPY, since this neuropeptide is involved in several physiological functions, including cognition, anxiety, and motor activity.[39,78–82] The hippocampus plays a significant role in the processing of spatial information and memory and, in association with the amygdala, in anxiety behavior. Thus, NPY overexpression in the hippocampus could result in anxiolytic-like effects, impairment of spatial memory, and reduction of long-term potentiation (LTP).[83–85] In this regard, we showed that rats injected with rAAV1/2-NPY using the NSE promoter in the CA1 area of the septal hippocampus had impaired LTP.[85] Long-term potentiation is a process that depends on high-frequency neuronal activity and thus can be affected by the release of NPY from transfected neurons: indeed, NPY may suppress LTP in CA1 by inhibiting voltage-gated Ca^{2+} channels via Y2 receptors, thus reducing glutamate release.[41,42,85] Rats also exhibited a transient learning deficit[33] suggesting a delayed process of learning. Interestingly, in subsequent studies,[30,63] no learning deficits were observed in naive rats injected into the hippocampus with rAAV-NPY vectors containing the CBA promoter. These different results likely depend on the hippocampal region where NPY was overexpressed, namely, the septal pole in the first study and both the septal and temporal poles in the second study. Thus, the effects of NPY on cognition depend on the hippocampal region where the peptide levels are increased.[86,87] Another possible explanation involves the use of different promoters, that is, NSE or CBA, which might have preferentially induced transgene expression in distinct neuronal populations (see also ref. 21), thus resulting in different functional outcomes. In this respect, it is possible that interneurons and GABAergic transmission could be affected by NPY overexpression. Interneurons contribute to oscillatory network activity arising during information and memory processing of the hippocampus; therefore, changes in their afferent synapses induced by NPY may alter memory function. In support of this possibility, it has been shown recently that both inhibitory and excitatory afferent transmission on cholecystokinin-expressing basket cells, a subclass of GABAergic interneurons residing in the hilus granule cell border, was changed by exogenous NPY via Y2 receptors activation.[88] Whether viral overexpression of NPY induces similar effects, and the physiological implications of NPY-induced modulation of hippocampal interneurons, are still unknown, but it could be an alternative explanation for the differential effect on memory behavior observed in naive rats.

It is noteworthy that cognitive function is often compromised in epileptic patients;[89,90] thus, one should test it to determine if the gene therapy approach could exacerbate this condition. Recent findings have demonstrated that the attenuation of LTP found in hyperexcitable kindled rats is not further compromised by NPY overexpression[67] (Fig. 89–1), thus suggesting that seizure-induced cognitive impairment may not be worsened by rAAV-NPY treatment. Overexpression of NPY in the hippocampus does not appear to alter basal synaptic transmission in both naive and hyperexcitable kindled rats, and only minor changes in the short-term synaptic plasticity of excitatory glutamatergic synapses have been observed.[33,67]

Finally, no behavioral impairments were observed in naive rats injected intrahippocampally with rAAV1-CBA-NPY vector[63] when they were tested for anxiety and motor functions.[79]

These data suggest that rAAV-NPY gene therapy might be safe and well tolerated, although

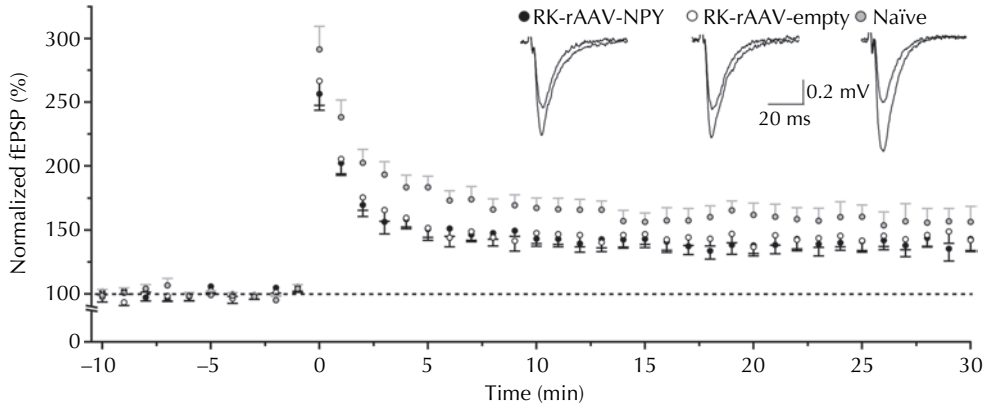

Figure 89–1. Long-term potentiation (LTP) is attenuated by rAAV-NPY treatment but is not further compromised in hyperexcitable kindled rats. Electrophysiological field recordings were obtained in slices from adult male rats after they were injected with rAAV-NPY (NSE promoter and serotype 1/2) or rAAV-empty vector and exposed to rapid kindling (RK). Stable field excitatory postsynaptic potentials (fEPSPs) were acquired as a baseline before high-frequency stimulation, delivered at 100 Hz for 1 s, was applied to slices (at 0 min) to induce LTP. Long-term potentiation was significantly and equally compromised in slices from RK-rAAV-NPY and RK-rAAV-empty animals compared to slices from non kindled naive animals (repeated measures ANOVA [$p < .05$] and the Mann-Whitney test [$p < .001$]). Initial slopes of fEPSPs are normalized to average baseline values and plotted against time (average of four fEPSPs per minute) for RK-rAAV-NPY (n = 11 slices, 7 rats), RK-rAAV-empty (n = 11 slices, 9 rats), and naive (n = 10 slices, 7 rats) groups. Data are shown as mean ± SEM. Representative fEPSPs (average of four traces) acquired during baseline and 20 min after high-frequency stimulation for each group. Modified from ref. 67.

these conditions should be further studied in the context of the epileptic pathology.

CONCLUSION

Focal-onset drug-resistant epilepsy, such as TLE, is potentially eligible for gene therapy since the therapeutic gene can be delivered into a specific epileptogenic brain area, thus obtaining local and sustained release of a therapeutic molecule. Recombinant AAV vectors have already been used in clinical trials for neurological disorders, with encouraging results, for example, in patients with Parkinson's disease.[7]

Experimental findings in rat models of seizures and epilepsy provide proof-of-principle evidence that the transfer of a gene expressing an endogenous anticonvulsant peptide in chronic epileptic tissue may offer a novel therapeutic intervention to control seizures.

However, further development of gene therapy strategies is still needed, such as improving gene delivery methods (i.e., brain targeting of the transgene using systemic delivery[91]), using inducible gene expression regulation, which would allow the transgene to be switched off when required,[92] and studying in greater depth the safety profile and possible side effects of gene therapy in epileptic conditions.

A protocol for a Phase 1 clinical trial based on rAAV-NPY is under consideration at the U.S. Food and Drug Administration (http://www4.od.nih.gov/oba/RAC/meetings/Sept2004/RACagenda092304.pdf): the target population is mesial TLE patients with pharmacoresistant chronic seizures who are candidates for surgical resection of the epileptogenic focus. If this alternative treatment strategy undergoes successful clinical trials, it will open new avenues for the treatment of seizures that offer potential cures rather than focusing on the symptoms.

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The authors have no conflict of interest to disclose

REFERENCES

1. Rogawski MA, Loscher W. The neurobiology of antiepileptic drugs. *Nat Rev Neurosci*. 2004;5(7):553–564.
2. Schachter SC. Currently available antiepileptic drugs. *Neurotherapeutics*. 2007;4(1):4–11.
3. Yoon HH, Kwon HL, Mattson RH, Spencer DD, Spencer SS. Long-term seizure outcome in patients initially seizure-free after resective epilepsy surgery. *Neurology*. 2003;61(4):445–450.
4. Duncan JS, Sander JW, Sisodiya SM, Walker MC. Adult epilepsy. *Lancet*. 2006;367(9516):1087–1100.
5. Pitkanen A, Sutula TP. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. *Lancet Neurol*. 2002;1(3):173–181.
6. Engelhardt B, Wolburg-Buchholz K, Wolburg H. Involvement of the choroid plexus in central nervous system inflammation. *Microsc Res Tech*. 2001;52(1):112–129.
7. Kaplitt MG, Feigin A, Tang C, et al. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet*. 2007;369(9579):2097–2105.
8. Fiandaca M, Forsayeth J, Bankiewicz K. Current status of gene therapy trials for Parkinson's disease. *Exp Neurol*. 2008;209(1):51–57.
9. Marks WJ, Jr., Ostrem JL, Verhagen L, et al. Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. *Lancet Neurol*. 2008;7(5):400–408.
10. Tuszynski MH, Thal L, Pay M, et al. A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nat Med*. 2005;11(5):551–555.
11. McPhee SW, Janson CG, Li C, et al. Immune responses to AAV in a phase I study for Canavan disease. *J Gene Med*. 2006;8(5):577–588.
12. Worgall S, Sondhi D, Hackett NR, et al. Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. *Hum Gene Ther*. 2008;19(5):463–474.
13. Monahan PE, Samulski RJ. Adeno-associated virus vectors for gene therapy: more pros than cons? *Mol Med Today*. 2000;6(11):433–440.
14. Kaplitt MG, During MJ. *Gene therapy in the central nervous system. From bench to bedside.* San Diego, CA: Elsevier; 2006.
15. Terzi D, Zachariou V. Adeno-associated virus-mediated gene delivery approaches for the treatment of CNS disorders. *Biotechnol J*. 2008;3(12):1555–1563.
16. Buning H, Perabo L, Coutelle O, Quadt-Humme S, Hallek M. Recent developments in adeno-associated virus vector technology. *J Gene Med*. 2008;10(7):717–733.
17. Shi W, Yu M, Qian Q. AAV-based targeting gene therapy. *American Journal of Immunology*. 2008;4(4):51–65.
18. Geisert EE, Jr., Del Mar NA, Owens JL, Holmberg EG. Transfecting neurons and glia in the rat using pH-sensitive immunoliposomes. *Neurosci Lett*. 1995;184(1):40–43.
19. Delgado-Escueta AV. Advances in lafora progressive myoclonus epilepsy. *Curr Neurol Neurosci Rep*. 2007;7(5):428–433.
20. Mandel RJ, Manfredsson FP, Foust KD, et al. Recombinant adeno-associated viral vectors as therapeutic agents to treat neurological disorders. *Mol Ther*. 2006;13(3):463–483.
21. Haberman R, Criswell H, Snowdy S, et al. Therapeutic liabilities of in vivo viral vector tropism: adeno-associated virus vectors, NMDAR1 antisense, and focal seizure sensitivity. *Mol Ther*. 2002;6(4):495–500.
22. Raol YH, Lund IV, Bandyopadhyay S, et al. Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. *J Neurosci*. 2006;26(44):11342–11346.
23. Boison D. Adenosine augmentation therapies (AATs) for epilepsy: prospect of cell and gene therapies. *Epilepsy Res*. 2009;85(2–3):131–141.
24. Epps S, Venable D, Faingold C, Wilson S, Coleman J. Effects of alteration of GAD by lentivirus mediated gene transfer on seizure severity in genetically epilepsy prone rats. *Exp Neurol*. 2006;198(2):568.
25. Kanter-Schlifke I, Georgievska B, Kirik D, Kokaia M. Seizure suppression by GDNF gene therapy in animal models of epilepsy. *Mol Ther*. 2007;15(6):1106–1113.
26. Paradiso B, Marconi P, Zucchini S, et al. Localized delivery of fibroblast growth factor-2 and brain-derived neurotrophic factor reduces spontaneous seizures in an epilepsy model. *Proc Natl Acad Sci U S A*. 2009;106(17):7191–7196.
27. Lerner JT, Sankar R, Mazarati AM. Galanin and epilepsy. *Cell Mol Life Sci*. 2008;65(12):1864–1871.
28. McCown TJ. Adeno-associated virus vector-mediated expression and constitutive secretion of galanin suppresses limbic seizure activity. *Neurotherapeutics*. 2009;6(2):307–311.
29. Vezzani A, Sperk G, Colmers WF. Neuropeptide Y: emerging evidence for a functional role in seizure modulation. *Trends Neurosci*. 1999;22(1):25–30.
30. Noe' F, Pool AH, Nissinen J, et al. Neuropeptide Y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. *Brain*. 2008;131(Pt 6):1506–1515.
31. Hokfelt T. Neuropeptides in perspective: the last ten years. *Neuron*. 1991;7(6):867–879.
32. Hokfelt T, Bartfai T, Bloom F. Neuropeptides: opportunities for drug discovery. *Lancet Neurol*. 2003;2(8):463–472.
33. Sorensen AT, Kanter-Schlifke I, Carli M, et al. NPY gene transfer in the hippocampus attenuates synaptic plasticity and learning. *Hippocampus*. 2008;18(6):564–574.
34. Mazarati A, Langel U, Bartfai T. Galanin: an endogenous anticonvulsant? *Neuroscientist*. 2001;7(6):506–517.
35. Vezzani A, Sperk G. Overexpression of NPY and Y2 receptors in epileptic brain tissue: an endogenous neuroprotective mechanism in temporal lobe epilepsy? *Neuropeptides*. 2004;38(4):245–252.
36. Zini S, Roisin MP, Langel U, Bartfai T, Ben-Ari Y. Galanin reduces release of endogenous excitatory amino acids in the rat hippocampus. *Eur J Pharmacol*. 1993;245(1):1–7.
37. Noe' F, Nissinen J, Pitkanen A, et al. Gene therapy in epilepsy: the focus on NPY. *Peptides*. 2007;28(2):377–383.
38. Furtinger S, Pirker S, Czech T, Baumgartner C, Ransmayr G, Sperk G. Plasticity of Y1 and Y2 receptors and neuropeptide Y fibers in patients with temporal lobe epilepsy. *J Neurosci*. 2001;21(15):5804–5812.

39. Redrobe JP, Dumont Y, St-Pierre JA, Quirion R. Multiple receptors for neuropeptide Y in the hippocampus: putative roles in seizures and cognition. *Brain Res*. 1999;848(1–2):153–166.
40. Nadler JV, Tu B, Timofeeva O, Jiao Y, Herzog H. Neuropeptide Y in the recurrent mossy fiber pathway. *Peptides*. 2007;28(2):357–364.
41. Colmers WF, Lukowiak K, Pittman QJ. Neuropeptide Y action in the rat hippocampal slice: site and mechanism of presynaptic inhibition. *J Neurosci*. 1988;8(10): 3827–3837.
42. Klapstein GJ, Colmers WF. On the sites of presynaptic inhibition by neuropeptide Y in rat hippocampus in vitro. *Hippocampus*. 1993;3(1):103–111.
43. Paredes MF, Greenwood J, Baraban SC. Neuropeptide Y modulates a G protein-coupled inwardly rectifying potassium current in the mouse hippocampus. *Neurosci Lett*. 2003;340(1):9–12.
44. Tu B, Timofeeva O, Jiao Y, Nadler JV. Spontaneous release of neuropeptide Y tonically inhibits recurrent mossy fiber synaptic transmission in epileptic brain. *J Neurosci*. 2005;25(7):1718–1729.
45. Parker RM, Herzog H. Comparison of Y-receptor subtype expression in the rat hippocampus. *Regul Pept*. 1998;75–76:109–115.
46. Baraban SC. Neuropeptide Y and epilepsy: recent progress, prospects and controversies. *Neuropeptides*. 2004;38(4):261–265.
47. Woldbye DP, Nanobashvili A, Sorensen AT, et al. Differential suppression of seizures via Y2 and Y5 neuropeptide Y receptors. *Neurobiol Dis*. 2005;20(3): 760–772.
48. El Bahh B, Balosso S, Hamilton T, et al. The antiepileptic actions of neuropeptide Y in the hippocampus are mediated by Y2 and not Y5 receptors. *Eur J Neurosci*. 2005;22(6):1417–1430.
49. Woldbye DP, Larsen PJ, Mikkelsen JD, Klemp K, Madsen TM, Bolwig TG. Powerful inhibition of kainic acid seizures by neuropeptide Y via Y5-like receptors. *Nat Med*. 1997;3(7):761–764.
50. Woldbye DP, Madsen TM, Larsen PJ, Mikkelsen JD, Bolwig TG. Neuropeptide Y inhibits hippocampal seizures and wet dog shakes. *Brain Res*. 1996;737(1–2): 162–168.
51. Smialowska M, Sopala M, Tokarski K. Inhibitory effect of intrahippocampal NPY injection on amphetamine-induced behavioural activity. *Neuropeptides*. 1996;30(1):67–71.
52. Vezzani A, Michalkiewicz M, Michalkiewicz T, et al. Seizure susceptibility and epileptogenesis are decreased in transgenic rats overexpressing neuropeptide Y. *Neuroscience*. 2002;110(2):237–243.
53. Baraban SC, Hollopeter G, Erickson JC, Schwartzkroin PA, Palmiter RD. Knock-out mice reveal a critical antiepileptic role for neuropeptide Y. *J Neurosci*. 1997;17(23):8927–8936.
54. Patrylo PR, van den Pol AN, Spencer DD, Williamson A. NPY inhibits glutamatergic excitation in the epileptic human dentate gyrus. *J Neurophysiol*. 1999;82(1): 478–483.
55. Klemp K, Woldbye DP. Repeated inhibitory effects of NPY on hippocampal CA3 seizures and wet dog shakes. *Peptides*. 2001;22(3):523–527.
56. Reibel S, Nadi S, Benmaamar R, et al. Neuropeptide Y and epilepsy: varying effects according to seizure type and receptor activation. *Peptides*. 2001;22(3): 529–539.
57. Woldbye DP. Antiepileptic effects of NPY on pentylenetetrazole seizures. *Regul Pept*. 1998;75–76: 279–282.
58. Stroud LM, O'Brien TJ, Jupp B, Wallengren C, Morris MJ. Neuropeptide Y suppresses absence seizures in a genetic rat model. *Brain Res*. 2005;1033(2):151–156.
59. Morris MJ, Gannan E, Stroud LM, Beck-Sickinger AG, O'Brien TJ. Neuropeptide Y suppresses absence seizures in a genetic rat model primarily through effects on Y receptors. *Eur J Neurosci*. 2007;25(4):1136–1143.
60. Mazarati A, Wasterlain CG. Anticonvulsant effects of four neuropeptides in the rat hippocampus during self-sustaining status epilepticus. *Neurosci Lett*. 2002;331(2):123–127.
61. Allen JM. Molecular structure of Neuropeptide Y and regulation of expression of its gene. In: Mutt V, Fuxe K, Hokfelt T, Lundberg JM, eds. *Neuropeptide Y*. New York: Raven Press; 1989:33–41.
62. Richichi C, Lin EJ, Stefanin D, et al. Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. *J Neurosci*. 2004;24(12):3051–3059.
63. Noe' F, Vaghi V, Balducci C, et al. Anticonvulsant effects and behavioural outcomes of rAAV serotype 1 vector-mediated neuropeptide Y overexpression in rat hippocampus. *Gene Ther*. 2010;17(5):643–652.
64. Klein RL, Hamby ME, Gong Y, et al. Dose and promoter effects of adeno-associated viral vector for green fluorescent protein expression in the rat brain. *Exp Neurol*. 2002;176(1):66–74.
65. Pheng LH, Dumont Y, Fournier A, Chabot JG, Beaudet A, Quirion R. Agonist- and antagonist-induced sequestration/internalization of neuropeptide Y Y1 receptors in HEK293 cells. *Br J Pharmacol*. 2003;139(4):695–704.
66. Foti S, Haberman RP, Samulski RJ, McCown TJ. Adeno-associated virus-mediated expression and constitutive secretion of NPY or NPY13–36 suppresses seizure activity in vivo. *Gene Ther*. 2007;14(21):1534–1536.
67. Sorensen AT, Nikitidou L, Ledri M, et al. Hippocampal NPY gene transfer attenuates seizures without affecting epilepsy-induced impairment of LTP. *Exp Neurol*. 2009;215(2):328–333.
68. Bertram EH, Cornett JF. The evolution of a rat model of chronic spontaneous limbic seizures. *Brain Res*. 1994;661(1–2):157–162.
69. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N Engl J Med*. 2000;342(5):314–319.
70. Dudek FE. Commentary: a skeptical view of experimental gene therapy to block epileptogenesis. *Neurotherapeutics*. 2009;6(2):319–322.
71. Ravizza T, Gagliardi B, Noe F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. *Neurobiol Dis*. 2008;29(1):142–160.
72. Peden CS, Burger C, Muzyczka N, Mandel RJ. Circulating anti-wild-type adeno-associated virus type 2 (AAV2) antibodies inhibit recombinant AAV2 (rAAV2)-mediated, but not rAAV5-mediated, gene transfer in the brain. *J Virol*. 2004;78(12):6344–6359.
73. Sanftner LM, Suzuki BM, Doroudchi MM, et al. Striatal delivery of rAAV-hAADC to rats with preexisting immunity to AAV. *Mol Ther*. 2004;9(3):403–409.
74. Lowenstein PR, Mandel RJ, Xiong WD, Kroeger K, Castro MG. Immune responses to adenovirus and adeno-associated vectors used for gene therapy of brain diseases: the role of immunological synapses

in understanding the cell biology of neuroimmune interactions. *Curr Gene Ther*. 2007;7(5):347–360.
75. Brantly ML, Spencer LT, Humphries M, et al. Phase I trial of intramuscular injection of a recombinant adeno-associated virus serotype 2 alphal-antitrypsin (AAT) vector in AAT-deficient adults. *Hum Gene Ther*. 2006;17(12):1177–1186.
76. Stroes ES, Nierman MC, Meulenberg JJ, et al. Intramuscular administration of AAV1-lipoprotein lipase S447X lowers triglycerides in lipoprotein lipase-deficient patients. *Arterioscler Thromb Vasc Biol*. 2008;28(12):2303–2304.
77. Mueller C, Flotte TR. Clinical gene therapy using recombinant adeno-associated virus vectors. *Gene Ther*. 2008;15(11):858–863.
78. Wettstein JG, Earley B, Junien JL. Central nervous system pharmacology of neuropeptide Y. *Pharmacol Ther*. 1995;65(3):397–414.
79. Karl T, Burne TH, Herzog H. Effect of Y1 receptor deficiency on motor activity, exploration, and anxiety. *Behav Brain Res*. 2006;167(1):87–93.
80. Redrobe JP, Dumont Y, Quirion R. Neuropeptide Y (NPY) and depression: from animal studies to the human condition. *Life Sci*. 2002;71(25):2921–2937.
81. Thorsell A, Heilig M. Diverse functions of neuropeptide Y revealed using genetically modified animals. *Neuropeptides*. 2002;36(2–3):182–193.
82. Tschenett A, Singewald N, Carli M, et al. Reduced anxiety and improved stress coping ability in mice lacking NPY-Y2 receptors. *Eur J Neurosci*. 2003;18(1):143–148.
83. Zhou Z, Zhu G, Hariri AR, et al. Genetic variation in human NPY expression affects stress response and emotion. *Nature*. 2008;452(7190):997–1001.
84. Thorsell A, Michalkiewicz M, Dumont Y, et al. Behavioral insensitivity to restraint stress, absent fear suppression of behavior and impaired spatial learning in transgenic rats with hippocampal neuropeptide Y overexpression. *Proc Natl Acad Sci U S A*. 2000;97(23):12852–12857.
85. Sorensen AT, Kanter-Schlifke I, Lin EJ, During MJ, Kokaia M. Activity-dependent volume transmission by transgene NPY attenuates glutamate release and LTP in the subiculum. *Mol Cell Neurosci*. 2008;39(2):229–237.
86. Ishida H, Shirayama Y, Iwata M, et al. Infusion of neuropeptide Y into CA3 region of hippocampus produces antidepressant-like effect via Y1 receptor. *Hippocampus*. 2007;17(4):271–280.
87. Flood JF, Baker ML, Hernandez EN, Morley JE. Modulation of memory processing by neuropeptide Y varies with brain injection site. *Brain Res*. 1989;503(1):73–82.
88. Ledri M, Sorensen AT, Erdelyi F, Szabo G, Kokaia M. Tuning afferent synapses of hippocampal interneurons by neuropeptide Y. *Hippocampus*. 2011;21(2):198–211.
89. Helmstaedter C, Kurthen M, Lux S, Reuber M, Elger CE. Chronic epilepsy and cognition: a longitudinal study in temporal lobe epilepsy. *Ann Neurol*. 2003;54(4):425–432.
90. Elger CE, Helmstaedter C, Kurthen M. Chronic epilepsy and cognition. *Lancet Neurol*. 2004;3(11):663–672.
91. Gray SJ, Blake BL, Criswell HE, et al. Directed evolution of a novel adeno-associated virus (AAV) vector that crosses the seizure-compromised blood-brain barrier (BBB). *Mol Ther*. 2010;18(3):570–578.
92. Haberman RP, Samulski RJ, McCown TJ. Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. *Nat Med*. 2003;9(8):1076–1080.

Chapter 90

Adenosine Augmentation Therapy

Detlev Boison

The ribonucleoside adenosine is based on the purine base adenine, which was most likely already present on the prebiotic primitive Earth.[1] Being the core molecule of the energy metabolite adenosine-5′-triphosphate (ATP) as well as being an integral component of both DNA and RNA, adenosine likely played an important role in early evolution as an ideally positioned negative feedback regulator to adjust cellular activity (DNA, RNA) to available energy supplies (ATP). Adenosine has therefore evolved as an important modulator of function in brain, but also in heart, skeletal muscle, kidney, and adipose tissue, in the sense of a "retaliatory metabolite" that protects the cell against excessive external stimulation.[2]

Adenosine was first recognized as an endogenous modulator of neuronal excitability in 1980.[3] Today it is well recognized that adenosine exerts potent anticonvulsant[4] and neuroprotective[5] functions in brain that are largely mediated via the activation of pre- and postsynaptic G protein-coupled adenosine A_1 receptors (A_1Rs) providing presynaptic inhibition and stabilization of the postsynaptic membrane potential, respectively.[6,7] Furthermore, a rise in endogenous adenosine has been documented during ongoing seizure activity in patients with epilepsy,[8] and adenosine has consequently been identified as an endogenous mediator of seizure arrest and postictal refractoriness.[8,9] In addition, A_1Rs, which are the dominant receptor subtype in the limbic system, are crucial in preventing the spread of seizures,[10] and lack of the receptor is associated with spontaneous electrographic seizures[11] and with increased susceptibility to seizure-[11] or trauma-[12] induced mortality. Apart from the direct role of A_1Rs in regulating the susceptibility to seizures, stimulatory A_{2A}Rs, which in brain have their highest expression levels in striatum, appear to be involved in the regulation of several pathways thought to be implicated in epileptogenesis. Notably, A_{2A}Rs interact directly with various transmitter systems to modulate both inflammatory and cognitive processes.[13,14]

Clinical evidence, in particular the widespread use of the adenosine receptor antagonist theophylline as a bronchodilator, suggests that methylxanthines can cause seizures in patients without known underlying epilepsy.[15] Conversely, pharmacological activation of A_1Rs

provides effective seizure control in animal models,[16] albeit accompanied by significant, largely cardiovascular, side effects that are common after systemic use of adenosinergic drugs.[17] Importantly, pharmacological activation of A_1Rs effectively suppressed seizures in mice that were resistant to treatment with conventional antiepileptic drugs (AEDs).[18] Despite 30 years of research on adenosine and epilepsy, only the recent advent of novel molecular tools has led to the discovery of adenosine-related pathogenetic mechanisms in epilepsy and the translation of those findings into novel therapeutic approaches.

ADENOSINE DYSFUNCTION IN EPILEPSY

Although astrogliosis is a pathological hallmark of the epileptic brain, the important role of astrocytes in the control of neuronal excitability has only recently been appreciated.[19,20] Via the formation of tripartite synapses, astrocytes control synaptic transmission and neurovascular coupling.[21] Importantly, purinergic[22] as well as glutamatergic[23] signaling from astrocytes was shown to influence neuronal networks, and an astrocytic basis of epilepsy has been proposed.[23]

Astrocytes and Adenosine Kinase Are Key Regulators of Synaptic Adenosine

Synaptic levels of adenosine are largely controlled by an astrocyte-based adenosine cycle.[6,24] The major source of synaptic adenosine is its precursor, ATP, which can be released by astrocytes via regulated vesicular transport[22] or via hemichannels.[25] Once in the synaptic cleft, ATP is rapidly degraded into adenosine by a cascade of ectonucleotidases.[26] Proof that the astrocytic release of ATP plays a crucial role in regulating neuronal excitability via adenosine was provided using inducible transgenic mice that express a dominant-negative *N*-methylmaleimide-sensitive fusion protein receptor (SNARE) domain selectively in astrocytes to block the astrocytic release of ATP. Using these mice, it was shown that by releasing ATP, which accumulated as adenosine, astrocytes tonically suppressed synaptic transmission.[22] In contrast to classical neurotransmitters, which are removed from the extracellular space via specific energy-driven reuptake transporters, astrocyte membranes contain two types of equilibrative transporters for adenosine that rapidly equilibrate extra- and intracellular levels of adenosine.[27] Due to the lack of a classical transporter-regulated reuptake system for adenosine, the intracellular astrocyte-specific enzyme adenosine kinase (ADK) has likely adopted the role of a metabolic reuptake system for adenosine. Thus, by phosphorylation of adenosine into 5′-adenosine monophosphate (AMP), ADK drives the influx of adenosine into the astrocyte, thereby reducing the concentration of synaptic adenosine.[6,24,28] The notion that ADK is the key enzyme for the regulation of ambient adenosine in brain is further supported by the following evidence: (1) In adult brain, ADK expression is restricted to astrocytes.[29] (2) Inhibition of ADK in hippocampal slices increases endogenous adenosine and depresses neuronal firing, whereas inhibition of ADA has no effect.[30] (3) Pharmacological inhibition of ADK suppresses seizures in models of epilepsy.[31] (4) Genetic disruption of ADK induces an increase in adenosine.[32–35] (5) Overexpression of ADK triggers seizures by reducing ambient adenosine.[36] (6) A substrate cycle between AMP and adenosine, which involves ADK and 5′-nucleotidase, enables minor changes in ADK activity to translate rapidly into major changes in adenosine.[37]

ADK Is a Target for Seizure Prediction and Prevention

The crucial role of ADK in regulating the synaptic availability of adenosine and thus neuronal excitability has led to the proposal of the *adenosine kinase hypothesis of epileptogenesis*.[24] In an acute response to injury to the brain, a surge in micromolar levels of adenosine is triggered[38] that is further potentiated by acute downregulation of ADK during the first hours after experimental stroke[39] or status epilepticus.[40]

The acute injury-induced surge of adenosine increases the neuroprotective tone during the

first hours following an insult to the brain and seems to be a ubiquitous endogenous protective mechanism.[24] However, this acute surge in adenosine is also able to trigger several downstream pathways that can all contribute to the initiation of epileptogenesis. Several downstream mechanisms can be influenced by an acute surge of adenosine: Adenosine is an important modulator of inflammation.[41] Activation of A_1 as well as A_{2A} receptors triggers the proliferation of microglial cells.[42] In addition, adenosine A_{2A} receptor activation promotes inflammatory responses that are specific to the central nervous system (CNS).[43] Furthermore, increased levels of adenosine can induce downregulation of A_1Rs but upregulation of A_{2A}Rs on astrocytes.[44] Increased occupancy of astrocytic A_{2A}Rs was shown to increase astrocyte proliferation and activation.[45,46] Therefore, any injury-induced surge in adenosine can be a direct trigger for subsequent astrogliosis.

Based on the mechanisms discussed above, it is not surprising that astrogliosis is a common pathological consequence of different types of brain injury, such as status epilepticus, trauma, or stroke, but is also a common pathological hallmark of a variety of neurodegenerative and neuropsychiatric disorders, including Alzheimer's disease and autism (Fig. 90–1). As will be discussed in more detail below, astrogliosis is accompanied by overexpression of ADK, thereby inducing focal adenosine deficiency and spontaneous electrographic seizures. Adenosine deficiency can also explain a wide spectrum of comorbidities that are common in epilepsy or after brain injury (Fig. 90–1). Evidence that ADK, expressed by astrocytes, is a key molecular link between astrogliosis and neuronal dysfunction in epilepsy has been obtained in seizure models as well as through genetic and pharmacological approaches.

EVIDENCE FROM SEIZURE MODELS

Temporal lobe epilepsy is characterized by a complex pathophysiology including mossy fiber sprouting, granule cell dispersion, neuronal cell loss, ectopic neurons, and astrogliosis. Given this complex situation, it is almost impossible to attribute specific cause-effect relationships between those pathological changes and the development of seizures. To dissect out the specific contribution of astrogliosis to seizure generation, a mouse model was created that isolates astrogliosis from other components of the epileptogenic cascade.[47] In this model, a single unilateral injection of the excitotoxin kainic acid (KA) into the basolateral amygdala triggers status epilepticus that is terminated after 30 min with an intravenous infusion of lorazepam. This manipulation results in neuronal cell loss that is restricted to the ipsilateral CA3 area of the hippocampal formation.[47] The induced acute injury constitutes a trigger for subsequent epileptogenesis, and 3 weeks after KA injection the ipsilateral CA3 is characterized

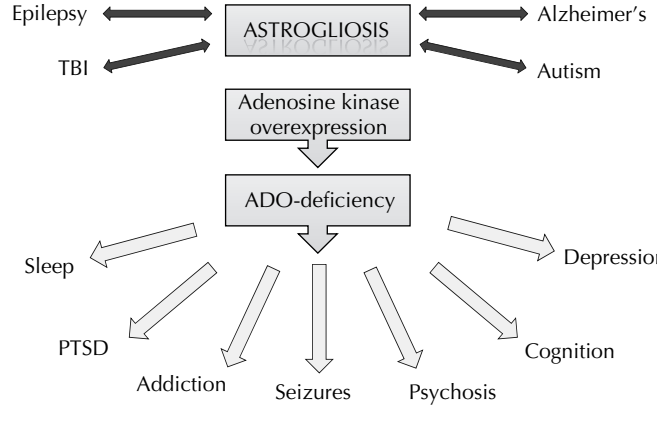

Figure 90–1. Astrogliosis and adenosine deficiency link diverse pathologies with co-morbidities. Astrogliosis—a common pathological hallmark of conditions as diverse as epilepsy, traumatic brain injury (TBI), Alzheimer's disease, and autism—is accompanied, at least in several models, with overexpression of ADK and resulting adenosine deficiency. Adenosine deficiency can likely explain several comorbidities between the conditions shown above, including sleep disorders, posttraumatic stress disorder (PTSD), addiction, seizures, psychosis, cognitive impairment, and depression.

by prominent astrogliosis and overexpression of ADK.[47] Remarkably frequent spontaneous electrographic seizures (approximately four seizures per hour, with a duration of 20 s per seizure) result that are restricted to the ipsilateral CA3.[47] These findings demonstrate spatial colocalization of astrogliosis, overexpressed ADK, and spontaneous seizures, and document that astrogliosis and/or overexpression of ADK per se, in the absence of any other component of the epileptogenic cascade, is sufficient to trigger seizures. Remarkably, during epileptogenesis in this model, astrogliosis, overexpression of ADK, and the first emergence of spontaneous seizures also coincide temporally at around day 12 following the KA injection, further highlighting the possible causal relationship between these events.[11]

EVIDENCE FROM GENETIC APPROACHES

The seizure model described above does not allow determination of whether astrogliosis or overexpression of ADK is required for seizure generation. To address this question, transgenic mice were engineered with global brain-wide overexpression of an *Adk*-transgene in addition to deletion of the endogenous *Adk* gene (Adk-tg mice). These animals are characterized by spontaneous hippocampal seizures in the absence of astrogliosis or any other histopathological alterations.[11] These data demonstrate that overexpression of ADK in the absence of any histopathological alteration usually associated with an epileptic brain is sufficient to trigger seizures. More recently, an injection of an adeno-associated virus (AAV) overexpressing a cDNA of *Adk* selectively in astrocytes was shown to trigger the same type of spontaneous seizures in otherwise healthy wild-type mice, whereas the injection of an AAV expressing an *Adk* antisense construct into the CA3 of Adk-tg mice was able to abolish almost completely spontaneous seizures in those animals.[48] Together these data define ADK as a rational target for therapeutic intervention.

EVIDENCE FROM PHARMACOLOGICAL APPROACHES

The identification of overexpressed ADK and the resulting adenosine deficiency as a major inducer of seizures implies that adenosine augmentation therapies (AATs) should be highly effective in seizure suppression. Indeed, adenosine A_1R agonists effectively inhibit neuronal activity, suppress seizures,[49] and have been the subject of intense drug development efforts.[50,51] Although A_1R agonists are effective in a variety of models, including one of pharmacoresistant epilepsy,[18] the systemic application of those drugs leads to profound cardiovascular and sedative side effects.[52] Sedative side effects of systemic adenosine augmentation can best be explained by the sleep-promoting effects of A_1R activation.[53] Since any type of injury or stress to the brain leads to an increase in endogenous adenosine,[8,38,54] agents (e.g., the ADK inhibitor ABT-702[55,56]) that amplify this site- and event-specific increase in adenosine could provide antiseizure activity comparable to that of adenosine receptor agonists.[31,57] Consequently, pharmacological inhibition of ADK is effective in inhibiting epileptic seizures,[31,40] with an improved therapeutic window compared to A_1R agonists.[58] However, systemic ADK inhibitors might not be a long-term therapeutic option for epilepsy due to their interference with methionine metabolism in liver[59,60] and the risk of brain hemorrhage.[61,62]

FOCAL ADENOSINE AUGMENTATION

Rationale for Focal Drug Delivery Approaches in Epilepsy

Side effects of systemic adenosine augmentation can most effectively be avoided by focal treatment approaches. In general, spatially restricted therapies are considered to be safe and feasible alternatives for systemic drug use, and given the focal nature of many epilepsies, focal interventions might be preferable.[63] The identification of neurochemical deficits that are specific for an epileptogenic focus provide a direct rationale for focal intervention (Fig. 90–2). Endogenous "AEDs" such as gamma-aminobutyric acid (GABA),[64–66] adenosine,[67–69] galanin,[70–72] or neuropeptide Y (NPY)[73,74] are therefore logical candidates for therapeutic intervention. Tools for focal drug delivery have been reviewed recently[75–78]

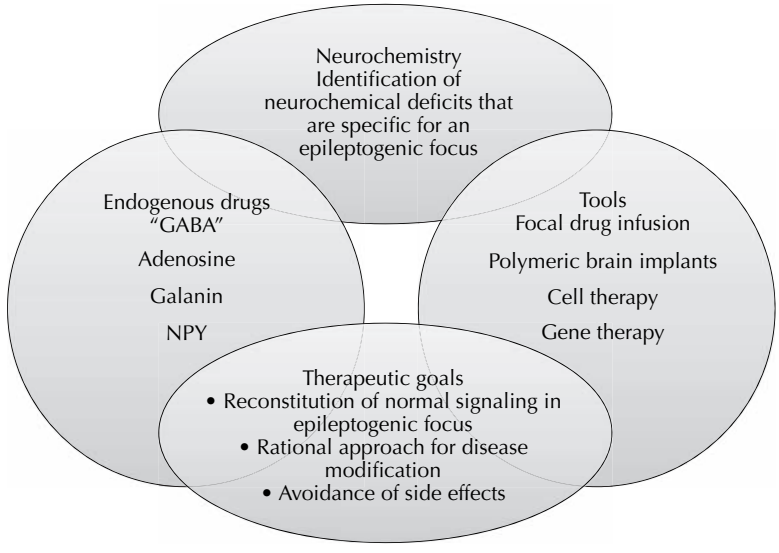

Figure 90–2. Rationale for local drug delivery in epilepsy.

and include polymeric brain implants,[79,80] cell therapy,[81–84] and gene therapy.[85–87] In contrast to conventional systemically used AEDs, the rational focal use of endogenous anticonvulsants is uniquely posed to reconstitute normal signaling within an epileptogenic focus and thereby not only to suppress seizures but also to affect disease progression in the absence of systemic side effects (Fig. 90–2). As outlined above, manipulation of ADK and focal adenosine augmentation are considered to be an effective and rational strategy for epilepsy therapy.

Molecular Approaches to Induce Adenosine Augmentation

The most effective strategy to increase levels of ambient adenosine is disruption of metabolic adenosine clearance. Pharmacologically, it has been demonstrated that inhibition of ADK is more effective in raising adenosine levels and in enhancing presynaptic inhibition than blockade of adenosine deaminase (ADA).[30] Likewise, engineered fibroblasts lacking ADK released 2.3 times more adenosine compared to fibroblasts lacking ADA.[35] Consequently, ADK is an effective target to induce cellular adenosine release. Two molecular strategies have been used to augment the adenosine system: (1) gene targeting to disrupt the endogenous *Adk* gene[34] and (2) RNA interference (RNAi) to knock down ADK expression.[88]

Using a gene-targeting construct to disrupt the endogenous *Adk* gene by homologous recombination in murine embryonic stem cells (ESCs) and subsequent biochemical selection for ADK deficiency, ESCs were isolated with a biallelic disruption of their endogenous *Adk* gene.[34] Upon directed differentiation in vitro into either neuronal or glial cell populations, the ADK-deficient cells released up to 40 ng adenosine per hour per 10^5 cells, an amount considered to be of therapeutic relevance. To induce therapeutic adenosine release in adult stem cells, an RNAi approach was used to induce a knockdown of ADK in human mesenchymal stem cells (hMSCs). This was achieved by constructing a lentivirus expressing an artificial micro-RNA directed against ADK. Transduction of hMSCs with the virus yielded cell populations in which ADK expression was reduced by up to 80%; this manipulation resulted in a release of about 1 ng adenosine per hour per 10^5 cells.[32] Control cells, transduced with a lentivirus expressing a scrambled control sequence, did not release detectable amounts of adenosine. These results demonstrate that although complete genetic disruption of the *Adk* gene is more effective in inducing adenosine release, RNAi-based strategies can effectively be used to engineer stem cells to release adenosine.

Seizure Suppression and Prevention by Focal Adenosine Augmentation

PROOF OF PRINCIPLE

The first proof of principle that focal adenosine augmentation might be effective for seizure control was demonstrated in the rat kindling model. Synthetic ethylene vinyl acetate copolymers were engineered to release approximately 20 to 50 ng adenosine per day.[89] Individual polymers were implanted into the lateral brain ventricles of rats that had been kindled in the hippocampus. Recipients of adenosine-releasing polymers were characterized by a strong reduction of stage 5 seizures for at least 7 days and by a reduction of epileptiform electrical afterdischarges for up to 3 days. In line with a transient delivery of adenosine, the antiepileptic effects gradually decreased and were no longer evident 2 weeks after polymer implantation. Control implants loaded with BSA failed to display any therapeutic effects. This was the first published demonstration that a focal release of adenosine in doses of 20 to 50 ng adenosine per day can suppress epileptic seizures.[89] Subsequently, robust but transient seizure suppression in kindled rats was demonstrated using intraventricular implants of encapsulated fibroblasts engineered to release adenosine.[35] The proof of principle that focal adenosine delivery can be of therapeutic benefit was further validated by an independent research group using intracranial adenosine injections in a rat seizure model.[69]

STEM CELL-BASED ADENOSINE DELIVERY

In the adult brain the subgranular zone of the hippocampal formation, as well as the subventricular zone, contain primitive cell sources and stem cells capable of repairing the injured brain.[90] Therefore, stem cell therapies are a logical choice for regeneration and repair of the epileptic hippocampal formation. While stem cell-derived brain implants may indeed repair the injured hippocampus in epilepsy and may be of therapeutic value by modifying circuitry through synaptic interactions,[91] stem cell-based brain implants may also exert direct anticonvulsant activity via the paracrine release of adenosine.[92] Based on the therapeutic efficacy of paracrine adenosine delivery,[35,92] stem cell-based adenosine delivery can be achieved effectively by injecting the cells into the infrahippocampal fissure of rodents, thereby avoiding interference with hippocampal circuitry.[93] Infrahippocampal implants of neural precursor cells derived from murine or human ESCs as well as hMSCs survive within the infrahippocampal fissure for at least several weeks.[32,47,93–95]

Infrahippocampal implants of adenosine-releasing stem cells provided potent seizure control in several experimental paradigms: (1) Embryonic stem cell-derived neural precursor cells injected into rats prior to the onset of kindling provided robust suppression of kindling epileptogenesis.[93] (2) The same cells injected after intra-amygdaloid KA-induced status epilepticus in mice prevented the development of spontaneous seizures 3 weeks after the injury, a time point when recipients of control cells had developed recurrent electrographic CA3 seizures at the rate of about four seizures per hour[47]; remarkably, the animals with the adenosine-releasing implants were characterized by significantly reduced astrogliosis and almost normal expression levels of ADK. Together, these data suggest a novel disease-modifying and possibly antiepileptogenic effect of focal adenosine augmentation.[47] (3) Adenosine-releasing hMSC implants were demonstrated to provide neuroprotective as well as antiepileptic effects in the mouse intra-amygdaloid KA model of CA3-restricted epileptogenesis[32,95]; however, due to 40 times lower amounts of released adenosine, these effects were less profound compared to those observed in ESC recipients.

SILK-BASED ADENOSINE DELIVERY

Although stem cell-based adenosine delivery yielded robust antiepileptic and possibly antiepileptogenic effects in two different models of epilepsy, those approaches still seem far from future clinical implementation. To provide a platform for rapid clinical implementation of focal adenosine augmentation, the natural biopolymer silk was recently evaluated for the therapeutic delivery of adenosine.[79,96] Purified silk fibroin presents a unique option for therapeutic adenosine delivery, as it is biocompatible and biodegrades slowly.[97] Degradation kinetics can be regulated to allow control of release from weeks to years.[97] Both silk as well

as adenosine are approved by the U.S. Food and Drug Administration (FDA), and the frequent use of silk sutures in brain confirms the feasibility of implanting silk biomaterials into brain.

In the first therapeutic silk-based adenosine delivery approach, a hierarchically structured silk-based implant was engineered with target release rates of 0, 40, 200, and 1000 ng adenosine per day.[79] The devices were implanted into the infrahippocampal fissure of rats prior to the onset of electrical kindling. It was demonstrated that focal adenosine release from silk-based polymers dose-dependently retarded kindling epileptogenesis. Importantly, recipients of polymers releasing a target dose of 1000 ng adenosine per day did not display any behavioral seizures during the time period of active adenosine release, whereas control rats experienced convulsions during the same period. As soon as adenosine release from the polymers began to wear off, seizures gradually reappeared with progressive intensity.[79]

Likewise, when implanted after completion of kindling into fully kindled rats (reproducible stage 4 to 5 seizures), implants engineered to release 1000 ng adenosine for a defined time period of 10 days effectively suppressed seizures for 10 days in fully kindled rats. After expiration of adenosine release from the polymers, stage 4 to 5 seizures recurred.[98] To assess the potential antiepileptogenic effect of focal adenosine delivery, unilateral (ipsilateral to the side of kindling) implantations with the same adenosine-releasing or control polymers were performed prior to the onset of kindling. All animals received a total of 30 kindling stimulations between days 4 and 8 after polymer implantation. This treatment resulted in stable stage 5 seizure expression in control animals, whereas seizure stages in adenosine-treated animals did not progress beyond stage 1. The lack of convulsive seizures in the adenosine group can be attributed either to the antiepileptogenic effects of focal adenosine delivery or merely to seizure suppression by adenosine (masking potential antiepileptogenic effects). To distinguish between the two possibilities, kindling was discontinued to allow expiration of adenosine release from the polymers. On day 18, kindling was resumed. Whereas control animals continued with generalized stage 5 seizures, animals from the adenosine group resumed kindling at stage 0 to 1, indicating that epileptogenesis was indeed suppressed during the time period of active adenosine delivery.[98] Pharmacological control experiments with the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) further corroborated the disease-modifying effects of focal adenosine delivery: Whereas a dose of 1 mg/kg DPCPX had no effects in nonkindled control animals, DPCPX restored stage 5 seizures in fully kindled rats that were protected from transient adenosine delivery.[35] Importantly, DPCPX did not trigger seizures in kindled animals that received adenosine-releasing implants prior to the onset of kindling, a result that further substantiated a possible antiepileptogenic effect of focal adenosine delivery.[98]

Together these studies document the therapeutic potential of silk-based adenosine delivery, capitalizing on a biopolymer that fulfills crucial requirements for future clinical application, such as biocompatibility, delivery of defined doses of adenosine, safety, and therapeutic efficacy in a widely used preclinical model. Although the polymer design used in this study[79] precluded long-term applications due to adenosine depletion over a period of 2 weeks, the system would nevertheless be suitable for initial short-term clinical safety and feasibility trials.

CHALLENGES AND IMPACT

Data from the acute and chronic models of epilepsy discussed above suggest that focal AATs may combine anticonvulsive, neuroprotective, and possibly antiepileptogenic properties. The conceptual rationale for focal AAT development differs from that of classical AED development. Since current AED development follows largely a *neuro*centric concept, it is unlikely that the development of new AEDs acting on similar neuronal targets will lead to any significant improvement in antiepileptic therapy. In contrast, augmentation of adenosine as an upstream modulator of several downstream pathways is uniquely suited to affect neuronal excitability on the network level and therefore constitutes a new pharmacological principle that has not yet been exploited in clinical epilepsy therapy. In addition, adenosine is an *endogenous* anticonvulsant and therefore is subject to physiological clearance. Rather

Therapy	Benefits	Limitations
Stem cell-based AAT	• Paracrine release of adenosine • Additional benefit of network repair? • Widespread delivery possible • Cell injection possible	• Long-term effectiveness/viability? • Control of network interactions? • Immunosuppresion if not autologous cell source
Polymer-based AAT	• Release of defined doses • Release by defined kinetics • Precise local restriction • Safety	• Invasive surgical procedure • Local effects/limitatin to focal epilepsies • Long-term effectiveness

Figure 90–3. Benefits and limitations of stem cell-based or silk polymer-based AATs.

than leading to toxic accumulations of adenosine, adenosine augmentation is likely to restore adenosinergic equilibrium, thereby avoiding undue side effects.

The stem cell-based and silk-based focal AAT approaches discussed here have distinctive benefits and limitations that are summarized in Fig. 90–3. Before focal AATs can move into the clinical realm, several issues need to be resolved. These include but are not limited to (1) determination of the median effective doses (ED_{50}s) and median toxic doses (TD_{50}s) and the respective therapeutic index; (2) differentiation of antiepileptic efficacy in mechanistically different animal models; (3) determination of appropriate time points for therapeutic intervention; (4) determination of appropriate therapeutic target populations (e.g., temporal lobe epilepsy vs. cortical dysplasia); and (5) demonstration of long-term efficacy.

Adenosine augmentation therapies rationally utilize the brain's endogenous adenosine-based seizure-control system, thereby presenting significant therapeutic potential for epilepsy. Adenosine is already FDA-approved for the treatment of supraventricular tachycardia and has been used in intrathecal infusions in Phase 1 clinical trials for the treatment of chronic pain.[99] Based on their relative safety profile, focal AATs, in particular those involving silk-based adenosine delivery, could rapidly be translated from animal studies to clinical trials. One possibility for first safety and feasibility studies could be the infusion of adenosine into an epileptic temporal lobe during its surgical removal. When coupled to synchronous electroencephalographic recordings, a proof of principle could be established that adenosine is effective in pharmacoresistant human epilepsy. Given the potential disease-modifying effects of focal AAT, the implantation of a bio-resorbable adenosine-releasing silk polymer into an epileptogenic brain region could eventually provide lasting benefit even after expiration of adenosine delivery. To make the best possible use of those disease-modifying effects, it is of benefit to use a carrier system that can degrade with time without having any long-lasting residual impacts.

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REFERENCES

1. Miller SL, Urey HC. Origin of life. *Science*. 1959;130:1622–1624.
2. Newby AC, Worku Y, Holmquist CA. Adenosine formation: evidence for a direct biochemical link with energy metabolism. *Adv Myocardiol*. 1985;6:273–284.
3. Dunwiddie TV. Endogenously released adenosine regulates excitability in the in vitro hippocampus. *Epilepsia*. 1980;21:541–548.
4. Dragunow M, Goddard GV, Laverty R. Is adenosine an endogenous anticonvulsant? *Epilepsia*. 1985;26(5):480–487.
5. Dragunow M, Faull RLM. Neuroprotective effects of adenosine. *Trends Pharmacol Sci*. 1988;9:193–194.
6. Boison D, Chen JF, Fredholm BB. Adenosine signalling and function in glial cells. *Cell Death Differ*. 2010;17:1071–1082.

7. Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. Adenosine and brain function. *Int Rev Neurobiol*. 2005;63:191–270.
8. During MJ, Spencer DD. Adenosine: a potential mediator of seizure arrest and postictal refractoriness. *Ann Neurol*. 1992;32:618–624.
9. Dragunow M. Adenosine and seizure termination. *Ann Neurol*. 1991;29:575.
10. Fedele DE, Li T, Lan JQ, Fredholm BB, Boison D. Adenosine A_1 receptors are crucial in keeping an epileptic focus localized. *Exp Neurol*. 2006;200: 184–190.
11. Li T, Lan JQ, Fredholm BB, Simon RP, Boison D. Adenosine dysfunction in astrogliosis: cause for seizure generation? *Neuron Glia Biol*. 2007;3:353–366.
12. Kochanek PM, Vagni VA, Janesko KL, Washington CB, Crumrine PK, Garman RH, Jenkins LW, Clark RS, Homanics GE, Dixon CE, Schnermann J, Jackson EK. Adenosine A1 receptor knockout mice develop lethal status epilepticus after experimental traumatic brain injury. *J Cereb Blood Flow Metab*. 2006;26: 565–575.
13. Sebastiao AM, Ribeiro JA. Tuning and fine-tuning of synapses with adenosine. *Curr Neuropharmacol*. 2009;7:180–194.
14. Magen I, Avraham Y, Ackerman Z, Vorobiev L, Mechoulam R, Berry EM. Cannabidiol ameliorates cognitive and motor impairments in mice with bile duct ligation. *J Hepatol*. 2009;51:528–534.
15. Boison D. Methylxanthines, seizures and excitotoxicity. *Handb Exp Pharmacol*. 2010;200:251–266.
16. Benarroch EE. Adenosine and its receptors: multiple modulatory functions and potential therapeutic targets for neurologic disease. *Neurology.* 2008;70:231–236.
17. Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. *Annu Rev Neurosci*. 2001;24:31–55.
18. Gouder N, Fritschy JM, Boison D. Seizure suppression by adenosine A_1 receptor activation in a mouse model of pharmacoresistant epilepsy. *Epilepsia.* 2003; 44:877–885.
19. Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, Haydon PG. Glutamate-mediated astrocyte-neuron signalling. *Nature*. 1994;369:744–747.
20. Nedergaard M. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science.* 1994;263:1768–1771.
21. Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev*. 2006;86:1009–1031.
22. Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY, Takano H, Moss SJ, McCarthy K, Haydon PG. Astrocytic purinergic signaling coordinates synaptic networks. *Science*. 2005;310:113–116.
23. Tian GF, Azmi H, Takano T, Xu QW, Peng WG, Lin J, Oberheim N, Lou NH, Wang XH, Zielke HR, Kang J, Nedergaard M. An astrocytic basis of epilepsy. *Nat Med*. 2005;11:973–981.
24. Boison D. The adenosine kinase hypothesis of epileptogenesis. *Prog Neurobiol*. 2008;84:249–262.
25. Kang J, Kang N, Lovatt D, Torres A, Zhao Z, Lin J, Nedergaard M. Connexin 43 hemichannels are permeable to ATP. *J Neurosci*. 2008;28:4702–4711.
26. Zimmermann H. Extracellular metabolism of ATP and other nucleotides. *Naunyn Schmiedebergs Arch Pharmacol.* 2000;362:299–309.
27. Baldwin SA, Beal PR, Yao SY, King AE, Cass CE, Young JD. The equilibrative nucleoside transporter family, SLC29. *Pflugers Arch*. 2004;447:735–743.
28. Boison D. Adenosine kinase, epilepsy and stroke: mechanisms and therapies. *Trends Pharmacol Sci*. 2006;27:652–658.
29. Studer FE, Fedele DE, Marowsky A, Schwerdel C, Wernli K, Vogt K, Fritschy J-M, Boison D. Shift of adenosine kinase expression from neurons to astrocytes during postnatal development suggests dual functionality of the enzyme. *Neuroscience*. 2006;142: 125–137.
30. Pak MA, Haas HL, Decking UKM, Schrader J. Inhibition of adenosine kinase increases endogenous adenosine and depresses neuronal activity in hippocampal slices. *Neuropharmacology.* 1994;33:1049–1053.
31. Kowaluk EA, Jarvis MF. Therapeutic potential of adenosine kinase inhibitors. *Expert Opin Investig Drugs*. 2000;9:551–564.
32. Ren G, Li T, Lan JQ, Wilz A, Simon RP, Boison D. Lentiviral RNAiinduced downregulation of adenosine kinase in human mesenchymal stem cell grafts: a novel perspective for seizure control. *Exp Neurol*. 2007;208: 26–37.
33. Güttinger M, Padrun V, Pralong W, Boison D. Seizure suppression and lack of adenosine a_1 receptor desensitization after focal long-term delivery of adenosine by encapsulated myoblasts. *Exp Neurol*. 2005;193:53–64.
34. Fedele DE, Koch P, Brüstle O, Scheurer L, Simpson EM, Mohler H, Boison D. Engineering embryonic stem cell derived glia for adenosine delivery. *Neurosci Lett*. 2004;370:160–165.
35. Huber A, Padrun V, Deglon N, Aebischer P, Mohler H, Boison D. Grafts of adenosine-releasing cells suppress seizures in kindling epilepsy. *Proc Natl Acad Sci USA.* 2001;98:7611–7616.
36. Fedele DE, Gouder N, Güttinger M, Gabernet L, Scheurer L, Rulicke T, Crestani F, Boison D. Astrogliosis in epilepsy leads to overexpression of adenosine kinase resulting in seizure aggravation. *Brain*. 2005;128:2383–2395.
37. Arch JR, Newsholme EA. Activities and some properties of 5′-nucleotidase, adenosine kinase and adenosine deaminase in tissues from vertebrates and invertebrates in relation to the control of the concentration and the physiological role of adenosine. *Biochem J*. 1978;174:965–977.
38. Clark RS, Carcillo JA, Kochanek PM, Obrist WD, Jackson EK, Mi Z, Wisneiwski SR, Bell MJ, Marion DW. Cerebrospinal fluid adenosine concentration and uncoupling of cerebral blood flow and oxidative metabolism after severe head injury in humans. *Neurosurgery*. 1997;41:1284–1292; discussion 1292–1293.
39. Pignataro G, Maysami S, Studer FE, Wilz A, Simon RP, Boison D. Downregulation of hippocampal adenosine kinase after focal ischemia as potential endogenous neuroprotective mechanism. *J Cereb Blood Flow Metab*. 2008;28:17–23.
40. Gouder N, Scheurer L, Fritschy J-M, Boison D. Overexpression of adenosine kinase in epileptic hippocampus contributes to epileptogenesis. *J Neurosci*. 2004;24:692–701.
41. Hasko G, Pacher P, Vizi ES, Illes P. Adenosine receptor signaling in the brain immune system. *Trends Pharmacol Sci*. 2005;26:511–516.

42. Gebicke-Haerter PJ, Christoffel F, Timmer J, Northoff H, Berger M, Van Calker D. Both adenosine A1- and A2-receptors are required to stimulate microglial proliferation. *Neurochem Int*. 1996;29:37–42.
43. Yu L, Huang Z, Mariani J, Wang Y, Moskowitz M, Chen JF. Selective inactivation or reconstitution of adenosine A2a receptors in bone marrow cells reveals their significant contribution to the development of ischemic brain injury. *Nat Med*. 2004;10:1081–1087.
44. Cunha RA. Neuroprotection by adenosine in the brain: from A1 receptor activation to A2a receptor blockade. *Purinergic Signaling*. 2005;1:111–134.
45. Hindley S, Herman MA, Rathbone MP. Stimulation of reactive astrogliosis in vivo by extracellular adenosine diphosphate or an adenosine A2 receptor agonist. *J Neurosci Res*. 1994;38:399–406.
46. Brambilla R, Cottini L, Fumagalli M, Ceruti S, Abbracchio MP. Blockade of A2a adenosine receptors prevents basic fibroblast growth factor-induced reactive astrogliosis in rat striatal primary astrocytes. *Glia*. 2003;43:190–194.
47. Li T, Ren G, Lusardi T, Wilz A, Lan JQ, Iwasato T, Itohara S, Simon RP, Boison D. Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. *J Clin Invest*. 2008;118:571–582.
48. Theofilas P, Brar S, Li T, Stewart K-A, Sandau U, Poulsen DJ, Boison D. Adenosine kinase as a target for therapeutic antisense strategies in epilepsy. *Epilepsia*. 2011;52(3):589–601.
49. Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. *Nat Rev Drug Discov*. 2006;5:247–264.
50. Spedding M, Williams M. Developments in purine and pyridimidine receptor-based therapeutics. *Drug Dev Res*. 1996;39:436–441.
51. Williams M, Jarvis MF. Purinergic and pyrimidinergic receptors as potential drug targets. *Biochem Pharmacol*. 2000;59:1173–1185.
52. Monopoli A, Conti A, Dionisotti S, Casati C, Camaioni E, Cristalli G, Ongini E. Pharmacology of the highly selective A1 adenosine receptor agonist 2-chloro-N6-cyclopentyladenosine. *Arzneimittelforschung*. 1994;44:1305–1312.
53. Bjorness TE, Kelly CL, Gao T, Poffenberger V, Greene RW. Control and function of the homeostatic sleep response by adenosine A1 receptors. *J Neurosci*. 2009;29:1267–1276.
54. Fredholm BB. Adenosine, an endogenous distress signal, modulates tissue damage and repair. *Cell Death Differ*. 2007;14:1315–1323.
55. Kowaluk EA, Mikusa J, Wismer CT, Zhu CZ, Schweitzer E, Lynch JJ, Lee CH, Jiang M, Bhagwat SS, Gomtsyan A, McKie J, Cox BF, Polakowski J, Reinhart G, Williams M, Jarvis MF. ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl) pyrido[2,3-d]pyrimidine), a novel orally effective adenosine kinase inhibitor with analgesic and anti-inflammatory properties. II In vivo characterization in the rat. *J Pharmacol Exp Ther*. 2000;295:1165–1174.
56. Jarvis MF, Yu H, Kohlhaas K, Alexander K, Lee CH, Jiang M, Bhagwat SS, Williams M, Kowaluk EA. ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholinopyridin-3-yl)pyrido[2,3-d]pyrimidine), a novel orally effective adenosine kinase inhibitor with analgesic and anti-inflammatory properties: I. In vitro characterization and acute antinociceptive effects in the mouse. *J Pharmacol Exp Ther*. 2000;295:1156–1164.
57. McGaraughty S, Cowart M, Jarvis MF, Berman RF. Anticonvulsant and antinociceptive actions of novel adenosine kinase inhibitors. *Curr Top Med Chem*. 2005;5:43–58.
58. Jarvis MF, Mikusa J, Chu KL, Wismer CT, Honore P, Kowaluk EA, McGaraughty S. Comparison of the ability of adenosine kinase inhibitors and adenosine receptor agonists to attenuate thermal hyperalgesia and reduce motor performance in rats. *Pharmacol Biochem Behav*. 2002;73:573–581.
59. Boison D, Scheurer L, Zumsteg V, Rülicke T, Litynski P, Fowler B, Brandner S, Mohler H. Neonatal hepatic steatosis by disruption of the adenosine kinase gene. *Proc Natl Acad Sci USA*. 2002;99:6985–6990.
60. Mato JM, Martinez-Chantar ML, Lu SC. Methionine metabolism and liver disease. *AnnRev Nutr*. 2008;28:273–293.
61. Erion MD, Wiesner JB, Rosengren S, Ugarkar BG, Boyer SH, Tsuchiya M. Therapeutic potential of adenosine kinase inhibitors as analgesic agents. *Drug Dev Res*. 2000;50:S14–S26.
62. McGaraughty S, Jarvis MF. Purinergic control of neuropathic pain. *Drug Dev Res*. 2006;67:376–388.
63. Nilsen KE, Cock HR. Focal treatment for refractory epilepsy: hope for the future? *Brain Res Brain Res Rev*. 2004;44:141–153.
64. Thompson KW. Genetically engineered cells with regulatable GABA production can affect afterdischarges and behavioral seizures after transplantation into the dentate gyrus. *Neuroscience*. 2005;133:1029–1037.
65. Nolte MW, Loscher W, Herden C, Freed WJ, Gernert M. Benefits and risks of intranigral transplantation of GABA-producing cells subsequent to the establishment of kindling-induced seizures. *Neurobiol Dis*. 2008;314:342–354.
66. Gernert M, Thompson KW, Loscher W, Tobin AJ. Genetically engineered GABA-producing cells demonstrate anticonvulsant effects and long-term transgene expression when transplanted into the central piriform cortex of rats. *Exp Neurol*. 2002;176:183–192.
67. Boison D, Stewart K-A. Therapeutic epilepsy research: from pharmacological rationale to focal adenosine augmentation. *Biochem Pharmacol*. 2009;78:1428–1437.
68. Boison D. Adenosine augmentation therapies (AATS) for epilepsy: prospect of cell and gene therapies. *Epilepsy Res*. 2009;85:131–141.
69. Anschel DJ, Ortega EL, Kraus AC, Fisher RS. Focally injected adenosine prevents seizures in the rat. *Exp Neurol*. 2004;190:544–547.
70. McCown TJ. Adeno-associated virus vector-mediated expression and constitutive secretion of galanin suppresses limbic seizure activity. *Neurotherapeutics*. 2009;6:307–311.
71. Kanter-Schlifke I, Toft Sorensen A, Ledri M, Kuteeva E, Hokfelt T, Kokaia M. Galanin gene transfer curtails generalized seizures in kindled rats without altering hippocampal synaptic plasticity. *Neuroscience*. 2007;150:984–992.
72. Mazarati AM. Galanin and galanin receptors in epilepsy. *Neuropeptides*. 2004;38:331–343.
73. Noe F, Frasca A, Balducci C, Carli M, Sperk G, Ferraguti F, Pitkanen A, Bland R, Fitzsimons H, During M, Vezzani A. Neuropeptide Y overexpression using recombinant adeno-associated viral vectors. *Neurotherapeutics*. 2009;6:300–306.

74. Sorensen AT, Kanter-Schlifke I, Carli M, Balducci C, Noe F, During MJ, Vezzani A, Kokaia M. NPY gene transfer in the hippocampus attenuates synaptic plasticity and learning. *Hippocampus*. 2008;18: 564–574.
75. Schmidt D, Holmes GL. Commentary: novel delivery approaches for antiepileptic drugs: Hope and hurdles. *Neurotherapeutics*. 2009;6:381–382.
76. Rogawski MA. Convection-enhanced delivery in the treatment of epilepsy. *Neurotherapeutics*. 2009;6: 344–351.
77. Barcia JA, Gallego JM. Intraventricular and intracerebral delivery of anti-epileptic drugs in the kindling model. *Neurotherapeutics*. 2009;6:337–343.
78. Bennewitz MF, Saltzman WM. Nanotechnology for delivery of drugs to the brain for epilepsy. *Neurotherapeutics.* 2009;6:323–336.
79. Wilz A, Pritchard EM, Li T, Lan JQ, Kaplan DL, Boison D. Silk polymer-based adenosine release: therapeutic potential for epilepsy. *Biomaterials*. 2008;29: 3609–3616.
80. Kokaia M, Aebischer P, Elmér E, Bengzon J, Kalén P, Kokaia Z, Lindvall O. Seizure suppression in kindling epilepsy by intracerebral implants of GABA- but not by noradrenaline-releasing polymer matrices. *Exp Brain Res*. 1994;100:385–394.
81. Loscher W, Gernert M, Heinemann U. Cell and gene therapies in epilepsy—promising avenues or blind alleys? *Trends Neurosci*. 2008;31:62–73.
82. Shetty AK, Hattiangady B. Concise review: prospects of stem cell therapy for temporal lobe epilepsy. *Stem Cells*. 2007;25:2396–2407.
83. Raedt R, Van Dycke A, Vonck K, Boon P. Cell therapy in models for temporal lobe epilepsy. *Seizure*. 2007;16: 565–578.
84. Boison D. Cell and gene therapies for refractory epilepsy. *Curr Neuropharmacol*. 2007;5:115–125.
85. Riban V, Fitzsimons HL, During MJ. Gene therapy in epilepsy. *Epilepsia*. 2009;50:24–32.
86. Vezzani A. The promise of gene therapy for the treatment of epilepsy. *Expert Rev Neurother*. 2007;7: 1685–1692.
87. McCown TJ. The clinical potential of antiepileptic gene therapy. *Expert Opin Biol Ther*. 2004;4:1771–1776.
88. Boison D. Inhibitory RNA in epilepsy: research tool and therapeutic perspectives. *Epilepsia*. 2010;51(9): 1659–1668.
89. Boison D, Scheurer L, Tseng JL, Aebischer P, Mohler H. Seizure suppression in kindled rats by intraventricular grafting of an adenosine releasing synthetic polymer. *Exp Neurol*. 1999;160:164–174.
90. Wu C, Chang A, Smith MC, Won R, Yin X, Staugaitis SM, Agamanolis D, Kidd GJ, Miller RH, Trapp BD. Beta4 tubulin identifies a primitive cell source for oligodendrocytes in the mammalian brain. *J Neurosci*. 2009;29:7649–7657.
91. Hattiangady B, Rao MS, Shetty AK. Grafting of striatal precursor cells into hippocampus shortly after status epilepticus restrains chronic temporal lobe epilepsy. *Exp Neurol*. 2008;212:468–481.
92. Güttinger M, Fedele DE, Koch P, Padrun V, Pralong W, Brüstle O, Boison D. Suppression of kindled seizures by paracrine adenosine release from stem cell derived brain implants. *Epilepsia*. 2005;46:1–8.
93. Li T, Steinbeck JA, Lusardi T, Koch P, Lan JQ, Wilz A, Segschneider M, Simon RP, Brustle O, Boison D. Suppression of kindling epileptogenesis by adenosine releasing stem cell-derived brain implants. *Brain*. 2007;130:1276–1288.
94. Boison D. Engineered adenosine-releasing cells for epilepsy therapy: human mesenchymal stem cells and human embryonic stem cells. *Neurotherapeutics.* 2009;6:278–283.
95. Li T, Ren G, Kaplan DL, Boison D. Human mesenchymal stem cell grafts engineered to release adenosine reduce chronic seizures in a mouse model of CA3-selective epileptogenesis. *Epilepsy Res.* 2009;84: 238–241.
96. Pritchard EM, Szybala C, Boison D, Kaplan DL. Silk fibroin encapsulated powder reservoirs for sustained release of adenosine. *J Control Release*. 2010;144: 159–167.
97. Horan RL, Antle K, Collette AL, Wang Y, Huang J, Moreau JE, Volloch V, Kaplan DL, Altman GH. In vitro degradation of silk fibroin. *Biomaterials*. 2005;26: 3385–3393.
98. Szybala C, Pritchard EM, Wilz A, Kaplan DL, Boison D. Antiepileptic effects of silk-polymer based adenosine release in kindled rats. *Exp Neurol*. 2009;219: 126–135.
99. Eisenach JC, Hood DD, Curry R. Phase I safety assessment of intrathecal injection of an American formulation of adenosine in humans. *Anesthesiology*. 2002;96:24–28.

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