
Introductory Chapter: Mechanisms and Function of Synaptic Plasticity

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1. Introduction

Many everyday experiences such as reading a book like this one, classroom learning, drug taking, or stressful situations can result in changes of our brain at different levels. These changes can manifest themselves in altering both the structure and function of neural circuits. Neural circuits are built by neurons, which form points of contacts with each other, the synapses [1]. A given neuron can form thousands of synapses on its dendrites, cell body and axon, and through synaptic transmission, communicates information with other neurons in the nervous system. It is at the synapses that changes in brain function occur through modification of synaptic transmission termed synaptic plasticity (reviewed in [2]). Below, a description of synaptic plasticity is provided in terms of its historical context, mechanisms of its different forms, and directions of research on synaptic plasticity.

2. A brief history of synaptic plasticity

The term plasticity has its origin in science more than 100 years ago and has been attributed to the famous Spanish scientist and founder of modern neuroscience Santiago Ramón y Cajal [3, 4]. His idea that the brain can store information by modifying synaptic connections was expressed in 1894 [5], even 3 years before Charles Sherrington introduced the term synapse for connections between neurons [6, 7]. Subsequently, Ramón y Cajal discovered that neurons are unique entities and synapses are the points of communication between them, the neuron doctrine [8]. It was also Ramón y Cajal who insisted that small spiny protrusions of dendrites, dendritic spines, were not an artifact but real and that they have a key role in mediating synaptic connectivity [9].

The idea and concept of synaptic plasticity gained prominence in the late 1940s with pioneering work by the Polish neurophysiologist Konorski [10] and the Canadian psychologist Hebb [11]. Konorski described plasticity as “permanent functional transformations,” and Hebb attributed testable physiologic characteristics to synaptic plasticity [6]. Synaptic plasticity

means that the connections between nerve cells in the brain are not static but can undergo changes, they are plastic. Mammalian brains are remarkably plastic, which implies an ability to modify existing neural circuits and to alter future behavior, emotions, and responses to sensory input [12]. Synaptic plasticity refers to activity-dependent changes in the efficacy of synaptic communication and has been proposed to be critically involved in the remarkable capacity of the brain to translate transient experiences into apparently unlimited numbers of memories that can last for many years.

Even though the notion of synaptic plasticity dates back to the end of the nineteenth century, it took almost 80 years before experimental evidence was obtained to demonstrate that synapses are capable of long-lasting changes in synaptic strength [13]. Timothy Bliss and Terry Lomo experimentally induced an increase in the synaptic strength of neurons in the mammalian hippocampus as a result of electrical stimulation. Such an increase in postsynaptic responses is now called long-term potentiation (LTP). Further experimentation by Serena Dudek and Mark Bear [14] revealed the ability of synapses to change in two directions, namely to increase (LTP) or decrease (long-term depression, LTD) in strength, i.e., synapses undergo activity-driven bidirectional modification. Both LTP and LTD have been found in various brain regions, most prominently the hippocampus [2, 15], cerebellum [16], cerebral cortex [17–19], and the amygdala [20–25] where sensory input has been linked to motor output in fear conditioning paradigms.

3. Synaptic and neural plasticity

Principally, synaptic plasticity refers to the strengthening or weakening of synaptic contacts as a result of increasing or decreasing activity levels of the neurons involved in a particular neural circuit. Synaptic plasticity implies direct regulation of pre- and/or postsynaptic neurons through alterations of the synaptic machinery. Examples include changes (a) of the number of neurotransmitter receptors in the postsynaptic membrane, (b) in the quantity of neurotransmitters released from the presynaptic neuron into a synapse, or (c) in receptor sensitivity to the released neurotransmitters [26–29]. Synaptic plasticity has been found at synapses that convey glutamate-mediated excitation and at other synapses that mediate GABAergic inhibition [2, 30]. Synaptic plasticity takes place at different time scales, from tens of milliseconds to life-long changes in synaptic transmission. Therefore, synaptic plasticity can be classified as either short-term or long-term. Short-term synaptic plasticity occurs at time periods from sub-second to minutes whereas long-term synaptic plasticity changes the efficacy of synapses for hours to years and is thought to form lasting memories that are stored in brain circuits.

The terms neuroplasticity, neural plasticity, or brain plasticity are used in a broader context to indicate changes that occur throughout a person's life either at the synapse or whole neurons or even entire brain regions. The basic premise is the same, namely that certain aspects of the brain or brain function can be changed throughout life [31]. This was not always understood to be the case. Previous studies of the brain suggested the existence of a critical period early in life during which the brain is amenable to changes of structure and function (plastic) and would remain unchangeable thereafter (static) (reviewed in [30, 32]). Likewise, synapses were considered as simple relay stations for information transfer from one neuron to another or

from a neuron to a muscle cell. These relay stations were thought to be established during development and to remain in place throughout life with a relatively fixed synaptic strength of the connection. Neuroscience textbooks nowadays appreciate the extreme plasticity of most synapses such that they are able to change their strength as a result of either their own activity or through activity in another pathway [30].

4. Plasticity, memory, and learning

Plasticity is now known to be an intrinsic property of the brain such that it is not limited by its own genome but can adapt to external stressors, physiological alterations, and a person's experiences. Plasticity manifests itself as dynamic shifts in the strength of preexisting connections across distributed neural networks and as modifications of the mapping between behavior and neural activity that take place in response to changes in afferent input or efferent demand [32]. Not only can existing connections undergo rapid changes, the establishment of new connections through dendritic growth and arborization can follow [33–36]. Synaptic and/or neural plasticity is the mechanism for development and learning, but it is also the basis of much brain pathology as seen in various neurological disorders, and maladaptive synaptic plasticity may contribute to neuropsychiatric disorders [2].

While synaptic plasticity is a key concept in itself for brain function and dysfunction, it has become central to our understanding of the mechanisms of learning and memory. Synaptic plasticity is intimately related to learning and memory because memories are thought to be represented by neural networks that are connected at synapses. One critical concept in this regard is the Hebbian theory [11], which proposes an explanation for neuronal adaptation during the learning process and is considered a basic mechanism for synaptic plasticity. Hebb postulated that coincident activity of synaptically connected neurons leads to lasting changes in the efficacy of synaptic transmission. Experimental evidence supports this hypothesis by demonstrating that modifiable synapses exist in brain and form the basis for learning and memory. Under conditions when a presynaptic neuron repeatedly and persistently stimulates a postsynaptic neuron, i.e., when both neurons are active, synaptic connections are modifiable in their efficacy. Hebb's theory has been summarized in a more colloquial way by Siegrid Löwel's phrase: "Cells that fire together, wire together [37]." One important aspect of Hebb's theory relates to the exact timing of activity of the presynaptic neuron in relation to postsynaptic activity. The presynaptic cell needs to generate action potentials just before the postsynaptic cell and not at the same time, a concept known as spike-timing-dependent plasticity [38].

It is now generally accepted that memories are stored as alterations in the strength of synaptic connections between neurons [30]. Alterations in synaptic efficacy have been traced for hours to months, and therefore, LTP is both the most widely studied and the most popular candidate cellular mechanism for storing information in neural circuits over long-time periods. Irrespective of the usefulness of LTP and LTD as examples of long-lasting synaptic plasticity, some authors have cautioned that it is not clear how LTP and LTD relate to memory, i.e., the causal link between LTP and memory has not been demonstrated convincingly (reviewed

in Ref. [30]), especially for hippocampal LTP. Other forms of memory and plasticity have allowed linking cellular events and circuitry to behavior, e.g., classical conditioning in the invertebrate model *Aplysia*, eye-blink conditioning, and amygdala-dependent fear conditioning [30, 39, 40]. Particularly, cerebellar LTD and amygdalar LTP are considered to directly underlie memory-associated behavioral changes [41, 42].

5. Endocannabinoids as mediators of synaptic plasticity

Over the past two decades, a new set of signaling molecules has been implicated in synaptic plasticity, namely, endogenously generated cannabinoids, the endocannabinoids (eCBs) [2, 43–54]. Two endocannabinoids, N-arachidonoyl ethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) have been found to be the natural agonists of cannabinoid receptors in the brain, CB1R [46]. These signaling molecules are unusual neurotransmitters because they are not stored in synaptic vesicles in synaptic terminals. Instead, endocannabinoids are made on-demand from membrane lipids of activated neurons and are released nonsynaptically. Nevertheless, they have been shown to be involved in synaptic plasticity in many neural systems in both short-term and long-term plasticity, learning and memory such as extinction of aversive memories [52–56]. Endocannabinoids are known to play a role in synapse formation, neurogenesis, and a number of bodily functions such feeding [57, 58], anxiety, pain reception, and recovery after brain injury [59–62]. Endocannabinoids serve as intercellular messengers in the brain [46]. They act in a retrograde fashion at synapses and presynaptically regulate both glutamatergic and GABAergic synapses to alter release-probability in synaptic plasticity. Endocannabinoids mediate short-term synaptic plasticity through a form of neuronal communication known as DSI, Depolarization-induced Suppression of Inhibition (reviewed in [46, 53, 54]). During DSI, when a principal neuron is activated through experimental current injection or activation of metabotropic glutamate or acetylcholine receptors, the inhibitory input onto that principal neuron is transiently reduced or abolished. When a postsynaptic principal neuron experiences a brief increase in intracellular calcium concentration, it synthesizes and releases endocannabinoids that travel to the presynaptic neuron and bind to cannabinoid receptors triggering an intracellular messenger cascade. The result is a transient decline of incoming inhibitory signals in the form of GABA arriving from presynaptic neurons. During DSI, endocannabinoids travel from the postsynaptic cell to the presynaptic GABA-releasing one and through activation of CB1R turn off neurotransmitter release. Endocannabinoids, thereby, act as retrograde-signaling molecules. DSI works as a transient local effect because endocannabinoids are lipids that cannot diffuse widely in the extracellular watery space of neurons. DSI allows neurons to disconnect briefly from other neurons or alter the strength of synapses made onto them through relieve of their inhibition [46]. DSI is a regulatory process allowing neurons to control their own synaptic excitability in an activity-dependent manner. A corresponding form of short-term synaptic plasticity has been described in the cerebellum, DSE, Depolarization-induced Suppression of Excitation, which reduces synaptic excitation by suppressing presynaptic glutamate release [44].

In addition to serving a role in mediating short-term synaptic plasticity, endocannabinoids have been shown to be critical in several forms of long-term synaptic plasticity. In the hippocampus, endocannabinoids evoke long-term depression at inhibitory, but not excitatory, synapses [63]. Endocannabinoid-mediated LTD (eCB-LTD) was described in the cerebellum [64], in the glutamatergic synapses onto medium spiny neurons in the striatum [65, 66] and at synapses between layer V pyramidal neurons in the neocortex [67]. Here, eCB-LTD does not depend on postsynaptic activation of metabotropic glutamate receptors but requires coincident activation of presynaptic ionotropic glutamate (NMDA) receptors. eCB-LTD in both the dorsal and the ventral striatum with the nucleus accumbens requires postsynaptic activation of group I metabotropic glutamate receptors [2, 68–70]. Differences exist regarding a requirement for concomitant presynaptic activity [71], the known involvement of anandamide as the endocannabinoid [72] and the presence of postsynaptic D2 dopamine receptors [73, 74] in the dorsal striatum.

6. Developments and directions of synaptic plasticity research

Synaptic plasticity has become an overriding theme of brain research in order to understand the nervous system in its function and dysfunction. Over the past several decades, researchers have attempted and succeeded in deciphering molecular and cellular synaptic changes that are the basis for behavior and disease [75–77]. However, even though our understanding of synaptic plasticity has grown tremendously, pivotal questions regarding plasticity and its function remain to this day, e.g., how do the different forms of synaptic plasticity compliment or interfere with each other [55, 78].

Technical advances in neuroscience research are also a major catalyst for progress in synaptic plasticity research. Most recently, among these advances are genetic, optical, and optogenetic methods that allow researchers to manipulate single cells or neural circuits with subcellular precision, at microsecond timescales or through longitudinal electrophysiological and optical recordings [79–89]. Novel experimental and conceptual approaches will pave the way to a more complete understanding of the functional consequences of synaptic plasticity and its implication for health and disease.

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Conflict of interest

The author declares that there is no conflict of interests regarding the publication of this chapter.

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Post-Transcriptional Mechanisms of Neuronal Translational Control in Synaptic Plasticity

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Additional information is available at the end of the chapter

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Abstract

The dynamic complexity of synaptic function is matched by extensive multidimensional regulation of neuronal mRNA translation which is achieved by a number of post-transcriptional mechanisms. The first key aspect of this regulatory capacity is mRNA distal trafficking through RNA-binding proteins, which governs the transcriptomic composition of post-synaptic compartments. Small non-coding microRNA and associated machinery have the capacity to precisely coordinate neural gene networks in space and time by providing a flexible specificity dimension to translational regulation. This RNA-guided subcellular fine-tuning of protein synthesis is an exquisite mechanism used in neurons to exert control of synaptic properties. Emerging evidence also implicates brain-enriched long non-coding RNA and novel circular RNA in posttranscriptional regulation of gene expression through the modulation of both mRNA and miRNA functions, thereby exemplifying the complex nature of neuronal translation. Herein, we review current knowledge of these regulatory systems and analyse how these mechanisms of transcriptomic regulation may be linked together to achieve high-order spatiotemporal control of post-synaptic translation.

Keywords: translation, mRNA trafficking, messenger ribonucleoprotein, microRNA, P-bodies, circular RNA

1. Introduction

The early phases of long-term potentiation (LTP) and long-term depression (LTD) are characterised by post-translational modification and trafficking of AMPA glutamate receptors, modifying reactivity of the post-synaptic membrane following an appropriate inducing stimulus [1]. While this is an effective means of altering synaptic transmission in the short term, consolidation of these changes into late-phase synaptic plasticity requires a substantially

deeper response, driven by novel transcription and translation of messenger RNA (mRNA) [2]. Translation is a particularly interesting aspect of this system as discrete foci of polyribosomes have been observed in dendritic spines engaging in protein synthesis, indicating a role for localised distal translation in dendritic function which has since proven critical in synaptic plasticity, and indeed, learning and memory [2–5].

Considering the importance of dendritic translation in strengthening alterations to the post-synaptic compartment, the role of mRNA in connecting neuronal transcription and distal translation is crucial and understandably subject to significant modulation. To this extent, neurons exploit a variety of post-transcriptional regulatory mechanisms to ensure an array of over 2500 unique, dendritically localised mRNAs are only translated when appropriate [5]. At present, it is clear that a variety of mRNA-binding proteins (RBPs) act to selectively distribute mRNAs to individual dendritic spines and may additionally act as enhancers or inhibitors of translation. Non-coding RNA, specifically microRNA (miRNA), provides a potent and specific dimension to translational control, which themselves may be regulated by other novel, brain-enriched, non-coding RNA.

Despite extensive investigation of neuronal mechanisms of translation control, we still lack a clear understanding of how these systems work both individually and synergistically to regulate the localisation and appropriate timing of translation. This chapter therefore explores recent advances in the field of neuronal post-transcriptional translational regulation, with particular focus on miRNA and how these small non-coding RNAs interact with a variety of intermediates to exert precise control over neuronal protein synthesis.

2. Intracellular trafficking supplies dendrites with mRNA

2.1. Cis-acting factors

Neuronal subcellular compartments such as dendritic spines rely on distal trafficking of mRNA from the soma to provide a stockpile of transcripts for protein synthesis. Considering the functional similarity and close proximity of these compartments, precise localisation of mRNA is critical for modulation of the dendritic transcriptome, which influences translation in response to stimuli such as synaptic transmission. This raises a key question; how can thousands of unique mRNAs successfully localise in functionally similar subcellular neuronal compartments?

Part of the answer lies within the mRNA itself as its 3' UTR is encoded with *cis*-acting sequences known as localisation elements (LE), which signal the desired site of transcript localisation. LEs were initially observed in chicken embryonic myoblasts whereby isoform-specific 3' UTR sequences were shown to direct the accumulation of α -actin and β -actin in peripheral and perinuclear regions, respectively [6]. Dendrite-specific LEs have since been identified in key mediators of synaptic plasticity, including AMPA receptors, MAP2, α CaMKII and BDNF [7–10], indicating LEs play a critical role in the post-synaptic localisation of specific mRNAs. The AMPA receptor is a particularly interesting example as “flip” and “flop” splice variants have been shown to respectively localise in dendrites and the soma [7], revealing alternative

splicing may serve to edit LE expression and subcellular localisation through removal of undesirable LEs in specific transcript splice variants. Furthermore, MAP2 long and short 3' UTR variants generated by alternative polyadenylation have been shown to, respectively, target the dendrites and soma [9], implicating polyadenylation as another possible mechanism through which LE expression may be altered.

Following the discovery of LEs, a subset of studies has focussed on identifying a mechanistic basis through which these sequences facilitate selective mRNA trafficking. Considering the role of proteins in physically transporting mRNA (covered next), a distinct possibility is “digital” LEs form secondary structures to facilitate “analogue” recognition by RNA-binding proteins (RBP) [11]. This proposed mechanism of LE function is strongly supported by the identification of 3' UTR stem-loop LEs required for the transport of K10, ASH1 and Oskar mRNAs in non-neuronal models [12–14]. In the context of synaptic function, a 5' UTR stem-loop motif has recently been shown to direct sensorin mRNA to the synapse in *Aplysia*, both demonstrating the role of stem-loops in moderating synaptic mRNA localisation and revealing that LEs may not be purely confined to the 3' UTR [15].

2.2. Trans-acting factors

While encoding mRNAs with LEs constitutes an elegant mechanism by which transcripts may be directed to subcellular compartments, neurons require an efficient physical mechanism of mRNA distribution to ensure all dendrites and their spines are adequately supplied with transcripts. Such a system is provided by a specialised group of RBPs, the neuronal microtubule network and associated motor proteins, all of which act in concert to facilitate mRNA trafficking from the soma to far distal regions of the neuron.

Current evidence heavily implicates neuronal messenger ribonucleoprotein (mRNP) granules as the primary vehicles of neuronal mRNA trafficking as observed through their translocation in neurites and mRNA/protein enrichment [16, 17]. These granules are composed of aggregated mRNA-RBP complexes, which are hypothesised to form upon *cis-trans* interaction between RBPs and target mRNA LEs (**Figure 1**), conferring specificity to this system while additionally providing a means of translationally repressing mRNAs during transport [18, 19]. At present, the neuronally enriched fragile X mental retardation protein (FMRP) and Stauf family proteins are the chief candidate RBPs involved in neuronal mRNA trafficking as evidenced by their enrichment within mRNP granules while complexed with a variety of plasticity-relevant mRNAs including CaMKII α , MAP1b, β -actin and poly(A)-binding protein (PABP) [20–25]. An important aspect of these studies is that neuronal RBPs clearly target a variety of unique mRNAs, therefore suggesting RBPs recognise a number of different LEs, or alternatively, LEs for the same subcellular compartment share similar structural properties. Regardless, the key advantage of this system is the need for relatively few unique RBPs relative to the number of mRNAs requiring transport. In contrast to this observation, zip-code-binding protein 1 (ZBP1) specifically engages in activity-dependent trafficking of β -actin mRNA to the axon and dendrites through recognition of a 54nt LE within the 3' UTR [26, 27]. While other binding partners may exist, it is possible that ZBP1 acts as a highly specific mediator of β -actin expression due to the high demand for this protein in neuronal cytoskeletal remodelling [28].

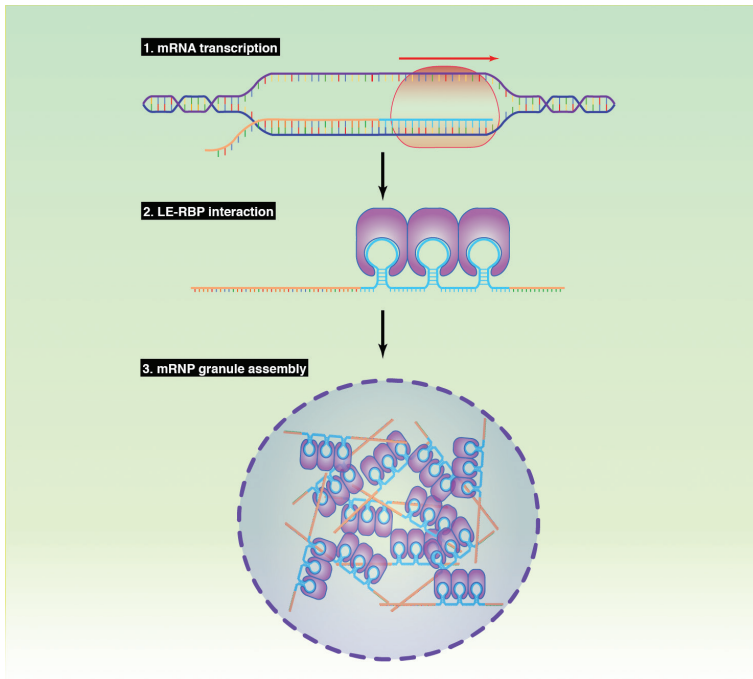


Figure 1. Assembly of neuronal messenger ribonucleoprotein granules. (1) During transcription, mRNAs are inherently encoded with cis-acting localisation elements in the 3' UTR. (2) These sequences form secondary structures such as stem-loops, facilitating recognition by RNA-binding proteins. (3) Aggregation of multiple mRNA-RBP complexes results in the formation of neuronal mRNP granules, which transport mRNAs to distal locations in a translationally repressed state.

The overall significance of RBPs in neuronal mRNA trafficking is further exemplified by their hypothesised role as adaptors, linking transcripts to motor proteins for distal transport via microtubules. Supporting this view, mRNP granule-associated FMRP has been shown to directly interact with KIF3c and KIF5 kinesin motor protein subunits [22, 29], while similarly, ZPB1 directly binds the tail domain of KIF11 while complexed with β -actin mRNA [30]. The role of microtubules in this process has been further elucidated by the observed microtubule-dependent translocation of Staufen granules to the dendrites in response to synaptic activity as seen through GFP-fusion experiments [31]. It is interesting to note that Dynein is another candidate motor protein involved in mRNA microtubular trafficking; however, its role is currently only described in *Drosophila* embryonic extracts [32].

2.3. “Synaptic Tag and Capture” and “Sushi Belt” theories

Evidence pertaining the activity-dependent migration of neuronal mRNP granules to the dendrites implies that these granules bear functional significance in the regulation of activity-dependent translation [31, 33]. However, despite our extensive knowledge of neuronal mRNA trafficking, it is still unclear how specific neuronal mRNP granules are successfully delivered

to individual dendritic spines following stimuli such as synaptic transmission. A commonly accepted model regarding this question was initially proposed in 1997 by Frey and Morris, who presented the “Synaptic Tag and Capture” theory in the context of LTP [34, 35] which has since received significant attention, and indeed, refinement [36–38]. At the core of this theory, strong synaptic stimuli capable of inducing late-phase LTP “tags” specific dendritic spines, signalling for the uptake of plasticity-related proteins and mRNAs from dendritic shafts and the soma [34]. While evidence obtained since the original proposal of this model has supported synaptic tag and capture, the identity of the tag, whether a single molecule or state of the synapse, has yet to be elucidated [38].

More recently, Doyle and Kiebler [18] have proposed an alternate “Sushi Belt” theory of dendritic mRNA trafficking with stronger emphasis on neuronal mRNP granules. This theory suggests that mRNPs constitutively circulate dendritic shafts through microtubules and motor proteins, analogous to a sushi conveyor belt. Application of a potentiating stimulus to any particular spine “tags” it, resulting in recruitment of mRNP granules to that spine through the action of dynamic microtubules when novel translation is required. Importantly, this theory accounts for both basal and activity-dependent mRNP transport in addition to anterograde and retrograde mRNP translocation, which has previously been observed [16]. Considering current knowledge of mRNA dendritic transport, however, it remains a distinct possibility that facets from both of these models may likely underlie the true mechanism behind both basal and activity-dependent neuronal mRNA trafficking.

3. microRNAs specifically and potently regulate post-synaptic translation

MicroRNAs (miRNAs) are a class of short, ~22nt non-coding RNAs that function as target-specific guides in the degradation and translational repression of mRNA. Since their initial discovery in 1993 [39, 40], the role of miRNA in providing combinatorial specificity to translational regulation has been highlighted through the discovery of more than 3700 unique miRNA participating in over 366,000 experimentally supported miRNA-target interactions [41]. Profiling studies have further identified the CNS as a source of significant miRNA enrichment whereby a subset of miRNA and associated factors regulate synaptic plasticity and are subject to activity-dependent turnover, indicating a key role in the maintenance of synaptic function [42]. Consequently, dysregulation of miRNA function is thought to contribute to the pathogenesis of complex neurological diseases, in particular, neuropsychiatric conditions characterised by impairments in learning and memory [43].

3.1. miRNA biogenesis and target interaction

miRNAs are derived from the transcription and processing of miRNA genes (**Figure 2**) located in a diverse array of genomic locations, including coding, non-coding and intergenic regions [44, 45]. Transcription of miRNA genes by RNA polymerase II yields primary miRNA (pri-miR), which house one (monocistronic) or multiple (polycistronic) miRNA species and are post-transcriptionally supplemented with 5' methylguanosine cap and 3' poly(A)

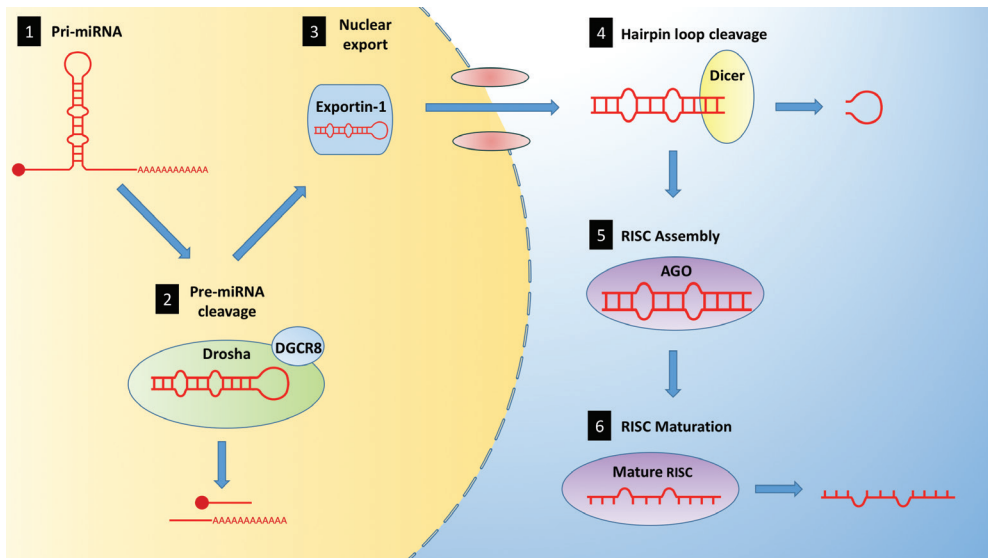


Figure 2. miRNA biogenesis. (1) Transcription of miRNA genes yields pri-miRNA, which adopt a hairpin secondary structure and contain 5' cap and 3' poly(A) tail similarly to mRNA. (2) These structures are cleaved from the pri-miRNA by Drosha and cofactor DGCR8, yielding a ~70nt pre-miRNA which is (3) transported to the cytosol through exportin-5. (4) Further processing by dicer cleaves the pre-miRNA hairpin loop, leaving a miRNA duplex which is (5) loaded into one of four human argonaute proteins. (6) The RISC is considered mature following selection of a guide strand and subsequent ejection of the passenger strand.

tail structures [46–48]. Complementary base pairing between the miRNA sequence and its antisense counterpart forms a secondary hairpin structure which is liberated from the primary transcript via cleavage, mediated by the nuclear microprocessor Drosha and co-factor DGCR8 [49]. The resultant ~70nt miRNA precursor (pre-miR) is exported to the cytoplasm through the action of Exportin-5 in a Ran-GTP-dependent manner [50, 51] wherein the cytoplasmic RNase III Dicer cleaves the hairpin loop structure from this pre-miR [52, 53]. This produces a ~22nt double-stranded miRNA duplex which associates with one of four orthologues of the Argonaute (AGO) protein family, thereby forming an immature RNA-induced silencing complex (RISC) [54]. Maturation of this complex is achieved through positive selection of a 5' uridine containing “guide” [55] strand from the miRNA duplex and subsequent ejection of the antisense “passenger” strand from the RISC [56–58]. Incomplete Watson-Crick base complementarity between the miRNA seed region (nucleotides 2–8) and miRNA recognition elements encoded in the mRNA 3' UTR (and occasionally, 5' UTR and CDS) subsequently acts as a targeting mechanism for the RISC, facilitating translational repression and degradation of specific mRNAs [59–62]. This redundancy in miRNA-mRNA interaction is a particularly critical characteristic of miRNA function as it enables a single miRNA to regulate a many unique transcripts, eliminating the need for excessively large numbers of very specific miRNA.

3.2. miRNA regulation of gene expression

3.2.1. 5' to 3' exonucleic decay

The initial stages of cap-dependent mRNA translation are primed by interaction between 5' bound eukaryotic initiation factors (eIF) and 3' poly(A)-binding proteins (PABP), which both circularise mRNA for efficient translation and promote assembly of a translation initiation complex in the 5' UTR [63]. The mRNA 5' methylguanosine cap and 3' poly(A) tail are therefore considered as two critical *cis*-acting factors in translation initiation and are thus ideal targets for translational repression and degradation by miRNA and the RISC [64].

An extensive body of research has since revealed that the RISC induces activation of the 5' to 3' mRNA decay pathway to facilitate degradation of target mRNAs. The first step of this pathway is characterised by recruitment of the PAN2-PAN3 and CCR4-NOT deadenylation complexes to target mRNA, which trigger sequential cleavage of the 3' poly(A) tail [65, 66]. Co-immunoprecipitation and structural analysis suggest that the RISC indirectly recruits these deadenylation factors through the action of GW182 proteins, which bind both AGO family members in addition to the PAN3 and NOT1/9 deadenylase subunits [67–70]. Erosion of the poly(A) tail subsequently stimulates the removal of the 5' methylguanosine cap via DCP2 and associated decapping factors, recruited to the target mRNA via CCR4-NOT [71]. The exposed mRNA is then subject to 5' to 3' digestion by XRN1 exonuclease [71].

3.2.2. Inhibition of active translation

RISC-mediated degradation of mRNA has long been recognised as the canonical mechanism through which miRNAs specifically modulate the transcriptome and therefore translation. Emerging evidence suggests the RISC may also function to repress translation through a number of mechanisms (**Figure 3**), foremost of which is inhibition of active translation. This form of translational repression arose as a potential facet of RISC function following the initial discovery of miRNA in association with polysomes [72]; however, no evidence was uncovered regarding a link between miRNA binding and arrest of active translation. A similar discovery has also been observed for the dendrite-abundant translational repressor FMRP, which has been shown to form translationally active complexes with ribosomes in mouse cortical preparations [73]. Further studies have identified CDS-bound phosphorylated FMRP is associated with stalled ribosomes, leading to the current hypothesis that FMRP halts active protein synthesis through binding actively translating ribosomes [74–76]. This holds significance for neuronal miRNA function since FMRP has been shown to interact with AGO1 [77], AGO2 [78], MOV10 (RISC RNA helicase) [79] and specific microRNAs [80], raising the possibility that miRNA and the RISC may act as a targeting mechanism for FMRP. Considering these interactions together in conjunction with the dendritic enrichment of FMRP, miRNA- and FMRP-mediated stalling of actively translating ribosomes could constitute an exciting dimension to miRNA function particularly relevant to synaptic translation.

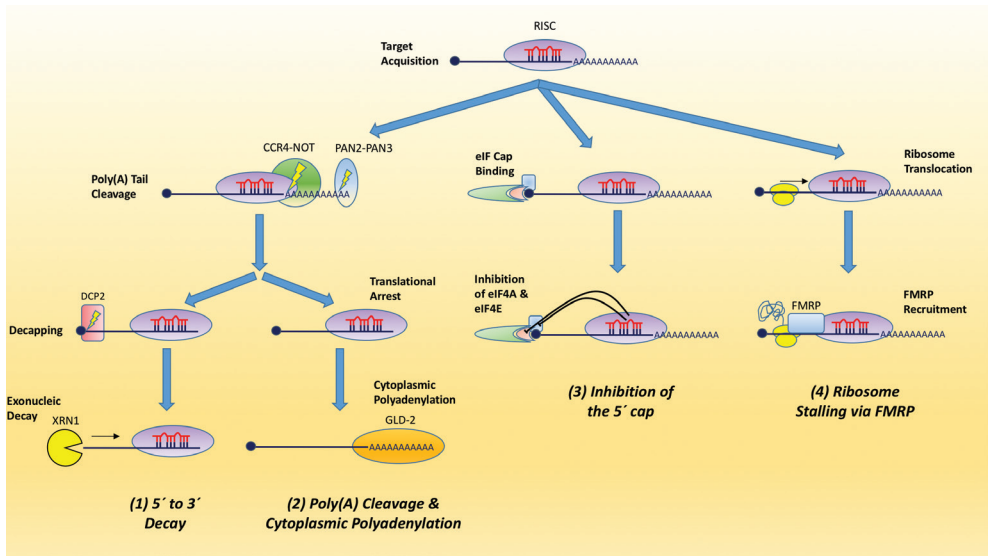


Figure 3. Mechanisms of miRNA-mediated translational repression. The RISC acquires target mRNAs through complementary base pairing, triggering one of a number of repressive pathways. (1) Transcript degradation is stimulated through RISC recruitment of deadenylase complexes to cleave the mRNA poly(A) tail, followed by decapping and exonucleic decay. (2) Cleavage of the poly(A) tail reduces the capacity for target mRNA to act as a substrate for protein synthesis, leading to translational repression likely reversible by cytoplasmic poly(A) polymerases. (3) The RISC may additionally stimulate dissociation of eIFs from the mRNA 5' cap, or alternatively, (4) recruit FMRP to halt active synthesis through ribosome stalling.

3.2.3. Poly(A) tail erosion and cytoplasmic polyadenylation

While RISC induction of mRNA decay constitutes an effective mechanism through which translation may be fine-tuned for cellular needs, in the neuronal context, this mechanism relies heavily on both transcriptional activity and mRNA trafficking to maintain transcriptomic supply at dendrites. Consequently, a system by which the translational competency of mRNAs could be modulated during periods of quiescence and in response to plasticity-inducing stimuli would significantly relieve pressure applied by RISC degradation of transcripts. A major candidate mechanism through which this may be achieved is modulation of mRNA poly(A) tail length, since this structure and associated PABPs facilitate 5'–3' interactions required for translation initiation [63]. The manipulation of reporter mRNA in HeLa cultures [81] and cell-free systems [82] has indeed revealed let-7 translational arrest of reporter luciferase depends on the expression of a poly(A) tail, emphasising the role of the poly(A) tail as a conduit through which translational competency may be altered by miRNA. A requirement for such a system to exist, however, is the functional decoupling of poly(A) tail erosion and mRNA decay. Recent studies illustrate that this occurs in embryonic culture systems such that deadenylated targets of miRNA, including miR-35-42, miR-51-56 and miR-155, have been shown to accumulate in a stable manner [83, 84], suggesting miRNA-mediated poly(A) tail cleavage does not necessarily instigate mRNA decay.

This potential dimension to miRNA function becomes particularly interesting in the neural context, as cytoplasmic polyadenylation has the potential to reinstate the translational activity of poly(A) deficient mRNAs. Poly(A) polymerase GLD-2 is a promising contender for this function due to its neuronal enrichment, cytoplasmic localisation and participation in translational enhancement via poly(A) tail extension [85, 86]. In addition to these features, GLD-2 function increases the abundance and stability of poorly translated mRNAs and is also required for the induction of LTP, suggesting that cytoplasmic polyadenylation could play a significant role in synaptic plasticity [85, 87, 88]. Regardless, the role of GLD-2 and deadenylase counterpart PARN (poly(A)-specific ribonuclease) in bidirectional regulation of poly(A) tail length and neuronal translation as part of the cytoplasmic polyadenylation complex illustrates the significance of cytoplasmic polyadenylation in neuronal translational control [85].

3.2.4. Inhibition of translation initiation

Further emerging evidence implicates miRNA and the RISC in direct inhibition of translation initiation through interaction with 5' cap-bound eIFs responsible for mediating ribosomal assembly in the mRNA 5' UTR [63]. At present, AGO1-RISC has been shown to indirectly stimulate disassociation of eIF4E via GW182, and eIF4A through a mechanism independent of GW182, inhibiting the assembly of an eIF4F translation initiation complex [89, 90]. While it is clear that this interaction acts to prevent translation of target mRNAs, there is uncertainty regarding whether this is another mechanism of translationally arresting mRNA or simply a precursor to mRNA degradation. The recent discovery of an RNA-independent association between CCR4-NOT subunit NOT1 and eIF4A2 RNA helicase involved in RISC degradation of target mRNA [91] suggests the latter is likely but does not rule out the possibility of eIF interference as a means of transcript repression.

3.3. microRNAs in synaptic function

Considering the array of confirmed and emerging miRNA functions, miRNA degradation and/or repression of transcripts constitutes an effective mechanism for fine-tuning highly dynamic translation at the post-synaptic compartment. While miRNAs are thought to be largely stable mRNA species, new evidence suggests that miRNAs are subject to activity-dependent synthesis and rapid turnover in dendritic spines [42]. Dendritic synthesis of miRNA became particularly apparent following the discovery of pre-miRs [92] and their inactive processing enzyme Dicer at the post-synaptic density (PSD) whereby activity-dependent stimulation of Calpain I was sufficient to induce Dicer function [93]. These data were supported by the detection of mature miRNA species enriched in mouse forebrain synaptoneuroosomes [92]. Activity-dependent degradation of miRNA has also recently been observed in mouse retinal neurons; however, the exact mechanism of degradation remains elusive [94]. Interestingly, exosome release may act as a means of post-synaptic miRNA clearance, as MAP1B-enriched neuronal exosomes released in response to depolarisation have recently been shown to contain miRNA (miR-638, miR-149*, miR-4281 and let-7e) normally downregulated by depolarisation in the dendritic compartment [95].

Supporting the critical role of miRNA in synaptic function, microarray analysis and deep-sequencing have revealed that subsets of miRNA are subject to differential expression up to 24-h post-induction of hippocampal synaptic plasticity [96, 97]. These data have since been extended through the development of a conditional *Dicer 1* knockout in the adult mouse fore-brain shown to result in enhanced LTP, learning and memory [98]. Reflecting on these discoveries, it is perhaps not surprising that a number of neuron-enriched miRNA have been described in terms of the regulation of synaptic function.

Arguably the most studied miRNA in the context of synaptic function, miR-132 is widely regarded for its role in the regulation of synaptic plasticity in brain regions such as the visual cortex [99] and hippocampus [100]. Following neuronal activity, miR-132 is subject to a rapid increase in CREB-mediated (cAMP response element-binding protein) transcription, resulting in stimulation of dendritic growth and branching through suppression of p250GAP (p250 GTPase activating protein) [101, 102]. These modifications to dendritic morphology are characterised by expression of stubby, mushroom-like dendritic spines which act to potentiate synaptic transmission [80]. miR-132 expression additionally directs the upregulation of NR2A, NR2B and GluR1 glutamate receptor subunits following BDNF stimulation [103], revealing this miRNA may direct synaptic plasticity through guiding both morphological and physiological change. A particularly interesting aspect of miR-132 function, however, is its relationship with FMRP, which provides novel insight regarding potential interplay between miRNA and FMRP homologues. Specifically, miR-132 effects on dendritic morphology are abolished following FMRP knockdown, uncovering a functional relationship between these molecules likely to be responsible for miR-132-associated regulation of plasticity-relevant transcripts [80]. A similar relationship has been observed for miR-125b whereby FMRP deficits inhibit miR-125b negative regulation of both dendritic spine morphology and NR2A [80], further supporting the role of FMRP in neuronal miRNA function.

Aside from miR-132, several recent studies have implicated a variety of other neuron-enriched or specific miRNAs in the modification of synaptic function. miR-134 is a key example which modulates dendritic spine volume through the regulation of *Limk1* expression, resulting in dendritic spine volume deficits when miR-134 is overexpressed [104, 105]. This function of miR-134 in the negative regulation of dendritic spine morphology is reflected in the impairment of mouse contextual fear learning following miR-134 overexpression in the CA1 hippocampus; however, post-transcriptional miR-134 regulation of the CREB transcription factor appears to significantly contribute to this deficiency [106]. Another miRNA involved in the inhibition of synaptic plasticity is miR-137 which decreases dendritic complexity and length through downregulation of mind bomb-1 ubiquitin ligase while additionally inhibiting pre-synaptic vesicle release [107, 108]. miR-137 also directly influences AMPA receptor expression through the regulation of GluA1, leading to synaptic silencing and deficits in hippocampal learning when overexpressed in mice [109].

Considering these examples alone, it is evident that miRNAs modulate a variety of synaptic characteristics through post-transcriptional regulation of a number of unique mRNA species, emphasising the importance of miRNA in coordinating a number of gene expression networks to fine-tune synaptic plasticity. Moreover, miRNA species appear to provide the basis through which the induction of plastic alterations to the synapse may be bidirectionally

regulated. This not only implicates specific miRNA species such as miR-132 in the enhancement of synaptic plasticity, but also additionally implies miRNA including miR-134 and miR-137 act as a breaking mechanism to prevent excessive potentiation or depression of individual synapses. Despite this extensive understanding of miRNA function, many are yet to be fully characterised in relation to synaptic and indeed neuronal function.

4. P-bodies and mRNP granules: linking mRNA transport, translation and repression

miRNA regulation of gene expression is sequestered within subcellular granules known as processing bodies (P-bodies), proposed to form through aggregation of translationally arrested mRNA and mRNA degradation machinery [110]. These granules were initially described as cytoplasmic “speckles” termed GW-bodies, observed as aggregates of GW182 complexed with mRNA [111]. P-bodies have since been characterised as foci of mRNA degradation due to their enrichment of translationally repressive RBPs such as Argonaute proteins, DCP1 & DCP2 decapping enzymes, Pat1 & edc3 decapping factors and XRN1 exonuclease [110]. Considering these granules are both enriched at the post-synaptic density and actively migrate to post-synaptic compartments following neuronal activity [112–114], P-bodies are currently heavily implicated in post-synaptic translational regulation.

Neuronal mRNP granules similarly form by the aggregation of messenger RNPs to provide a vehicle for mRNA distal transport while maintaining mRNAs in a translationally arrested state [18]. Interestingly, *Drosophila* Staufen and FMRP mRNP granules are enriched with homologues of mRNA decay machinery including DCP1, XRN1 and AGO2 [24], demonstrating these granules bear similarity to P-bodies. In conjunction with this observation, an exciting interaction between these granules has recently been reported whereby 50% of dendritic P-bodies have been shown to dock with neuronal mRNPs [113], raising the prospect that these granules exchange their contents.

Drawing from these observations, a fascinating scenario is the potential for neuronal RNP granules to unload their mRNA and mRNA degradation cargo to P-bodies for repression until translation is appropriate, thereby acting as a means of mRNA storage with the potential for degradation if specific transcripts are not required. In support of this possibility, mapping of mRNA localisation during yeast glucose starvation and tetracycline transcriptional suppression indicates mRNA can associate with polysomes from P-bodies [115], revealing mRNA may be subjected to translation following sequestration in P-bodies. Adding to this evidence, cationic amino acid transporter-1 mRNA has recently been observed undergoing release from miR-122 repression and subsequently associating with polysomes from P-bodies during cellular stress [116], further implicating miRNA in repressing translation without necessarily instigating mRNA decay. While the potential underlying mechanisms driving mRNA de-repression in P-bodies remains unclear, *Drosophila* GLD-2 poly(A) polymerase has been observed to co-localise with FMR1 in neuronal mRNP granules [87] and may therefore be delivered to P-bodies, lending to the possibility of cytoplasmic poly(A) tail lengthening in de-repressing mRNA.

In addition to their mRNA, P-bodies themselves respond to cellular stimuli as synaptic input induces as much as a 60% decrease in dendritic P-body abundance [113], while artificial stimulation of cap-dependent translation decreases P-body biogenesis in PNS sensory neurons [117]. Overall, these observations imply that these repressive granules could dissociate in a pro-translational environment to release a collection of mRNAs for novel protein synthesis. A subset of dendritic P-body-like granules containing miRNAs repressed mRNAs and lacking XRN1, which respond to synaptic activation may represent a subcategory of P-bodies specifically designed for such a purpose [112]. Stress granules, which contain translationally stalled mRNAs, translational enhancers (such as eIFs and PABPs) and interact with P-bodies, are another key candidate for mRNA storage; however, their functional relevance is limited to periods of exposure to stressful cellular stimuli [118].

5. Emerging non-coding RNAs in post-transcriptional regulation of synaptic translation

5.1. Long non-coding RNA

Long non-coding RNA (lncRNA) is a broad category of >200nt non-coding transcripts characterised by widespread genomic distribution and involvement in a variety of regulatory functions [119]. Like miRNA, lncRNAs inhabit a range of genomic regions including intergenic, telomeric and gene regulatory sequences [119]. Protein-coding genes have also been identified as a source of lncRNA expression wherein these RNA may reside in antisense, overlapping, intronic or bidirectional configurations relative to protein-coding sequences [120]. From these genes, lncRNAs are transcribed by RNA polymerase II following which many are subject to posttranscriptional modifications including alternative splicing, 5' capping and polyadenylation [121]. Despite this similarity to mRNA, recent studies have revealed the genomic origin of lncRNA dictates the functional role of individual lncRNA species, which may serve as regulators of translation, transcription and epigenetic modifications, or act as miRNA precursors or sponges [120, 122]. Translational regulation is a particularly interesting aspect of lncRNA function due to the range of emerging post-transcriptional mechanisms through which this may be achieved. This holds significance in the context of neuronal function as the adult brain has recently been identified as a source of substantial lncRNA enrichment, reflected in the discovery of 849 (of 1328 examined) lncRNAs subject to specific patterns of expression in mice [123]. Accordingly, a number of lncRNAs have exhibited roles in the regulation of synaptic translation and synaptic function, adding further complexity to the nature of neuronal translation.

The rodent lncRNA *Bc1* and its primate orthologue *BC200* are prime examples of neuronal lncRNAs, which modulate post-synaptic translation of plasticity-associated mRNA, including *Arc*, *CaMKII* and *MAP1B* [124]. Current studies indicate that *Bc1/BC200* instigates translational repression at somatodendritic compartments through inhibition of eIF4A and eIF4B activity at the mRNA 5' cap, thereby suppressing translation initiation [125]. In conjunction with this observation, *BC200* alone has been shown to contain *cis*-acting adenine-rich elements which

potentially facilitate interaction with PABPs [126], indicating this lncRNA may additionally interfere with 5' to 3' mRNA interactions required for translation initiation. FMRP may further contribute to the multidimensional nature of *Bc1/BC200* lncRNA function as oligonucleotide masking of an mRNA recognition motif within the *Bc1* lncRNA primary sequence inhibits FMRP interaction with MAP1B mRNA [124]. This suggests that *Bc1* lncRNA may potentially act as a guide in FMRP association with plasticity-associated mRNA analogous to miRNA and the RISC; however, the exact functional mechanism of these relationships is yet to be elucidated.

In addition to *Bc1/BC200*, other emerging lncRNAs have been implicated in synaptic regulation through multiple post-transcriptional mechanisms. lncRNA Malat1 is one such example, which modulates synaptic density through positive regulation of neuroligin 1 and SynCAM1 mRNA expression and alternative splicing of CaMKIIB [127]. Interestingly, this occurs within nuclear speckles, known for their role as sites of mRNA storage and processing, revealing this lncRNA modulates synaptic properties from the nucleus via a post-transcriptional mechanism [127]. lncRNA Tsx has additionally been shown to enhance short-term hippocampal memory in mice through an elusive mechanism, suggesting a role for this lncRNA in the regulation of synaptic plasticity [128].

Considering these examples together, it is evident that current understanding of the exact mechanisms through which various lncRNAs post-transcriptionally modulate synaptic properties is limited. Despite this gap in the literature, lncRNAs appear to bidirectionally regulate post-synaptic translation, suggesting lncRNAs may act to fine-tune protein synthesis rather than strongly influence gene expression networks. Supporting this theory, mRNAs with roles in synaptogenesis including *CamkIIa* and *Dag1* have been observed in complex with their antisense lncRNA counterparts in synaptoneuroosomes of the adult mouse forebrain [129]. This further raises the possibility that lncRNAs could block translation via binding target mRNAs while additionally inhibiting miRNA-mediated mRNA decay through masking miRNA recognition elements. This distinct prospect further underscores the complex and multidimensional nature of post-transcriptional lncRNA function in the regulation of synaptic properties and emphasises the need for extensive characterisation of individual lncRNA species.

5.2. Circular RNA

Circular RNAs (circRNAs), as their name implies, are a class of circular long non-coding RNAs produced by exonic or intronic splicing which lack 5' cap and 3' poly(A) tail structures and exhibit exonuclease-resistance [130]. Unlike miRNA and RBPs which generally serve to regulate translation through direct modulation of mRNA characteristics, emerging evidence implicates circRNA as miRNA sponges, serving as a regulatory mechanism by which miRNA translational regulation may be dampened. A well-characterised example is the brain-enriched circRNA ciRS-7 (also known as CDR1as) which contains as many as 74 conventional seed-binding sites for miR-7 that allow this circRNA to function as a more potent miRNA sponge than traditional anti-miRs used for miRNA knockdown [131, 132]. While this circRNA demonstrates highly specific miRNA sponging, it is unclear whether this is a unique example or common among other species. For comparison, circHIPK3 and circRNA mlb, respectively, knockdown 9 and 2 unique miRNA [133, 134].

circHIPK3 is a particularly interesting example as overexpression of this species triggers a reduction of midbrain size in developing zebrafish [133], indicating circRNA may exert substantial influence over neuronal structure and function. Considering this, the function of this RNA class bears significant implications in the neuronal context since circRNAs exhibit substantial brain enrichment and accumulate during nervous system development and with age [135, 136]. circRNA may also act to fine-tune plasticity-associated translation as evidenced through their subcellular enrichment within dendritic compartments of cortical pyramidal neurons and interneurons, and differential expression of at least 42 distinct species during bicuculline-induced synaptic plasticity [134]. Together, these studies therefore implicate circRNA in both neuronal development and plasticity and provide an exciting added dimension to neuronal translational control. Further characterisation of individual circRNA will no doubt be integral in both determining the status of miRNA sponging as a primary circRNA function and the functional significance of these RNA in neuronal structure and function.

6. Towards a unified model of neuronal post-transcriptional regulation of translation

Since the initial discovery of extrasomatic neuronal translation, it has become evident that a number of regulatory systems fine-tune neuronal protein synthesis in an exquisitely coordinated manner to control structural and physiological characteristics associated with synaptic plasticity. Since these regulatory systems are continually exhibiting varying degrees of overlap, deciphering the mechanisms through which these systems both function in isolation and interact is of critical importance in revealing the intricate details of neuronal post-transcriptional regulation of translation.

A key example is the interplay between neuronal transport mRNP granules and P-bodies at the post-synaptic compartment [113]. The possibility of mRNP granules delivering cargo to P-bodies is an enticing prospect further exemplified by the potential for P-bodies to isolate and store repressed mRNAs for translation under suitable conditions [115]. What remains to be considered is the potential for P-bodies to export cargo to neuronal mRNP granules, perhaps acting as a means of mRNA recycling especially interesting in the context of the proposed “Sushi Belt” theory of neuronal mRNA transport [18]. As P-bodies, neuronal mRNP granules, polysomes and stress granules display multiple degrees of functional interaction, an overlying mechanism of mRNA transport, storage, repression and translation is likely at play to exert considerable influence over the available pool of translatable mRNA at the post-synaptic compartment.

With regard to miRNA function in the neuronal context, an increasing body of evidence underscores the fact that miRNAs are not simply mediators of mRNA decay. For neurons in particular, the existence of reversible systems of translational repression would present an energetically favourable mechanism of translational control capable of reducing total reliance on novel transcription and extra-somatic mRNA trafficking to dendrites. Indeed, the role of cytoplasmic poly(A) polymerases such as GLD-2 in enhancing activity-dependent translation [85] shows potential for cytoplasmic poly(A) tail modulation as a reversible mechanism

of neuronal translational control, warranting investigation of the theoretical decoupling of miRNA repression and degradation of mRNA in the neuronal context. Furthermore, uncovering the functional relevance of FMRP in mediating miRNA ribosome stalling will likely reveal a neuron-specific aspect to miRNA function which may prove critical in characterising the role of FMRP in the biogenesis of neuronal disease. Further miRNA studies in conjunction with high-throughput ribosome profiling [137], poly(A) tail sequencing [138] and co-immunoprecipitation sequencing will prove critical in revealing the existence and functional significance of these repressive systems in coordinating post-synaptic neuronal translation.

From the presented examples miR-132, miR-134 and miR-137, plasticity-relevant miRNA species clearly regulate dynamic morphological and physiological changes at the post-synaptic compartment through interaction with a variety of targets. However, it is important to note that these miRNA constitute well-described examples subject to intensive research. Additional miRNAs, such as miR-9, miR-22, miR-124 and miR-188, among others [139], have all displayed an association with synaptic plasticity and therefore stress the need for further characterisation of specific miRNA and how together they exquisitely coordinate gene expression networks in regulation of synaptic plasticity. This holds true for lncRNAs, which appear to regulate synaptic plasticity via an assortment of posttranscriptional mechanisms [119], stressing the need for further investigation of individual lncRNA species and the mechanisms through which they act. Considering the potential role of circRNA in regulating miRNA [131–134] and their post-synaptic enrichment, emphasis should no doubt be additionally placed on characterising these non-coding RNA in the context of neuronal translational control. Expression of circRNA is likely to partially account for the distinct complexity of neuronal translation and could act as novel therapeutic target or biomarker in neurological disease.

Continual advancement in high-throughput technology and analysis will substantially further our ability to characterise and integrate these regulatory systems into a unified model of neuronal posttranscriptional regulation of neuronal translation. Increasing our knowledge of this aspect of the central dogma of molecular biology in the neuronal context will subsequently facilitate characterisation of the molecular events underlying synaptic plasticity, fueling our understanding of learning and memory.

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Mitochondrial Regulators of Synaptic Plasticity in the Ischemic Brain

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Additional information is available at the end of the chapter

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Abstract

Synaptic plasticity is a process by which neurons adapt or alter the strength of information transfer, and it is known to play a role in memory formation, learning, and recovery after injury. In this chapter, we describe how ischemic insults alter neuronal intracellular mechanisms and signaling pathways, and we discuss how, after neuronal injury, synaptic plasticity is regulated prior to and during death or rehabilitation and recovery. In addition, recently described regulators of synaptic plasticity will be introduced.

Keywords: ischemia, mitochondrial metabolism, neuroprotection, Bcl-xL, ATP synthase

1. Cellular mechanisms after cerebral ischemia

Cerebral ischemia occurs as a result of a lack of, or insufficiency of, blood supply to the brain, which results in the failure to meet neuronal metabolic demands. Thrombotic or embolic stroke (focal), and cardiac arrest or cardiac surgery (global) are common causes of cerebral ischemia. The loss of oxygen and glucose flow to the brain eventually leads to neuronal energy deficits. These energy deficits result in the failure of adenosine triphosphate (ATP)-dependent ion pumps expressed on the neuronal plasma membrane, permitting an unregulated surge of ion influx into the neuronal cytoplasm [1–3]. Calcium influx induces the release of neurotransmitters from presynaptic neurons and activation of postsynaptic glutamate receptors such as N-methyl-D-aspartate (NMDA) receptors and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [4–6]. Uncontrolled opening of postsynaptic receptors provokes failure of intracellular ion homeostasis, resulting in excessive postsynaptic entrance of calcium or sodium through NMDA- or AMPA-regulated channels; this initiates signaling pathways.

Calcium is a trigger for a number of important cell-signaling pathways. Increased levels of intracellular calcium activate phospholipase C, which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP₂) and forms diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP₃) [1]. The hydrophobic DAG molecule is expressed on the cell membrane, and recruits protein kinase C (PKC) from the cytosol. PKC regulates synaptic function by phosphorylating ion channels [7–9] and glutamate receptors [10, 11], and enhancing neuronal outgrowth [12, 13]. On the other hand, hydrophilic IP₃ travels into the cytosol and binds with the IP₃ receptor expressed on the endoplasmic reticulum (ER). The ER membrane-embedded IP₃ receptor releases calcium from the ER to mitochondria, depolarizing mitochondrial inner membranes and further compromising ATP production.

Ischemic conditions also activate death receptors (e.g., Fas, tumor necrosis factor (TNF) α R, DR) that cause caspase activation and ultimately lead to neuronal apoptosis [14, 15]. Death receptor-ligand binding releases caspase 8, which directly cleaves either caspase 3 (which can activate downstream death-inducing enzyme pathways) or BH3 interacting-domain death agonist (Bid) to form truncated tBid [16, 17]. tBid translocates to the mitochondria and initiates activation of the pro-apoptotic proteins Bax and Bak. Oligomerization of Bax on the mitochondrial membrane causes cytochrome c release. Cytochrome c forms an apoptosome complex made up of cytochrome c, apoptotic protease-activating factor 1 (APAF 1), and caspase 9. Caspase 9 cleaves and activates effectors such as caspase 3 and caspase 6 which results in neuronal apoptosis. Numerous proteins are subjected to caspase-mediated cleavage [18] including regulators for synaptic function such as glutamate receptors [19, 20], synaptic adhesion molecules [21], ion channels [2], neuronal growth/pruning regulators [22, 23], and inflammatory cytokines [24, 25].

In addition, ischemic stimulation increases the permeability of the blood-brain barrier, and activates neuroinflammatory responses in the brain. Inflammatory infiltration such as the entrance of leukocytes (e.g. neutrophils, macrophages, and lymphocytes) activates microglia and astrocytes which then release inflammatory regulators, including cytokines (e.g. interleukin-1 β (IL-1 β), IL-6), tumor necrosis factor α , chemokines (e.g. chemokine C-C motif ligand 2 (CCL2), CXC-chemokine ligand 1 (CXCL1)), nitric oxide, reactive oxygen species (ROS), and growth factors [26–29]. Neuroinflammation is a dual-purpose response that can hasten neuronal death or facilitate repair depending on the circumstances. For example, TNF- α is one of the most well-studied pro-inflammatory cytokines increased by ischemic events; it is clearly responsible in large part for ischemia-induced brain injury [30]. However, TNF- α also enhances synaptic strength by increasing the expression of AMPA receptors [31] and through the regulation of the transcription factor, NF κ B [32], increasing the expression of anti-apoptotic proteins (Bcl2, and Bcl-xL) and facilitating the production of neurotrophic factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) [33, 34] which play a role in neuroprotection [35]. Taken together, ischemia triggers a multifunctional and complex process in the brain that can lead to neuronal death or facilitate the defense system to rescue the brain against neurotoxic stimulation.

2. Synaptic plasticity in the ischemic brain

The process of early neuronal growth and neurite elongation is critical for synapse formation and neuronal network development. Projection of filopodia, where actin and microtubules

become polymerized and attach to substrates, anchors a growth cone and guides a peripheral domain within the thin outer edge of the growth cone known as the lamellipodium. After the lamellipodium contacts the substrate, the central domain of the growth cone, where actin is reorganized and microtubules predominate, moves toward its target [36–38]. These steps occur repeatedly during neurite sprouting and branching. When the tip of the axon reaches its target (either dendrite, soma, or another axon), it differentiates to become suitable for neurotransmission. Presynaptic terminals contain a high density of mitochondria, presynaptic vesicles, and endosomes to enhance communication within the synapse. The target of presynaptic contact is the postsynapse, which matures by the expression of neurotransmitter receptors. Neurexin-neurologin, N-cadherin, ephrin, and synaptic cell-adhesion molecules play a role in the interaction between presynaptic and postsynaptic neurons [37, 39–42].

Neuronal development is far from static in the mature brain. Recent studies have found that neurons are capable of plasticity during the entire human lifespan [43–46]. Mature neurons have the ability to repair their synaptic network after neurotoxic insults. Stroke induces changes in numerous genes including the ones involved in axonal sprouting in both young and aged animals [47]. Alteration of neuronal connectivity and degradation of neurites are well described after ischemic stroke [47–49], and strategies to strengthen the synaptic network to regain neuronal function and enhance brain repair after episodes of brain injury have been reported [47, 50]. Our study demonstrates examples of the dynamic and adaptive changes that occur in neurites after glutamate neurotoxic challenge. Neurite branches initially become fragmented and damaged (**Figure 1A and D**), but then neurites regain structure (**Figure 1B and C**) over time, or undergo degradation if severely damaged; in some case collateral sprouting occurs to compensate for lost neurites and to regain synaptic connectivity with healthy neighboring cells (**Figure 1D–F**).

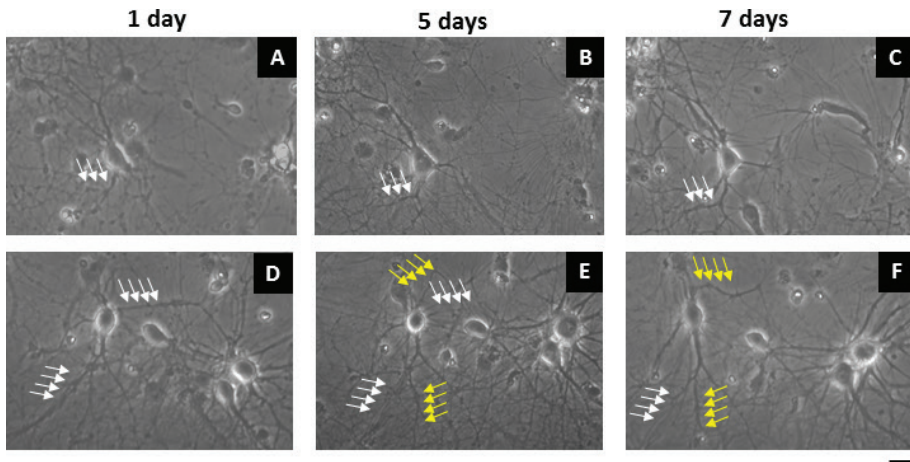


Figure 1. Adaptation of neurite sprouting and pruning after glutamate-induced neurotoxicity. Primary hippocampal neurons were treated with 20 μ M glutamate and then imaged at days 1, 5 and 7 after introduction of the insult. Bar, 20 μ m. (A) Damaged and fragmented neurite at 1 day after insult (white arrows). (B) Early recovery of injured neurite at day 5 (white arrows). (C) Thickening of recovering neurite at day 7 (white arrows). (D) Fragmented and damaged neurites at day 1 (white arrows) are cleared at day 5 (E, white arrows), but strengthening of collateral branches was found at day 7 (F, yellow arrows) to supplement synaptic networking.

Numerous studies have reported the modification of synaptic plasticity during or after ischemic events. Some studies describe synaptic modification as a part of death signaling; on the other hand, other studies suggest changes in synaptic function as a mechanism of protection or rehabilitation. Synaptic plasticity plays a role in the decision for the neuron to live or die. Synaptic transmission demands high levels of energy production [51]. Failure to control neurotransmitter release [6, 52], and abnormalities of synaptic transmitter release due to lack of energy supply after ischemic insult are well described previously [53]. Regulation of the postsynaptic receptor is critical to control synaptic plasticity [54, 55], and studies have reported that NMDAR or AMPAR are subject to alterations after ischemic events. Earlier studies have shown that brains of animals undergoing four vessel occlusion (4VO)-induced global ischemia show impaired voltage-dependent NMDAR responses, display NMDA-mediated hyperexcitability and loss of long-term potentiation (LTP), and manifest morphological changes of pyramidal neurons prior to the onset of delayed death in the CA1 region of hippocampus [56, 57]. Recent studies expand on these mechanisms and distinguish roles for individual ion channel subunits [58–61]. Studies have reported that ischemia is responsible for the alteration of AMPA receptor expression, especially the GluR2 subunit, transforming a non-calcium permeable into a calcium-permeable AMPA receptor, thereby further mediating calcium entry into CA1 neurons after global ischemia. Although this role contributes to delayed neuronal death in the globally ischemic rodent brain [62–65], these changes may also occur during normal events in synaptic plasticity [54, 66]. Despite the close relationship between ischemia-induced neuronal death and NMDA receptor activation, the application of NMDAR antagonists fails to prevent stroke-related brain injury in clinical trials [67] perhaps indicating involvement of NMDAR in neuronal survival. Indeed, functioning NMDARs are required for synaptogenesis, and the NMDAR plays a neuroprotective role against apoptotic stimulation and oxidative stress [61, 68–70]. In summary, synaptic changes that occur during ischemia may be protective or detrimental depending on the severity and temporal sequence of ischemic events.

2.1. Synaptic failure may lead to ischemic death

Structural damage including degradation of axons or dendrites, and loss of synaptic connectivity associated with synaptic dysfunction are described in various cerebral ischemic models [53, 71]. Animals undergoing ischemic surgery exhibit a reduction of the total neuronal population and an increased appearance of degenerating neurons and apoptotic cells [72–76]. Shy of frank cell demise, axonal morphological changes after brain ischemia have been reported within a variety of brain regions including cortex, striatum, and hippocampus. Degenerating axons exhibit swelling and the appearance of varicosities in both an acute and chronic manner; changes are observed over a period between 6 h and 4 weeks after ischemia reperfusion [49]. Cerebral ischemic insults also lead to cytoskeletal disruption in neurites such as a reduction in the amount of microtubule-associated protein (MAP2), an enhancement in neurofilament proteolysis and an alteration of tau in some neurons [77–79]. Ischemia causes an increase in intracellular calcium levels and ROS production [15] and triggers neuronal death by mechanisms such as enhancing mitochondrial permeability which can facilitate both apoptotic and necrotic death and neuritic degeneration [80]. Therefore,

ischemia is one of the major causes of structural and functional failure of both somata and neuronal processes during stroke.

2.2. Synaptic repair after ischemic events

Although ischemic stroke induces neuronal death that leads to functional disability, studies have reported evidence for synaptic plasticity contributing to recovery after stroke [81]. Rats undergoing neocortical ischemia had induction of the growth-associated protein 43 (GAP-43) which is enriched in the growth cone, promoting synaptogenesis and behavioral recovery [82, 83]. Axonal sprouting and plasticity in the intracortical circuitry was observed in post-ischemic brain regions [84, 85]. Plasticity was not limited to neurons but also occurred in other cells including reorganization of vascular structures. Enhancement of the dendritic network such as increased dendritic density has been reported in post-ischemic brain [86, 87]. Moreover, the brain is capable of generating new neurons. The subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus are reported to be the site of neurogenesis, exhibiting therapeutic potential for the treatment of brain diseases [88]. In particular, studies have shown that stroke increases neuronal progenitor cell populations in the brain [89, 90], and enhances cell proliferation in the SVZ [91] and in the ischemic penumbra of stroke patients [92]. These studies indicate that ischemia-induced signaling, even denervation itself, may act as a stimulus for the functional and structural recovery of synapses.

2.3. Preconditioning in synaptic potentiation

Despite the possibility of severe ischemia to trigger adaptive responses on its own, studies have also reported that non-noxious, lower levels of injury (preconditioning) may augment ischemic tolerance. Moderate levels of neurotoxic stimuli such as glutamate, ROS, or inflammation initiate survival mechanisms without impairing brain function. Thus, preconditioning builds a latent neuroprotective environment in the brain and provides for a reprogrammed defense response when the truly injurious stimulation occurs [93, 94].

Zukin's group has reported that ischemic preconditioning downregulates AMPA receptors, and blocks mitochondrial release of death molecules such as Smac and DIABLO without altering the pro-survival inhibitor of apoptosis (IAP) family, therefore attenuating ischemia-induced damage in rodent hippocampal neurons [95, 96]. Neuroprotective mechanisms of preconditioning are further evidenced as a change in dynamics of mitochondrial proteins. Preconditioning prevents translocation of the pro-apoptotic protein Bad, enhances the availability of the pro-survival protein Bcl-xL, blocks activation of caspase 3, decreases release of Smac, DIABLO, and cytochrome *c*, and prevents large conductance mitochondrial channel activity, eventually rescuing hippocampal neurons after ischemic insult [72]. In addition, mitochondrial Bcl2 protein family [97–99], redox regulators [100, 101], and transcription factors such as hypoxia-inducible factor (HIF) [102, 103], NFκB [104, 105], c-Fos [106], CREB [97, 105], Nrf2 [101, 107], and AP1 [108] are involved in gene regulation after preconditioning, further modifying neuronal functions such as neurotransmitter release, channel activity, and synaptic networking by regulating the expression of new proteins.

3. Regulators of mitochondrial function and synaptic plasticity

Ischemic insults damage the mitochondrial electron transport chain, decrease mitochondrial ATP production, impair ATP-dependent transporters, and allow intracellular calcium entrance that triggers opening of the mitochondrial permeability transition pore (mPTP) [80, 109, 110]. Ischemia also impairs electron transfer and causes ROS generation from mitochondrial proteins such as complex I and III [111]; ROS greatly contributes to mPTP-mediated responses in the mitochondrial membrane [112]. On the other hand, ischemia alters proteins that reside in the mitochondria, and changes levels of transcription factors that target the promoter regions of mitochondrial and nuclear genes. Thus, mitochondria are an essential organelle in ischemia-mediated neuronal responses. There are several regulators that modify mitochondrial function and plasticity to either enhance synaptic recovery after injury or signal for synaptic decline prior to neuronal death. We describe these individually and then show how they work together to support synaptic function during stress.

3.1. Mitochondrial reactive oxygen species

Oxidative phosphorylation is the metabolic process that produces ATP, but it is also the main source of production of superoxide in the mitochondria that can be converted into hydrogen peroxide. Due to the high metabolic rate of neurons, ROS is highly produced in the brain. However, the brain is also more vulnerable than other organs to ROS-induced damage, because it is rich in polyunsaturated fatty acids, and lacking in catalase activity [113, 114]. Indeed, ROS is one of the main causes of mPTP opening, release of mitochondrial death molecules and cell death in ischemic brain [15]. Although numerous studies have reported that an ischemia-induced surge of ROS causes functional and structural injury to neurons, ROS plays multifunctional roles. Physiological levels of ROS regulate synaptic signaling. Approaches that block production or enhance clearance of superoxide using depletion of NADPH oxidase, or overexpression of superoxide dismutase (SOD), respectively, failed to allow for LTP in hippocampal slice [115–117]. Moreover, SOD-overexpressing mice demonstrate defective hippocampal memory formation as measured by water maze learning and fear conditioning [117, 118].

Nitric oxide synthase (NOS) is an enzyme that generates nitric oxide (NO) gas from arginine. NO is considered to be a ROS, due to its ability to generate highly reactive peroxynitrite [119]. Indeed, 4VO-induced global ischemia causes induction of NO in various regions of the brain including hippocampus, cortex, striatum, and cerebellum [120]. Excessive NO production is reported to be neurotoxic as it damages mitochondria, and exacerbates excitotoxicity [121–123]. However, NO is also required for synaptic transmission and synaptic plasticity [124, 125]. NADPH oxidase (NOX) is a cell membrane-bound enzymatic complex that generates superoxide from NADPH, and it also expresses in the mitochondria [126]. NOX contributes to ROS-induced neuroinflammation and apoptosis [127, 128]. Studies reported an upregulation of NOX mRNA and protein level in response to experimental stroke [129, 130]. Inhibition of NOX using the pharmacological inhibitor apocynin improved ischemia-induced brain damage and mortality [131, 132]. However, mice lacking NOX subunits had impaired long-term

potentiation and manifested hippocampus-mediated memory deficits, indicating that a physiological level of NOX and ROS may be required for synaptic plasticity and memory formation [114, 115].

3.2. Mitochondrial permeability transition pore (mPTP) and ATP synthase

Ischemia-induced death signaling opens a calcium-sensitive inner mitochondrial membrane pore called mPTP, causing permeabilization of the mitochondrial inner membrane [80, 109, 110]. Loss of mitochondrial inner membrane integrity leads to leakage of intermembrane molecules such as cytochrome *c*, Smac, and DIABLO into the cytoplasmic space, facilitates death-signaling cascades, and results in the impairment of mitochondrial outer membrane structure and cell death. Thus, mPTP is recognized as an important target for neuroprotection; inhibition of mPTP opening may delay or prevent mitochondrial-mediated cell death. Since the discovery of the calcium-induced mitochondrial membrane permeability transition (PT) [133, 134], several molecular participants in mPTP structure or formation have been reported. However, the identification of mPTP is still under investigation.

Cyclophilin D (Cyp D) is a peptidyl-prolyl *cis/trans* isomerase that is localized to the mitochondrial matrix. Cyp D has been considered as a main element of mPTP. However, recent studies have reported that depletion of Cyp D did not eliminate mPTP [135–137] indicating it may not be the critical component of pore opening. One target of Cyp D is a complex of proteins that includes the voltage-dependent anion channel (VDAC), localized to the mitochondrial outer membrane, and the adenine nucleotide translocase (ANT), an ADP/ATP translocator localized to the mitochondrial inner membrane. Therefore, this protein complex was widely studied for its possible role in forming mPTP [138]. Studies showed that mitochondrial swelling after stimulation facilitates the formation of VDAC and ANT complexes along with Cyp D binding, leading to opening of pores in the mitochondrial inner and outer membranes [139–143]. Studies have continuously reported new participants of Cyp D-ANT-VDAC complex: the mitochondrial phosphate carrier binds to Cyp D and ANT [144], spastic paraplegia 7 forms a heterooligomeric complex with VDAC [145], and hexokinase binds to VDAC [146, 147]. However, a recent study revealed that animals lacking VDAC genes did not show improvement of mPTP-mediated mitochondrial stress. They exhibited equivalent levels of cytochrome *c* release, caspase activation, and cell death compared to control animals [135]. In addition, mitochondria from mice lacking ANT also displayed mPTP and cytochrome *c* release at similar rates compared to wild-type controls [148]. These studies indicate that further investigations are required to clarify the roles of VDAC and ANT in mPTP activation.

In contrast to the studies of VDAC and ANT, knockdown of the membrane-embedded portion (*c*-subunit) of the F_1F_0 ATP synthase does regulate the ability of mitochondria to undergo PT. Several recent studies have reported that F_1F_0 ATP synthase is an important candidate to form mPTP [149–157]. F_1F_0 ATP synthase is localized in the mitochondrial inner membrane (F_0 unit) and matrix (F_1 unit), and produces ATP by using the potential energy of the hydrogen ion gradient. F_1F_0 ATP synthase gained attention as a putative candidate for mPTP when it was found that it binds CypD [152] and when it was reported to regulate the efficiency of mitochondrial energy metabolism via controlling an inner mitochondrial membrane ion leak

[158]; the c-subunit of the ATP synthase was found to be required for mPTP [149]; the c-subunit of ATP synthase was revealed to form a voltage-sensitive channel, the opening of which is correlated with PT and neuronal death [155]. Bonora and Alavian also showed that shRNA-mediated c-subunit depletion protects neurons from PT-induced cell death, such as excitotoxic and oxidative stress. Together, the findings suggest that the c-subunit forms the pore component of mPTP [155]. Moreover, the depletion of c-subunit of F_1F_0 ATP synthase causes resistance to calcium-induced mPTP opening, while overexpression of c-subunit accelerates calcium-mediated responses, decreases mitochondrial potential, and promotes mitochondrial fragmentation [149, 155]. In addition to the role of the ATP synthase monomer, dimerization of F_1F_0 ATP synthase in mPTP has been correlated with the onset of mPT [151, 159–161].

3.3. B-cell lymphoma-extra large (Bcl-xL) and other Bcl2 family proteins regulate the synapse

Bcl-xL is a member of the Bcl-2 family of proteins. It is traditionally known for anti-apoptotic properties through its role to inhibit the activation/oligomerization of pro-apoptotic Bax and Bak on mitochondrial membranes [162, 163], and its ability to block mitochondria-mediated cytochrome c release and cell death [164]. However, recent studies have reported multifunctional roles of Bcl-xL in the brain. Bcl-xL facilitates reorganization and biogenesis of mitochondria by regulation of fission and fusion [165–167]. Inhibition of Bcl-xL decreases neuronal ATP levels but increases oxygen flux, indicating that Bcl-xL enhances the efficiency of mitochondrial metabolism by preventing the wasteful leak of H^+ ions through the inner membrane [158, 168]. Inefficient leakage of H^+ through a mitochondrial inner membrane pore prevents ATP production. These latter studies provided the first evidence for a functional role for Bcl-xL at the mitochondrial inner membrane and F_1F_0 ATP synthase. Bcl-xL interacts with α - and β -subunits [158, 169] of F_1F_0 ATP synthase in the mitochondrial matrix. These protein-protein interactions may cause conformational changes of the Bcl-xL- F_1F_0 ATP synthase complex, favoring closure of the c-subunit channel (putative mPTP), enhanced mitochondrial energy metabolism, and increased ATP production with minimal oxygen use (decreased inner membrane uncoupling).

It is therefore not surprising that Bcl-xL is an important player in energy-demanding processes such as neuronal outgrowth [74, 170] and synapse formation [167, 171]. Depletion of Bcl-xL in primary hippocampal neurons impairs neuronal branching and elongation [74]. Abnormalities of neurite sprouting caused by Bcl-xL depletion do not induce immediate cytotoxicity, but cause delayed neuronal death, presumably as more synapses fail. Despite their low propensity toward death in the absence of stress, neurons depleted of Bcl-xL are significantly vulnerable to hypoxic insult compared with control neurons, presumably due to failure of synaptic connections and impaired metabolism. In contrast to depletion, Bcl-xL overexpression increases levels of pre- and postsynaptic markers on axons and on the opposing dendrites and enhances the number of mitochondria and synaptic vesicles in the presynaptic bouton [167]. Bcl-xL also enhances synaptic vesicle recycling during presynaptic plasticity, by forming a complex of clathrin, Bcl-XL, and Drp1 which is necessary for normal or enhanced endocytosis [171]. In addition, Bcl-xL is reported to have multiple binding partners besides those of traditional Bcl2 family proteins that regulate apoptotic pathways (**Table 1**); thus, additional functions of Bcl-xL in synaptic plasticity need to be further investigated.

Protein	Roles	References
<i>Bcl2 family protein</i>		
Bak	Regulates apoptosis	[163, 208]
Bax	Regulates apoptosis	[209–211]
Beclin 1	Prevents autophagy	[212]
Bad	Regulates apoptosis and cell cycle	[213–215]
Bim	Regulates apoptosis	[216, 217]
Bid	Regulates apoptosis	[217]
PUMA	Regulates apoptosis	[209, 218]
<i>Non-Bcl2 family proteins</i>		
Apoptotic protease-activating factor 1 (Apaf-1)	Regulates caspase 9-mediated apoptosis	[219, 220]
Apoptosis regulatory protein Siva (Siva-1)	Sequesters Bcl-xL and induces apoptosis	[221]
F ₁ F ₀ ATP synthase	Regulates mitochondrial energy metabolism	[158, 169]
Aven	Stabilizes Bcl-xL and regulates caspase-dependent apoptosis	[222, 223]
Dynamin-related protein 1 (Drp1)	Regulates synaptic vesicle endocytosis	[165, 167, 171]
IP3 receptor	Regulates calcium signaling and apoptosis	[224, 225]
Phosphoglycerate mutase family 5 (PGAM5)	Enhances Keap1-dependent degradation	[226, 227]
PTEN-induced putative kinase 1 (PINK1)	Regulates phosphorylation of Bcl-xL to prevent its cleavage	[228]
Voltage-dependent anion channel (VDAC)	Regulates mitochondrial calcium, cytochrome c release, ATP release	[164, 229, 230]
Tumor protein p53	Regulates cytochrome c release and apoptosis	[231, 232]

Table 1. List of proteins that bind to Bcl-xL.

In contrast to its neuroprotective properties, Bcl-xL is also capable of decreasing synaptic strength [172, 173] and inducing neurotoxicity [73, 174]. Bcl-xL is subject to caspase-mediated fragmentation [174–176], and forms N-terminus truncated Δ N-Bcl-xL. The N-terminally localized BH4 region has been reported as the functional domain that carries out the anti-apoptotic role of Bcl-xL [177, 178], and cleavage of Bcl-xL to remove this domain gives pro-apoptotic characteristics to this molecule. Δ N-Bcl-xL is reported to induce large channel activity in the synaptic mitochondria [172], to cause decline of the amplitude of post synaptic potentials [173], and to increase cytochrome c release [179]. Studies show that transient global ischemia induces Δ N-Bcl-xL formation prior to delayed neuronal death in the CA1 region of hippocampus [72, 73]. The strategies to block Δ N-Bcl-xL formation such as administration of pharmacological inhibitors, or mutation of the caspase cleavage site protect rodent brains against ischemic injury [73, 172].

Bcl-2-associated x protein (Bax) is a pro-apoptotic member of the Bcl2 family containing BH1, BH2, and BH3 domains but lacking the BH4 domain found in many anti-apoptotic

family members. Bax forms channel activity in lipid bilayers [180], induces cytochrome c release [162, 181], and cooperates with mPTP candidates such as ANT and VDAC [138, 164]. Interestingly, although it is mostly known as a pro-apoptotic protein, it also has important functions in healthy synapses undergoing plasticity. Injection of Bax protein into the presynaptic terminal induces enhanced neurotransmitter release, similarly to effects of pro-survival Bcl-xL, indicating that Bax is capable of supporting normal synaptic plasticity in unstressed neurons [182]. In healthy hippocampal neurons, Bax is necessary for the formation of synaptic plasticity known as NMDA receptor-dependent LTD. Despite comparable expression of NMDA receptors in Bax knockout animals, these animals fail to demonstrate hippocampal LTD induction [183].

Bid is a pro-apoptotic BH3 only protein. Bid is normally expressed in the cytoplasm, but during cytotoxic stimulation, caspase cleaves Bid into truncated Bid (tBid) which activates other members of the pro-apoptotic Bcl2 family [184] or antagonizes anti-apoptotic Bcl2 proteins [17]. tBid contributes to the mobilization of cytochrome c by Bax and alters mitochondrial cristae independent of its function to activate Bax, and it opens mitochondrial intermembrane spaces [185]. Bid enhances mitochondrial membrane permeabilization, cooperates with mPT or Bax, and mediates large-channel conductances [186]. Studies show that Bid is an important activator in ischemia-induced brain injury [187, 188]. Cleavage of Bid was also found after middle cerebral artery occlusion (MCAO)-induced stroke in mouse. Bid knockout animals show decreased levels of cytochrome c release and infarct volume [187, 188].

3.4. Hypoxia-inducible factor 1 (HIF1- α)

HIF1- α is a transcriptional factor activated in response to hypoxia. In normoxic conditions, HIF1- α is generally degraded by prolyl hydroxylases (PHD)-mediated ubiquitination, but hypoxia inhibits PHD activity and leads to stabilization of HIF1- α which then is translocated to the nucleus and regulates gene expression. Although HIF1- α is not generally considered as a mitochondrial protein, HIF1- α is reported as an important player in mitochondrial function [189–191]. HIF1- α directly binds with the promoter region of Bcl-xL [192] and targets the expression of BNIP3, a BH3-only protein member of the Bcl2 family that mediates hypoxia-induced mitochondrial autophagy [193–195]. HIF1- α regulates a subunit of cytochrome c oxidase [196] which is an essential member in the mitochondrial electron transport chain. In addition, a recent study showed localization of HIF1- α to both the nucleus and mitochondria after hypoxia [197].

Since ischemia is closely related to hypoxic stimulation, there are studies reporting functions of HIF1- α in models of cerebral ischemia. HIF1- α is enhanced by MCAO-induced stroke in rodent brains, and co-regulated with death-signaling molecules such as caspases, inflammatory cytokines, and apoptotic molecules [198–202]. On the other hand, the neuroprotective role of HIF1- α has been studied by several groups during the past decade [203–205]. Impairment of dopaminergic differentiation and reduction of vascular endothelial growth factor are reported in a HIF1- α knockout model [206]. Tomita et al. reported that neuron-specific-HIF1- α knockout mice have reduced numbers of neurons and impaired spatial memory [207]. Therefore, understanding both physiological and pathological roles of HIF1- α and its targets is important to understand synaptic plasticity in cerebral ischemia.

4. Conclusion

Mitochondria are the center of intracellular energy production, and the executor of cellular fate. Ischemic injury triggers or is caused by mitochondria-mediated signaling pathways. We have discussed in this chapter the intracellular signals activated during and after episodes of ischemia, the alteration in the dynamics of structural components of the synapse, and how these elements play a role in attenuation of synaptic plasticity or recovery of synaptic responses. Since after ischemia, increased energy demands are inherent in the formation of new synapses and repair of the normal operation of existing synapses, we are particularly focused on mitochondrial components that regulate mPTP and neuronal energy metabolism. Although details of the structure of mPTP are still in question, it is clear that prevention of mPT and conservation of energy are critical in management of ischemia-induced damage in the brain. We have also highlighted non-canonical roles of mitochondrial Bcl2 family proteins in the synapse besides their known functions in apoptosis; these roles should be further studied to elucidate the crucial functions of mitochondria in synaptic plasticity.

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Molecular Mechanisms of Drug-Induced Plasticity

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Additional information is available at the end of the chapter

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Abstract

Substance-use disorders (SUDs) are a significant societal burden. Like many other conditions, SUDs are characterized by chronic relapse after periods without symptoms. Alterations in synaptic plasticity have been documented after acute and addiction-related behavioral exposures to drugs of abuse. These synaptic alterations are persistent and similar to the chronic relapse state of patients with SUDs, suggesting that reversing these synaptic alterations may prevent relapse. Additionally, many of these synaptic features of addiction mirror those found in other forms of learning and memory. These features suggest that the etiology of this disorder stems from persistent modifications in corticolimbic synaptic plasticity mediated by drug-induced alterations in addiction-related gene (ARG) expression. Understanding the regulation of addiction-related genes and their impact on synaptic plasticity and behavior may inform new pharmacological treatments that reverse these aberrant drug-evoked forms of plasticity in favor of remission.

Keywords: addiction, substance-use disorders, Fos, CREB, ADAR, BDNF, Mef2, miR-212, miR-9, FMRP, HuD, ELAVL

1. Introduction

Substance-use disorders (SUDs), or more colloquially known as drug addiction, are a significant societal burden both financially, incurring an estimated \$193 billion in costs (www.justice.gov/ndic, 2011), and emotionally devastating to patients and their families. In opposition to more controlled, casual drug use, SUDs are characterized by uncontrollable bouts of drug consumption in spite of negative life consequences. Patients with this disorder appear to lose interest in normal prosocial activities, instead directing their energy toward the pursuit of these substances. Since addiction is a chronic, relapsing condition, this disorder has been notoriously difficult to manage. A complete review of pharmacological mechanisms of drugs of

abuse understanding of the molecular, physiological, and behavioral mechanisms involved in the disorder is necessary for the discovery and implementation of more effective treatments.

The mesolimbic dopamine system is the most well-studied and implicated circuit associated with the disorder. Naturally, this neurocircuit may have evolved as an information-processing center to direct behavior toward environmentally important stimuli predicting the presence of vital resources, for example, food and water. Synaptic plasticity within these regions is implicated in associative learning of these stimuli to guide behavior necessary to receive these rewarding resources. Depending on the frequency and accuracy that a particular stimulus may predict a natural reward, goal-directed behavior toward this stimulus with the intention of obtaining the reward may become habitual. Thus, if an exogenous substance were to recruit this same neurocircuitry, this may elicit similar goal-directed behavior toward obtaining these compounds.

Structurally, drugs of abuse are highly varied and have many specific protein targets within the body (for a review of pharmacological mechanisms of drugs of abuse, see [1]). However, all pharmacological mechanisms of drugs of abuse cause excessive dopamine release originating from the ventral tegmental area within the nucleus accumbens (VTA, NAc; [2, 3]). Not only are these regions involved in this disorder, they also appear to be crucial mediators of appropriate goal-directed behavior toward natural rewards. Dopaminergic projections from VTA neurons are important in regulating motivation to perform integral, evolutionarily conserved behaviors such as feeding and reproduction [4, 5]. Drug-elicited synaptic plasticity also occurs within this region, possibly through similar mechanisms as natural rewards. Mirroring a classical symptom of addiction, the synaptic alterations induced by drugs of abuse inhibit the ability of other non-drug-related experiences to elicit synaptic plasticity [6, 7]. As such, it has been hypothesized that drugs of abuse cause a heightened form of plasticity-dependent associative memory leading to habit formation and addiction-like behaviors [8].

Although drug use is required for the development of addiction, it is most certainly not sufficient to cause this disorder. For example, around 176.6 million Americans from the age of 12 and up drink alcohol but only 17 million are classified as having an alcohol-use disorder (SAMSHA, 2013). Drugs are metabolized and eventually cleared from the body, thus, the drug's pharmacological effect is not sufficient. In addition, SUDs are classically characterized by chronic relapse after periods without symptoms [9]. This may occur many years after a patient's last reported use of a drug, suggesting that some stable functional alteration has occurred within the brain. Repeated drug exposure is required for an accumulation of molecular events that alter plasticity within specific regions affecting the functioning of the entire circuit. These synaptic alterations are persistent and similar to the chronic relapse state of patients with SUDs, suggesting that reversing these synaptic alterations may prevent relapse [9, 10]. Additionally, they appear to be protein-synthesis-dependent forms of plasticity [11–13]. Thus, the molecular mechanisms regulating gene expression ending with the translation into functional protein may play a role in the etiology of this disorder and thus may stem from persistent modifications in region-specific synaptic plasticity mediated by drug-induced alterations in addiction-related gene (ARG) expression.

Regulation of these molecules can occur at many different steps. The DNA in the genome is organized into small compact three-dimensional units assembled by histones into nucleosomes, which form the structure of chromatin. As such, basal transcription is necessarily stagnant in the absence of the regulation of gene accessibility. Modifications to the accessibility of genes, by regulation of these structures, are termed epigenetic regulation and have also been found to be an integral component in the development of addiction. Given that the epigenetic control of drug addiction has been elegantly reviewed in previous work [14–17], this chapter focuses on other aspects of gene expression control starting at the level of transcription.

2. Transcriptional regulation of addiction-related genes

Transcriptional regulation occurs through transcription factors, which are proteins that respond to intracellular changes leading to association with specific elements in target genes. Since the promoter region where the transcription factor binds to is outside of the coding region of that gene, these sequences are also termed cis-regulatory elements.

2.1. The Fos family transcription factors are differentially altered by drugs of abuse and contribute to addiction-related plasticity and behavior

Although altered transcription of various genes had been suggested by earlier work [18], it was not until the discovery that morphine caused increased striatal expression of a transcription factor, c-Fos, that it was shown that transcription factors may regulate this drug-induced gene expression [19]. Although first found to be regulated by acute morphine treatment, it was found that other drugs of abuse cause specific, dopamine-dependent, alterations in the expression of this transcription factor within addiction-related regions [20–22]. Important for the study of relapse, it was found that c-Fos was upregulated in mesolimbic structures following withdrawal, suggesting that it may play a role in the intense drug craving present in this disorder [23]. c-Fos is part of the Fos family of transcription factors, including FosB and Δ FosB, which all heterodimerize with Jun to form the AP-1 transcription factor complex [24]. This complex then associates its specific cyclic AMP response element (CRE), the AP-1 binding site, leading to increased transcription of genes downstream of these sequences.

Fos genes are considered immediate early genes (IEGs), or factors that are basally expressed at low levels but elevated rapidly by specific stimuli (first reviewed in the context of neuronal cells [25]). Since it was upregulated by other plasticity and memory-inducing events, it was hypothesized that c-Fos, and possibly other IEGs, may alter gene expression involved in memory formation [26]. As expected, the upregulation of c-Fos, FosB, Δ FosB, and Jun leads to increased AP-1-mediated transcription within addiction-related regions such as the NAc rapidly after acute drug administration [27]. Due to its regional specificity, it was hypothesized that alterations in these genes may provide a mechanism for reorganization of functional interactions between these brain regions in addiction [28]. Although c-Fos is rapidly upregulated and returns to basal levels quickly, AP-1 binding continues in the absence of elevated c-Fos mRNA or protein expression suggesting that other Fos proteins may replace

c-Fos and accumulate during chronic use of drugs. This may provide a molecular trace of chronic drug use in the absence of drug itself. This tolerance to initial c-Fos upregulation appears to be drug agnostic, as acute alcohol exposure blocked the previously observed acute cocaine upregulation of c-Fos, also suggesting that this molecular trace persists for the use of all different drugs [29].

To more specifically test the role of specific transcription factors in addiction, transgenic mice with specific mutations were engineered. The first experiment to utilize this approach used a transgenic mouse with a mutant *fosB* gene that prevented the production of Δ FosB. It is important to consider that this mutant *fosB* gene was not targeted to any specific cell type or even to the brain, but throughout the body. These mutant mice showed attenuated chronic induction of AP-1-binding activity, suggesting that the previously observed AP-1 binding without c-Fos upregulation was probably due to Δ FosB [30]. Additionally, this study was the first to link transcriptional regulation with altered addiction-like behavior. These researchers used a model of drug-seeking behavior termed conditioned place preference (CPP), where animals are trained to associate one environment with an experimenter-administered drug and a separate environment with saline [31]. Over time, animals will seek out the drug-paired cues associated with the environment where they received the drug. These *fosB*-mutant mice showed increased CPP compared to wild-type controls, suggesting that normal Δ FosB AP-1 binding might be involved in a compensatory or adaptive response to drugs. Fos family members have also been found to be upregulated after exposure to drug-paired cues in the absence of the drug itself, suggesting that this CPP effect may be due to the interaction between drug and cues soliciting the availability of the drug, an importance facet in addiction research [32].

Although certain molecular factors may cause increased addiction-like behavior or plasticity within one set of cells, it may cause an opposite effect on these measures in another. Cellular specificity is an important point that has been discussed in neuroscience for some time. In an experiment complimentary to this performed by Schroeder and colleagues, researchers developed an inducible system to specifically overexpress Δ FosB within D1 neurons in the striatum [33]. Neurons within the striatum are primarily GABAergic medium-spiny neurons that mostly express D1 or D2 dopamine receptors. Since D1 receptors are G_s GPCRs, they activate the neuron leading to increased GABA release, while D2 receptors are G_i and are inhibited by dopamine binding. Since drugs of abuse cause hyperdopaminergic conditions within the striatum, both D1 and D2 containing neurons play important but dissociable roles in the development of addiction. Targeted Δ FosB overexpression in D1 neurons showed a similar effect on increased CPP behavior at low doses (5 and 15 mg/kg), but normal CPP behavior at higher doses (20 mg/kg). This suggests that cocaine-induced Δ FosB expression within these cells is necessary and sufficient to promote addiction-like behaviors. Furthermore, GABAergic inhibition from these cells may play a role in CPP behavior. Finally, this is physiologically relevant, as drugs of abuse and to a lesser extent natural rewards cause upregulation of Δ FosB in this specific neuronal subtype [28, 34]. In a similar experiment, nonspecific deletion of Δ FosB throughout the body caused the same behavioral alteration, increased CPP behavior to lower doses of cocaine [30]. Although this finding may seem contradictory, when taken together with previous data, this points to the existence of other regions or possibly neuronal subtypes that act in opposition to D1 neurons. Following up on this, overexpression of Δ FosB within D2

expressing neurons in the striatum produced no effects, illustrating the cellular specificity of these drug-induced alterations [35]. Thus, Δ FosB within these regions or cells may not play a role, or possibly inhibit, the plasticity required for the development of these behaviors.

Another behavioral model frequently used to study addiction is self-administration of drugs [36]. In this model, an intravenous catheter will infuse cocaine into the animal once it has completed a specific behavioral response, usually pressing an active lever in opposition to an inactive lever that produces no effect. This can be used to study motivation to consume drugs, as the requirement for lever pressing can be made harder by increasing the number of presses required to receive an infusion of drug. Animals with a similar inducible Δ FosB genotype as used by Kelz and colleagues showed increased motivation to self-administer cocaine [37]. This was also the first experiment to show that this alteration specifically affected motivation toward drugs and not naturally rewards such as a food pellet.

Transcription factors elicit changes in the expression of genes; thus this work suggests that there must be some downstream targets of Fos proteins that are mediating these effects on addiction-related behavior. Kelz and colleagues first described a downstream target that may alter plasticity involved in these behavioral alterations [33]. Striatal neurons are normally quiescent, even with dopamine input, and thus require glutamate which is an important neurotransmitter in addiction-like behaviors to alter their firing (reviewed in [38]). Kelz and colleagues found that Δ FosB overexpression caused an increase in a specific AMPAR subunit, GluR2 which could lead to altered plasticity [39]. Additionally, they could recapitulate their effects on behavior by viral-mediated overexpression of this subunit in the striatum. Overall, many Δ FosB targets are genes that appear to be previously linked to addiction-like behaviors such as CDK5 and NFkB.

2.2. CREB, a transcription factor well established in learning and memory associated plasticity, plays a role in addiction

Drugs of abuse can cause acute regulation of molecular-signaling cascades that can modify transcription factor activity. For example, many drugs of abuse can cause acute upregulation of the second messenger cAMP within many addiction-associated brain regions [40–46]. cAMP response element-binding protein (CREB) is phosphorylated in response to cAMP upregulation by a kinase sensitive to cAMP, protein kinase A (PKA; [47, 48]), calcium-calmodulin dependent kinases (CaMK) and other kinases [49]. The role of CREB in other forms of plasticity-associated memory has been well elucidated [48]. Once phosphorylated, CREB can enter the nucleus and bind to its response element, the cyclic AMP response element (CRE). CREB was first implicated in drug-induced alterations in response to acute and chronic morphine treatment within the locus coeruleus, another brain region associated with addiction [50]. Later, it was found that chronic, but not acute, morphine caused a decrease in CREB within the NAc [51]. Cocaine self-administration was found to be sensitive to PKA inhibition, an upstream regulator of CREB, by increasing lever pressing [52]. Following this experiment, it was later found that NAc overexpression of CREB caused a decrease in cocaine-CPP behavior [41]. Conversely, overexpression of a mutant CREB containing a single-point mutation, alanine for serine at residue 133, that prevents its phosphorylation and blocks its ability to

regulate transcription acting as a dominant negative form of CREB, caused an increase in cocaine-CPP behavior. Further correlating the role of this transcription factor in the association of drug cues, researchers found that re-exposure to the original drug-paired environment in CPP without drug caused increased phosphorylation of CREB [53]. Highlighting the differences of behavioral tasks, it was found that NAc overexpression of CREB caused an increase in self-administration behavior [54]. This gene has also been studied in human patients. Researchers found that opioid-dependent patients were more likely to have a polymorphism in a protein that associates with CREB, CREB-binding protein, within a region associated with its activation by phosphorylation [55]. Altogether, it appears that CREB expression is involved in many aspects of addiction-related behavior.

CREB is a nuclear transcription factor that binds to CRE upstream of target genes. CREB regulation has been found to cause upregulation of ARG mRNA such as *c-fos*, *Fosb*, brain-derived neurotrophic factor (*BDNF*), and *TrkB* (e.g., in methamphetamine self-administration [56]). *BDNF* and its receptor *TrkB* have a very long history in the study of addiction-related behaviors as well as general plasticity occurring within the brain [57]. Further implicating CREB as a regulator of plasticity, it has been found to be involved in late-phase forms of long-term potentiation (LTP [58]). As mentioned before, NAc neurons are normally quiescent and require glutamate activation with or without dopamine stimulation to fire. CREB has been associated with the regulation of this feature of NAc neuronal membrane excitability [59]. It was found that increased CREB activity leads to enhanced NMDAR-mediated currents, an important receptor for causing action potentials as well as for learning and memory. This regulation increases NAc neuronal-firing probability through enhancement of the membrane excitability. This may partially explain the effect of NAc CREB on behaviors such as self-administration. Thus, CREB regulates the expression of many genes associated with plasticity and addiction-related behaviors.

CREB and Δ FosB are very well-documented addiction-related transcription factors but, undoubtedly, there are many more transcriptional factors that alter addiction-related plasticity and ultimately behavior. Myocyte-enhancing factor 2 (MEF2) was found to be involved in cocaine-induced structural plasticity [60]. Interestingly, it was necessary and sufficient to increase the number of dendritic spines. Structural plasticity or the alteration of the physical synaptic shape has been associated with electrophysiological measurements of plasticity [61–63] specifically in addiction [63]. Additionally, it was found that MEF2 regulates a wide network of plasticity-associated genes opening up further exploration into its role in addiction-related plasticity and behavior [64]. Overall, transcriptional regulation of ARG expression can lead to modifications in drug-evoked synaptic plasticity and behavior [65, 66].

3. Posttranscriptional regulation of plasticity and addiction-related transcripts can promote addiction-like behaviors

Posttranscriptional regulation occurs after transcription until the mRNA is translated into a functional protein. Posttranscriptional regulation takes many forms such as mRNA processing, splicing, editing, stability, and translation. mRNA stability and translation have been

the most well-characterized posttranscriptional mechanism in plasticity, especially in addiction. Additionally, it appears that mRNA stability may be especially important in the brain, as nearly 20% of brain-expressed genes have been predicted to be controlled through this mechanism [67].

3.1. miRNAs destabilize specific mRNAs which could lead to diminished expression of genes involved in addiction

Stability can be influenced by direct binding of factors to discrete recognition sites. One group of factors involved in this type of mRNA stability are small noncoding RNAs, termed microRNAs (miRNAs). miRNAs are ~20–22 oligonucleotide molecules that are encoded within the genome and make up nearly 2–3% of transcribed genes but are not translated into protein. miRNAs target specific mRNAs through binding of complementary sequences within the miRNA, termed seed regions, to the 3' untranslated region (UTR) of the mRNA. Association of miRNAs with the 5'UTR of mRNA has also been discovered [68] leading to different cellular effects, but most studies within the brain have focused on the 3'UTR. Accordingly, the rest of this section focuses on this mechanism.

Although the miRNA may associate directly with the mRNA target, the aftereffects of this binding are mediated through a large, supporting protein complex, the RNA-induced-silencing complex or RISC. Through associating with the RISC, an miRNA can target specific mRNAs for degradation or translational repression [69]. This complex contains a multitude of auxiliary proteins, with the most important being Argonaute (Ago; [70]). Ago is the component most directly involved in regulating the mRNA. There are many Ago isoforms, but generally they are separated into those containing nuclease “slicer” activity and those without. Thus, Ago is important for the well-characterized miRNA-induced destruction of mRNA.

Although one miRNA has one specific seed region to bind to mRNAs, mRNAs may have numerous sites complementary for multiple miRNAs. This suggests that a single miRNA targets many mRNAs, affecting a myriad of cellular processes and pathways. This is reminiscent of transcriptional regulation, as one transcription factor may regulate a specific sequence that is upstream of various types of genes. This opens the possibility for an miRNA that may regulate a network of plasticity-associated genes. For example, miR-9-3p was found to diminish *Dmd* and *Sap97* mRNA [71]. These genes are negatively associated with LTP expression, thus miRNA-induced silencing of these mRNAs would reverse this effect. A hippocampal LTP-dependent behavior, the Morris water maze, was also found to be regulated by the expression of this miRNA.

Similar to a group of plasticity- and addiction-associated transcription factors, miRNA expression patterns appear to be similar to IEGs in their temporal- and activity-dependent regulation [72–74]. As demonstrated before, many of these IEGs play integral roles in general plasticity and addiction-related plasticity. Similar to IEGs, miRNAs have been associated with neural development, synaptic plasticity, and even behavior [73–79].

The role of miRNA-induced posttranscriptional regulation in addiction was first studied in the regulation of the large-conductance-calcium-and-voltage-activated potassium channel (BK). This channel is integral to plasticity by affecting excitability, firing, and transmitter release

from neurons [80]. It is also one of the many protein targets of alcohol within the brain, for the most part potentiating its conductance [81]. This channel has been well studied in its role in tolerance to alcohol in the striatum, requiring larger amounts to exert similar effects [82]. This seems to be mediated by decreased BK channel expression in the membrane. A single gene can produce multiple mRNAs with the same coding region but with different 3'UTRs through the inclusion or exclusion of different exons [83]. Research has shown that the alpha subunit of this BK channel is alternatively spliced both in the coding region and in the 3' UTR in response to neuronal activity [82, 84]. These researchers also found that alcohol caused a decrease in one specific BK mRNA that has an miR-9-binding site in its 3' UTR. Given that this isoform of the BK channel mRNA is the most abundant in the striatum and miR-9 expression is increased by alcohol exposure, alcohol tolerance seems to be mediated at least in part by this miRNA [82]. As mentioned before, miRNAs target many mRNAs that contain a complementary sequence in the 3'UTR. Thus, these authors also proposed that miR-9 targets many other mRNAs such as the beta subunit of the GABA receptor, GABRB2, and the D2 receptor. Overall, this suggests that alcohol-mediated upregulation of miR-9 leads to the development of an alcohol-specific regulation of mRNAs associated with plasticity and addiction.

When animals are given extended access to drugs of abuse in a self-administration model, they undergo a specific behavioral response termed escalation of drug use. Uncontrollable use of large amount of drugs is an important facet in the human condition. With this, researchers sought out an miRNA that may be increased by this behavior and found that miR-212, an activity-dependent miRNA regulated by CREB, was upregulated in the dorsal striatum of these animals after extended access [85]. One of the targets of miR-212 is Sprout-related, EVH1 domain containing 1 (SPRED1), a corepressor of serine-threonine protein kinase Raf1, which increases the activity of adenylate cyclase by promoting its phosphorylation. Thus, upregulation of miR-212 during escalated drug use diminishes SPRED1 levels, potentiating cAMP production and CREB activity leading to increased miR-212 expression. When miR-212 was overexpressed within the dorsal striatum, rats in extended access showed decreased levels of cocaine infusions. Oppositely, blocking of miR-212 by a locked nucleic acid (LNA) caused an increase in cocaine infusions. Thus, extended access to cocaine may recruit miR-212 leading to a positive feedback loop that effectively diminishes stereotypical escalation of drug intake. Overall, this suggests miR-212 acts on a pathway involving CREB to enhance its signaling, possibly in a compensatory, protective manner.

3.2. RNA-binding proteins bind to mRNAs and can bidirectionally regulate addiction-associated mRNA translation into protein

RNA-binding proteins (RBPs) are another group of regulators of mRNA stability and translation. Some RBPs recognize discrete sequences in the 3'UTR, such as the AU-rich instability conferring elements (AREs [86]). As with miRNAs, there is potential for a single RBP to affect multiple downstream processes leading to miRNAs and RBPs to be termed "master switches" of gene regulation [87, 88]. This is especially important in the brain, as around 15–20% of brain-specific transcripts contain AREs suggesting these genes are regulated by this type of posttranscriptional regulation [67]. Fragile x-mental retardation protein (FMRP)

is an RBP that regulates the translation of nearly 850 brain mRNAs, many associated with synaptic function [89]. One of its regulatory targets are the group I metabotropic glutamate receptors mGluR1 and mGluR5 [90–92]. These receptors have been associated with cocaine-evoked behaviors [93, 94]. Thus, Smith and colleagues tested the role of FMRP in addiction-related behaviors [95]. They found that *Fmr1* KO mice were deficient in cocaine locomotor sensitization and conditioned place preference. The effect on conditioned place preference was reversed by genetic reduction in mGluR5, suggesting that FMRP's effect on drug-evoked behaviors was due to mGluR5 activity. Finally, they found that FMRP caused a reduction in cocaine-induced structural plasticity suggesting that these behavioral deficits may be caused by this aberrant plasticity.

Although FMRP is the only RBP specifically studied in addiction-like behaviors, many more have been associated with these behaviors [96]. One such family of RBPs is the Hu, or embryonic lethal abnormal vision-like (ELAV-L) proteins. Hu proteins are brain and neuronal specific and are homologous to the *Drosophila* protein ELAV protein [97, 98]. HuD expression and function is activity dependent, suggesting it may regulate IEGs and other activity-dependent genes associated with plasticity [99–101]. In confirmation of this idea, our laboratory has shown that HuD is critical for stabilizing U-rich containing mRNAs during neuronal development and synaptic plasticity [101]; work from our laboratory reviewed in [102, 103]. HuD is a critical regulator of multiple plasticity-associated genes, thus suggesting that it may be involved in addiction-related altered gene expression, plasticity, and ultimately behavior. Overall, post-transcriptional regulation may emerge as an important player tying drug-evoked molecular alterations with behavior.

3.3. mRNA editing can alter the sequence or expression of an addiction-related mRNA leading to alterations in addiction-related plasticity and behavior

mRNA editing is another posttranscriptional regulatory mechanism. The most common mechanism for RNA editing is through covalent modifications of a specific nucleotide, without the alteration of the original DNA-encoded gene. This would generate multiple alternative forms of a protein which may be important in different cellular contexts.

The most common mRNA modification leading to editing is the removal of an amino group and its replacement with an oxygen, termed deamination. This occurs on the N6 position of adenosine nucleotides, converting this nucleotide into an inosine. This position directly contributes to conventional Watson-Crick base pairing, thus allowing inosine to pair with cytosine similar to guanosine. Translationally, this leads to the original adenosine to be read as a guanine, effectively causing an A→G site-directed mutagenesis. This modification is catalyzed by adenosine deaminase that acts on RNA (ADAR) enzymes [104–108].

Specific mRNA editing in a neurophysiological context was first described in the GluRA2 subunit of AMPAR [109]. As mentioned before, editing of mRNA is a rapid mechanism to generate multiple alternative forms of a protein depending on environmental circumstances. These receptors are integral to excitatory synaptic signaling, especially in plasticity. Thus, AMPAR editing generates channels that are impermeable to calcium and displays

faster recovery rates from desensitization. In terms of drug-induced plasticity, a recent study demonstrated that GluA2 editing by ADAR2 in the NAc of rats regulates cocaine seeking [110]. These researchers studied reinstatement, a specific stage of drug self-administration [36]. Animals first need to learn the required instrumental behavior to receive a reward in self-administration. When the animal reaches an experimenter-determined criterion, then the animal is said to have “acquired” self-administration behavior. If the animal is then presented with the same context and levers, but rewards are withdrawn, the animal will eventually override the previously learned reward-driven behavior. Eventually, the animal will no longer perform the task required for the reward, which is termed extinction. Finally, when an animal is exposed to cues associated with the reward or the reward itself, the animal will reinstate its behavior to receive the reward. Reinstatement is thought to be analogous to relapse in human-addiction patients. In this study, they found that abstinence from cocaine was associated with a decrease in NAc-edited calcium-impermeable AMPAR as well as ADAR2. Next, Schmidt and colleagues overexpressed ADAR2 within the NAc. They found that this diminished cocaine-primed reinstatement of self-administration behavior, suggesting that ADAR2 within the NAc is important for the regulation of relapse-like behavior. Overall, there are many avenues for mRNA editing in drug-induced plasticity and addiction-like behaviors [111].

Another posttranscriptional mRNA modification is the covalent modification of adenosine to N6-methyladenosine (m⁶A). This modification is carried out by a methyltransferase complex, containing WTAP, METTL14, and KIAA1429. Hence, this complex is usually thought of as the “writers” of m⁶A modifications. In opposition to the activity of this complex, enzymes have been discovered that promote the demethylation of mRNA, or the “erasers” of these modifications. These modifications have been studied for many years as a stable, unalterable modification. However, with the discovery of m⁶A demethylases this shifted opinion to suggest that this modification is dynamic and thus could be regulated by physiological conditions (reviewed in [112]). For example, it was reported that regulation of m⁶A was important to the normal functioning of the circadian rhythm in mammals [113]. Thus, m⁶A is physiologically relevant in behavior.

The first m⁶A demethylase to be discovered was the fat mass and obesity-associated protein (FTO). As the name suggests, it was identified as the strongest genetic variation to predispose patients to obesity [114–117]. At the opening of this chapter, evidence was presented to show that addiction-related neurocircuitry originally evolved to elicit goal-directed behavior toward food, suggesting this gene may also be involved in other forms of goal-directed behavior. The first study to link these findings was performed in alcoholic patients, finding that the genotype associated with obesity (rs9939609) was inversely correlated with general alcohol consumption, measures of alcohol dependence, as well as cigarette use [118].

Later work found that this gene is expressed in dopaminergic neurons within the midbrain and is increased by acute cocaine administration (20 mg/kg [119]). Cocaine, and to a lesser extent amphetamines, inhibits the dopamine transporter (DAT), leading to increased synaptic dopamine. This in turn leads to hyperactivation of dopamine receptors on both sides of the synapse. The presynaptic terminal contains D2 and D3 receptors, which are inhibitory GPCRs. Thus, the presynaptic dopaminergic neuron will show increased suppression of firing in response to cocaine. In the next set of experiments, these researchers found that Fto-deficient

dopaminergic neurons showed diminished cocaine-induced suppression of firing rate, suggesting that signaling through D2/D3 receptors is regulated by Fto. As expected, it was found that protein expression of these downstream targets (DRD3, GIRK2, and NMDAR1) was reduced by Fto deficiency. Functionally, Fto deficiency led to a reduction in GIRK currents, possibly detailing a mechanism for the diminished cocaine-induced suppression of firing rate in dopaminergic neurons. Finally, Fto-deficient mice showed attenuated acute cocaine-induced locomotor activity, suggesting that these genetic and electrophysiological measures translate into this behavioral modification.

4. Conclusion

As shown here, there are many avenues for regulation of ARGs at the transcriptional or the posttranscriptional level. These regulatory mechanisms all have been implicated in the development of this disorder and thus may be involved in the vulnerability of some individuals to substance-use disorders. Understanding the regulation of addiction-related genes and their impact on synaptic plasticity and behavior may inform new pharmacological treatments that reverse these aberrant drug-evoked forms of plasticity in favor of remission.

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The Ghrelin Receptor Regulates Dendritic Spines and the NMDA Receptor–Mediated Synaptic Transmission in the Hippocampus

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Additional information is available at the end of the chapter

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Abstract

Increasing evidence suggests the involvement of ghrelin (an orexigenic hormone) and its cognate receptor growth hormone secretagogue receptor (GHSR1a, also known as the ghrelin receptor) in extra-hypothalamic functions such as hippocampal learning and memory. However, cellular and molecular mechanisms underlying the ghrelin-regulated hippocampal neuron activity are poorly understood. In this chapter, we show the following: (1) ghrelin promoted phosphorylation of the *N*-methyl-*D*-aspartate receptor (NMDAR) subunit 1 (GluN1) in a PKC/PKA-dependent manner and amplified NMDAR-mediated excitatory postsynaptic currents, (2) ghrelin stimulated phosphorylation of CREB (cAMP response-element-binding protein), and (3) ghrelin increased phalloidin binding to F-actin, suggesting possible reorganization of dendritic spines; all occurred through the activation of GHSR1a in the CA1 pyramidal cell of the hippocampus in cultured slice preparations. Interestingly, the ghrelin's effects on GluN1 and CREB phosphorylation were negatively modulated by exogenous application of endocannabinoids, 2-arachidonoylglycerol (2-AG), and anandamide (ANE), in type 1 cannabinoid receptor (CB1R)-dependent and -independent manners, respectively. It is suggested that ghrelin and the ghrelin receptor regulate synaptic transmission and plasticity in the hippocampus, interacting with the endogenous cannabinoid system, which may be essential and necessary for successful acquisition of metabolic state-dependent learning and adaptive appetitive behavior.

Keywords: GluN1 phosphorylation, NMDAR-EPSC, ghrelin binding, GHSR1a KO mice, Phalloidin, CREB, endocannabinoid, CB1R

1. Introduction

We empirically know that we can learn things more easily, accurately, and quickly when we are interested, motivated, and reward-driven. Numerous animal models of learning have placed experimental subjects under fasted conditions in order for successful acquisition of specific tasks by using food as a reward [1]. This suggests the possibility that a molecule, which plays a critical role in appetitive behavior and/or reward-related feeling, may also be involved in the acquisition of learning.

1.1. Hippocampus in reward-related learning

Although the mesolimbic dopaminergic system is central to the study of motivational and reward-related learning, the neurobiological basis of reward-related learning cannot be explained completely without the participation of the hippocampus. The hippocampus is a primary site of activity-dependent plasticity and neuromodulation that have been hypothesized to be the neuronal substrate for learning and memory. The hippocampus lies upstream of the dopaminergic reward circuit and sends a major output to the reward system. More specifically, the hippocampal glutamatergic outputs regulate reward responses in the nucleus accumbens [2]. Therefore, cellular and synaptic plasticity within the hippocampus alters the transfer of information throughout the brain's reward system.

1.2. Ghrelin in reward-related learning

Ghrelin is a unique acylated 28 amino acid peptide hormone that is released from the stomach when it is empty. Ghrelin was originally identified as an endogenous ligand for the growth hormone secretagogue receptor (GHSR1a, now known as the ghrelin receptor) [3]. Activation of GHSR1a initiates a release of growth hormone from pituitary glands. Activation of GHSR1a also stimulates feeding center in the hypothalamus [4]. Indeed, the hypothalamus shows the highest localization of GHSR1a [4]. However, increasing evidence suggests that ghrelin may have numerous physiological functions in the brain outside the hypothalamus. For example, ghrelin stimulates the brain's reward center [5]. Ghrelin improves memory retention [6]. Thus, the accelerated acquisition of learning under fasted conditions suggests the potential importance of ghrelin as a key molecule for cellular and molecular mechanisms of reward-related learning and memory [7–9].

1.3. Source of ghrelin: brain or stomach?

Systemic ghrelin can cross the blood-brain barrier and enter the hippocampus [10]. Thus, peripheral ghrelin could be a source to be utilized by hippocampal neurons. On the other hand, in the mouse model, acylated ghrelin was readily transported across the blood-brain barrier in the brain-to-blood direction, but the quantity of its transport in the blood-to-brain direction appeared negligible [11]. Furthermore, vagotomy prevented peripheral ghrelin's effect on the hypothalamus [12], suggesting that ghrelin's direct effect on the brain may be of intrinsic origin [13, 14]. Neurons in the hypothalamus and septum are reported to be immunopositive to ghrelin [4] and likely to release ghrelin [15]. The septal neurons project

directly to the hippocampus making monosynaptic connections [16]. Thus, centrally produced ghrelin in the septal neuron could be a source of ghrelin for hippocampal synapses, independently of systemic ghrelin, and contributes to neuron plasticity leading to contextual learning.

1.4. Investigating hippocampal GHSR1a in isolation

The hippocampus receives hypothalamic and arcuate projections directly from the fornix, while being situated centrally for functional interactions with other limbic cortexes by exchanging reciprocal synaptic connections. Thus, GHSR1a in the hippocampus is likely a direct target of hypothalamic and limbic cortical inputs including those from the septum. Each of these inputs may provide ghrelin independently or collectively to the hippocampal GHSR1a. Identifying the source(s) of ghrelin that affects hippocampal neuron function is an important scientific issue. Unfortunately, it is beyond the scope of this chapter because our goal is to first identify cellular and molecular mechanisms underlying the GHSR1a-involved process of hippocampal learning. Here, we review our progress in determining direct effects of ghrelin and GHSR1a-mediated cellular signaling in the hippocampal neuron synapse transmission and plasticity in isolation, without secondary modulation originating in the extra-hippocampal network activity, by using the hippocampal slice culture model and exogenous application of ghrelin.

2. Localization of GHSR1a in the hippocampus of cultured slices

2.1. Fluorescent ghrelin binding

We demonstrate the localization of GHSR1a in the cultured hippocampal slices with the use of three different methods. First of all, an octanoylated form of FITC-conjugated ghrelin (1 μ M) was used in the rat hippocampal slice culture. Ghrelin can bind to GHSR1a only when it is octanoylated [17]. Thus, FITC-conjugated octanoylated ghrelin is a useful and reliable molecule that specifically binds to the receptor [18]. Non-octanoylated form of FITC-conjugated ghrelin was used as a control in order to confirm the specificity of octanoylated ghrelin binding. After 1 h of incubation followed by fixation (with 4% paraformaldehyde), intense binding was detected in the pyramidal cell layer of all CA fields with the given focal plane shown in **Figure 1a** [19]. However, by imaging FITC signals in many different focal planes in the same specimen using a confocal microscope (Fluoview 1000, Olympus), we learned that the actual binding ranged from dentate gyrus granule cell layer to CA1, CA2, and CA3 pyramidal cell layers, suggesting that GHSR1a has ample expression in the cultured hippocampus and is widely distributed throughout the dentate gyrus and Ammons horn.

2.2. eGFP-tagged GHSR1a expression in transgenic mouse hippocampus in slice culture

According to the eGFP-tagged reporter gene mapping of the whole mouse brain, GHSR1a is reported to be highly localized in the hippocampus [20]. However, it is not determined whether transgenic mouse hippocampus retains the expression of functional GHSR1a in the

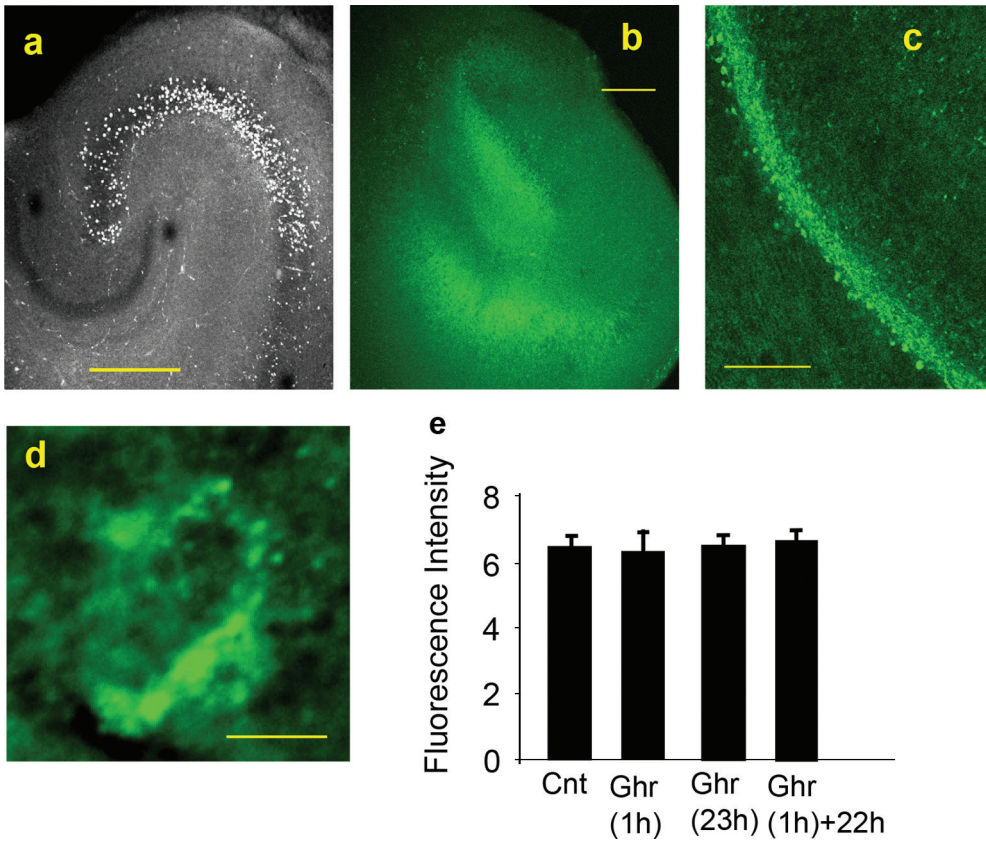


Figure 1. Localization of GHSR1a in cultured hippocampus in slices. **a.** Binding of the octanoylated form of FITC-conjugated ghrelin. **b.** eGFP signals from GHSR1a-expressing cells in mouse hippocampal slices. **c.** eGFP signals from CA1 pyramidal cell layer. **d.** GHSR1a immunoreactivity. **e.** GHSR1a expression was quantified in an arbitrary scale in control, 1-h incubation in ghrelin, 23-h incubation in ghrelin, and 1-h incubation in ghrelin followed by 22-h incubation in control media without ghrelin. Calibrations: (a) and (b) 500 μ m, (c) 200 μ m, and (d) 5 μ m. (Modified with permission from Muniz and Isokawa [19]).

in vitro culture. Hippocampal slices were prepared from eGFP-tagged GHSR1a-expressing transgenic mouse brains, where the eGFP gene was inserted downstream of the GHSR1a promoter (Jackson Lab, B6;129S7-Ghsr<tm2Rgs</J/Stock# 019908). Up to 3 weeks in culture, the localization of GHSR1a was identified by live-imaging eGFP fluorescence. **Figure 1b** shows eGFP signals that are visible in the infra-pyramidal blade of the dentate gyrus and the pyramidal cell layer of the CA1 region. With higher magnification, the CA1 pyramidal cell layer became clearly detectable with resolution that is sufficient to identify individual neurons (**Figure 1c**). Under different focal planes, eGFP signals were detected in other CA fields (i.e., CA2 and CA3) in the pyramidal cell layer and from the supra-pyramidal blade of the dentate gyrus granule cell layer.

2.3. Immunohistochemistry of GHSR1a

In addition to receptor binding and eGFP tagging, we investigated the localization of GHSR1a immunohistochemically in the cultured rat hippocampal slices using two different antibodies raised against GHSR1a (rabbit polyclonal anti-GHSR1a from Phoenix Pharmaceutical, Burlingame, CA, and from Santa Cruz Biotechnology Lab, Santa Cruz, CA). With both antibodies, GHSR1a immunoreactivity was successfully detected in the dentate gyrus and the Ammons horn of our rat hippocampal slice culture. More specifically, fluorescent signals collected from secondary antibody (Alexa 488, Life Technologies, Grand Island, NY) by confocal imaging revealed that GHSR1a was localized primarily as numerous aggregates surrounding the soma (**Figure 1d**). Together, these observations provide cellular and molecular evidence that the ghrelin receptor, GHSR1a, is expressed and localized in the cultured rat and mouse hippocampus in slices in a similar manner to what was reported in the *in vivo* whole brain specimen.

2.4. Receptor internalization in the hippocampal slice culture

GHSR1a exhibits an unusually high constitutive activity, which is the ability to propagate the intracellular signal in the absence of agonist [21, 22]. Thus, it may be possible that the downstream-signaling level, determined by constitutive activity, could reflect membrane expression of the receptor. In addition, agonist-induced internalization is a widely acknowledged process among all G-protein-coupled receptors including GHSR1a [23]. Thus, we were concerned whether GHSR1a in our hippocampal slice culture might have undergone such a process during the application of ghrelin in our experiments. Live slice cultures were incubated in ghrelin (100 nM) for either 1 or 23 h. At the end of the incubation, the specimens were fixed and processed for immunohistochemistry. GHSR1a immunoreactivity was imaged with a confocal microscope and quantified using image analysis software (IPLab, BD Bioscience, San Jose, CA). We did not find any difference in the magnitude of GHSR1a immunoreactivity between 1-h incubation and 23-h incubation, when compared with control (**Figure 1e**) [24]. We also conducted a “second” control, which was to incubate the slices in ghrelin for 1 h and the additional 22 h in control media (without ghrelin). At the end of the experiment, slices were fixed and processed for immunohistochemistry. There were no differences in the GHSR1a immunoreactivity between control and the “second” control. This finding suggested that GHSR1a did not appear to be internalized by the exogenous application of agonist to a significant extent even if a long-term incubation of 23 h was employed. There was a report that GHSR1a hardly desensitized with the nM range of concentrations of ghrelin [25], which is in agreement with our observation and supports the present finding.

3. Phosphorylation of GluN1 by ghrelin

Subunit phosphorylation is a critical step to facilitate the NMDAR channel function and to induce plasticity in excitatory synapses [26]. Among various phosphorylation sites in the NMDAR, we focused on the phosphorylation of Ser 896 and Ser 897 in the GluN1 subunit. The phosphorylation of Ser 896 is dependent on protein kinase C, and the phosphorylation

of Ser 897 is dependent on protein kinase A [27]. The reason for investigating these two phosphorylation sites are as follows: (1) GHSR1a is a Gq-coupled receptor, so that the activation of GHSR1a initiates inositol trisphosphate-mediated-signaling pathways leading to the activation of protein kinase C [28]; and (2) GHSR1a can cause a robust activation of cAMP/PKA-signaling cascade, which has been reported in the process of procuring sufficient energy [29].

3.1. Dose-dependent increase of pGluN1

Phosphorylation of GluN1 was studied using a goat polyclonal anti-pGluN1 at Ser 896/897 (Santa Cruz Biotechnology, Santa Cruz, CA) and Alexa 488 secondary antibody. Phosphorylated GluN1 (pGluN1) was quantified based on the fluorescent signal, captured by a confocal microscope. Representative pGluN1 signals were manually selected as a strongly fluorescing small puncta (examples are shown by yellow arrows in **Figure 2a**). A total of 236 representative puncta were manually selected from 30 confocal images, and the area and intensities of these puncta were measured using IPLab (BD Bioscience, San Jose, CA). Based on the measurement, selection criteria were established and applied to 2024 confocal images taken from 157 hippocampal slices. Among them, 35513 pGluN1 immunoreactive puncta, taken from 1714 confocal images, satisfied the selection criteria, and were used for data analysis.

Exogenous application of ghrelin (1–1000 nM) increased the magnitude of phosphorylation in GluN1 in a dose-dependent manner. The ghrelin-induced increase in pGluN1 immunoreactivity peaked at a concentration of 10 nM ($r^2 = 0.899$, $p < 0.0001$, $n = 56$; analysis of variance (ANOVA)) (**Figure 2b**). The ghrelin-induced changes in pGluN1 immunoreactivity were sensitive to and inhibited by the GHSR1a antagonist, D-Lys3-GHRP6 (1 μ M), and an inverse agonist, substance P-analog (10 μ M), when they were applied with ghrelin (100 nM). However, the application of D-Lys3-GHRP6 or substance P-analog alone (without ghrelin) did not have any effect as compared with control ($p < 0.001$, $n = 47$, unpaired t -test) (**Figure 2c and d**). This suggested the possibility that (1) there were few endogenous ghrelin molecules present in our cultured hippocampal slices, or (2) constitutive activation of GHSR1a (if any) did not appear to affect the phosphorylation state of GluN1 in cultured hippocampus in slices.

3.2. Effect of ghrelin on pGluN1 in GHSR1a knockout mouse

In cultured hippocampal slices prepared from homozygous GHSR1a knockout (-/-) mice, exogenous application of ghrelin (100 nM) failed to cause any change in pGluN1 immunoreactivity (**Figure 2e and f**) when compared with the wild-type GHSR1a (+/+) mouse hippocampus ($p < 0.005$, $n = 31$, unpaired t -test) (**Figure 2e and f**). Slices prepared from heterozygous GHSR1a knockout (+/-) mouse hippocampus also failed to show any change in pGluN1 immunoreactivity in response to ghrelin (100 nM) (**Figure 2e and f**). This suggested that a small reduction in the expression of GHSR1a could negate the ghrelin's effect on the GluN1 subunit phosphorylation at Ser 896/897. Furthermore, we found that the baseline pGluN1 was elevated in homozygous GHSR1a knockout (-/-) mice as compared with wild-type GHSR1a (+/+) mice in both control and ghrelin. This suggested that the NMDAR channel may be hyperactive in the hippocampus of homozygous GHSR1a knockout (-/-) mouse. It might be

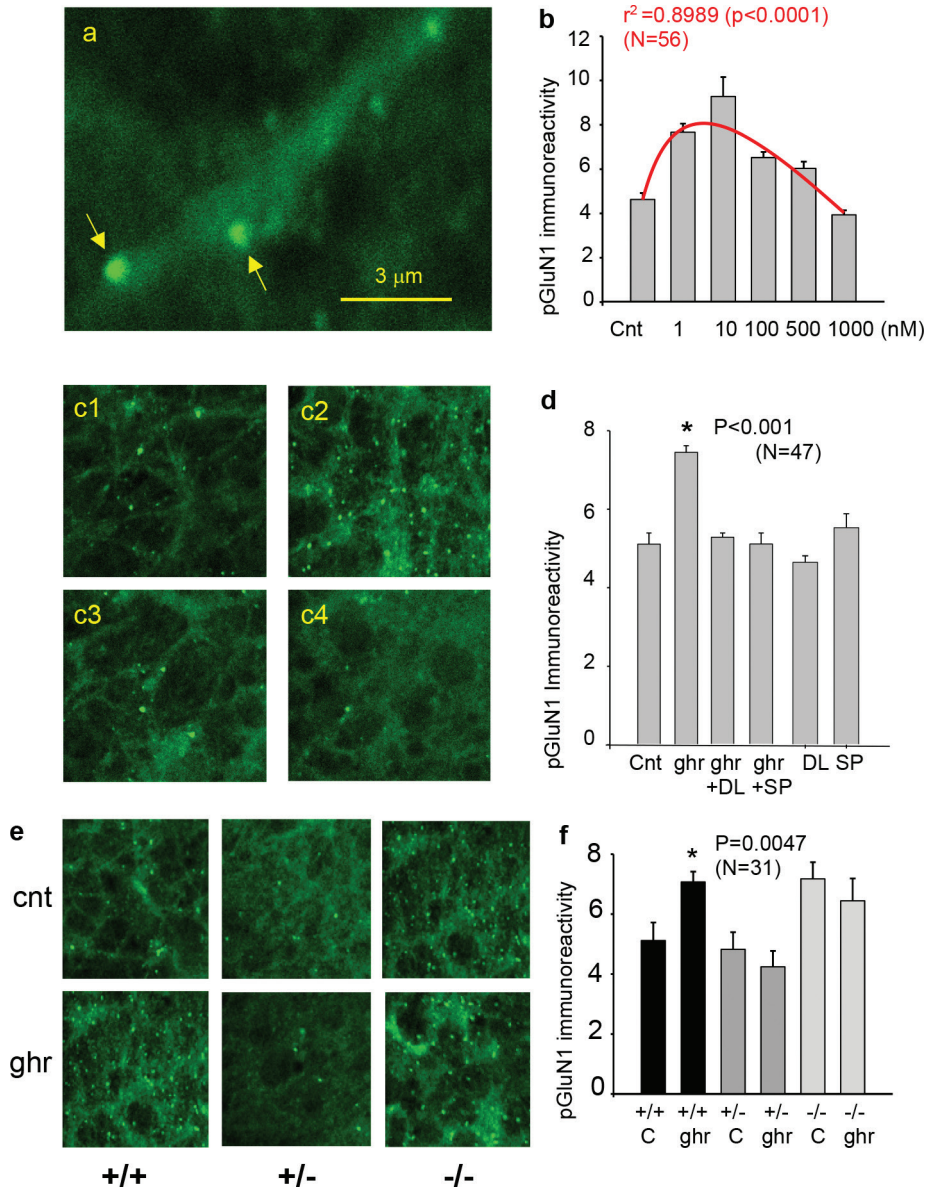


Figure 2. pGluN1 immunoreactivity in cultured rat hippocampal slices. **a.** Representative pGluN1 signals are indicated by yellow arrows. **b.** Dose-dependent changes in pGluN1. **c1–c4.** pGluN1 in control (c1), ghrelin (c2), ghrelin and D-Lys3-GHRP6 (c3), and ghrelin and substance P-analogue (c4). **d.** Quantification of pGluN1 in control (c), ghrelin (ghr), ghrelin and D-Lys3-GHRP6 (ghr + DL), ghrelin and substance P-analogue (ghr + SP), D-Lys3-GHRP6 alone (DL), and substance P-analogue alone (SP). **e.** pGluN1 in control (cnt) and ghrelin (ghr) in wild type (+/+), heterozygous GHSR1a knockout mice (+/-), and homozygous GHSR1a knockout mice (-/-). **f.** Quantification of pGluN1 in wild-type and GHSR1a knockout mice. (Modified with permission from Muniz and Isokawa [19]).

possible that some compensatory mechanisms had switched on under the total absence of GHSR1a, in order to readjust homeostatic balance in the NMDAR channel activity.

3.3. Endocannabinoids negatively modulate ghrelin's effect on pGluN1

Endocannabinoids (eCB) and the type 1 cannabinoid receptor (CB1R) have been implicated essentially in regulating a feeding behavior. They stimulate hypothalamic orexigenic neurons, enhance appetite, and initiate food consumption [30]. Interestingly, there is a report to suggest that ghrelin may exert its orexigenic effect through the endogenous cannabinoid system by producing eCBs in the hypothalamus [31]. However, to date, there is no evidence in the hippocampus that a similar interaction might occur between ghrelin and the endocannabinoid system. We tested if eCBs such as 2-AG (2-arachidonoylglycerol) and anandamide might modulate the effect of ghrelin on the phosphorylation of GluN1 [32].

Hippocampal slices were incubated in 20 nM of R(+)-methanandamide (non-hydrolyzing form of anandamide) together with 100 nM of ghrelin. The magnitude of pGluN1 immunoreactivity remained unchanged when compared to the control ($p = 0.939$) (**Figure 3a**), suggesting that the effect of ghrelin on pGluN1 was negated by R(+)-methanandamide. Interestingly, the inhibitory effect of R(+)-methanandamide was not blocked by the CB1R antagonist, AM251 (5 μ M), which suggested that R(+)-methanandamide exerted its effect independently of CB1R. We then tested the antagonist of TRPV1 (transient receptor potential vanilloid type 1), since anandamide is also an agonist of this receptor [33]. Neither capsazepine (caps, 5 μ M) nor iodoresiniferatoxin (IRTX, 10 nM), antagonists of TRPV1, was effective of blocking the inhibitory action of R(+)-methanandamide (**Figure 3a**). It may be possible that R(+)-methanandamide directly inhibited the NMDAR channel, since anandamide was reported to directly interact and inhibit nonspecific cation-permeable receptor channels [34].

Next, we tested the effect of 2-AG. Similar to the result of R(+)-anandamide, the application of 2-AG (10 μ M) negated the stimulatory effect of ghrelin on the phosphorylation of GluN1 (**Figure 3b**). A negative effect of 2-AG was blocked by the CB1R antagonist, AM251 (5 μ M), suggesting that the effect of 2-AG was exerted through the activation of CB1R. A synthetic agonist of CB1R, WIN 55,212 (4 μ M), also blocked the ghrelin's stimulatory effect on pGluN1 in the CB1R-dependent manner. We then applied 150 mM KCl in the attempt of depolarizing neurons and mobilizing endogenous 2-AG (instead of exogenously applying 2-AG). The application of KCl mimicked the inhibitory effect of 2-AG on the ghrelin-mediated enhancement of GluN1 phosphorylation. The magnitude of pGluN1 remained unchanged in the presence of ghrelin during KCl application and was comparable to control. KCl-mediated inhibition of the ghrelin's stimulatory effect was blocked by the CB1R antagonist, AM251 (5 μ M), suggesting that the application of KCl successfully mobilized endogenous 2-AG. Finally, we used an inhibitor of MAGL (monoacylglycerol lipase), JZL184 (100 nM). MAGL is the degradation enzyme for 2-AG. Thus, JZL184 slows down the rate of 2-AG degradation while maintaining an elevated concentration of ambient 2-AG and making the effect of endogenous 2-AG longer and more intense. As shown in **Figure 3b**, JZL184 was effective of negating the ghrelin's action on the phosphorylation of pGluN1 ($p < 0.0001$), and this effect was reversed by the CB1R antagonist, AM251 ($p < 0.05$). Although we cannot estimate the

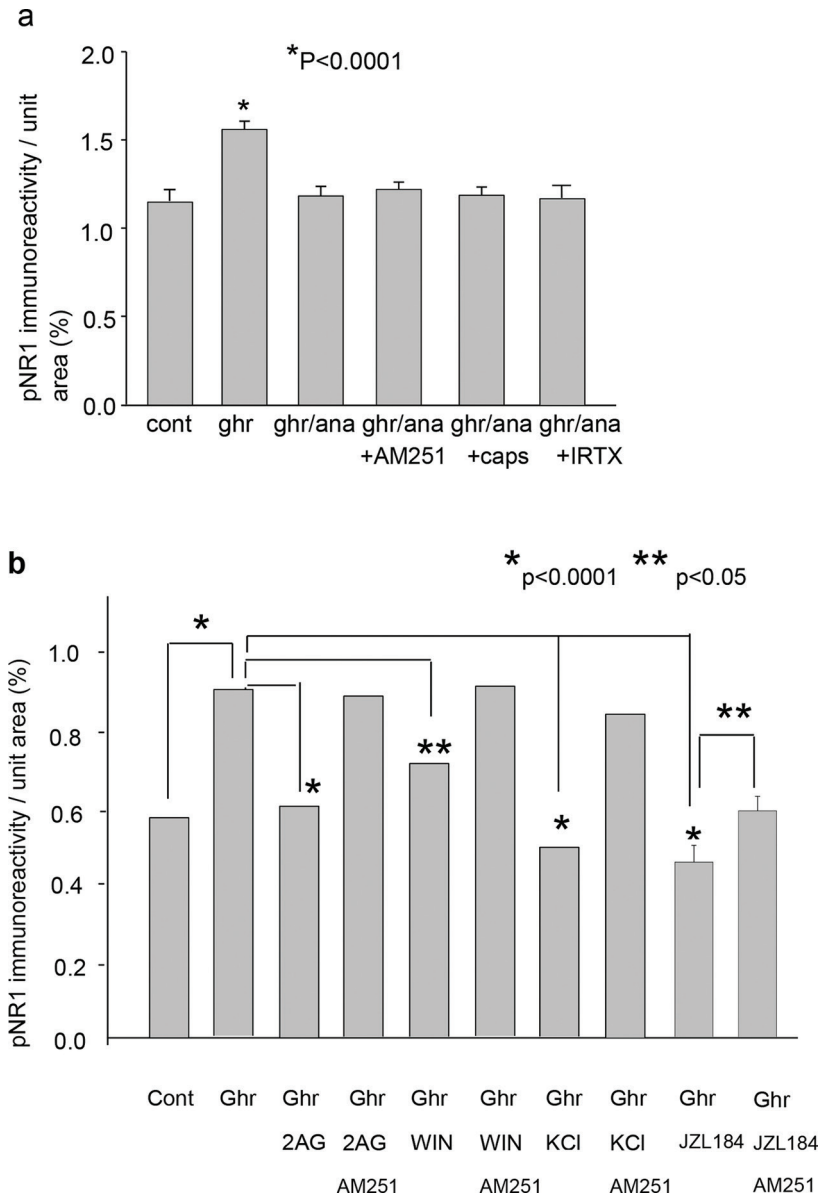


Figure 3. Effects of endocannabinoids on ghrelin-induced increase in pGluN1. **a.** Co-application of ghrelin (200 nM) and R(+)-methanandamide (20 nM) blocked the stimulatory effect of ghrelin on the phosphorylation of GluN1. This inhibitory effect of R(+)-methanandamide was independent of CB1R and TRPV1. **b.** Co-application of ghrelin (200 nM) with 2-AG (10 μ M), WIN55,212 (4 μ M), KCl (150 mM), and JZL 184 (100 nM), all negated the stimulatory effect of ghrelin on the phosphorylation of GluN1. The CB1R antagonist, AM251 (5 μ M), blocked the inhibitory effect of 2-AG, WIN55212, KCl, and JZL 184 on the phosphorylation of GluN1, indicating that these compounds exerted their effects through the activation of CB1R. (Modified with permission from Cuellar and Isokawa [32]).

intrinsic concentration of 2-AG and the rate of increase by JZL184 in our hippocampal slice culture, a physiological range of fluctuation in the concentration of eCBs appears to be sufficient to interact and modulate the ghrelin-signaling cascade on the GluN1 subunit.

4. Ghrelin amplifies NMDAR-mediated synaptic currents

4.1. Ghrelin on evoked NMDAR-EPSCs

NMDAR-EPSCs (*N*-methyl-D-aspartate receptor-mediated excitatory postsynaptic currents) were isolated with a patch electrode in the whole-cell voltage-clamp configuration in single CA1 pyramidal cells at a holding potential of +40 mV (Axopatch 200A and pClamp 10, Molecular Devices), while stimulating the stratum radiatum every 20 s. Extracellular solution (ACSF: artificial cerebrospinal fluid) contained 50 μ M picrotoxin and 10 μ M NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[F] quinoxaline) in order to block GABAergic induced pluripotent stem cells (IPSCs) and AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor-mediated EPSC component. A brief local application of ghrelin (100 nM) enhanced NMDAR-EPSCs ($*F = 29.12$, $p < 0.001$, $**F = 28.39$, $p < 0.005$, $n = 30$, *one-way repeated measure ANOVA*) (**Figure 4a**). This increase was sensitive to D-Lys3-GHRP-6 (GHSR1a antagonist, 100 μ M). Subsequent bath application of D-Lys3-GHRP6 reduced the amplitude of NMDAR-EPSCs ($*t = 10.18$, $p < 0.0006$, $**t = 14.96$, $p < 0.0002$, $n = 41$, *unpaired t-test*). Complete blockade of EPSCs by APV (100 μ M, the antagonist of NMDAR) supported our interpretation that the recorded EPSCs were indeed generated solely by the activation of the NMDAR.

Interestingly, in the absence of exogenous ghrelin, NMDAR-EPSCs were reduced in the peak amplitude in response to D-Lys3-GHRP6 (**Figure 4c**) ($*F = 63.58$, $p < 0.001$, $n = 37$, *one-way repeated measure ANOVA*). This reduction was reversible. Upon washout of the antagonist of GHSR1a, the amplitude of NMDAR-EPSCs recovered. We suggest that, in cultured hippocampal slices, (1) GHSR1a is likely to be constitutively active and (2) endogenous ghrelin might be present and available for the NMDAR. Finally, none of the responses presented above with ghrelin and the GHSR1a antagonist, D-Lys3-GHRP6, were generated in slices prepared from GHSR1a knockout mice ($F = 0.772$, $p > 0.77$, $n = 18$, *one-way repeated measure ANOVA*).

4.2. Ghrelin on spontaneous NMDAR-EPSCs

Spontaneously occurring NMDAR-EPSCs (sEPSCs) responded to exogenous application of ghrelin and the GHSR1a antagonist similarly to evoked NMDAR-EPSCs. The amplitude of sEPSCs was 100 pA in average in control ACSF (**Figure 4d1**). However, it was increased by twofolds in response to exogenous application of ghrelin (**Figure 4d2**). The increase was recovered to the control level following the bath application of D-Lys3-GHRP6 (100 μ M) (**Figure 4d3**). Although the amplitude of sEPSCs changed in response to the application of agonist and antagonist of GHSR1a, the frequency of sEPSCs did not change significantly, suggesting that the effect of ghrelin and GHSR1a signaling was likely postsynaptic.

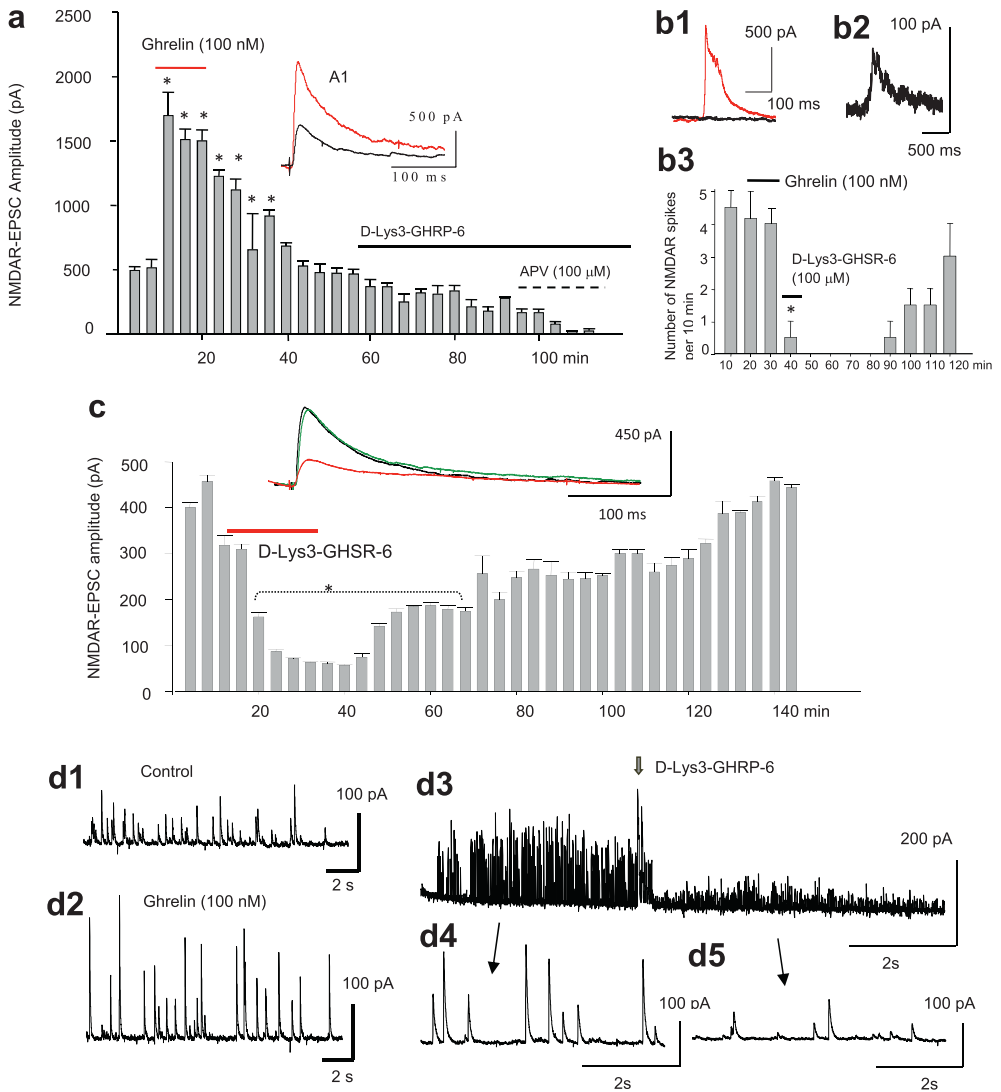


Figure 4. Effect of ghrelin on the NMDA receptor–mediated EPSCs. **a**, Single pyramidal neurons in the CA1 of cultured rat hippocampal slices were voltage clamped at +40 mV in the presence of picrotoxin and NBQX. Stratum radiatum was stimulated every 20 s in order to avoid a rundown. Recording pipette contained cesium-based solution with QX-314 and MgATP. Local brief puff application of ghrelin (100 nM) increased the EPSC amplitude transiently, which was blocked by the GHSR1a antagonist, D-Lys3-GHRP6 (100 μM), and was totally eliminated by the NMDAR antagonist, APV (100 μM). **b**, NMDA spike currents in control (red trace in **b1**), in the presence of D-Lys3-GHRP6 (black trace in **b1**), and in wash (**b2**). Bar graph in **b3** represents the occurrence of NMDA spikes per 10 min for a total of 120 min of gap-free recording. **c**, Reversible inhibition of NMDAR-EPSCs by D-Lys3-GHRP6 in the absence of ghrelin. **d1–4**, Spontaneous NMDAR-EPSCs. (Modified with permission from Muniz and Isokawa [19]).

4.3. Ghrelin on NMDA spike currents

NMDA spikes are spontaneously generated local electrical signals at dendritic branches [35] where NMDARs are highly localized [36]. The generation of NMDA spikes is promoted by glutamate spillover at any single point in the entire dendritic tree [37] that may involve extra-synaptic receptors [38]. In our rat hippocampal slice culture, NMDA spike currents were insensitive to exogenous application of ghrelin. However, the generation of NMDA spike currents was blocked by the bath application of GHSR1a antagonist, D-Lys3-GHRP6, in the absence of ghrelin (**Figure 4d**) (*chi-square* = 20.135, $p < 0.045$, $n = 12$, *Friedman one-way repeated measure ANOVA*). The blockade of NMDA spike currents by the GHSR1a antagonist was reversible, and upon washout of D-Lys3-GHRP6, NMDA spike currents recovered. Although we cannot rule out the possibility that D-Lys3-GHRP6 interacted with the NMDAR directly, inhibiting its function independently of GHSR1a [39], our finding of the inhibitory effect of D-Lys3-GHRP6 on the NMDA spike suggests that GHSR1a-mediated intracellular signaling modulates the activity of extra-synaptic NMDARs and supports our interpretation that GHSR1a is likely present on pyramidal cell dendrites with physical proximity to the NMDA receptor.

5. Ghrelin-induced phosphorylation of CREB

The family of CREB (cAMP response element-binding protein) transcription factors is involved in a variety of biological processes including the plasticity of the nervous system [40]. In order for CREB to be active, it needs to be phosphorylated before being translocated to the nucleus. Thus, the identification of a phosphorylated CREB is a reliable assay for predicting the occurrence of plasticity, learning, and memory in neurons. We previously reported in the *in vivo*-fasting model in rats that metabolic demand stimulated and upregulated the phosphorylation of CREB by twofolds in the hippocampus together with other limbic cortexes such as piriform cortex, the entorhinal cortex, and the cortico-amygdala transitional zone [41]. Here, we discuss the NMDA receptor-mediated and ghrelin-enhanced phosphorylation of CREB in our cultured hippocampal slices.

5.1. Ghrelin-stimulated phosphorylation of CREB

CREB activity was assayed immunohistochemically using a rabbit polyclonal antibody against phosphorylated CREB (pCREB at Ser 133) (Cell Signaling, Danvers, MA) (**Figure 5a–c**). pCREB immunoreactivity was quantified using an auto-segmentation tool provided by IPLab imaging software. Low concentrations of ghrelin in 50 and 100 nM did not have any effect on pCREB expression. However, 200 nM and above concentrations of ghrelin increased the expression of pCREB by fourfolds compared to control ($p < 0.01$) (**Figure 5d**). The magnitude of pCREB expression was not different among 200, 500, and 1000 nM. A steep change in response to differing concentrations of ghrelin may be explained by the unique process of ghrelin des-octanoylation [42]. Finally, the effect of ghrelin was mediated by GHSR1a, since the antagonist of GHSR1a, D-Lys3-GHRP6 (100 μ M), blocked the ghrelin-mediated increase in the expression of pCREB (**Figure 5e**).

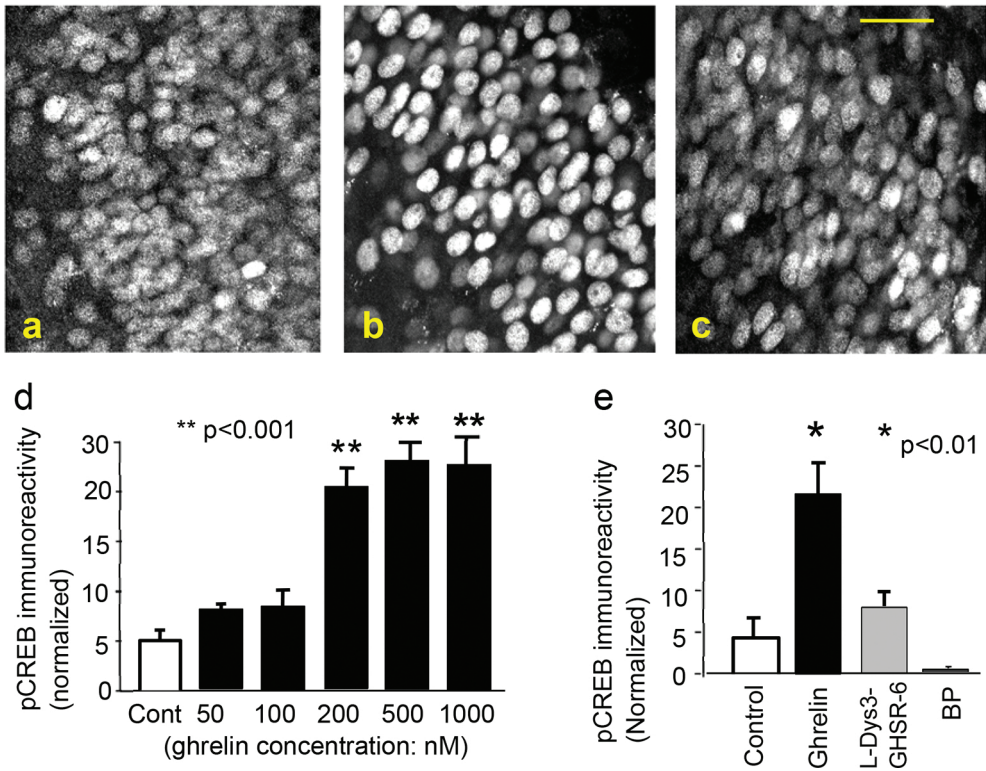


Figure 5. Effects of ghrelin on pCREB. **a–c.** pCREB immunoreactivity in control (**a**), ghrelin (**b**, 200 nM), and in *D*-Lys3-GHRP6 (**c**, 100 μM). **d.** pCREB in response to ghrelin doses. **e.** Summary graph of pCREB in control, ghrelin, and *D*-Lys3-GHRP6. Calibration: 30 μm. (Modified with permission from Cuellar and Isokawa [32]).

5.2. Effect of endocannabinoids on ghrelin-mediated upregulation of pCREB

Synergistic involvement of the endogenous cannabinoid system is suggested in the ghrelin-mediated CREB phosphorylation in the hypothalamus [31]. However, in the hippocampus, the contribution of endocannabinoids and the cannabinoid receptor in short- and long-term plasticity has been explained independently of ghrelin and GHSR1a. Furthermore, in Section 3.3, we discussed that ghrelin-mediated enhancement of GluN1 subunit phosphorylation appeared to be negatively modulated, instead of synergistically amplified, by eCBs. Here, we examined potential interactions of the endogenous cannabinoid system to ghrelin-induced hippocampal plasticity at the level of CREB phosphorylation.

A low concentration (20 nM) of R(+)-methanandamide, a nonhydrolyzing form of anandamide, inhibited ghrelin-induced increase of pCREB (**Figure 6**). This inhibitory effect of R(+)-methanandamide was not blocked by the CB1R antagonist AM251 (5 μM) or the TRPV1 antagonist capsazepine (5 μM), suggesting that the action of R(+)-methanandamide on the ghrelin-mediated phosphorylation of CREB may be independent of the CB1R or TRPV1.

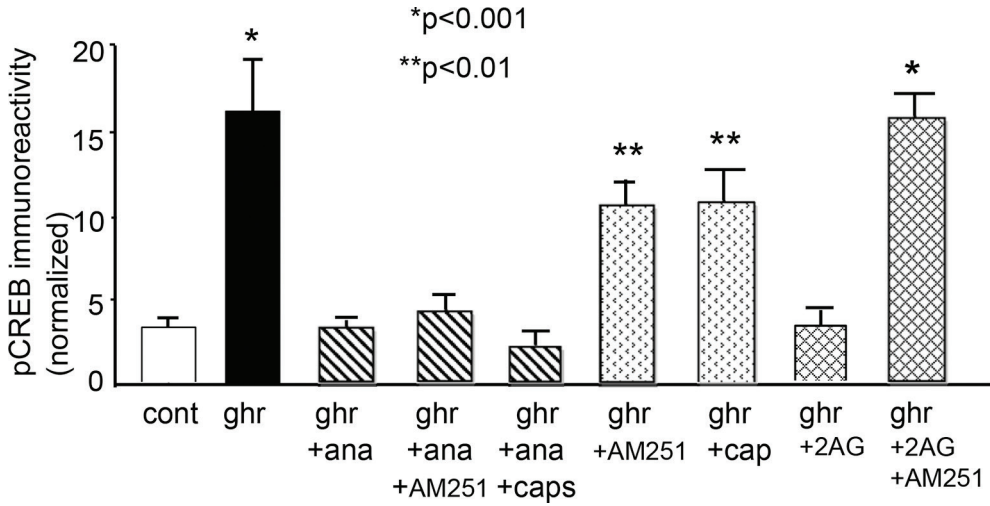


Figure 6. Effects of eCBs on ghrelin-mediated phosphorylation of CREB in the hippocampus. (Modified with permission from Cuellar and Isokawa [32]).

Furthermore, incubation of slices in AM251 alone (without anandamide) or in capsazepine alone (without anandamide) did not block a ghrelin-induced increase in CREB phosphorylation. These results suggested that neither CB1R nor TRPV1 appeared to be involved in the negative effect of R(+)-methanandamide on the ghrelin-induced phosphorylation of CREB.

We next examined the effect of 2-AG on the ghrelin-induced upregulation of pCREB. Similar to R(+)-methanandamide, 2-AG (10 μ M) inhibited ghrelin-induced increase in pCREB (Figure 6). However, in contrast to R(+)-methanandamide, the inhibitory effect of 2-AG was blocked by CB1R antagonist AM251, suggesting that the action of 2-AG was mediated through the activation of CB1R.

Although we cannot rule out the possibility that eCBs negatively modulated the ghrelin's stimulatory effect on CREB phosphorylation independently of the phosphorylation of the NMDAR GluN1 subunit, our interpretation is that the target of the negative effect of eCBs is the NMDAR, because (1) GluN1 phosphorylation by ghrelin was negated by both 2-AG and anandamide in the identical manner to CREB phosphorylation and (2) the NMDAR is situated upstream of the signaling cascade of CREB activation, having the NMDAR as a necessary molecule in the induction of hippocampal synaptic plasticity.

6. Ghrelin and dendritic spines

CREB-induced gene expression includes reorganization of cytoskeletal proteins. Diano et al. [10] reported that ghrelin upregulated the number of spine synapses in the hippocampus. However, it is elusive whether the increase in synapse occurred on existing spines or on newly generated spines. We examined changes in the number of dendritic spines with a

hypothesis that ghrelin might stimulate the generation of dendritic spines. Polymerized actin (F-actin) is highly localized in dendritic spines. Thus, we used phalloidin, a mushroom toxin that has a high affinity to F-actin, as a marker for the identification of dendritic spines. Alexa 488-conjugated phalloidin was visualized and relative changes in fluorescence puncta were quantified using confocal microscope and imaging software (IPLab) (**Figure 7a**).

6.1. Short-term effect of ghrelin on dendritic spines

Ghrelin was applied for 60 min with a concentration of 200 nM to cultured rat hippocampal slices. At the end of the incubation, the slices were fixed with 4% paraformaldehyde and treated with fluorescent phalloidin for confocal visualization of dendritic spines. In control, the average spine density, measured as phalloidin fluorescence was 0.302/unit area \pm 0.039 standard error of mean (SEM) ($n = 30$ images taken from 10 slices). Ghrelin increased the average spine density to 0.499/unit area \pm 0.058 SEM ($n = 30$, $p < 0.001$) (**Figure 7b**). The antagonist of GHSR1a, D-Lys3-GHRP6 (100 μ M), blocked the ghrelin's stimulatory effect on spine generation, and the average spine density returned to the control level (0.333/unit area \pm 0.041 SEM, $n = 30$). These results suggested that ghrelin may increase the number (or the size) of spines by activating GHSR1a and the downstream-signaling molecules, and that 60 min of incubation in ghrelin is sufficient to induce the generation of "new" spines.

6.2. Long-term effect of ghrelin on dendritic spines

Ghrelin was applied for 23 h at a concentration of 200 nM to cultured rat hippocampal slices. At the end of the incubation, the slices were fixed and treated with fluorescent phalloidin for confocal visualization. Similar to the 60-min application, ghrelin-treated slices expressed a higher density of dendritic spines compared with the control (0.618/unit area \pm 0.043 SEM, 30 images from 10 slices, $p < 0.001$) (**Figure 7c**). Spine density remained elevated at the end of the 23-h application. Pre-application of slices with D-Lys3-GHRP6 for 2 h before the application of ghrelin blocked the increase of phalloidin fluorescence, and the spine density stayed at a control level (0.322/unit area \pm 0.024 SEM, $n = 30$).

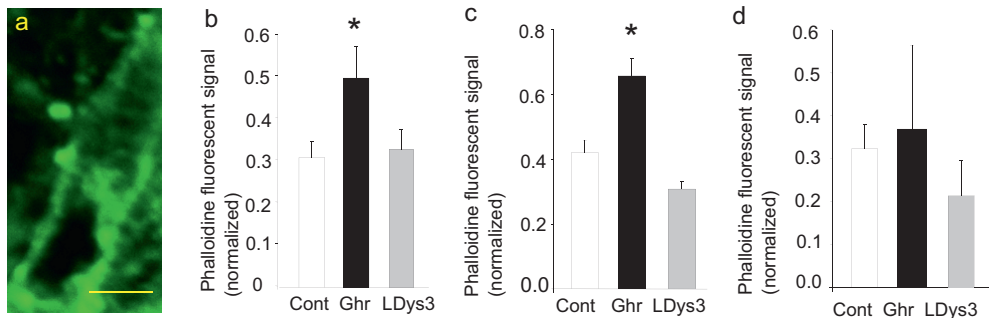


Figure 7. Dendritic spines visualized with Alexa 488-conjugated phalloidin. **a.** Representative phalloidin image. **b.** Phalloidin signals in response to 60-min incubation in ghrelin. **c.** Phalloidin signals in response to 23-h incubation in ghrelin. **d.** Phalloidin signals in response to 22-h incubation in control media after 60-min application of ghrelin. (Modified with permission from Berrout and Isokawa [24]).

6.3. Ghrelin is required to maintain “newly added” spines

Our results indicated that a 60-min application of ghrelin was sufficient to increase spine density. Our results also showed that spine density remained elevated after 23-h application of ghrelin. A question raised from this result is whether the maintenance of elevated spine density in 23 h of incubation with ghrelin really required 23 h of continual availability of ghrelin (since 60-min application was sufficient to increase spine density). In order to answer the question, we incubated hippocampal slices in ghrelin-containing culture media for 60 min, then removed the slices from ghrelin-containing media and incubated in control media for additional 22 h without ghrelin. At the end of the incubation period (of 1 h with ghrelin and the subsequent 22 h without ghrelin), the slices were fixed and treated with fluorescent phalloidin for confocal visualization of dendritic spines. At the end of this combined treatment, spine density was 0.370/unit area \pm 0.193 SEM, which was comparable to a control level (0.314/unit area \pm 0.057 SEM) (**Figure 7d**). It appears that spine density can increase in response to ghrelin within 60 min and remain elevated for up to 23 h as long as ghrelin is present. However, once ghrelin is removed and no longer available to activate GHSR1a, “newly added” spines retract and the spine density recovers to a control level. In conclusion, ghrelin can add “new” spines to hippocampal neuron dendrites, and that continual availability of ghrelin is a prerequisite together with non-desensitizing activity of GHSR1a for this form of spine plasticity.

7. Concluding remarks

The hippocampus plays a critical role in employing food-searching strategies. Ghrelin is thought to be essential in order to retain memories regarding the spatial localization of food sources [43]. Food search is typically initiated when metabolic demand increases, and the search typically does not end until the metabolic demand is fulfilled. During fasting, a serum ghrelin level increases and stays increased until fasting ends. The rate of ghrelin crossing the blood–brain barrier also increases in a ghrelin concentration-dependent manner [10]. Although it is not known whether the concentration of intrinsic ghrelin in the hippocampus (if any) may fluctuate with metabolic demand, ghrelin can be a key molecule for metabolic demand–induced neuron plasticity in the hippocampus, which serves as a cellular and molecular substratum for food-related memories and learning. Ghrelin-dependent maintenance of plasticity and the loss of plasticity in the absence of ghrelin may nicely explain when and how long such plasticity is required in order for organisms to successfully exercise adaptive appetitive behavior for survival.

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Plasticity in Damaged Multisensory Networks

Karolina A Bearss and Joseph FX DeSouza

Additional information is available at the end of the chapter

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Abstract

This chapter opens by discussing functional and anatomical locations as well as neural networks of unimodal senses: vision, somatosensation, audition, gustation and olfaction. How and where these unimodal sensory systems intersect and interact with multimodal sensory processes to provide a holistic view of how experiencing complex external objects and events lead to a single multimodal percept. Reviews of current neuropsychological research on damage occurring within both unimodal and multimodal sensory networks further explain the association between these networks and how they operate together in perception. Current research reviews on cross-modal plasticity reveal the neural changes that occur in multisensory areas following brain damage and the potential benefits of this plastic reorganization of the cortex.

Keywords: plasticity, multisensory networks, neural damage, unimodal, multimodal integration

1. Introduction

When we actively engage and interact with objects in our surroundings, our brains inherit an enormous amount of information pertaining to the complexity of these objects and features of the external environment itself. Information from the environment is initially collected by our multiple senses, and then processed and interpreted before motor interaction are planned and executed, all occurring within an instance. Perception has been traditionally viewed as a unimodal sensory process with the different sensory modalities operating as independent processes. However, it is obvious that multisensory interactions must occur in various perceptual tasks and events in order to allow one to have proper interactions with the environment. There are numerous brain areas and pathways for multisensory interaction that will be briefly overviewed within this chapter.

External environmental information is received through sensory modalities such as vision, somatosensation, audition, olfaction and gustation. Each of these sensory modalities is comprised of an organ that contains specialized receptive cells, with corresponding receptive fields that respond to external stimuli. The stimulation of a neuron's receptive field initiates a response related to the size and shape of the stimulus; this information travels via sensory pathways sent to the corresponding primary receiving areas of the cerebral cortex. In general, sensory pathways are neurons that link the sensory receptors at the periphery with the spinal cord, brainstem, thalamus and cerebral cortex.

It is essential to clarify how individual pathways work in unison in order to understand the neuronal changes that arise from damage within these systems as a whole. Plasticity of the spared senses can shed light on how the brain changes as a result of damage, providing important insight related to time management and rehabilitation for a variety of neuropsychological disorders.

Next, we will discuss how the senses combine in multisensory networks that enhance our sensory experiences and reactions to external stimuli. Various neuroanatomical techniques are documented in the existing literature, which have been used on nonhuman primates to pinpoint key multimodal cortical sites such as the superior temporal sulcus (STS), intraparietal sulcus, posterior insula, parietoprecipital cortex, frontal brain regions including the prefrontal, premotor and anterior cingulate (AC) cortex [1–4], and subcortical sites including superior colliculus (SC), claustrum, medial pulvinar nucleus of the thalamus, suprageniculate, hippocampus and the amygdaloid complex [5–7]. The exact functions of these areas and their connections with other multisensory networks will be further examined.

Extensive studies on both animals and humans have shown that during the early stages of development, any environmental alteration or disruption of processing in a single sense results in an enormous fluctuation in favor of neuronal plasticity between multiple senses [8–11]. However, less is known about the potential neuronal changes that arise after damage has occurred to multisensory areas later on in life. Thus, this section will discuss research in cases where damage has occurred within multisensory networks and areas involved in polysensory integration. First, we will describe a study on a patient with Balint's syndrome [12], followed by a report on a case study examining the multisensory effects of damage via infarct in the right primary visual cortex [13]; we will then describe a study which looks at patients with hemianopia and/or neglect in a visual detection task [14]. Lastly, we will describe research on plasticity in multisensory dorsal stream networks involved in nonvisual processing of action [15].

Damage to heteromodal or unimodal sensory areas reduces the effects of multisensory integration that give rise to holistic perception. However, spared multisensory networks have shown to be associated with behavioral benefits through cross-modal plasticity, where the spared heteromodal areas attempt to reconnect the multisensory system and bypass injured areas [9, 11, 14–17].

The final sections of the chapter will discuss some of the research on neuroplastic reorganization that we are currently conducting in our laboratory. In one study, we investigated the effects of long-term professional ballet training on tract fractional anisotropy (FA) lateralization and

extent of tract FA. Research indicates that experience and training modulate brain structural parameters including volume and FA [18]; FA is a diffusion tensor imaging (DTI) derived index of water molecule diffusion sensitive to the collective effect of microstructure properties. The effect of long-term ballet training characterized in terms of tract FA lateralization, extent of tract FA and global volume has not been previously reported. We localized these FA parameters in expert dancers relative to controls using DTI, and results indicate that dancers had greater leftward lateralization in the anterior thalamic radiation (ATR), whereas the non-dancers had greater rightward lateralization and that dancers also had higher FA localized to the left cortical spinal tract (CST). Higher dancer FA implies heightened axonal ability to communicate. The large percentages of variability shared by dancer years of training and the structural metrics FA and global volume implicate a substantive impact of dance training on brain structure.

In another study, we investigated the structural and functional plasticity associated with dance expertise in a cross-sectional pilot study, comparing ballet dancers to controls. Using functional magnetic resonance imaging (fMRI), whole-brain functional activation maps of dancers and controls were compared, while they engaged in motor imagery of dance movements. Anatomically the results reveal that dancers exhibited greater cortical thickness in areas such as the inferior occipital gyrus, inferior frontal gyrus and superior temporal gyrus. We also found years of dance training to be positively correlated with cortical thickness in various regions, including the fusiform gyrus and parahippocampal gyrus. These preliminary results suggested that dance expertise is associated with a functional reorganization that corresponds to reduced activity reported in other motor expertise groups.

Lastly, our lab has investigated the impacts that dance intervention has on people with Parkinson's disease (PwPD) and healthy controls. Dance has been shown to have a positive effect on motor functioning in PwPD, but less well understood are the effects of dance on mood and associated brain activity observed within PwPD. Our aim was to examine the effects of dance on both motor and nonmotor functioning and correlate these potential effects to onsite recordings of resting state electroencephalogram (rsEEG) within the alpha rhythm (6–12 Hz), collected immediately before and after a single dance class. Precisely, we aim to examine and potentially construct brain-related plasticity mechanism(s) as a function of dance. As expected, the preliminary results show an overall improvement in motor impairment after a single dance class in PwPD. We also found differential effects of dance on negative and positive mood for both PwPD and HC. Finally, we show an increase in global alpha power after a single dance intervention. These findings imply that dance promotes changes in affect through its overwhelming positivity, use of imagery, use of imagination, the presence of music and a sense of bonding through partner work. These results are of importance as their implications may allow researchers to better correlate and understand the positive behavioral and physiological benefits of dance for PD, and perhaps can aid in the implementation of dance as a form of rehabilitation for PwPD. We are currently leading a follow-up study examining the results of participation in multiple dance classes overtime in conjunction with our ongoing rsEEG research, which will provide an account for the observed neural changes in global alpha power.

Taken together, these investigations on neural plasticity following dance will supplement the earlier studies discussed in the section on damaged multisensory networks. They provide compelling evidence that plasticity occurs in both healthy and clinical populations following learning, exposure to training and greater experience with a particular task.

2. Unimodal and multimodal sensory systems

2.1. Visual perception

The eye is considered the primary sensory organ for the modality of vision. All external information from both eyes has to travel to the very back of the brain, the primary visual receiving area known as the occipital lobes, before it begins to process toward conscious vision. Libet's famous study, deriving from direct stimulation of the somatosensory cortex (postcentral gyrus), suggests that at threshold intensity for a sensation, visual information can guide actions within one-fifth of a second (200 ms) and that takes about half a second (500 ms) for us to see an object consciously [19, 20].

Most neurons from the retina and lateral geniculate nucleus (LGN) of the thalamus terminate onto the striate cortex (area V1) where initial cortical processing of all visual information first occurs in the perceptual process. Two retinal ganglion cells exist and create two separate pathways, magnocellular (M) and parvocellular (P). M and P pathways become segregated within the LGN, where the bottom two layers consist of M cells, and the upper four layers are made up of P cells. Neural signaling is converted faster by the M cells for the functions of visual motion perception and eye movements, whereas P cells contribute more toward object recognition and face recognition and thus represent more constant stimulus presence. The M and P pathways remain segregated beyond the striate cortex where the M pathway continues along the dorsal stream of the cortex and the P pathway along the ventral stream of the extrastriate cortex (V2) [21]. The dorsal stream passes through area V2, then area V4, and leads to the posterior parietal cortex (PPC), Brodmann's areas 5 and 7, and the middle temporal area (MT). Research on monkeys has shown that MT is responsible for motion processing, representation of object location, control of the eyes and arms for action and is thus referred to as the "where pathway" [21, 22]. Ventral stream begins at V1 then travels through V2–V4 and finally terminates in the inferior temporal area (IT). The ventral stream is devoted to fine analysis of the visual scene and to the perception of color, features and form on an object and is thus referred to as the "what pathway" [21, 22].

Finally, there is still unsatisfactory evidence as to whether these two separate systems converge onto a mutual pathway that explains the neurological basis of visual perception. One way to explore this further is to examine multimodal processing areas and their association with each of the senses.

2.2. Somatosensory systems

Somatic sensibility has four major modalities that provide us with information of objects in the external world: touch (via physical contact with the skin), proprioception (through the

position and movement of our body), nociception (such as pain and itch) and temperature (such as feeling warmth and cold) [23]. Each of these modalities begins at a somatosensory organ with distinct receptors and pathways that lead to the brain; however, all share common sensory neurons known as the dorsal root ganglia neurons (DRG). Tactile and limb proprioception are transmitted to the ventral posterolateral (VPL) nucleus of the thalamus via the dorsal column of the spinal cord known as the medial lemniscus pathway. These neurons project to the primary somatosensory cortex (SI) in the postcentral gyrus located on the parietal lobe where information is processed regarding the perception of your body and the external environment [24], whereas pain and temperature information terminate in the intralaminar nuclei of the thalamus through the anterolateral pathway [25]. These afferents also project to SI, specifically the dorsal anterior insular cortex and to the anterior cingulate gyrus where both deep pain and dull pain are processed [23].

SI performs the initial stage of cortical processing, and an overlap of information from any of the four somatosensory modalities may intermingle in higher cortical areas leading to complexity in neural responses. SI is subdivided into 4 Brodmann's area: 3a, 3b, 1 and 2. Most of the thalamic afferents project to areas 3a and 3b and these areas in turn extensively innervate their axons to areas 2 and 1 [23]. These four regions are differentiated according to their functionality; areas 3b and 1 receive somatosensory information from areas on the skin, whereas areas 3a and 2 receive proprioceptive information from receptors that belong to muscles and joints [23]. Research on monkeys has shown that much of SI projections innervate a secondary brain region, the secondary somatic sensory cortex (SII), which projects to areas responsible for tactile memory within the temporal lobe [26]. Lastly, Brodmann's area 5 receives input from SI and pulvinar and is known to be responsible for the integration of tactile and proprioceptive information [23]. Projections from the PPC innervate motor areas in the frontal lobe which play a role in the sensory initiation and guidance of movement [23].

2.3. Audition

Auditory perception gives rise to the ability to perceive sound in our environments by detecting vibrations and changes in pressure in the air. The ear is considered the organ for auditory perception; however, it also assists us in determining balance of our bodies [23]. The eighth cranial nerve travels and branches onto the cochlear nuclear complex located within the brainstem. From here, axons project to the inferior colliculus within the midbrain and to the nucleus of the lateral lemniscus located within the pons where further processing of sound occurs [23]. All of these afferents then move to the medial geniculate nucleus of the thalamus and end up in the superior temporal gyrus a part of the primary auditory cortex (Brodmann's areas 41 and 42). Once action potentials reach here, the conscious perception and processing of sound occur.

2.4. Olfaction and gustation

The olfactory system, also known as the sense of smell, aids humans in distinguishing an enormous amount of odors that are categorized into various groups; spicy, floral, burnt, resin, fruit and putrid [27]. Mitral and tufted cells act as relay cells from the olfactory bulb to the

olfactory cortex which is subdivided into five areas: (1) anterior olfactory nucleus (AON), an area responsible for the processing of odors [28]; (2) amygdala for memory associated with specific odors; (3) olfactory tubercle mediating multisensory integration of olfactory information; (4) piriform cortex, responsible for olfactory processing; and finally (5) entorhinal cortex (EC) for preprocessing familiar odors [23]. The latter four parts relay information to the orbitofrontal cortex (OFC) via the thalamus and are an area responsible for decision making for expected rewards or punishments given a certain situation. In addition, the olfactory cortex makes direct connection to the frontal cortex itself for value judgments of odors [23].

Gustatory perception, otherwise known as taste, begins with its essential organ, the tongue. There are a total of five basic tastes that the gustatory system distinguishes, and a combination of these basic tastes gives rise to more complex, established tastes: sour, salty, sweet, bitter and umami [23]. Chorda tympani (cranial nerve VII) innervate the anterior two-third of the tongue and soft palate, the glossopharyngeal (cranial nerve IX) innervates the posterior one-third of the tongue, and lastly, both the vagus (cranial nerve X) and glossopharyngeal nerves innervate the epiglottis and the pharynx [23]. These afferent nerves enter the solitary tract and synapse with secondary neurons in the gustatory area of the medulla [23]. These second order afferent neurons project to the ventral posterior medial nucleus of the thalamus [23]. Finally, they project to the gustatory cortex, between the anterior insula and frontal operculum in the ipsilateral cerebral cortex, to provide conscious perception and discrimination of taste [23].

2.5. Multisensory interaction: anatomical and synaptic levels

Objects and situations that we experience in our everyday lives are embedded within rich and complex environment that contain an enormous amount of information. Perceiving, planning and responding to these complex scenarios involve more than a single, isolated sense. Instead a holistic gathering of information across multiple sensory modalities must occur in order to have successful interactions with the external world. This ability to combine sensory information across different modalities enhances both detection and discrimination of external objects [29]. For example, visual sensitivity can be enhanced with the presence of an auditory or tactile stimulus in healthy participants [30]. In this part of the chapter, we will discuss the neural pathways from primary unimodal sensory areas to multimodal association areas involved in sensory integration. After each multimodal description, we will present cases where damage occurred within these areas to provide explanations of their particular functions.

Unimodal association areas discussed above project to multimodal sensory association areas including the parietotemporal and prefrontal cortices, cingulate gyrus, hippocampus and amygdala [23, 31]. In order to plan and compute a movement toward an external object, the multimodal sensory association areas project to multimodal motor association areas where these converging sensory inputs are transformed into planned motor commands or movements [23, 31]. Execution of movement is initiated when the multimodal motor association areas project to premotor (motor preparation) and primary motor (movement execution) cortices [23].

Our knowledge of the nature and localization of neural mechanisms underlying multimodal sensory processing has stemmed from studies involving different animal species while using

a variety of neuroscience techniques. Studies on nonhuman primates using direct cortical recordings have shown convergence of unimodal afferents onto heteromodal cortical sites within the superior temporal sulcus (STS), intraparietal sulcus, posterior insula, parietoprecipital cortex, and frontal regions including the prefrontal, premotor and anterior cingulate (AC) cortices [1–4]. Heteromodal sites have also been found in subcortical structures such as the SC, claustrum, medial pulvinar nucleus of the thalamus, supragenulate, hippocampus and the amygdaloid complex [5–7].

Each multisensory neuron located within the SC contains a map of sensory space corresponding to the senses of audition, vision and tactile sensation. These multimodal sensory maps overlap each other, eliciting activation in the same region of the SC where different sensory modalities innervate the same spatial location on the SC [32]. Research has also shown that multisensory neurons within the SC not only respond to multiple sources of sensory information but are also capable of combining them into an integrated form, when two or more afferent sensory neurons appear in close temporal and spatial proximity. The firing rate of these multisensory neurons sums the impulses for each individual modality and thus increases the firing rate of these cells, in turn resulting in a multimodal neuronal response [33]. In contrast, studies have shown that responses to a stimulus from another sensory modality can substantially lessen a vigorous unimodal sensory response in a different sensory modality [34]. Thus, the SC, both at the population and synaptic level, has shown cross-modal integration mediating attentive and orientation behaviors to external stimuli [33].

Neuroimaging studies in humans have been a bit more convoluted with respect to their findings as the description of the different networks involved in multisensory perception has shown to depend on the type of stimuli being integrated and the demands of the task [35]. Multisensory cortical areas in humans include superior temporal sulcus (STS), intraparietal sulcus (IPS) including the ventral (VIP) and lateral (LIP) intraparietal sulcus, parieto-occipital cortex, posterior insula and frontal regions which include the premotor and prefrontal cortices, parietal cortex and the lateral occipital tactile-visual area [36]. With regard to the subcortical level, multisensory activity has been shown in thalamus [37], ventral and dorsal regions of the cochlear nucleus [38], SC [39] and basal ganglia (BG) [40].

Studies in monkey parietal cortex have shown that area VIP receives multimodal sensory inputs from visual, somatosensory, auditory and polysensory areas [41]; in addition, area LIP connects with areas dealing with spatial vision (visual area MT and the auditory caudiomedial) along with the frontal eye field (FEF) [42] and with inferotemporal cortex (ventral visual pathway) [43]. Also, studies on STS show its connection with the visual occipital cortex and with the auditory association area [44]. In addition, the prefrontal cortex receives projections from auditory and visual cortices, essentially playing the role in temporal integration [45]. Lastly, studies on monkeys have shown interconnected multisensory networks in the putamen, VIP, premotor cortex, and parietal area 7b in the perception of visual, tactile and auditory stimuli presented in peripersonal space (a part of external space that is close to the body or a particular body part) [39, 46].

3. Damage in multisensory networks and plasticity

In this part of the chapter, we will introduce patient cases where damage has occurred in particular areas within multisensory networks at both the local brain regions and synaptic levels that will shed light on the mechanisms behind neural plasticity and cortical reorganization.

One study conducted on a patient with a rare affliction known as Balint's syndrome, observed the effects of spatial visuotactile interactions [12]. Balint's syndrome is a very rare neuropsychological impairment resulting from two or more strokes in the parietal lobes in each hemisphere. The symptoms present themselves as a severe disturbance of external space representation with clinical signs of simultanagnosia (inability to perceive the visual field as a whole), oculomotor apraxia (difficulty in fixating the eyes) and optic ataxia (inability to move hand extremities to a specific external object guided by vision) [47]. Within this study, visuotactile interactions were examined during a tactile discrimination task where spatially congruent or incongruent visual cues were simultaneously presented in either the same or the opposite side as the tactile object near the patient's hand. The results for healthy comparison subjects showed that irrelevant visual events elicited a strong and involuntary orientation of spatial attention during concurrent discrimination of tactile stimuli at the same location that was not observed in the Balint's syndrome patient [47]. This finding explains how the posterior parietal cortex (PPC) contributes to spatial processing of exogenous shifts in attention. In the patient with Balint's syndrome, severe spatial deficits were observed affecting the left hemispace in the visual tasks. More precisely, when stimulating the patient's left hand in the left side of visual space, visuotactile interactions were not modulated by spatially congruent conditions. However, when the right hand was stimulated on the right side of space, performance was affected where improvement in responses occurred when the visual cue was presented on the right side, whereas a visual stimulus near the opposite hand caused an interference [47]. In addition, to dissociate the effects on somatotopic and spatiotopic coordinates, the patient crossed their hands during unimodal tactile discriminations. The results indicated that tactile performance of the left hand was improved when it was crossed over into the right hemispace, whereas no significant changes were found with the crossed over left hand into the right hemispace. The results of the study suggested that from the bilateral PPC damage what was lost was the spatial selectivity of the visuotactile effects, the effects produced from the crossing over of the hands suggest a deficit in egocentric spatial coding with respect to the left tactile stimulus. These results indicate the critical role that the PPC has in the integration of both visual and tactile sensory information.

A case study was conducted on a patient with damage to their right primary visual cortex sustaining loss in their left visual hemifield, left hemianopia [13]. In this study, the researchers performed a visual detection task to compare abilities while varying the position of the patients left arm in space. Variation in the presentation of visual stimuli in space included a baseline condition where the patient's left hand was on their lap, while other conditions presented visual stimuli in various reaching space locations, in locations well out of reach, and lastly a condition where the patient held a tennis racket in their hand in order to extend their reach. The results indicated that the patient's ability to detect visual stimuli in their left blind field was significantly improved with the extension of their contralesional arm into the blind

field [13]. This arm-mediated visual enhancement was restricted to visual stimuli being presented in reaching distance of the hand. Similar results have been reported in cortical activity of VIP and LIP regions in the monkey, regions related to bimodal visuotactile integration of the hand [1]. In humans, these bimodal neurons contain receptive fields that are located directly on the surface of the skin and extend outward into peripersonal space. Thus, any external stimuli approaching the body, for example the hand, will elicit responses in cells that are responsible for that particular receptive field. This recruitment of activity is present even without the person observing the object coming in closer proximity to the hand [15]. Together these findings indicate that the extension of the arm in space enhances visual processing in the presence of degraded visual information.

As mentioned earlier in the chapter, primary visual cortex projects onto several other areas of the brain. Thus, any loss of neurons in the primary visual cortex could be compensated for by the activity of other higher-order visual association areas that remain stimulated by visual input in the presence of damage to V1, such as in hemianopia. In fact, researchers proposed the existence of compensatory synaptic changes for the neuronal loss seen in primary visual cortex where some patients tend to exhibit blindsight. The hypothesis of blindsight is that subcortical pathways bypass the primary visual cortex and directly project onto secondary visual areas such as V5 (for motion detection), thalamus, brain stem, hypothalamus and/or the amygdala (for emotional response). In fact, this hypothesis was confirmed based on anatomical data acquired from fMRI studies where extrastriate activations in the damaged hemisphere of a hemianopic patient were observed during a forced-choice task known to elicit blindsight [48]. The results of this study support the notion of possible changes occurring at the synaptic level between spared and damaged visual networks based on clinical patients.

Another study investigated whether bimodal audiovisual interactions affect visual processing in patients with hemianopia, visuospatial attention deficit (i.e., neglect) and with both conditions presented visual stimuli in the impaired field in two conditions [14]. The patients underwent a visual detection task with unimodal (i.e., vision only) and cross-modal conditions, with the latter presenting a simultaneous auditory stimulus with the visual target that was either spatially congruent or incongruent. The results of the study showed that in patients with hemianopia or neglect, temporally congruent audiovisual stimuli improved the ability to consciously detect the contralesional visual stimuli in comparison with unimodal visual stimuli only. However, these results were not seen in patients that exhibited both hemianopia and neglect [14]. These findings can be explained by considering the functional characteristics of the multisensory neurons found within SC. As described previously, stimuli from multiple sensory modalities interact at close spatial proximity within the SC, which in turn, produces an enhancement of multisensory integration and responses relative to when stimuli are spatially disparate in the environment and SC receptive fields, and there is no integration. The lack of enhancement in patients with both hemianopia and neglect could be explained by the idea that an auditory cue alone can produce improvement in visual detection, but if both hemianopia and neglect are simultaneously present, the effect of the auditory cue may be inhibited. Also, when the lesion was within fronto-temporo-parietal areas (seen in patients with neglect) or to the occipital cortex (seen in patients with hemianopia), both visual and auditory stimuli were integrated. However, in patients with hemianopia

and neglect that have damage to both anatomical areas, the visual and auditory stimuli were not integrated [14].

Using functional magnetic resonance imaging (fMRI) and psychophysical methods, researchers have investigated action control and space perception in congenitally blind and sighted adults while performing active and passive hand movements without any visual feedback information. Congenital blindness is defined as an absence of vision from the time of birth and can be caused by a number of factors including environment, genetic or improper development [17]. In this study, participants were blindfolded in the fMRI scanner, while they performed kinesthetic guided hand movements in a delayed recognition task. Participants were asked to draw three different line patterns one after the other with a stylus, in their right hand, and maintain a mental representation of their hand movements across a variable delay, while their right hand rested. After the delay, a movement recognition trial was initiated where participants were asked to trace a single probe line pattern and press one of the two buttons with their left hand to indicate whether the last line pattern matched one of the stimulus items [16]. The results indicated that both groups did not differ in their task performance. Interestingly, however, kinesthetically guided hand movements activated the bilateral primary somatosensory cortex, left anterior intraparietal sulcus and the left superior parietal lobe [16]. As explained in the previous section on multisensory integration within the brain, this area is part of the dorsal stream pathway which is responsible for visually guided movements. The fact that this pathway was activated in congenitally blind patients indicates that the functions pertaining to this stream arise even in the absence of visual experience [16]. Sighted participants showed greater activation in areas that are responsible for tactile object localization and processing of spatial coordinates (i.e., precuneus) and pre-supplementary motor area associated with higher-order motor control in comparison with congenitally blind participants. This finding indicates that sighted participants have less experience with in nonvisual movement control creating higher task-related demands on these networks [16]. Unlike the sighted participants, congenitally blind participants rely heavily on their remaining senses to guide their movements, and this is evident in the findings where stronger activation in the extrastriate cortex and auditory cortex was present in congenitally blind participants while performing the kinesthetically guided hand movements. These coactivations imply heteromodal plasticity in the auditory cortex due to visual loss [16]. Taken together, these results suggest that the dorsal stream pathway is not only responsible for visual action directed movements but also for somatosensory guidance of movements and that spared sensory areas may manage the loss of function from the preexisting damaged areas.

In summary, there are a number of studies that indicate the integration of multisensory modalities within particular damaged brain regions. Damage to heteromodal association areas, such as the PPC, and independent unimodal sensory cortices, such as the occipital cortex, indicates how these areas are essential for multisensory integration and the clinical signs that occur postdamage to them, among other polysensory areas described above. Damage to either any of the heteromodal sensory areas or unimodal sensory areas reduces the effects of multisensory integration that give rise to holistic perception. However, spared multisensory networks have shown behavioral benefits by cross-modal plasticity, both at the cortical level and

synaptic level, where spared heteromodal areas attempt to reconnect the multisensory system via bypassing existing injured brain areas thus creating alternative routes in the system.

4. Plasticity research in the lab

In this section of the chapter, we will discuss some of the research on neuroplastic reorganization that we are currently conducting in our laboratory.

4.1. Leftward heightened lateralized fractional anisotropy measures in professional ballet dancers

In one study, we investigated the effects of long-term professional ballet training on tract fractional anisotropy (FA) lateralization and extent of tract FA [49]. Sensorimotor and cognitive training have been associated with altered tract properties as well as altered brain volume. Research indicates that experience and training modulate brain structural parameters including volume and FA [50]. FA is a diffusion tensor imaging (DTI) derived index of water molecule diffusion highly sensitive to the collective effect of microstructure properties, is a putative index of anatomical connectivity and thus is an indicator of microstructural tissue changes.

A single study involving professional ballet dancers and healthy age-matched controls studied whether brain plasticity in either reflex and/or perceptual vestibular processing had discrete neural basis [51]. The authors emphasized that studying brain plasticity while observing changes in gray matter (GM) and white matter (WM) within the vestibular system is of importance as both reflexive and perceptual processing can be assessed while investigating the potential effects of training. Here, the authors correlated GM density and WM microstructure in both groups while simultaneously measuring vestibular psychophysical parameters [51]. Results showed reduction in GM volume in the posterior bilateral vestibular cerebellum negatively correlated with years of dance experience where dancers demonstrated reduced GM volume relative to controls [51]. These results suggested that brain changes within the vestibular cerebellum as a function of dance training affect processing of vestibular perception [51]. Due to the fact that this study reported changes in global volume, our study aimed to investigate changes in FA specifically while secondarily observing global volume changes within dancers and healthy controls.

The effects of long-term ballet training characterized in terms of tract FA lateralization (calculated by `tbss_sym` script was used [52]), extent of tract FA and global volume have not been previously reported in existing literature and thus was the primary concern for this study. We localized tract FA lateralization and extent of tract FA in expert ballet dancers ($n_{male} = 9$, $M_{age} = 23.00$, $SD = 10.21$) relative to healthy controls ($n_{male} = 5$, $M_{age} = 24.89$, $SD = 1.70$) using DTI. The results indicated that dancers had greater leftward lateralization in the anterior thalamic radiation (ATR), whereas healthy controls had greater rightward lateralization and that dancers also had higher FA localized to the left cortical spinal tract (CST). Refer to **Table 1** for significantly lateralized FA voxels; p -values; and effect sizes (Cohen's d).

Voxels	Max X	Max Y	Max Z	Structures	<i>d</i>	<i>p</i> < 0.10
260	-15	-56	28	JHU: 11% left Ci; JH: 10% left CB WM	2.00	0.052
49	-17	22	41	HOC: 8% left SFG WM	1.89	0.079
48	-21	-55	39	JHU: 3% left ATR	1.83	0.038
29	-18	33	31	JHU: 3% left IFOF; 3% left ATR	2.25	0.059
25	-7	-67	34	JHU: 3% left Ci	2.34	0.069
5	-19	27	33	JHU: 3% left ATR; HOC: 4% WM of left SFG	1.19	0.099
1	-14	47	27	JHU: 29% left FMi; 3% left ATR	1.65	0.100

ATR = anterior thalamic radiation; CB = callosal body; Ci = cingulum; *d* = Cohen's *d*; FMi = forceps minor; IFOF = inferior fronto-occipital fasciculus; ILF = inferior longitudinal fasciculus; SFG = superior frontal gyrus; HCs = healthy controls. Percentages represent the probability identity of a structure as estimated by the HOC = Harvard-Oxford Cortical, JHU = JHU-ICBM White-Matter Tractography and JH = Juelich Histological Atlases; WM = white matter.

Table 1. MNI coordinates of dancer leftward FA asymmetry.

In addition to the FA lateralization found in our expert ballet dancers, we also showed that substantive variability in FA was shared with ballet training (32–35%) implicating a relatively large effect of training on tract alteration and enhanced tract conductivity. Overall, the results of our study imply that higher dancer FA reflects heightened axonal ability to communicate. The large percentages of variability shared by dancer years of training, and the structural metrics FA and global volume implicate a substantive impact of dance training on change in brain structure.

4.2. Functional and neural correlates of dance expertise

In a different study, we investigated structural and functional plasticity associated with dance expertise in a cross-sectional pilot study, comparing ballet dancers to healthy controls [53]. Using functional magnetic resonance imaging (fMRI), whole-brain functional activation maps of dancers and controls, while they engaged in motor imagery of dance movements, were compared. Brain plasticity has been studied through a wide variety of experimental paradigms. With respect to humans, one of the most influential models to study experience-related plasticity has been that of probing the structural and functional changes that occur as a result of motor skill learning and expertise [54, 55] through complex motor skills that take extensive time and practice to learn, focused on expert groups whose motor expertise

is commonly restricted to finger or single limb movements. Thus, there has been a noted lack of studies, particularly in the context of functional neuroimaging, that have investigated the plasticity associated with motor expertise in skills that require whole-body movements [56, 57]. An expert group that may be used to investigate questions on plasticity associated with expertise in complex whole-body movements is that of professional dancers, which were investigated within our current study.

To investigate the above structural and functional neural changes, we had both ($n = 17$) expert ballet dancers ($n_{\text{male}} = 11$, $M_{\text{age}} = 19.00$, $SD = 1.17$, dance experience $M = 11.25$, $SD = 3.21$ years) and ($n = 5$) controls ($n_{\text{male}} = 3$, $M_{\text{age}} = 26.00$, $SD = 10.07$, with no self-reported dance experience) perform motor imagery of dance movements while undergoing 8 min of functional neuroimaging. Due to dance being a highly complex motor and cognitive task, both groups were expected to recruit an extensive functional network that included motor-related cortical and subcortical areas, as well as frontoparietal regions during motor imagery [57]. We also investigated the structural correlates associated with dance expertise by comparing cortical thickness between dancers and controls. Various studies have reported motor learning and expertise to be associated with alterations in gray matter, with the common finding being an increase in gray matter in regions believed to be task relevant [58, 59]. Prior to the fMRI scanning procedure, participants received a 20–45 min tutorial on motor visualization, in which they learned the difference between visualizing movements from an internal (kinesthetic motor imagery) and external (visual motor imagery) perspective; this was done to ensure that the participants engaged in the motor imagery task from an internal perspective. Participants were then placed in the MRI scanner and were instructed to visualize themselves dancing to the music from an internal perspective. Scanning followed a block design, consisting of five 60-s-long dance imagery task blocks, interleaved by six 30-s long rest blocks [68].

Anatomically the results revealed that dancers exhibited greater cortical thickness in areas such as the inferior occipital gyrus, inferior frontal gyrus and superior temporal gyrus ($p < 0.01$). We also found years of dance training to be correlated with cortical thickness in various regions, including positive correlations being reported in the fusiform gyrus and parahippocampal gyrus ($p < 0.01$).

Functionally, controls were found to exhibit significantly greater activity in areas such as superior frontal and medial gyrus, anterior cingulate cortex, hippocampus, precuneus and left cerebellum during motor imagery of dance movements when compared to the expert dancers.

Dance training involves learning and correction of movements, thus leading to the consistent recruitment of regions that are related to functions such as motor control, timing and synchronization, visuomotor imagery, spatial transformations, and action observation and imitation; our results showed cortical differences between dancers and controls in brain regions whose functions are those listed above. It is possible that dancers, who are skilled imaginers due to their training extensively recruit regions such as the precuneus when they are engaged in visual imagery of dance movements. The precuneus in particular has been shown to be implicated in various types of mental imagery tasks, including in motor imagery, where it is believed to be involved in the processing of spatial relationships for body movement control.

These preliminary results suggest that dance expertise is associated with a functional and structural reorganization that corresponds to the reduced activity reported in other motor expertise groups.

Lastly, final studies in the lab investigate the impacts that dance intervention has on people with Parkinson's disease (PwPD) and healthy controls [60–66]. Dance has been shown to have positive effects on motor functioning in PwPD [65, 67], but less well understood are the neural effects and changes of dance on motor skills and nonmotor skills and associated brain activity observed within PwPD. Our aim was to examine the effects of dance on both motor and non-motor functioning and correlate these potential effects to onsite recordings of resting state electroencephalogram (rsEEG) alpha rhythm recordings, collected immediately before and after participation in a single dance class. Precisely, we aimed to examine and potentially construct brain-related plasticity mechanism(s) as a function of dance. We compared changes in motor (using the standardized MDS-UPDRS Part-III), non-motor (using PANAS-X) and rsEEG in both PwPD ($n = 17$; $N_{Males} = 12$, $M_{age} = 68.82$, $SD = 8.95$) and healthy controls ($n = 19$; $N_{Males} = 6$, $M_{age} = 52.78$, $SD = 17.30$) before (PRE) and after (POST) voluntary participation in a single 1.25-h dance class for the dance with Parkinson's program at Canada's National Ballet School (NBS) [60].

As expected, our preliminary results showed overall motor impairment improved after a single dance class in PwPD ($p < 0.001$). We also found differential effects of dance on negative and positive mood for both PwPD and HC ($p < 0.01$). We are in the continual process of data collection and correlating these behavioral effects with rsEEG recordings.

Thus far, these findings imply that dance promotes changes in motor functioning and affects through its overwhelming positivity, use of imagery, use of imagination, the presence of music and a sense of bonding through partner work. These results are of importance as its implications may allow researchers to better correlate and understand the positive behavioral and physiological benefits of dance for PD, and perhaps will aid in the implementation of dance as a form of rehabilitation for PwPD. Currently, we are leading a follow-up study examining these potential results from participation in multiple dance classes overtime. This is in conjunction with our ongoing rsEEG research and will provide a better understanding behind the observed neural changes seen in global alpha power. Further examination of whether the music being played at multiple frequencies would somehow influence these changes in neural alpha band rhythms observed after class is needed to help explain the increases in alpha power observed in our findings.

5. Conclusion and future directions

Overall, it seems reasonable to conclude that in the context of multisensory or unimodal damage or deprivation, the brain recognizes these losses in function and reorganizes to exploit the remaining intact unimodal or multimodal senses at its disposal. The presented set of reviewed literature on plasticity in multisensory networks may have important implications regarding teaching, learning and rehabilitation strategies in persons with damage to the above described

brain areas. These findings indicate that the brain is capable of plastic changes throughout the lifespan, and even in healthy individuals, the brain seems to be always changing as a function of training and expertise.

However, it is essential to make note that plasticity changes are intrinsic properties of the central nervous system, and thus, neural plastic changes do not always lead to a behavioral gain, but instead could be deleterious. Thus, more research should be focused on modulation of neural plasticity for optimal behavioral gain across all different types of individuals.

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Synaptic Plasticity by Afferent Electrical Stimulation

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Additional information is available at the end of the chapter

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Abstract

The effect of afferent electrical stimulation on synaptic plasticity within the sensorimotor cortex will be discussed. Afferent electrical stimulation induces a down regulation of inhibitory neural circuits and plays a critical role in strengthening excitatory synapses. Synaptic modifications such as long-term potentiation (LTP) mechanisms could be a crucial mechanism underlying this stimulation-induced cortical plasticity. LTP and long-term depression (LTD) of synaptic transmission are crucial factors for activity-dependent changes in the strength of synaptic connections. Many studies demonstrated that these pathways play an important role in cortical synaptic plasticity. Repeated activation of excitatory synapses induces both short-term potentiation (STP) and LTP. Both types of synaptic potentiation affect N-methyl-D-aspartate glutamate receptors leading to the formation of new synapses or the unmasking of excitatory amino acid receptors on motor neurons. This increased excitability localized within the sensorimotor cortex may reflect an increase in neuronal activity as a result of a dynamic interaction of various synaptic and cellular mechanisms due to the local processing of afferent electrical input to the sensorimotor cortex. The chapter reviews also the large number of studies using fMRI and TMS to examine the effects of afferent electrical input from the hand on the excitability of human sensorimotor cortex.

Keywords: neuromodulation, afferent electrical stimulation, long-term potentiation (LTP), sensorimotor cortex, stroke rehabilitation

1. Introduction

In the development of neurobiology, it was generally thought that synapses simply transfer information between one neuron and another neuron or between one neuron and a muscle cell. Further, it was thought that established connections during development are relatively

fixed in their strength. However, up to now, the current opinion in neurobiology is that most synapses are highly plastic and are able to change their strength depending on their own activity or through synaptic input from another pathway. It is generally accepted that synaptic plasticity is the basic mechanism for learning and memory and reorganization in brain damage.

Synaptic plasticity can be divided into an intrinsic and extrinsic synaptic plasticity. Intrinsic synaptic plasticity refers to changes in the strength of a synapse by its own activity. Extrinsic synaptic plasticity is a change in the strength of a synapse by synaptic input through another pathway. For this change in the strength of a synapse through input from another pathway, there is a widely accepted model in neurobiology called long-term potentiation (LTP) that links synaptic plasticity with memory and long-term depression (LTD). The chapter will discuss the effect of afferent electrical (AE) stimulation on synaptic transmission and synaptic plasticity in general and especially within the sensorimotor cortex with a special protocol using whole-hand afferent electrical stimulation with a wire glove. Results from AE stimulation provide evidence for the induction of synaptic plasticity within the sensorimotor cortex leading to a reduced short interval intracortical inhibition (SICI) and an increased intracortical facilitation (ICF) and consequently to an increased motor cortex excitability, as verified with functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS) techniques.

AE stimulation has been proven to induce changes in synaptic transmission and synaptic plasticity within the sensorimotor cortex [1]. An increased blood oxygen level dependent (BOLD) response after 30 min of AE stimulation within the sensorimotor cortex has been shown by fMRI [2]. 30 min of AE stimulation is able to modulate the corticospinal excitability as well as the activity of intracortical inhibitory and excitatory circuits that can be detected by TMS [3]. These conditioning effects could be measured up to 2 h post stimulation [2]. This exploration has strengthened the understanding that electrical peripheral nerve stimulation is a powerful tool to induce sustained excitability increases as well as rapidly evolving neuroplastic changes of the human sensorimotor cortex. Up to now, the optimal set of parameters for afferent electrical stimulation for the modulation of the corticomotor output is not exactly known [4]. Stimulation intensity appeared to have the strongest relationship to motor cortical excitability, whereas frequency has been shown to modulate motor-evoked potential (MEP) amplitudes [5]. Furthermore, different levels of afferent electrical stimulation were investigated—sham, sub-threshold (below the threshold for sensory perception)/50 Hz, sensory (above the threshold for sensory perception)/2 and 50 Hz, and motor level/2 Hz.

2. Synaptic plasticity by afferent electrical stimulation in BOLD imaging

2.1. Neurophysiology of afferent electrical stimulation

Afferent electrical stimulation generates synchronous tonic input to the brain due to depolarization of a large diameter group, Ia and Ib afferents, and to a lesser extent group II afferents of the hand, as it is the case in functional and neuromuscular electrical stimulation [6–10]. The

electrical input is transmitted to the spinal cord posterior column nuclei, the ventral posterolateral nucleus of the thalamus, and to the Brodmann areas 3a, 2, and 4 of the brain cortex [11–14]. The hand is a rich source of proprioceptive input to the brain via the afferents because the hand's intrinsic muscles have high-density muscle spindles [15, 16], a large number of joint receptors, as well as Golgi tendon organs [17, 18] with a portion of the tendons within the hand but belonging to the forearm muscles [10]. In addition, there are definite experimental findings that confirm that proprioceptive and exteroceptive somatosensory afferents of groups Ia (primary large muscle afferents), Ib (afferents from Golgi organs), and group II (slow and rapidly adapting skin afferents, secondary thin muscle afferents) have short latency projections to the contralateral sensorimotor cortex, particularly BA 3a, 1, 2, and 4 [14, 19, 20]. For the afferent route to the primary motor cortex M1, a projection from BA 3a is discussed [21]. By applying afferent electrical stimulation there will be afferents involved that “sense the body's own movement” [22]. In several positron emission tomography (PET) and fMRI studies, it was confirmed that vibration to the hand palm of healthy adult humans activates the contralateral primary sensorimotor (SM1), the supplementary motor area (SMA), and the secondary somatosensory cortex S2 bilaterally [23–25].

2.2. Methodology of afferent electrical stimulation

Continuous whole-hand afferent electrical stimulation with a wire glove (WG, **Figure 1**) is a potential tool that can induce neuromodulatory effects within the sensorimotor cortex. For ipsilateral neuromodulatory effects, cortical projections of Ia, Ib, II afferents, and transcallosal projections from the contralateral brain cortex are supposed [26, 27].

The wire glove is connected to a two-channel transcutaneous electrical nerve stimulation (TENS) stimulator. The WG acts as the anode, and carbon film surface electrodes above the tendons of the forearm flexors and extensors just proximal to the wrist act as cathodes (**Figure 1**). Before fitting the hand to the WG, conductive jelly should be applied over the whole hand. A train of 50-Hz stimuli with a pulse width of 300 μ s is used for stimulation (**Figure 2**).

Depending on the skin resistance, the amplitude for the threshold of sensation lies between 2.0 and 4.0 mA and the level for supra-threshold stimulation is set to 120% of the threshold of sensation level. For sham, the stimulator is set to 0 mA, and healthy volunteers are told to be stimulated below the level of sensation. AE stimulation is applied for 30 min to the relaxed right or left hand [3]. Pulse width is set to 300 μ s (**Figure 2**).

The level for sub-threshold stimulation is set to 80% of the threshold of sensation level. At the sensory level, (120% of the threshold of sensation level) electromyography (EMG) did not show any muscle contractions. For somatosensory AE stimulation, the frequency is set at 50 Hz and for motor AE stimulation at 2 Hz. The current stimulus amplitude for 50 Hz ranges from 2.0 to about 5.0 mA. For the motor level, the intensity is increased from the sensory threshold level until slight motor contractions of all small hand muscles are visible. The current stimulus amplitude for the motor level is about 10.0 mA. The sham stimulation is carried out identically, but the stimulation amplitude is set to 0 mA. Subjects are not informed about the stimulation level and are instructed to distract attention from the stimulation.

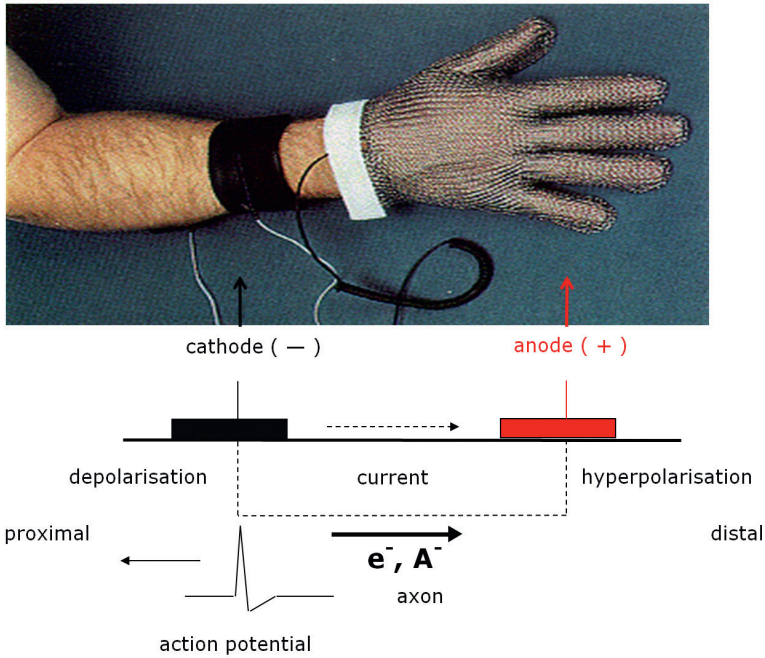


Figure 1. Wire glove: a two-channel stimulator delivers a train of 50-Hz stimuli (pulse width 300 ms) with the amplitude for somatosensory and motor stimulation, ranging from 2.0 to 10.0 MA. The wire glove acts as a common anode. The cathodes are placed over the tendons of the forearm flexors and extensors. Beneath the electrodes over the forearm flexors and extensors, the action potentials for the AE stimulation are elicited.

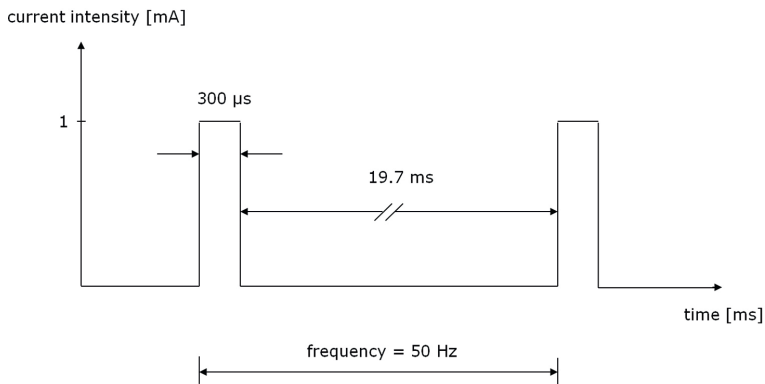


Figure 2. Current pulse: with a rectangular pulse with a width of 300 μ s and a frequency of 2/50 Hz, the AE stimulation is continuous for 30 min. The current intensities range between 2 (somatosensory threshold) and 10 mA (motor threshold).

2.3. BOLD modulation by afferent electrical stimulation

Neuromodulatory effects of afferent electrical stimulation are already proven by fMRI [2, 28] applying self-paced simple finger movements. During finger-to-thumb-tapping with a frequency of about 2 Hz in fMRI movement-related BOLD responses of several brain areas are well known in the contra and ipsilateral hemisphere within the pre- and postcentral gyrus, the medial and superior frontal gyrus, and on both cerebellar hemispheres with a dominance ipsilaterally to the active left hand (Figure 3).

Neuronal activation within these areas is expected and has been reported by other investigators who studied the activity of human cortical motor areas during self-paced finger movements [29–31]. In a classical pre-/post-study design, a baseline finger-to-thumb tapping paradigm is run in fMRI (Figure 4).

Post 30 min of whole-hand afferent electrical stimulation of the tapping hand, the finger-to-thumb tapping paradigm in fMRI shows an increase of brain activation measured by the corresponding BOLD response on both hemispheres within the pre- and post-central as well as the medial frontal gyrus (Figure 5).

The left SMA shows augmented brain activation as well. The finding that an increase of movement-related responses is absent when the sham paradigm is applied further confirms the validity of these results. Obviously, afferent electrical stimulation can change motor cortex representation bilaterally within the primary and secondary sensorimotor cortex and consequently has the potential to induce neuroplasticity in neurorehabilitation. The detected increased BOLD responses reflect an increased neuronal activity due to augmented afferent proprioceptive and exteroceptive inputs to the sensorimotor cortex [32]. Logothetis could demonstrate a strong correlation between spatially localized BOLD response and local field potentials. AE stimulation of group Ia and Ib afferents and their

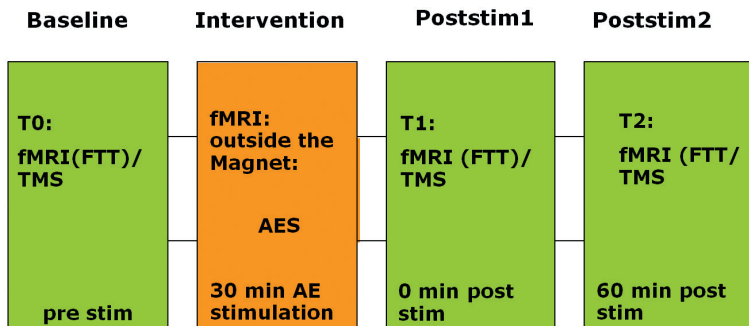


Figure 3. Classical pre-/post-design for studying synaptic plasticity: at T0 baseline measurement (finger-to-thumb tapping in fMRI, TMS) prior to 30-min AE stimulation, at T1 first measurement (fMRI/TMS) post stimulation (post stimulation 1) and at T2 60-min post-AE stimulation second measurement (fMRI/TMS) post stimulation (post stimulation 2).

direct transcallosal projections induce augmented local field potentials (LFP) for at least several minutes within the sensorimotor cortex that was already proven in somatosensory-evoked potential studies [13]. Augmented LFPs change intramotorcortical excitability with a larger recruitment by a motor task. AE stimulation addresses especially group Ia and Ib and to a lesser extent group II afferents and thus should augment sensorimotor LFPs. AE stimulation in stroke patients after a daily stimulation training program over several weeks improved motor performance [33–35], obviously by an increased motoneuron recruitment due to augmented motorcortical excitability leading to synaptic plasticity with intracortical facilitation and unmasking of preexisting silent synapses [36–41]. Horizontal connections transversing the superficial layers of the sensorimotor cortex are capable of both increases and decreases in strength and synaptic efficacy [42, 43]. A persistent high-frequency input enhances the motoneuron recruitment with probably a synaptic modification through long-term potentiation (LTP). Post-tetanic potentiation is unlikely because the neuromodulation lasts at least 2 h (**Figure 6**).

However, in the literature, there is evidence for a cortical origin of the modulation of cortical motoneuron excitability by afferent electrical input [1, 26, 44]. The multimodal integration cortex in the superior (SPL) and inferior (IPL) parietal lobule receives sensory information of

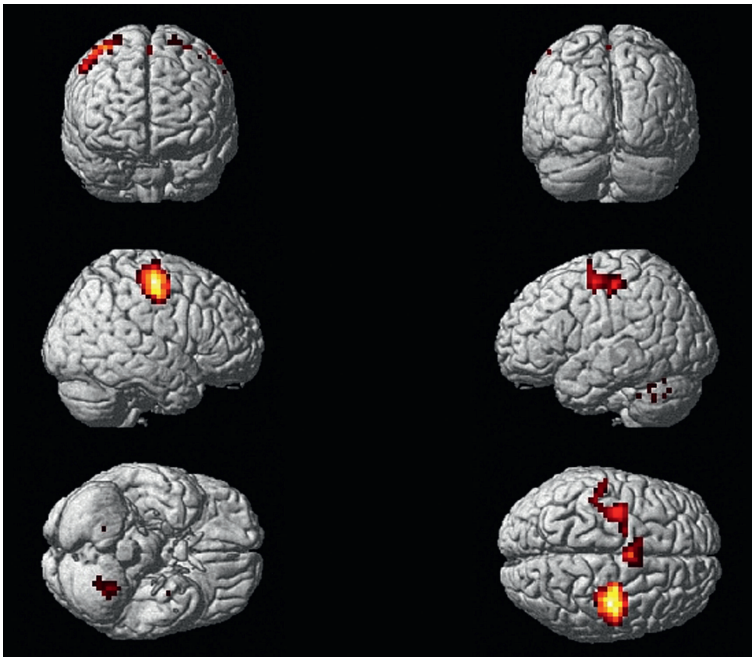


Figure 4. BOLD response during the test motor task (TMT): contra and ipsilaterally within the SM1, premotor area (PM), and SMA as well as in both cerebellar hemispheres with a dominance of the left hemisphere ipsilaterally to the stimulated hand.

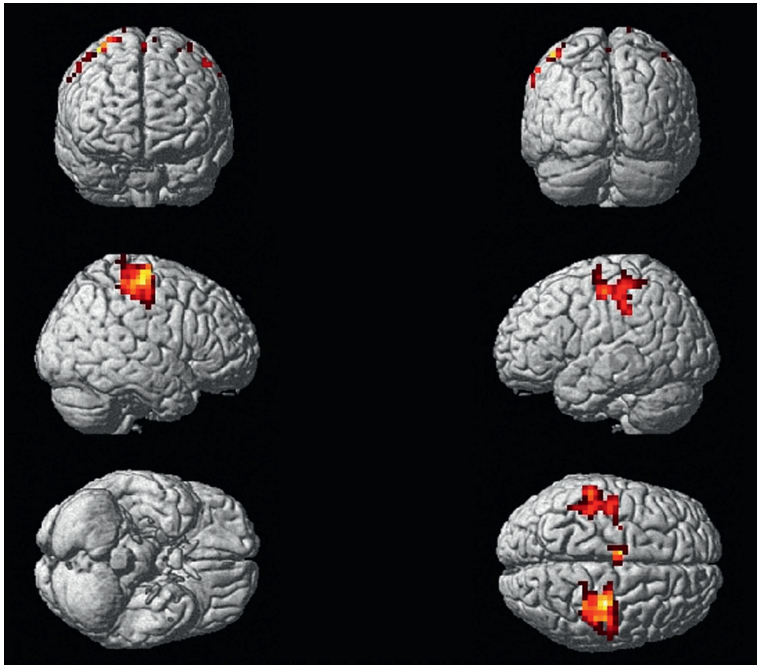


Figure 5. Between-condition analysis in fMRI: conditioned motor task at T1 (CMT1)—test motor task at T0 shows an increase of BOLD response during CMT1 of the contralateral hemisphere within SM1, PM, and IPL and of the ipsilateral hemisphere within SM1, PM, IPL, SMA, and cingulate gyrus (GC).

different modalities and transforms it into information for proper action. In IPL, proprioceptive, exteroceptive, premotor, and visual information converges during grasping with the hand. IPL is involved in sensorimotor transformations to convert retinal signals of target locations into a pattern of peripheral motor output to muscles to move the hand to the target [45, 46]. The information for these target movements will be processed primarily by the group Ia and Ib afferents, which are especially addressed by the afferent electrical stimulation. Thus, the IPL activity in the conditioned motor task immediately after the AE stimulation (CMT1) indicates direct input bilaterally to IPL that is very important for the neurorehabilitation for visually guided movements and the eye-hand coordination (**Figure 3**). Increased IPL activity after daily AE stimulation for 3 months in stroke patients concurs also with an improvement of neglect [33, 34]. Increased proprioceptive and exteroceptive input to the brain has also the potential of lowering muscle tone, which is in agreement with described beneficial effects of AE stimulation on spasticity [33, 34, 45, 46].

The increased BOLD responses were supposed to be due to a precapillary vascular response or to reduced sensorimotor network thresholds. Wu et al. [47] demonstrated in 2005 that median nerve stimulation elicited an enduring increase in task-related perfusion and BOLD responses in the cortical thumb representation in the absence of changes in baseline blood

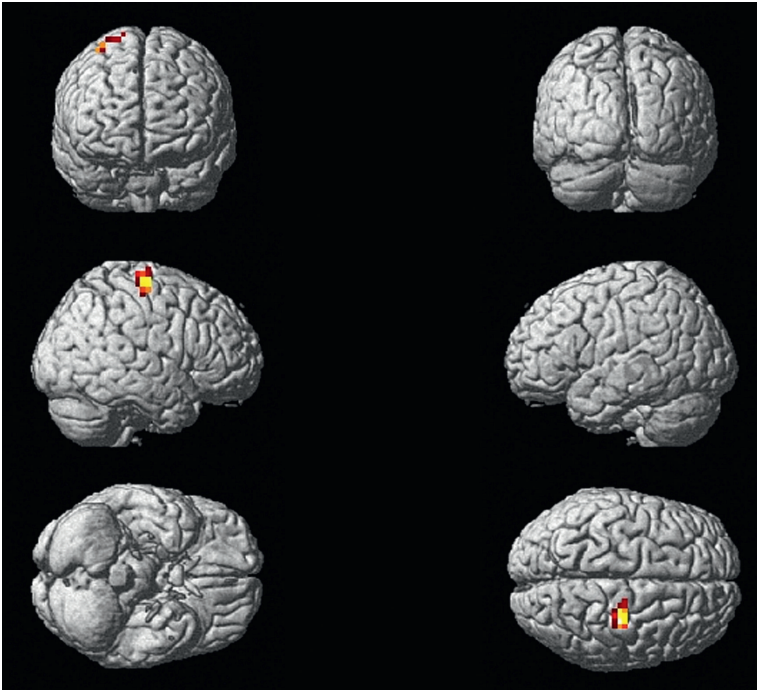


Figure 6. Between-condition analysis in fMRI: conditioned motor task at T2 (CMT2)—test motor task at T0 shows still an increase of BOLD response 2 h post stimulation with brain activation declining nearly to TMT level.

flow. Consecutively, the increased BOLD response was associated with increased cortical excitability but was still unclear.

3. Synaptic plasticity by afferent electrical stimulation in TMS

3.1. Background

With transcranial magnetic stimulation (TMS, excitatory and inhibitory circuits of the human motor cortex can be studied. Many studies have used TMS to investigate the effect of AE stimulation of the hand on motor cortex excitability with the paired-pulse technique, demonstrating a reduction of SICI [48, 49]. By a preceding electrical stimulus to a mixed or cutaneous nerve Motor Evoked Potentials (MEPs) are affected and show a smaller amplitude [48, 50–52]. AE stimulation of the hand showed conflicting results with no effect on MEP amplitudes, MEP amplitude facilitation, MEP amplitude inhibition, or both [12, 53–61], depending on the parameters used [62–65] on disparate effects of stimuli on different motoneuron pools, on different experimental settings (e.g., single pulses versus stimulus trains, various stimulus intensities, relaxed versus contracted target muscles), and on different stimulation and recording sites. Low-amplitude vibration of a muscle increased MEP amplitudes and decreased the effectiveness of SICI [66, 67].

BOLD signal intensity changes prior to and after constraint-induced movement therapy (CIMT) within the sensory and motor cortex proved a close correlation with SICI and ICF in paired-pulse TMS [68]. With TMS effects of AE stimulation on the motor system, its duration can be investigated very easily, yielding to information about the excitability of the motor cortex, and they help to clarify the physiological basis of variations in BOLD responses by AE stimulation. In case of a specific vascular response of the precapillary microvasculature independent from neuronal effects, TMS parameters should not change after afferent electrical stimulation.

3.2. TMS methodology

Again, a classical pre-/post-design is implemented (**Figure 4**) with a baseline TMS assessment (T0), AE stimulation for 30 min, a further TMS assessment (T1), a resting period for 1 h, and a third TMS assessment (T2). The experimental setup includes four different AE stimulation levels in randomized order: (1) sham, (2) sub-threshold with 50 Hz (sub-threshold/50 Hz), (3) sensory with 50 Hz (sensory/50 Hz), and (4) AE stimulation at motor level with 2 Hz (motor/2 Hz). At motor level, 2 Hz is chosen to avoid painful tetanic muscle contraction. Between sessions, there is at least an interim time of 5 days. Paired-pulse TMS is performed using a bi-stimulation module. A figure-of-eight coil with external loop diameter of 90 mm is applied for motor responses in the right first dorsal interosseus (FDI) muscle at the lowest motor threshold (MT, **Figure 7**).

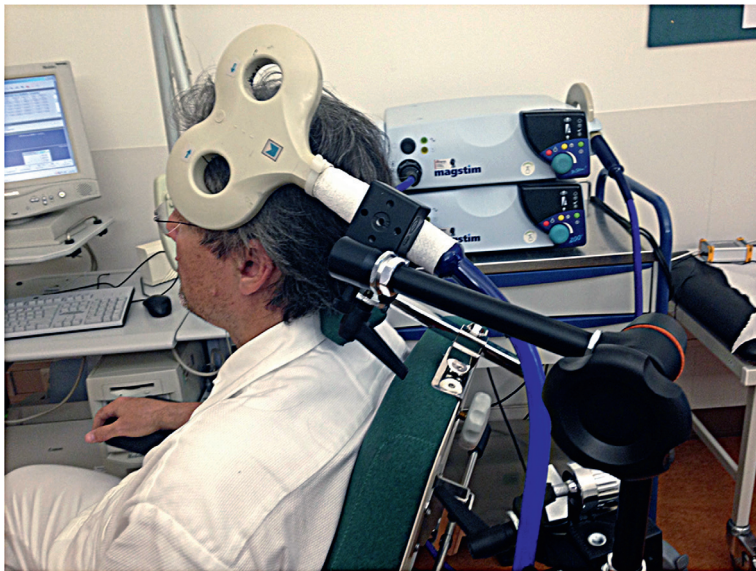


Figure 7. TMS experimental setup: a figure-of-eight coil (external loop diameter of 90 mm) is held over the left motor cortex at the optimum scalp position to elicit motor responses in the right FDI muscle at the lowest MT. The intersection of the coil is placed tangentially to the scalp with the handle pointing backward and laterally at a 45° angle away from the midline to induce postero-anterior current flow. Surface muscle responses are obtained via two 9-mm diameter of Ag-AgCl electrodes with the active electrode applied over the motor point of the muscle and the reference on the metacarpophalangeal joint of the index finger. Muscle responses are amplified and filtered (bandwidth 8–2000 Hz).

The coil is held tangentially to the scalp with a 45° angle away from the midline for postero-anterior current flow. Muscle responses are recorded with 9-mm diameter of Ag-AgCl electrodes over the belly of the first dorsal interosseus (FDI) and the metacarpophalangeal joint of the index finger and are amplified and filtered (bandwidth 8–2000 Hz). The minimum stimulus intensity that produces an MEP at rest of 50 μ V in three out of five trials defines the resting motor threshold (RMT). Then MEP recruitment curve (RC) with TMS intensities of 90, 100, 110, 120, 130, 140, 150, and 160 of the MT is measured. RC is recorded in T0, T1 and T2 measurements. For each stimulator, output intensity of five pulses is delivered with randomized stimulus intensity. The first MEP for each trial is discarded because of startle and reflex responses. Short interval cortical inhibition (SICI) is performed according to the technique of paired magnetic stimulation [69]. The conditioning and the test stimuli are set at 80 and 120% of MT, respectively. Inhibitory interstimulus intervals (ISIs) of 3 ms and facilitatory ISIs with 13 ms are applied. Conditioned and unconditioned trials are randomized. If MT after T0 for the paired-pulse measurement is changed, the stimulus intensity is adjusted to the corresponding MT in T1 and T2. The actual amplitudes after correction relate to those before AE stimulation. Usually paired-pulse TMS studies are carried out in 20–30 subjects with half of the subjects assigned to the verum and half to the control group. The verum group undergoes afferent electrical stimulation and the control group undergoes sham stimulation. Subjects are seated in a comfortable reclining chair during TMS measurements, afferent electrical stimulation, and at rest. Both hands are placed relaxed on soft supports beside the body [3].

3.3. Modulation of TMS parameters by afferent electrical stimulation

MT in TMS is thought to reflect the neuronal membrane excitability because it is increased by drugs that block voltage-gated sodium channels but not by drugs influencing neuronal synaptic transmission [70–72]. MT measured at baseline varies between 37 and 45% of maximum stimulator output among subjects. As shown in **Figure 8**, AE stimulation of 30 min has a significant decreasing effect on MT. This effect is obviously related to the strong effect of AE stimulation since the control group does not show any differences in MT. Post-hoc comparisons shows reliable MT decreases immediately after and 1 h after AE stimulation [3, 73].

Compared to MT, the MEP recruitment curve assesses neurons that are intrinsically less excitable or spatially further away from the center of activation by TMS. Increasing the TMS intensities from 90, 100, 110, 120, 130, 140, 150 to 160% of the MT determined for each subject MEP recruitment curves that show an increasing left-sided shift with increasing current intensity after the 30-min period of AE stimulation. **Figure 9** presents the effect of AE stimulation on MEP recruitment curves measured at T0 and T1.

No other effects are found reliable. However, whereas the control group shows any effects on amplitudes after time and no interaction effects of “stimulus intensity” and “time”, the group with verum AE stimulation shows the important reliable main effect of “time” with increased amplitudes after AE stimulation. The interaction effect does not reach significance. In both post-stimulation conditions (T1 and T2), the recruitment curve is increased compared to T0. Post-hoc comparisons at each intensity level reveal that at lower and mid-range intensities, MEP increases are significant both in T1 and T2 compared to T0. At higher stimulus

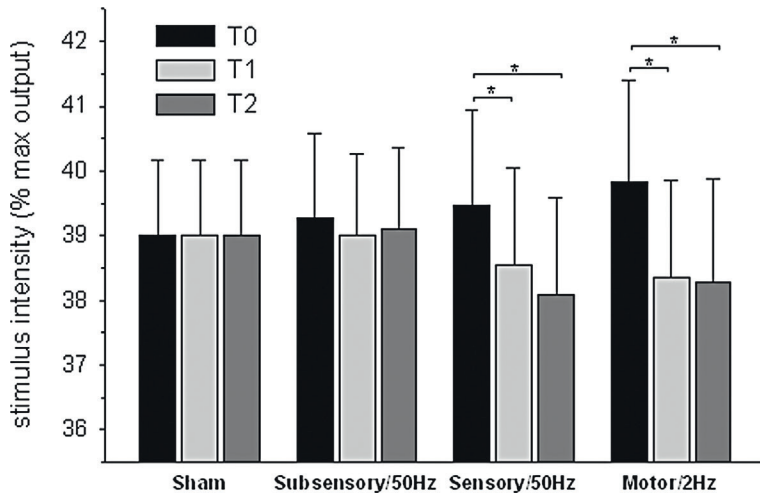


Figure 8. Resting motor threshold expressed as percentage of maximum stimulator output for the FDI muscle before (T0), immediately after (T1), and 1 h after (T2) afferent electrical stimulation with sub-sensory, sensory/50 Hz, and motor level electrical stimulation intensity as well as sham stimulation. Values plotted as mean (S.E.M.). * indicates significant difference ($p < 0.05$) from T0. For sham stimulation there is no change of the resting motor threshold at T1 and T2 compared to the baseline measurement.

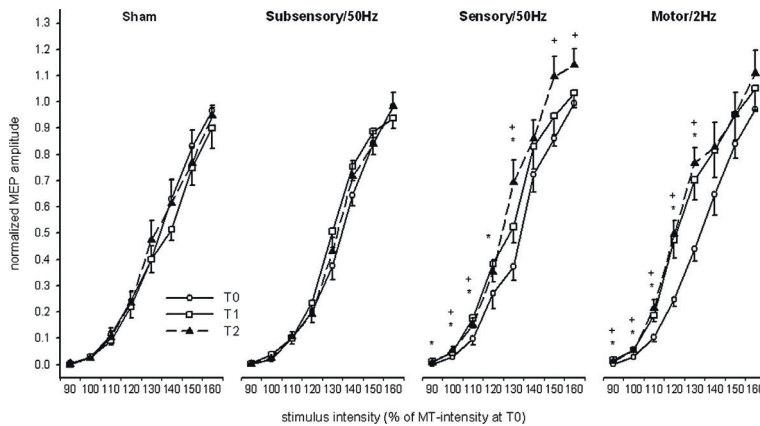


Figure 9. MEP recruitment curves before (T0), after (T1), and 1 h after (T2) AE stimulation at sub-sensory, sensory/50 Hz, motor level, and sham stimulation. MEPs are normalized to the mean MEPmax at T0. Mean (S.E.M.) of the normalized MEP amplitude is plotted for each stimulus intensity. * indicates significant difference ($p < 0.05$) between T1/T0 and (+) significant difference ($p < 0.05$) between T2/T0. A left-sided shift can be seen with increasing current intensities.

intensities (140–160% of MTT0 intensity), the tendency is partly kept although not reaching significance level (**Figure 9**). Additionally, post-hoc group comparisons of each intensity level are conducted and show reliable differences between AE and sham at T1 and T2. A two-factorial ANOVA with factors “group” and “stimulus intensity” is applied that does not reveal any difference in groups at T0.

Since paired-pulse TMS gives access to the motor cortex independently of spinal or peripheral mechanisms, it allows the evaluation of the intracortical circuits [3, 73]. There is good evidence that the interaction between a sub-threshold conditioning stimulus and a supra-threshold test stimulus at short interstimulus intervals (1–5 ms) relies on activation of c-aminobutyric acid (GABA)—in particular GABAA—circuits in the motor cortex. **Figure 10** shows the effect of AE stimulation on SICI and ICF.

Here, apart from the inherent effect of “interstimulus interval (ISI)”, the three-factorial ANOVA reveals also a main effect of “time” and an interaction effect between “time” and “group”. No other effects are found reliable. Follow-up ANOVAs for each group separately confirmed the effect of “ISI” for controls and AE stimulation group, respectively, but found a reliable effect of “time” only for the group who received verum AE stimulation. Other effects are not found reliable. Generally, in the verum group, the MEP inhibition at 3 ms is reduced and the facilitation at 13 ms is increased compared to T0 at T1 and T2. These changes do not reach significance for T1 but do so for T2 as post-hoc comparisons reveal. The circuit underlying intracortical facilitation is less well understood and is thought to be mediated by glutamate. Moreover, the downregulation of inhibitory neural circuits seems to play also a critical role in strengthening excitatory synapses. Current data suggest that afferent electrical stimulation also has a direct effect on the excitability of the intracortical circuits responsible for SICI and ICF at a cortical level. Conversely, no changes in spinal motor excitability (amplitude and persistence of F waves) are currently observed.

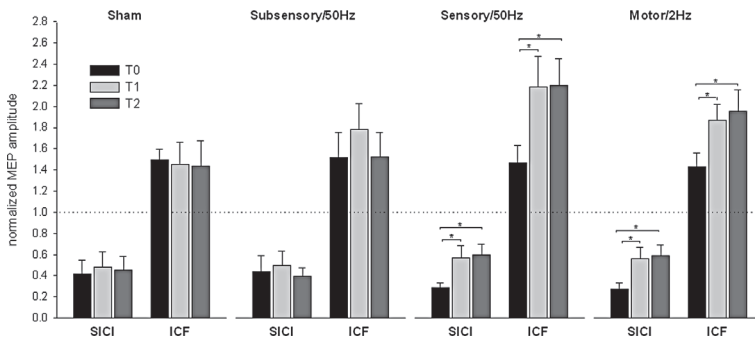


Figure 10. Paired-pulse TMS stimulation: before (T0), after (T1), and 1 h after (T2) AE stimulation at sensory/2 Hz level. The values for intracortical inhibition and ICF are normalized to their corresponding values in single-pulse stimulation for each condition and are then plotted as the mean (S.E.M). * indicates significant difference ($p < 0.05$) from T0. At T1 and T2, a decrease of SICI can be seen; ICF is significantly increased at T2.

4. Discussion

From these pre-/post-AE stimulation studies, we learn that changes in motor cortex excitability outlast AE stimulation up to 2 h and intracortical excitability is significantly enhanced 1 h after AE stimulation and not significantly increased immediately after the AE stimulation. Up to now, this late excitability enhancement remains unclear. Maybe an intracortical synaptic

reorganization LTP is the underlying mechanism for the delayed facilitation. The stimulation-induced cortical plasticity may be due to synaptic modification such as LTP. LTP, as well as LTD of synaptic transmission, had been suggested to be responsible for activity-dependent changes in the strength of synaptic connections and efficiency of synaptic signal transduction since its discovery in the early 1970s of the last century [74]. But the outcome of synaptic modifications on behavioral changes induced by stimuli that drive LTP- or LTD-like processes is not well known today, especially not in patients with brain lesions. STP and LTP are induced by repeated activation of excitatory synapses with N-methyl-D-aspartate glutamate receptors leading to the formation of new synapses or the unmasking of other excitatory amino acid receptors on motor neurons [75]. Also, remote modulation of motor cortex excitability may be involved including additional cortical and subcortical structures connected with the primary motor cortex. The increased excitability may reflect an increased neuronal activity due to dynamic interaction between various synaptic and cellular mechanisms that locally process the augmented afferent proprioceptive and exteroceptive input to the sensorimotor cortex [26]. The strongest neuromodulatory changes could be achieved with stimulation of the highest intensity (motor level). Defining proper stimulation parameters, in particular with regard to stimulation intensities and frequencies, will provide an important basis for further therapeutic applications of afferent electrical stimulation in neurorehabilitation, especially in stroke patients or patients after traumatic brain injury.

In conclusion, the increased cortical excitability leads to an extension of neuronal activity. The time course of neurophysiological effects, as measured by TMS, and also seen in fMRI BOLD responses, suggests a prolonged clinical efficacy of AE stimulation. Further studies should focus on the issue whether more specialized stimulation protocols in particular with regard to stimulation intensities and frequencies can prolong the modulatory effects on the sensorimotor cortex through plastic changes in synaptic efficacy and thus can sub-serve a long-term rehabilitation process of impaired motor functions of the hand after brain lesions [76]. This finding of increased motor cortical excitability after afferent electrical stimulation can help develop new rehabilitation strategies in combination with physical and occupational therapy.

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Plasticity of Dendritic Spines. Not Only for Cognitive Processes

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Additional information is available at the end of the chapter

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Abstract

Excitatory synaptic transmission is associated with the input of “new” information at synaptic junctions established by dendritic spines. The role that each type of spine plays in the transmission of the synaptic impulses is different. Indeed, there is a close relationship between the shape of spines and the differential processing of the excitatory synaptic information that is relayed to them, influencing in turn the transmission of synaptic information related to several psychoneural processes.

The vast majority of the experimental evidence shows that specific plastic interchanges of dendritic spines’ shapes are related to specific functional effects in the postsynapse, i.e., the acquisition or learning of new information (thin spines), or to the storage of information in memory (mushroom spines).

Several brain regions are involved in other functions different than those of a cognitive nature, and all projection neurons in these areas possess dendritic spines. However, the functional significance of the changes that the spines of these neurons express has not been studied. Thus, in this Chapter we will discuss experimental evidence supporting the claim that dendritic spines express plastic changes in some brain regions that are not directly related to cognition, and we will also preliminarily approach their possible functional meaning.

Keywords: plasticity, dendritic spines, synapse, cognition

1. Cognition and noncognition: basic concepts

Person A walks down the sidewalk alongside a fence when suddenly, a dog leaps out behind the fence and barks at Person A. Person A, caught off guard, jerks back and then kicks the fence. Coming to his sense, he realizes that the dog is a harmless little Chihuahua, still stuck behind the fence. Now conscious of the situation, he looks around and sees that various passers-by have been watching him and are laughing at his situation. Feeling exposed, Person A continues walking, now blushing furiously.

Looking at this situation, we might ask, if Person A was going to be embarrassed by his actions, why he reacted the way he did in the first place. Why he did not avoid the scare, and with it, the situation? Why did he kick an object without first working through what it was? This type of event—and Person A's type of reaction—is unrelated to cognition, which is the processing of information that permits the abstraction and manipulation of the environment by way of symbols (language and thought, for example) [1]. In this example, Person A could not place the origin of the stimulus nor relate it to as a previous experience, so his reaction had no cognitive component.

Certain *noncognitive* processes express themselves simultaneously with cognitive processes [2]. These processes arise consciously or unconsciously [3], automatically or implicitly [4]. These noncognitive processes do not require attention, perception, learning, memory, language, or thought for their integration; be that as it may, cognitive processes may be present for the regulation or modulation of those noncognitive ones [5].

Izdar [2] explores the role of noncognitive processes in emotional processing. He mentions that there exists a neural basis for the expression of the emotions that escape cognition and which precede the conscious experience of the stimulus. A series of experiments by LeDoux [6] provide experimental evidence for the two-way configuration for the expression of fear: one of them, the faster, permits the organism to generate a reaction to the stimulus without being fully aware of the situation. Person A's situation illustrates this case clearly.

Noncognitive processing has been the object of conceptual debate, since at one time it was thought that all nerve activity was aimed at the expression of processes related to cognition. Lazarus [7] writes that "cognition is the end of all cortical or subcortical activity." Likewise, Lazarus [8] defends that position by arguing that even simple perceptual phenomena (a type of which can be seen in Person A's case) depend on and create meanings or evaluations with respect to the stimulus. Frijda [9] argues that the nervous system is capable of generating emotions, but only when those emotions had been previously acquired through cognition. Frijda [10] argues that "emotions are the result of meaning, and that meaning is the result of inferences about causes and consequences." However, Zajonc [11] responds to Lazarus with experimental evidence wherein he illustrated the primacy of some noncognitive processes that do not themselves attribute meanings to stimuli. Zajonc mentions that even the integration of information from the retinohypothalamic tract is sufficient for the organism to produce a response, "leaving the attribution of the meaning of the stimulus a synapse away" [11].

Izdar [2] talks about the existing predisposition to argue that cognition is anything that goes hand in hand with learning, memory, and, in general, with "mental" life, leaving aside all

those instinctive behaviors or those which present without cognitive acquisition or previous experience. An example of this type of behavior is the sucking reflex, the response to aversive stimuli that presents in infants even without prior cognitive processing to give it meaning. Likewise, the expression of circadian rhythms like the sleep-wake cycle. Some of these behaviors come from ancestral information accumulated in the course of a species' evolution, which shapes the brain architecture in the absence of experience with the environment [12] and that establishes itself in the absence of cognitive processing, serving the latter as a potentiator and moderator in later stages of life. It is worth mentioning that the processing of information can take place even in infants without previous experience or learning, that is, without cognitive processing [13]. The above leads to the argument that the processing of information has as much a noncognitive component as a cognitive one.

One can make a distinction between cognitive and noncognitive processes: a cognitive process depends on experience, learning, and memory, whose neural basis is the function of the areas of association of the cerebral cortex, changing stimuli into abstractions, meanings, and manageable symbols [14]. On the other hand, noncognitive processes have as their neural basis the function of subcortical structures such as the hypothalamus [15], the amygdala [6], and the functioning of primary areas of the cerebral cortex [14] that do not depend on learning, on memory, or on previous experience for the expression of certain behaviors, like sexual ones, those based on the emotional fast track [6], and the execution of voluntary movement; among others.

Noncognitive processing generates controversy within psychological epistemology. It creates a heuristic conflict that requires a solution not only from psychology but also from those sciences that provide evidence about the determinants of behavior.

This chapter presents experimental evidence about: the expression of behaviors that do not depend on cognition, as is the case in sexual behavior, which is expressed by virtue of neurophysiological changes in hypothalamic nuclei as well as in the ventromedial nucleus; about the formation of biological rhythms, like the sleep-wake cycle, which depend predominantly on the function of the preoptical area and the suprachiasmatic nucleus of the hypothalamus; about the execution of voluntary motor activity that depends on the function of the primary motor area; and about the expression of emotions through a fast track that is integrated in the nuclei of the amygdala even in the absence of the participation of the cerebral cortex and, therefore, of conscious experience.

2. Neuronal plasticity

The plastic capacity of the structures related to the expression of some of the previously mentioned behaviors that are usually related to cognition. However, as the experimental evidence presented in this chapter will show, the neural structures involved in those cognitive processes also form a part of the repertoire involved in the variable expression "noncognitive" processes through their plastic capacity.

Transmission of excitatory information between neurons is mediated by the activation of receptors located on dendritic spines. These neural substructures exist in all projection neurons, and by virtue of participating in the functional integration of the afferent information by those neurons, they “add” a psychoneural attribute to the conformation of functions integrated into the corresponding neural circuits.

The dendritic spines are cytoplasmic protrusions that cover varying portions of the tubular surface of the dendrites. Depending on the excitatory afferents, the spines show varying densities and distributions along the length of the dendrites. Although the spines generally translate the excitatory information, the way in which they process it depends on their geometric structure. According to their shape, six types of spines have been described: (1) thin; (2) mushroom; (3) stubby; (4) wide; (5) branched; and (6) double [16, 17].

The primary characteristic of these structural distinctions lies in the presence or absence of a neck and a head. The thin spines have a long, narrow neck that results in a bulbous structure, or “head,” whose length is shorter than the neck. The mushroom spines have a short, narrow neck that leads to a head whose diameter and length are greater than those of its neck. Stubby spines are protoplasmic protrusions that show no difference between head and neck and whose length is less than their diameter. Similarly, wide spines show neither neck nor head, but their length is greater than their diameter. Branched spines, for their part, display a narrow neck that emerges from the dendrite and which divides into two similar necks before terminating in two similar heads. Double spines have a neck that emerges from the dendrite and forms a head, which then forms another neck, and which finally terminates in a second head.

Typically, the different types of spines show variable amounts between the neurons that host them, but their proportional density remains relatively similar in all neurons: thin > mushroom > fat > wide > branched > double.

Bioelectrically, thin spines have been linked to the rapid transmission of afferent information and are functionally related to the acquisition of new information (learning). Meanwhile, mushroom spines have been related to the slow transmission of afferent synaptic information and with the storage of the same (memory). The other types of spines have not been much studied. There is, however, evidence that suggests that stubby and wide spines could be related to the regulation of the excitability of postsynaptic neurons, while branched spines could be a transformation of other, larger spines (presumably mushroom spines) into two new (branched) spines—hypothetically thin ones. Finally, no functional evidence of the activity of the double spines has been uncovered, although their geometric structure suggests that they represent two independent sites of synaptic contact.

From this chapter’s perspective, thin and mushroom spines are particularly relevant. According to the evidence, both are related to the processing of cognitive information: learning from thin spines and memory from mushrooms. However, it is clear that certain lines of projection neurons—like those located in the primary motor cortex, some nuclei of the amygdala, and certain hypothalamic nuclei, like the ventromedial, the preoptic, and the suprachiasmatic—possess dendritic spines that have shown plastic changes, which have been induced experimentally.

3. Neuronal plasticity in cognitive and noncognitive processes

The hypothalamus is a structure that predominantly participates in the regulation of emotion/affective behavior, in the control of visceral functions, and in the maintenance of the body's homeostasis [18]. The hypothalamus is a structure associated with noncognitive functions. It is neuroanatomically divided into several nuclei, among which is the ventromedial nucleus, which participates in the regulation of sexual behavior in females [18–21]. This portion of the hypothalamus receives information primarily from the medial amygdala and passes the information along to other structures, like the periaqueductal gray and the medulla oblongata, provoking the display of female sexual behavior [21].

Research conducted on the ventromedial nucleus of the hypothalamus has shown the presence of adaptive plastic changes in the neurons of this structure. There is evidence that estrogenic activity reduces the density of dendritic spines on the projection neurons of the ventromedial hypothalamus, thereby facilitating lordosis behavior in female rats [22]. Studies in our laboratory [15] have shown plastic changes in projection neurons in the ventrolateral area of the ventromedial nucleus during different stages of the estrous cycle. Among other plastic changes in the neurons, we observed changes in the densities of spines and in the proportional densities of thin and mushroom spines. The density of spines was greater in diestrus, proestrus, and estrus with respect to metestrus, which was reflected in the greater proportional density of thin and mushroom spines in those stages in which the circulating levels of estradiol are higher. We suggest that these changes are associated with neuroendocrine mechanisms, and that they do not respond to any kind of activity related to cognition. Furthermore, these findings evidence that the functional role of the types of spines that have been classically linked to learning (thin spines) and memory (mushroom spines) would also be linked to other neuropsychophysiological events, beyond those related to the expression of cognitive functions.

The largest number of thin spines in stages like those above could be related to the rapid transmission of synaptic information that is, in fact, mediated by spines with the same geometric characteristics as the thin spines [23–25]. Thus, thin spines would then mediate those changes that in the short term influence female behavior in the shorter phases of the reproductive cycle. Meanwhile, the largest proportion of mushroom spines could be related to the changes that may occur slowly in the formation of patterns of sexual behavior by virtue of the fact that the transmission mediated by this type of spine triggers responses mediated by secondary messengers that, when transmitted to the nucleus, generate the synthesis of proteins [16, 17, 26] capable of modifying the psychophysiology of sexual behavior. Thus, the plasticity of dendritic spines in this region unrelated to cognitive activity might be more related to patterns of synaptic activity. Yes, like those related to cognition, but whose functional significance should be associated with the bioelectric activity that is most fundamental to synaptic activity. Consequently, the interpretation of the plastic changes mediated by dendritic spines should be attributed to the very psychophysiological activity of the noncognitive region in question.

Sensory input is of great importance in the deployment of sexual behavior. Olfactory information that has passed through regions such as the olfactory lobe, the amygdala, the stria terminalis, and the medial preoptic area or the hypothalamus has not yet been involved in nervous

centers that give that olfactory information a cognitive character. In all of these structures, it has been seen that exposing rodents to the smell of the opposite sex results in an increase in *fos*-protein, which is related to the corresponding increase in metabolic and cellular activity [27]. Thus, the brain generates a plastic response at the molecular level in the presence of relevant sexual stimuli. Studies of brain lesions have shown that massive damage to the medial preoptic area eliminates sexual behavior of male rats throughout the entire life of the individual [28]. In the case of females, the same occurs when the ventromedial hypothalamus is injured [29], a site that, as has already been mentioned, relates to the organization of female sexual behavior.

Despite those findings, there exists in the literature a vast quantity of work, which mentions the role of the prefrontal cortex—an area clearly associated with cognition—in relation to sexual behavior [30–32]. A study by Agmo et al. [33] reported that injuries to the prefrontal cortex and particularly to regions that receive information from the amygdala considerably delay the onset of sexual behavior. However, that same study reported that once male sexual behavior does begin, it then develops normally despite the injury. These data suggest that the prefrontal cortex could be playing an important role in the integration of the information necessary to initiate the approach during sexual arousal. Moreover, other structures participate in the emergence of sexual behavior as sensory receptors, as well as in the expression of copulatory behavior. It is important to highlight that there exists significant gender dimorphism in humans and rodents related to the structures involved in the expression of sexual behavior [27]. This might mark some tendencies about the way processes not directly associated with cognition could differ with the gender of the individuals.

There is experimental evidence that shows a direct relationship between good and bad performance in cognitive tasks and varying levels of hormones such as estradiol and progesterone during the menstrual cycle. It has been observed that in the execution of cognitive tests involving verbal fluency, perceptual speed, fine motor skills, verbal memory, and working memory, performance is higher during the follicular phase, when the greatest amount of estradiol is present in blood plasma. Likewise, when plasma progesterone levels reach their peak in the cycle—halfway through the luteal phase—performance improves on tests of visual memory, in comparison with the menstrual phase [34, 35]. This suggests that there is a differential modulation of cognitive processes by some ovarian hormones, depending on the variation of their concentrations throughout the menstrual cycle. Fernández et al. [36] conducted a longitudinal study that used fMRI to observe the brain activity of young women as they completed cognitive tests focused on language use during different phases of their menstrual cycle. They obtained data that suggested that the neural recruitment necessary to carry out such tasks is very sensitive to the hormonal fluctuations—progesterone and estradiol—of the menstrual cycle. The results likewise showed that the activity of the cortical areas associated with language varies through the different stages of the cycle, and that both progesterone and estradiol were capable of modulating neuronal plasticity of certain areas during the tests. The influence that hormones—which do not imply any cognitive process—have on tasks that are entirely associated with cognition is remarkable.

As in the case of the female reproductive cycle, other hypothalamic structures play a key role in the establishment of some biological rhythms. The suprachiasmatic nucleus and preoptic

area of the hypothalamus are associated with the expression of the sleep-wake cycle. This cycle is regulated by the activity of a number of genes, such as the *per*, *clock*, and *tim* genes, which are transcribed cyclically [37, 38]. From the viewpoint of synaptic plasticity, a study from Girardet et al. [39] on rats showed an increase of afferent glutamatergic synapses toward the suprachiasmatic nucleus of the retina, a phenomenon regulated by the input of photic information [40]. That working group also associated with the synaptic arrangements and the configuration of the glia in the suprachiasmatic nucleus with the entrance of photic information that regulates the sleep-wake cycle.

The suprachiasmatic nucleus is comprised of general bipolar small neurons whose dendrites may branch or not. Those dendrites display the presence of dendritic spines whose distribution is irregular [41] and which establish synaptic contact with 33% of all of the synapses in the said nucleus [42]. Despite the relevance of the eventual synaptic plasticity that could implicate the circadian regulation of the sleep-wake cycle, there are no studies that show variations in the synaptology of the suprachiasmatic nucleus, which strongly suggests that investigations ought to be performed.

Some studies discussed the relevance of the use of diverse techniques to establish which circuits are involved in insomnia in human adults [43, 44]. However, it seems clear that the participation of structures associated with cognition (like the prefrontal cortex) is also linked with the adequate establishment of those circuits that govern the sleep-wake cycle. Among these structures, it has been reported that the medial prefrontal cortex reduces its functional connectivity with the medial temporal cortex [45].

It has also been reported that the medial and inferior prefrontal cortex showed a decrease in activity when performing fMRI [46], and, particularly, it has been shown that pyramidal neurons from the infralimbic cortex layer III experience alternating plastic changes during both phases of the cycle: in the nocturnal phase, neurons show a pattern of dendritic arborization that is more profuse, and a greater density of spines in comparison with the diurnal phase, which could relate to the cyclical activity of the liberation of different neurotransmitters, growth factors, and corticosterone, in association with the afferent activities of the fibers of the suprachiasmatic nucleus [47]. Another study showed a decrease in the gray matter of the orbitofrontal cortex and in the parietal cortex [48]. In these studies, the results were associated with the presence of insomnia. Together, these investigations suggest that the sleep-wake cycle, although it is a biological rhythm, could also be regulated by structures associated with cognition.

Voluntary motor activity is the result of a series of mostly cognitive processes in which certain areas of the neocortex such as the prefrontal cortex, the premotor cortex, the parietal posterior cortex, and the primary motor cortex, as well as subcortical areas such as the basal ganglia, the thalamus, and the cerebellum participate [49, 50]. All these structures form different circuits for programming and establishing the commands necessary for the execution of movement. The information that is processed in them is sent through the spinal cord by the pyramidal tract from the Betz pyramidal neurons in the primary motor cortex. These last neurons perform the final step in circuits, integrating all the information that has been processed previously.

The pyramidal neurons in layer V of the primary motor cortex do not perform cognitive processing of the information they receive, but rather represent the final necessary filter for that information before motor function [49–51]. Little has been studied about the plastic capacity of these neurons. However, there is research that shows that after behaviors with a cognitive component, such as motor learning [52] and self-stimulation [53, 54], changes occur in the dendritic arborization of pyramidal neurons in layer V of the primary motor cortex. At the same time, preliminary studies conducted in our laboratory (submitted to publication) showed a greater density of dendritic spines (thin, mushroom, and branched spines) on neurons in the layer V of the motor cortex of mice subjected to forced motor activity over a week under differing levels of intensity using a treadmill device. The increase in branched spines corresponded directly to the increase in thin spines, a phenomenon that could be interpreted as a circumstantial demand for the integration of information coming to those neurons due to the increased demand for motor performance. For its part, the greater proportion of mushroom spines could be interpreted as the establishment of patterns of motor activity, which adjusted throughout the study to meet the increasing demand for physical effort on the part of the rats.

Another necessary component for the performance of voluntary movement is the adjustment of patterns of motor execution at the cerebellar level, a characteristic that, it has been suggested, is not associated with the processing of cognitive information. In this sense, it has been shown that Purkinje cells of the simple lobe of the cerebellum present plastic changes at the level of their dendritic spines during the performance of moderate motor activity. Such modifications consist of an increase in the stubby dendritic spines, which could be due to the input of excessive afferent synaptic information—inherent in the requirements of motor activity—which stimulates the postsynaptic components (the dendritic spines), thus causing the formation of the type of spines that would regulate the hypothetical hyperexcitability of the Purkinje neurons involved [55].

The role of the cerebellum during motor learning, a cognitive process, has also been approached. Those studies have investigated metabolic activity in the cerebellar cortex [56] and the plastic changes of the dendritic spines on the Purkinje neurons, particularly in the region that corresponds with the paramedian lobe [57]. In keeping with the role attributed to the spines in paradigms involving cognitive activity, the study found that there was a particular increase in thin spines (acquisition of new information, or learning) and in mushroom spines (consolidation of acquired information, or memory).

Overall, the results of research related to the functional activity of the cerebellum suggest that differential regions of this brain structure work in concert as much in cognitive processing—motor learning—as in noncognitive processing—motor adjustments. In both cases, there is evidence of plastic events at dendritic spines level underlying these processes.

As mentioned in a previous section, there exists a debate over the role of cognition in the emotions. There are certain brain structures involved in the neural circuits that lead to the expression of emotions, including the amygdala and the prefrontal cortex. In the noncognitive

processing of emotion, the amygdala plays a key role. This structure participates in two distinct ways. In the more direct way, the amygdala receives thalamic sensory afferents that provide the information necessary to generate emotional responses and, given their connections with the hypothalamus, that induce autonomic activation. Moreover, the connections of the amygdala with the periaqueductal gray matter and the medulla give way to the responses of “freezing” or fight/flight, respectively. The slower way for the amygdala to participate is an indirect route in which the prefrontal cortex processes information associated with the emotional significance of experiences, providing the amygdaloid complex with the information necessary to trigger the appropriate response [6].

From the above, it can be concluded that the expression of emotions depends on structures that are related both to the organization of cognitive functions—the prefrontal cortex, in the indirect route—and with noncognitive functions—the amygdala, in the direct route. It has been reported that chronic stress induces an increase in dendrite length, in dendritic arborization, and in the density and length of the spines of the neurons in the nuclei of the basolateral amygdala. In turn, acute stress provokes an increase in the density of spines, which could be associated with an increase in elevated circulating levels of glucocorticoids [58], and it is clear that the neuronal cytoarchitecture of the amygdala related to noncognitive processes is also subject to plastic changes.

It is common to think of us processing emotions either “consciously” or “unconsciously” [2]. From a neuroscientific point of view, these two terms are what we have in this chapter referred to as “cognitive” and “noncognitive.” A recent review of Lee et al. [59] discusses the importance of the noncognitive processing of information necessary for the expression of emotions, particularly in individuals diagnosed with anxiety disorders, schizophrenia, bipolar disorder, and stress. This paper makes a clear distinction between the perception of the emotions without the involvement of a cognitive process and the analysis of emotion that involves the assignation of meaning and emotional valence. It establishes that the perception of emotion that would involve structures such as the amygdala, the insular cortex, the anterior cingulate, and the primary visual cortex [60, 61] is a phenomenon in which information is processed about the stimulus that provoked an emotion without cognitive attribution [62] almost like an automatic processing of emotions without being aware of their meaning.

Although conflicting data regarding the structures that participate in this first step of the recognition of emotions [63] exists, it seems clear that the set of brain processes that involve emotional processing are not only limited to cognition, but that there exist other structures and previous, noncognitive processes that lead to emotional experience [2, 11, 13].

4. Conclusions

It should be clear that the processing of synaptic information in distinct regions of the brain is independent of the conceptual aspects of the neuropsychological process in question. Thus, the plastic events that underlie the functional organization of “cognitive” and “noncognitive” processes appear to present common neurophysiological and neuromorphological

bases. That is, they consist of adaptive changes in different levels of behavioral organization that, more than depending on the plastic events at a cellular level, depend on the structures involved and on the circuits that they establish among themselves in order to result in the expression of behavior.

Based on that, we would propose the following:

1. The intensification of experimental studies of neuronal plasticity related with “noncognitive” processes.
2. The broadening of the criteria of interpretation with regard to the functional significance of such plastic events.

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GABAergic Synapse Dysfunction and Repair in Temporal Lobe Epilepsy

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Additional information is available at the end of the chapter

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Abstract

Severe medial temporal lobe epilepsy (mTLE) is often associated with pharmacoresistant seizures, impaired memory and mood disorders. In the hippocampus, GABAergic inhibitory interneuron dysfunction and other neural circuit abnormalities contribute to hyperexcitability, but the mechanisms are still not well understood. Experimental approaches aimed at correcting deficits in hippocampal circuits in mTLE include attempts to replace GABAergic interneurons through neural stem cell transplantation. Evidence from studies in rodent mTLE models indicates that transplanted GABAergic progenitor cells integrate into the hippocampus, form inhibitory synapses, reduce seizures and improve cognitive deficits. Here, we review current work in this field and describe potential molecular mechanisms underlying successful transplantation.

Keywords: GABA, temporal lobe, epilepsy, hippocampus, GABAergic, interneurons, neuroligin, neurexin, seizures, inhibition, gephyrin, collybistin, cognition, spatial memory, behaviour, transplantation, therapy, stem cells

1. Introduction

Epilepsy is a brain disorder characterized by a predisposition to generate epileptic seizures that may have subsequent neurological, cognitive, psychological and social effects. Many patients with severe medial temporal lobe epilepsy (mTLE) experience intractable seizures and degenerative changes in the temporal lobes of the brain, particularly the hippocampus [1]. Pharmacological treatments for these patients may become ineffective [2–5], and chronic severe pharmacoresistant seizures in mTLE patients can lead to memory impairments, anxiety and depression. While removing epileptogenic foci provides better seizure control in these patients, surgery may not be feasible if the seizures are generated bilaterally or at

multiple foci. Moreover, one of the challenges of treating mTLE is that seizures trigger neuroplastic changes in the adult hippocampus, including axonal sprouting, rewiring and abnormal migration and growth of new dentate granule cells (GCs). Which of these changes are necessary and sufficient for generating recurrent seizures that can be corrected through cell-replacement therapies is not well known.

A major focus of current research is developing rigorous protocols for deriving human neural stem cells from pluripotent stem cells (PSCs) and directing their differentiation into the specific types of neurons, including subtypes of GABAergic interneurons. Additional studies are focusing on the circuit-level and molecular mechanisms that regulate incorporation of transplanted mouse or human GABAergic interneuron progenitors into host brains. Studies of the functional impact of transplanting GABAergic interneurons into the brain and spinal cord in models of different neurological disorders are still in their infancy [6], and relatively little is known about mechanisms guiding the survival, differentiation and synaptic integration of transplanted GABAergic interneurons in the adult brain. This review focuses on current work in this field of regenerative medicine and new directions for regenerating neural circuits.

2. Pathology of medial temporal lobe epilepsy

Dentate gyrus (DG) reorganization has been extensively studied in rodent models of mTLE, particularly the chemoconvulsant models that employ kainic acid or pilocarpine injections to induce status epilepticus (SE). Prominent features of DG reorganization are the loss of glutamatergic mossy cells and subsets of GABAergic interneurons. Depletion of these neuronal populations results in loss of feed-forward inhibition of DG GCs [7–11]. Some of the principal cells of CA1 and CA3, as well as adult-generated GCs of the DG born around the time of SE, sprout excitatory axon collaterals, increasing recurrent excitatory drive between neighbouring neurons [12, 13]. Many somatostatin (SOM) -expressing GABAergic interneurons degenerate in the DG and CA1 of the hippocampus in mTLE, and some residual GABAergic interneurons form compensatory synaptic connections [10, 14–18]. Axonal sprouting by surviving hippocampal GABAergic interneurons increases the number of inhibitory synaptic puncta above control values in chronically epileptic rodents, although this response is insufficient to counter the development of spontaneous recurrent seizures typical of mTLE [15, 19]. Together, these studies suggest that replacing hippocampal GABAergic interneurons in pharmacoresistant mTLE may be a promising strategy for suppressing seizures (**Figure 1**).

GCs are a type of excitatory glutamatergic neuron in the granule cell layer (GCL) of the DG of the hippocampus, and studies suggest that they are relatively more resistant to seizure-induced injury than many other cell types in the hippocampus [32]. They form axons called the mossy fibres that project to CA3 pyramidal neurons and other cell types [1, 33–35]. GCs are generated throughout life [28, 36–41]. In rodent models of mTLE and human patients, many GCs, born around the time of SE, develop altered morphology, excitability and connectivity. These adult-generated GCs form recurrent axon collaterals in the inner molecular layer, a form of neuroplasticity termed mossy fibre sprouting (MFS) [26]. Overgrowth of mossy

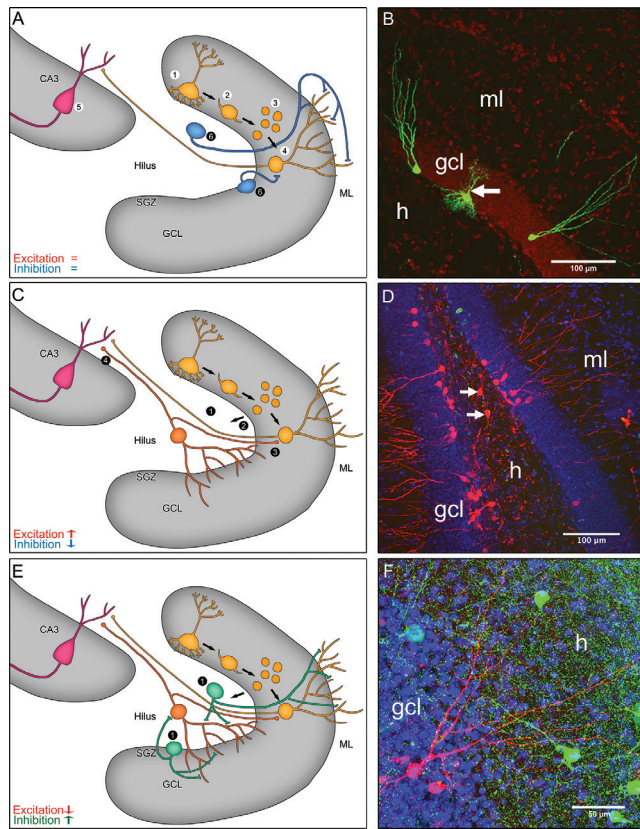


Figure 1. Studies of GABAergic interneuron transplantation are investigating whether it is feasible to replace populations of endogenous interneurons damaged by temporal lobe epilepsy. **(A)** In the non-epileptic mouse dentate gyrus (DG), self-renewing, quiescent Type 1 progenitor cells in the subgranular zone (SGZ) extend radial glial processes through the granule cell layer (GCL) (1). They divide asymmetrically to produce Type 2 progenitors (2) which further divide to generate pools of migratory Type 3 neuroblasts (3). As these neuroblasts mature, they differentiate into GCs, migrate into the hilus, extend their dendrites towards the molecular layer (ML)(4) and project axons to hilar interneurons and mossy cells and CA3 pyramidal cells (5). Inhibitory GABAergic interneurons (6) synapse with GCs and provide inhibition. GCs are shown in yellow, inhibitory interneurons in light blue and pyramidal cells in magenta. **(B)** High-resolution confocal image of retrovirally labelled GCs (green) and neuronal nuclei (NeuN, red) in the naive mouse hippocampus. White arrow indicates a normal Type 1 progenitor cell in the SGZ. **(C)** In mTLE, some of the hilar GABAergic interneurons die, resulting in an overall loss of inhibition to GCs (1). Additionally, some adult-generated GCs undergo abnormal migration into the hilus, becoming ectopic. They typically form abnormal dendrites and sprout recurrent axonal collaterals, forming excitatory feedback projections onto other GCs (3) as well as abnormal excitatory projections to the CA3 pyramidal cells (4) [20–30]. GCs are shown in yellow; an abnormal, ectopic GC in orange; and pyramidal cells in magenta. **(D)** High-resolution confocal image of retrovirally labelled GCs (red) in the hippocampus of a mouse with mTLE. Nuclei are marked using a Nissl stain (blue). White arrows show ectopic GCs born after induction of epilepsy located in abnormal locations in the hilus. **(E)** Studies employing transplantation of GABAergic progenitors into either the normal or epileptic hippocampus show that they migrate away from the site of injection and form dense axonal arbors throughout the hilus, GCL and molecular layer. These interneurons appear to form functional synapses with hyperexcitable GCs, including those with aberrant morphologies (1), increasing synaptic inhibition in the epileptic circuit. GCs are shown in yellow; abnormal, ectopic GC in orange; transplanted GABAergic inhibitory interneurons in green; and pyramidal cells in magenta [31]. **(F)** High-resolution confocal image of a retrovirally labelled GC (red) receiving dense synaptic contacts from transplanted MGE-derived GABAergic progenitors (green). Nuclei are labelled using Nissl staining (blue) [31].

fibre recurrent collaterals onto GCs and pyramidal cells contributes to a hyperexcitable dentate environment [27, 29, 42–44].

As demonstrated by computer simulations, a few hubs of highly interconnected GCs are sufficient to create a hyperexcitable network [30, 45]. Additional epilepsy-induced neuroplastic changes to the DG include GC dispersion, formation of GC basal dendrites and ectopic migration of GCs into the hilus of the DG [23–25, 46–50]. Many adult-generated GCs born in epileptic rodents also have reduced dendritic spines and hypertrophic cell bodies [20, 21, 44, 51]. These cells often have higher baseline firing rates than normally positioned GCs in epileptic animals and non-epileptic controls and more depolarized resting membrane potentials, predisposing them to hyperexcitability [22]. Although increasing adult neurogenesis is not sufficient to cause seizures, it contributes to hyperexcitability [52, 53].

3. Cognitive changes in medial temporal lobe epilepsy

Severe mTLE is linked to a number of comorbidities including cognitive deficits, heightened anxiety, increased aggression and depression [54, 55]. Rodents with mTLE show decreased social recognition, greater preference for closed arms in the elevated zero or plus maze test for anxiety and longer periods of immobility in the forced swim test for depression [56]. Additionally, a number of studies demonstrated severe learning deficits in the Morris water maze test of spatial memory [57, 58] and other spatial memory tasks [59]. Human mTLE patients exposed to virtual environments that test spatial memory also showed memory deficits [60, 61].

Changes in the properties of hippocampal and entorhinal cortex circuits may be responsible for cognitive changes in mTLE, but the nature of these changes is not well understood. Both human and rodent spatial memory formation and recall are dependent on place cells in the hippocampus and grid cells in the entorhinal cortex. These important cells exhibit distinct, spatially specific firing patterns, forming a topographical memory map of an area as an individual moves through space [62–65]. Hippocampal inhibitory interneurons, similar to place cells, show distinct, spatially specific discharges, implicating a role in the formation and fine tuning of spatial memories [66, 67]. Studies suggest that impairments in receptive field properties of the grid and place cells may occur in mTLE [68–71].

The regulation by inhibitory interneurons of various brain rhythms may also become altered, as rodents with mTLE show distinctly lower frequency and power of theta rhythms correlated with poor performance in spatial memory tasks [72–74]. Although the exact alterations occurring in the grid-place cell network are not yet clear, it is evident that the hyperexcitable firing of GCs and the overall disinhibition of the network by loss of inhibitory interneurons severely disrupts spatial memory formation [72, 75–79]. The disinhibition of the hippocampal networks following epileptogenesis and the subsequent development of spatial memory deficits suggest that the loss of inhibitory interneurons may disrupt place fields, providing a further rationale for cell-based therapies aimed at GABAergic interneuron transplantation.

4. Seizure suppression following transplantation of medial ganglionic eminence-derived neural progenitors

Initial studies established proof of concept for cell-based therapies for treating epilepsy by demonstrating that transplants of non-neural cells engineered to release GABA non-synaptically could increase seizure thresholds [80]. Increasingly, studies have aimed to identify the functional classes of interneurons that can migrate, integrate and suppress seizures in different models of epilepsy in rodents. The large variety of functional classes of cortical inhibitory interneurons and their sites of origin in the ventricular zones of the embryonic forebrain have been extensively studied. During development, forebrain GABAergic interneurons are born in the embryonic ventricular regions called the ganglionic eminences, including the medial, lateral and caudal ganglionic eminences (MGE, LGE, CGE). These transient proliferative zones lining the forebrain lateral ventricles generate different types of GABAergic interneuron progenitors, which then migrate to their final destinations in the forebrain, including the cerebral cortex, hippocampus and striatum [81–83]. Forebrain GABAergic progenitors expressing SOM or parvalbumin (PV) emerge from the MGE in early embryonic life and migrate tangentially into the cerebral cortex and hippocampus [84].

In naïve rodents and different epilepsy models, transplanted GABAergic interneuron progenitors from the embryonic MGE have been found to be highly migratory, a prerequisite for transplantation therapies aimed at repairing large brain areas. MGE-derived GABAergic progenitors transplanted into postnatal 3–4-day old mouse cerebral cortex differentiated into inhibitory interneurons expressing markers of mature GABAergic phenotypes, including PV, SOM, calretinin (CR) and neuropeptide Y (NPY), and displayed mature firing properties characteristic of inhibitory interneurons [85]. MGE-derived PV-positive interneurons transplanted into naïve postnatal 1–2-day old pups integrated into the endogenous circuitry and, upon maturation, displayed firing properties similar to endogenous PV-expressing interneurons and formed functional synapses onto pyramidal neurons [86]. In a mouse model of mTLE generated through neurotoxin-induced ablation of GABAergic interneurons, MGE transplantation significantly increased inhibitory postsynaptic currents (IPSCs) in CA1 pyramidal cells and reduced seizure frequency and severity [87]. These grafts contained high percentages of GABAergic interneurons that co-expressed PV, NPY or CR. Transplanting MGE cells into an epilepsy model caused by mutations of the Kv1.1 potassium channel also increased IPSCs in nearby endogenous pyramidal cells [88]. Additionally, MGE cell transplants into a cyclin D2 knockout model of hippocampal disinhibition restored lost inhibitory input and normalized hyperactivity and fear conditioning [89].

The efficacy of MGE cell transplantation for controlling seizures has also been studied in chemoconvulsant models of mTLE, including the kainic acid and pilocarpine (PILO) models. In an early ground-breaking study in the rat kainic acid model, Shetty and colleagues transplanted neurospheres derived from embryonic day-14 rat MGE progenitors and found that they reduced seizure duration, total time spent in seizures and seizure severity; however, these grafts failed to improve spatial memory deficits [90]. It is important to note that the

degree of cognitive impairment may differ between kainic acid or pilocarpine models, different species and even different strains of mice [91, 92].

The mouse PILO model shows a pattern of loss of hippocampal interneurons that is similar to human mTLE, making this model highly appropriate for preclinical studies investigating GABAergic interneuron transplantation [93]. Work from our laboratory showed that MGE cells transplanted into the hilus of the DG led to significant reductions in seizure frequency, duration and severity in the mouse PILO model [31]. The transplanted neurons matured into GABAergic interneurons that expressed CB, SOM or PV and formed dense networks of inhibitory synapses onto dentate GCs. Optogenetic experiments in hippocampal slices from these mice showed that light-induced depolarization of MGE transplants expressing channelrhodopsin (ChR2) triggered strong postsynaptic inhibitory currents in GCs, indicating that the transplanted neurons had integrated synaptically. These findings suggest that seizure suppression can be achieved with focal transplants into the DG. In this study, which employed continuous video-EEG recording for periods of up to 3 months, some of the mice show a reoccurrence of seizures several months after transplantation, suggesting that achieving enduring seizure suppression may require more widespread dispersion of the transplanted interneurons throughout different subfields of the hippocampus. Determining the optimal sites and cell types for permanent seizure suppression will be important for moving into clinical applications.

5. Transplantation therapy using human embryonic stem cell-derived progenitors

For treating patients with severe mTLE, sources of human interneurons are required. Previous work showed that differentiating human embryonic stem cells (hESCs) into GABAergic inhibitory interneuron progenitors can be achieved using specific combinations of signalling molecules and growth factors [94–100]. Carpentino et al. (2008) found that maturation of transplanted mouse or hESCs is highly dependent on the environment into which the cells are transplanted. For instance, in the mouse systemic kainic acid model, it was shown that ESC-derived neural progenitors transplanted in the CA3 area tended to migrate into the DG and differentiate into GCs, whereas those implanted into the fimbria tended to mature into astrocytes [101]. Lee et al. also transplanted undifferentiated hESCs into the CA3 region of the hippocampus in epileptic rats. Some of these differentiated into GABAergic interneurons (~21% of engrafted cells) and at 8 weeks post transplantation displayed immature morphology. Even with low numbers of GABAergic neurons, the animals with transplants showed reduced seizure frequency and seizure duration for 2–3 months. EEG recordings in these animals were limited to 60 hours per week, with a 2-week recording period, during daylight hours only. Additionally, when tested 3 months after transplantation, improvements in Morris water maze performance were not found [102]. More recent studies have focused on purified, fate-determined populations of hESCs for transplantation. Treating hESCs with the signalling molecule sonic hedgehog (SHH) or a sonic hedgehog agonist (SAG), in combination with modulating the WNT and FGF signalling pathways, can be used *in vitro* to induce ventral forebrain neural fates and MGE-like cell types [97–100, 103–106]. Ventralized hESC-derived progenitors have

identities similar to that of mouse MGE-derived GABAergic interneuron progenitors, potentially allowing the large-scale *in vitro* production of human cells for therapies to treat clinical disorders [94]. However, undifferentiated hESCs can cause teratomas, making it important to develop protocols for eliminating them prior to transplantation [107].

Evidence that fate-directed human GABAergic interneuron progenitors integrate into the epileptic circuitry of the hippocampus following transplantation into the hilus has emerged in several recent studies. To reduce immune rejection of cell grafts, human and mouse ESC transplantation studies generally use immunodeficient host animals. The nonobese diabetic (NOD)-severe combined immunodeficiency (SCID) mice are an immunodeficient mouse strain lacking mature T and B cells and with reduced natural killer (NK) cell activity. Another mouse strain, the Nod-scid-gamma (NSG) triple mutant, has a mutation at the interleukin-2 receptor (IL-2R) γ -chain locus. This strain shows the highest impairment in T-cell, B-cell and NK-cell development, resulting in low graft rejection [108]. Both strains have been used to study differentiation of ESC-derived GABAergic interneurons [109].

In a recent study in which hESC-derived progenitors were differentiated *in vitro* into MGE-like progenitors and transplanted into NSG mice, the transplanted cells differentiated into GABAergic neurons expressing SOM, PV, CB, CR or NPY after approximately 4 months. Additionally, optogenetic stimulation of the transplanted cells produced action potentials and resulted in IPSCs in endogenous hippocampal neurons, suggesting successful synaptic integration into the existing circuitry of the hippocampus. Video-EEG monitoring of these animals 3 months post-transplant showed reduced numbers of seizures in engrafted animals [110]. However, the EEG monitoring was only for short durations of 5–10 days, which is likely too brief a period to reliably evaluate seizures in rodent chemoconvulsant models, due to the clustered and periodic nature of the spontaneous recurrent seizures.

6. Ameliorating cognitive and behavioural abnormalities in epilepsy by transplantation of GABAergic interneurons

Inconsistent results regarding spatial memory improvement have been reported following GABAergic interneuron transplantation. In the Morris water maze test of spatial memory, C57BL/6 mice with PILO induced mTLE and received mouse MGE cell transplants showed significantly reduced escape latencies in training, significantly more platform crossings in the probe trial; improved path efficiency; and a greater amount of time spent in the target quadrant than epileptic controls [111]. In another study, rats with mTLE that MGE-derived stem cell grafts showed no improvements in the Morris water maze task 8 weeks post-engraftment relative to non-engrafted mTLE controls [90]. However, transplantation took place approximately 3 months following induction of epilepsy, a longer time interval than other studies. The lack of cognitive improvement at this later transplantation time point suggests a potentially limited time window in which transplanted GABAergic interneurons must integrate to confer cognitive improvements. In a third study in NSG mice with mTLE, engrafted hESC-derived GABAergic interneuron progenitors appeared to improve performance in the Y-maze test of spatial memory and memory in the novel recognition test [110].

Behavioural tests also suggested that mTLE mice receiving interneuron grafts were less hyperactive and aggressive, compared to mTLE controls with only intrahippocampal injections of media. In the handling test of aggression, in which mice are scored for aggressive reactions to a series of increasingly uncomfortable stimuli, TLE mice with hESC or foetal mouse MGE interneuron transplants scored significantly lower in aggression ratings than controls [109, 110]. These transplants also reduced hyperactive behaviour [110]. Taken together, these results suggest that both rodent and human GABAergic interneuron transplants may ameliorate some of the psychological comorbidities in rodents with mTLE. While the Morris water maze is currently one of the standard tests in the industry for spatial memory, rodents with mTLE often exhibit a phenomenon known as thigmotaxis, in which animals will locomote or swim adjacent to the walls of an apparatus or make repeated circles [91, 112]. In such animals, it is uncertain whether the data reflect poor spatial memory or an anxiety phenotype. Therefore, alternative spatial memory tests should be used to gain a more complete understanding of how GABAergic interneuron transplantation affects cognition. An alternative test of hippocampal-dependent spatial memory is a modification of the novel object recognition test in which animals must learn to recognize that a previously familiar object has changed location. This test, called novel object location task, takes advantage of the rodent preference for novelty and desire to explore changes in its environment [113, 114]. Another test of spatial memory is the Barnes maze, consisting of an elevated platform with closed holes around the circumference. One hole is available for escape into a dark box. Remaining on the platform is unpleasant to the rodent, due to bright lights, fans and/or loud ambient noise, encouraging a swift escape to the box. As this test measures a very natural desire to escape an unpleasant environment, it is considered an effective test of normal rodent behaviour and spatial memory [115]. The Barnes maze also has no walls, eliminating thigmotaxis, although care must be taken to prevent animals from falling from the raised platform. Additional tests of spatial memory include the Y-maze, T-maze and the radial arm maze, all of which measure the ability of a rodent to remember previously travelled areas [116–121]. This extensive array of spatial memory tests can provide a more complete picture of the behavioural improvements following GABAergic interneuron transplantation in rodents with mTLE.

Currently, most testing of aggression has been done using the handling test, which, while effective, is an unnatural stimulus to the rodent [110, 111]. In addition to the handling test, the resident-intruder test can be used to analyse the response of a rodent to more natural stimuli. Male rodents are territorial, and the resident-intruder test measures the aggressive reactions to a male rival within their space. Although care must be taken to avoid injury to animals, this test measures an innate animal response and can be an effective measure of aggression in mTLE animals with transplants [122].

Although heightened anxiety is a common and well-characterized comorbidity in rodent mTLE models and human patients, surprisingly little work has been done to examine the effects of GABAergic transplants on correcting anxiety phenotypes. As rodents with mTLE have a tendency to exhibit thigmotaxis, which skews results in tests such as the open field test or the Morris water maze [91, 112], paradigms such as the elevated plus maze, elevated zero maze or the light-dark box can be used to provide more accurate measures of anxiety in rodents with mTLE [123–128].

7. GABAergic synapse formation and stability: potential mechanisms of transplanted cell integration

Relatively few studies have examined the molecular mechanisms responsible for guiding synaptic integration of transplanted cells into mature neural circuits. Previous findings suggest that cell-cell interactions mediate the formation and stabilization of both excitatory and inhibitory synapses [129–131]. The synaptic scaffolding complex between GABAergic interneurons and their postsynaptic targets in the developing brain may also guide recruitment and stabilization of the new synaptic connections formed by transplanted interneurons (**Figure 2**).

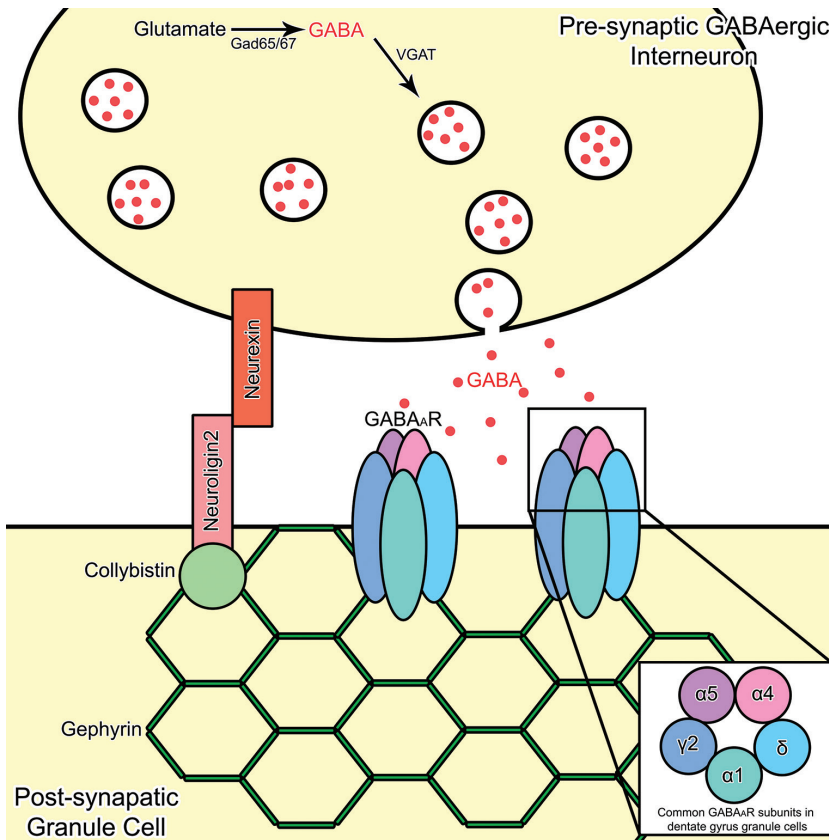


Figure 2. Interactions between cell surface molecules that are binding partners provide a potential mechanism for forming or stabilizing new synapses between transplanted GABAergic interneurons and endogenous neurons in the hippocampus. GABAergic synapse formation is coordinated by multiple molecules in the pre- and postsynaptic sites. Binding between presynaptic neurexin molecules and postsynaptic neuroligin2 (NLGN2) molecules may be important for initial formation or maintenance of GABAergic synapses. NLGN2 is associated with a postsynaptic complex containing collybistin, gephyrin and GABA_ARs, which are necessary in the formation of functional inhibitory circuitry. Collybistin, gephyrin, NLGN2 or GABA_AR subunit γ2 deficiency results in impaired inhibitory synapses [132–141].

The synaptic scaffolding protein gephyrin is a tubulin-binding protein that forms a lattice-work structure of hexagonal trimers that regulate GABA_A receptor clustering at synaptic sites [142, 143]. Gephyrin stabilizes inhibitory synapses and is required for proper function. Genetic reduction of the $\gamma 2$ subunit of GABA_A receptors, a primary binding partner of gephyrin in GABAergic synapses, also severely reduces gephyrin and GABA_A receptor clustering required for functional inhibitory synapses [132]. Repression of gephyrin expression causes a similar loss of clustering, revealing an interdependent relationship between the two synaptic binding partners necessary for proper inhibitory synapse formation and function [132, 144, 145]. Increases in endogenous gephyrin in response to compensatory surviving interneuron sprouting may also make the epileptic hippocampus a more receptive environment for new inhibitory synapses to form [146]. Gephyrin is significantly decreased in the first few weeks post-SE followed by a significant increase back towards normal levels at around 1 month post-SE [147]. Following transplantation, a majority of engrafted GABAergic interneuron synaptic boutons were associated with postsynaptic gephyrin clusters, indicating that this vital synaptic scaffolding component may be recruited to sites of new GABAergic synapse formation in the adult hippocampus [31].

Collybistin, another GABAergic synaptic scaffolding component, binds to both gephyrin and Neuroligin 2 (NLGN2) and may facilitate gephyrin-mediated clustering of GABA_A receptors. Collybistin is a GDP/GTP-exchange factor that interacts directly with gephyrin in the inhibitory synaptic scaffold [131, 133, 148–150]. Collybistin-deficient mice display reduced clustering of gephyrin and GABA_A receptors, reduced synaptic inhibition and altered synaptic plasticity [131, 141].

NLGN2 is part of a family of cell adhesion molecules implicated in synapse formation and stability. NLGN2 localizes only to GABAergic inhibitory synapses, where it is associated with neurexin, its presynaptic binding partner [151–153]. NLGN2 is part of the molecular scaffolding complex that includes collybistin and gephyrin [133]. NLGN2-deficient mice show decreased inhibitory function, as well as a variety of cognitive and behavioural comorbidities, such as increased anxiety, aggression and disruptions in spatial memory formation, similar to those seen in mTLE and other neurological disorders [129, 136, 137, 154–157]. Various studies have shown that binding between NLGN2 and neurexin induces inhibitory synapse formation [130, 158] and stabilization [135, 159], even in non-neuronal cell types [160].

GABA_A receptor subunit composition may also play a role in the integration and stabilizing influence of transplanted inhibitory interneurons. Composition of GABA_A subunits is impacted by the pathological changes induced in mTLE [161–163]. DG GCs, which are significant propagators of hyperexcitability in mTLE, are particularly enriched in the δ subunit of GABA_A receptors in the normal brain; these receptors have a very high affinity for GABA and are strongly involved in tonic inhibition [164–166] at extrasynaptic sites [167]. In general, hippocampal neurons express multiple subunits, including abundant α , β , δ and γ subunits, with δ primarily restricted to GCs of the DG, with additional expression of other subunits in the CA1 and CA3 areas [168]. As such, it is apparent that GABA_A receptors in the hippocampus are composed of a diverse pool of subunits that regulate inhibitory input. In mTLE,

the composition of GABA_A subunits becomes altered. Similar to the upregulation of gephyrin during the chronic phase of mTLE in response to compensatory interneuron sprouting, the $\gamma 2$ and α subunits also show increased expression in the hippocampus. Conversely, expression of the δ subunit decreases days after the initial epileptic event and remains depressed into the chronic stages of mTLE [169]. It is not known whether synapses formed by surviving inhibitory interneurons are capable of recruiting the necessary subunit composition for proper inhibition, considering the overall depletion of δ subunits compared to $\gamma 2$ and α . Moreover, whether transplanted, healthy GABAergic inhibitory interneurons can recruit all of the normal subunits to inhibitory synapses is not known. Further investigation of subunit composition within the epileptic hippocampus post transplantation will be necessary to investigate whether transplantation normalizes GABA_A receptor composition.

8. Conclusion

While safe and effective stem cell therapies for treating neurological disorders, including severe mTLE, may be years away from the clinic, recent work has increased scientific understanding of how to derive specific types of human neurons for transplantation and how to evaluate functional changes that result. Because human neuron maturation takes many months or years, transplantation studies in rodents are limited in the kinds of information they can provide about the potential therapeutic effects of these cells in clinical populations. Recent studies have utilized a wide range of experimental tools, including electrophysiology, immunohistochemistry, optogenetics, chemogenetics and behavioural assays to assess learning, memory, anxiety, social behaviour and depression. These approaches are aiding studies to evaluate synaptic integration and functionality of human neural stem cell transplants for treating epilepsy.

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Neuroplasticity in Bipolar Disorder: Insights from Neuroimaging

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Additional information is available at the end of the chapter

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Abstract

Background: Advances in neuroimaging techniques have produced evidence about disrupted frontolimbic circuits related to emotional regulation. These neuroimaging studies may suggest impairments in cellular plasticity in bipolar disorder (BD) patients. However, the long-term use of mood stabilizers may restore these dysfunctions by neurotrophic effects

Objectives: Review the major structures of the brain that underpin this disorder, synthesize the main findings in neuroimaging in BD, and debate neuroplastic effects of psychopharmacological treatment on findings from the neuroimaging studies.

Methods: We undertook a review from neuroimaging in BD. Search words entered were "bipolar disorder", "mania", "depression", "mixed states", "suicide", "psychosis" "lithium", "mood stabilizers", "neuroplasticity", magnetic resonance imaging, "functional magnetic resonance", "FDG-PET", "SPECT".

Results: The literature highlighted specific brain areas that support emotional regulation and processing. Moreover, there is heterogeneity across studies and some findings are controversial, but some results suggest neuroplastic effects from the long-term psychopharmacological treatment (particularly mood stabilizers) in bipolar disorder.

Conclusion: The findings in neuroimaging studies suggest there is fronto-limbic circuitry dysregulation in BD; changes in specific brain areas have been replicated in several studies, which may reflect impairments in neuroplastic phenomena. Evidence from neuroimaging studies have been also show that long-term treatment may be

associated with metabolic/functional compensation or structural restoration in bipolar responders.

Keywords: bipolar disorder, neuroimaging, treatment, neuroplasticity

1. Introduction

Bipolar disorder (BD) affects around 3% of the population [1] and is a serious multifactorial disease, caused by combination of genetic vulnerability and environmental stressors with abnormalities in neurotransmitter and neuroendocrine systems, and intracellular signaling pathways as well. Clinically, BD is characterized by recurrent changes of thought, behavior, cognition, mood, and desynchronization of circadian rhythm, which imply in affective phases—mania, hypomania, depression, and mixed states. As a result, BD is a condition often difficult to diagnose, since at least 50% of patients with BD have an initial episode of depression and 35% may have a delay in their diagnosis in up to 10 years [2]. In this context, samples more homogeneous in neuroimaging studies in BD may allow better understanding of BD pathophysiology, through the establishment of putative associations between areas and neuronal circuits and clinical phenotypes, and also to clarify the utility of the various neuroimaging methods for determining potential neurobiological markers of BD.

In this sense, some authors propose concepts in neuroimaging biomarkers for mood disorders, such as *prognostic biomarkers* that characterize the risk for onset or progression of the disease, *predictive biomarkers* associated with the likelihood of therapeutic response, and *pharmacodynamic biomarkers*, which show biological response related to drug treatment [3].

The majority of neuroimaging studies in BD have been demonstrated abnormalities in different cortical and subcortical areas involved in emotional processing and regulation, while *postmortem* histopathological studies of these regions have shown abnormal reductions of synaptic markers and glial cells in prefrontal cortex and hippocampus and point to a dysfunction of the complex intracellular mechanisms, which involve second messengers systems, regulation of the genic expression and synthesis of trophic factors [4]. Overall, these neuropathological and neuroimaging studies may suggest impairments in cellular plasticity and resilience in patients who suffer from mood disorders.

Conversely, a substantial body of evidence suggests that long-term psychopharmacological treatment with antidepressants and mood stabilizers—in particular lithium and valproate—may compensate for this dysfunction by reducing the pathological limbic activity subjacent to affective symptoms and by regulating gene expression of neurotrophic factors that exert neuroplastic effects within the pathways modulating emotional expression. Such effects may be associated with structural restoration or enlargement of specific brain areas in chronically treated BD patients evaluated in multiple neuroimaging studies, when compared to healthy controls [5–7].

Keeping these issues in mind, the purpose of this chapter is to review the major cortical and subcortical structures of the brain that underpin this disorder, describe the main findings in

structural and functional neuroimaging in BD, and synthesize impaired major cellular plasticity mechanisms and potential neuroplastic effects of mood stabilizers on structural and functional findings from the neuroimaging studies.

2. Circuits and neuronal models of emotion regulation

- Ventrolateral prefrontal circuit: Constituted by the ventrolateral prefrontal cortex (VLPFC)—Brodmann areas (BA) 10 and 47—which sends fibers to the ventromedial striatum (ventromedial caudate nucleus, ventral putamen, nucleus accumbens, and olfactory tubercle) and which projects to the globus pallidus (GP); pallidal fibers follow for ventral anterior and dorsomedial nuclei of the thalamus, which connects again to VLPFC. The anterior temporal cortex (areas comprising BA 20 and BA 38) maintains reciprocal connections with VLPFC and amygdala [8, 9].
- Ventromedial prefrontal circuit: Formed by the ventromedial prefrontal cortex (VMPFC), defined by BA 11 and 12, of which depart fibers to follow to the ventromedial striatum and ventral caudate nucleus. From these regions, projections run for ventral anterior, dorsomedial and ventrolateral nuclei of the thalamus, closing the circuit with thalamic pathways to the ventromedial cortex. It should be mentioned projections of these cortical areas also to the entorhinal cortex and amygdala—lesion in these areas produces impairment in the allocation of emotional valence of information, a process that facilitates storage of information—as well as reciprocal connections between the insula with the amygdala and the VMPFC [9].
- Anterior cingulate circuit: The ACC is the most part of BA 24, 25, and 32. These cortical areas maintain connections with the ventral medial striatum, of which follow fibers to the rostral lateral and ventral GP, which in turn sends projections for dorsomedial nucleus of the thalamus; fibers depart from this topography and return to the ACC, closing this circuit [8, 9].

The ACC is divided functionally into ventral or “affective” region (more anterior portions of BA 25 [paragenua] and BA 24 [subgenua]) and dorsal or “cognitive” region (posterior pre- limbic area [BA 32] and more posterior portions of BA 24).

The affective division of the ACC has connections with the amygdala, the periaqueductal gray matter, the anterior thalamic nuclei, the ventral striatum, and the insula; it contributes to the regulation of endocrine and autonomic functions, generation of appropriate social behavior, and part of the global emotional response by activation of somatic and visceral states relevant to emotional experience; cognitive division includes the posterior portions of BA 24 and 32 and connects to the periaqueductal gray matter and primary and associative cortical motor areas; it is associated with inhibition responses [10] and monitoring conflicts [11, 12].

- Dorsolateral prefrontal circuit (DLPFC) includes BA 10, 45, and 9/46 Brodmann, which covers part of the lateral surface of the frontal lobes. These regions depart fibers to the dorsolateral caudate nucleus, which in turn sends fibers to the GP and then to ventroanterior, dorsomedial, and ventrolateral nuclei of the thalamus; thalamic pathways from these nuclei return to DLPFC [8, 9].

Compounding these circuits, the cerebellum receives cortical projections from nuclei located in the base of the pons; the fibers of the pontine nuclei decussate and follow the middle cerebellar peduncle to specific cerebellar targets: while the motor cortex projects to the cerebellum (paravermian region) through lateral pontine nuclei, associative cortical areas of the prefrontal, parietal, and temporal regions as well as ACC reach the cerebellum through pontine nuclei medial [13].

The amygdala is divided into three major sections: basolateral, corticomедial, and central. The basolateral nucleus participates in the sensory information integration from external and internal environments, which are linked to learned information and are processed by associative cortical areas, with subsequent planning, selection, and implementation of the action; corticomедial nucleus contributes to the presence of emotional attributes related to sensory and nociceptive stimuli; and the central nucleus is the convergence site of all signs of the amygdala. The amygdala regulates the fight, flight, or freeze behaviors together with the periaqueductal gray matter and contributes to motor and autonomic responses to emotional stimuli [14].

Among several neural circuit models related to processes of emotional perception and regulation proposed in the literature, the Mary Phillips and coworkers' model highlights over others [15, 16]. This model proposes the existence of two neuronal systems: a ventral system comprising subcortical (the amygdala, the insula, the ventral striatum) and cortical structures (the hippocampus, the anterior cingulate, and prefrontal cortex) and would be linked to identification of the emotional meaning of a stimulus associated with generation of affective states and autonomic regulation; the dorsal system would be represented by dorsal regions of the anterior cingulate and PFC as well as the hippocampus and would support cognitive processes such as selective attention, planning, performance monitoring, and voluntary regulation of emotional states.

The assessment of neuroimaging findings in BD allows to corroborate the relevance of this model, from the identification of dysfunction in different cortical and subcortical areas—as already stated, structures involved in processing and emotion regulation—abnormal increase in activity of the amygdala during performance of emotional and non-emotional tasks; abnormal decrease in activity of the VLPFC and orbital frontal cortex (OFC); and abnormal decrease in functional connectivity between the amygdala and the prefrontal cortex during emotional regulation tasks. Moreover, in studies involving reward paradigms (anticipation of reward), there is abnormal increase in activity of the ventral striatum, the VLPFC and OFC [17, 18].

3. Main findings of neuroimaging in BD

3.1. White matter

The white matter (WM) hyperintensity is a change often described in BD patients, both in adult [19] and pediatric [20] samples. Among the WM association bundles, the corpus callosum (CC) is one of the structures of great interest in BD research. In this region, studies using diffusion tensor

technique (DTI) often show loss of structural integrity of the CC in its various segments (genu, body, or splenius) [21, 22]. Moreover, a recent study conducted by our group evaluated bipolar patients type I euthymic and showed reduction of CC in the areas of the genu and isthmus when compared to healthy controls, confirming data from other studies [23, 24], but with no significant difference between suicide and non-suicidal [25]; a meta-analysis documented the volume reduction of this structure in bipolar patients [26]. Finally, another study found that bipolar patients without suicide attempt had lower values of fractional anisotropy (FA) in the genu and body of the CC when compared to unipolar depressed and healthy controls, and bipolar suicide patients had reduction of FA in all regions of the CC when compared to healthy controls [27]. In addition, more recent studies have shown that in euthymic and non-euthymic bipolar patients with a history of psychotic symptoms was observed higher area of the rostrum of the CC [28] and lower FA in the body of the CC in bipolar euthymic or depressed patients [29]; importantly, changes of the CC have also been described in children and adolescents with BD [30] and in groups of risk for BD, such as first-degree relatives [31].

Furthermore, loss of functional integrity was verified in other associative bundles of white matter, for instance, uncinate fasciculus (which connects the orbital frontal cortex and areas of the ventromedial prefrontal cortex to the amygdala and hippocampus) was studied in some works, in which the results are inconsistent, with bilateral reduction of AF [32, 33] or increased AF to the left in this region [34]. Finally, lower FA in the left orbital frontal WM among patients with attempted suicide, a finding that correlated with higher impulsivity score [35].

Taken together, these findings suggest that WM abnormalities in BD may compromise the interhemispheric neuronal transmission and subsequent emotional processing/regulation—which may represent a potential anatomical biomarker of the disease—and precede the onset of bipolar disorder and predispose to brain development changes during the neurodevelopmental process of the central nervous system (CNS) in children and adolescents.

3.2. Frontal lobe

The anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), and orbital frontal cortex (OFC) represent the most widely studied frontal lobe areas in BD research.

Studies assessed the ACC through MRI showed volumetric reduction of the subgenual AC (sgACC) (areas 24 and 25 Brodmann), a finding confirmed in a meta-analysis [36]. In studies with proton magnetic resonance spectroscopy (^1H -MRS), reduction of the N-acetyl-aspartate (NAA) and increase of the choline in the ACC are the most consistent results [37]. Interestingly, some studies showed that long-term treatment with lithium increased the sgACC volume [6] and was associated to higher levels of NAA in this region [38, 39]. Additionally, Functional changes on PET studies were observed both in patients at resting state as in those undergoing activation tasks in the CC: most of them show hyperactivity ACC, notably the sgACC in patients in depressive [40] and manic [41] states.

Studies that evaluated the pregenual ACC (pgACC) also reported abnormalities, with reduction of the left pgACC volume, both in adults [42, 43] and in adolescents [44].

Another H¹MRS study of this region detected in patients with mania increased relationship glutamine/glutamate, a finding that may be related to impairment of glial-neuronal cell interaction in the interpretation of the authors [45].

Some studies have demonstrated reduction in DLPFC volume in adults [46, 47] and pediatric samples [48], whereas other studies using H¹MRS reported reduction of NAA in this region [49, 50]. At last, hypometabolism of the DLPFC with ¹⁸FDG-PET and functional magnetic resonance imaging (fMRI) in patients with BD was found, both in mania [51] and in depression [52–54].

Other studies evaluating the OFC showed reduction of its volume both in adults [46, 55–57] and in pediatric patients [58, 59], but the heterogeneity of their samples may limit the interpretation of results. A H¹MRS study of this region demonstrated the decrease of the NAA and the choline in hospitalized non-euthymic bipolar patients (mixed or manic episode), but a minority was in use of lithium [60], whereas our group reported normal metabolic levels in medial orbital frontal cortex in BD I euthymic outpatients with and without suicidal behavior [7]. In fact, in our sample, 30.2% of the subjects were prescribed the first mood stabilizer in the year after the first affective episode, 22% after the first year before the fifth year, and 47.8% 5 years after the first affective episode, and it was demonstrated a higher prevalence of suicide attempts in the latter group [61]. These results support the protective clinical effect of the use of mood stabilizers on the suicidal behavior.

Finally, in patients with mania, hypoactivation of the VLPFC and the OFC in fMRI studies was documented [62, 63].

3.3. Amygdala

Volumetric abnormalities of amygdala are among the most common findings, especially in adolescent samples, in which smaller volumes were reported, but with controversial results among adults. While the reduction of the amygdala among adolescents may represent an anatomical characteristic finding of this clinical subgroup, inconsistent results in adults may result from clinical course, the proportion of adult patients with early BD compounding the sample or neuroplastic effects associated with treatment [15].

The studies of fMRI suggest abnormality of the amygdala in response to a variety of experimental paradigms (resting state, processing of emotional stimuli, and cognitive tasks with or without emotional valence) in the context of the various affective states [11]. On the other hand, some authors reported the absence of hyperactivity of the amygdala during euthymia, which may reflect normalization of the amygdala function induced by long-term treatment, possible evidence of neuroplasticity [64].

3.4. Cerebellum

Most structural neuroimaging studies of the cerebellum showed a reduction in the volume of sub-regions of the cerebellar vermis [65–68], and reduction of the cerebellar volume may be associated with genetic predisposition to BD [69]. Additionally, reduction of the density of the gray matter of the cerebellar vermis has been reported in untreated BD patients, but not in

patients under treatment, which may suggest possible neuroprotective effects associated with psychopharmacological drug use [70].

3.5. Hippocampus

Data from a meta-analysis that summarized the results of 25 studies of hippocampal structure have found reduced hippocampal volume, especially in bipolar adolescent samples and reported apparent relationship between increased hippocampal volume and lithium therapy, which may explain the non-significant difference in hippocampal volume in most studies with samples of adult patients when compared to healthy controls [71].

4. Evidence of impairment of cellular resilience and plasticity in BD

There is growing evidence in literature of changes of neuroprotective processes and cellular plasticity and resilience pathways in BD from morphometric and neuropathological studies. Particularly, several mechanisms have founded to be involved as putative etiologic theories that underlie the neurobiological basis of BD, including proinflammatory cytokines, intracellular signaling cascades, and disrupted neurotrophic factor pathways.

More specifically, inflammatory mediators, such as interleukins, tumor necrosis factor alpha (TNF- α), and C-reactive protein, may influence several aspects of the pathophysiology of BD through changes in regulation of neuronal excitability, neuronal survival, synaptic transmission, and plasticity [72, 73]. Several studies have demonstrated a low-grade proinflammatory state in BD during euthymia [74–77], whereas both mania and depression seem to be associated with even more increased circulating cytokines [78, 79]. In addition, it has been suggested that proinflammatory cytokines may be one of the mechanisms of progression of BD, according to some studies [77, 80].

In terms of dysfunction of intracellular signaling cascades, there is a solid evidence of impaired regulation of calcium signaling and increased intracellular calcium levels, with subsequent loss of modulation of neuronal and glial activity, increased oxidative stress, and shortened survival cell [81–83]. Besides, Bcl-2, a protein with both antiapoptotic and neuroprotective properties highly expressed in the limbic system [84, 85], is associated with calcium regulation, reducing its release; Bcl-2 polymorphism AA was associated with both higher cytosolic calcium levels in lymphoblasts [86] and age-related decreases in brain gray matter volume [87].

Additional important signaling cascades involved in BD pathophysiology are those associated with members of the neurotrophin family, especially brain-derived neurotrophic factor (BDNF), which exerts its biological effects through activation of intracellular systems, including the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway [88].

BDNF is essential for neuroplastic phenomena, such as neurogenesis, neuronal survival, normal maturation of neural development pathways, and synaptic plasticity and dendritic

growth in adulthood as well [89], and it has been demonstrated circulating BDNF is reduced during manic and depressive states [90, 91], while ERK/MAPK pathway is an important intracellular mediator of biological effects of neurotrophic factors, acting on several proteins involved in cellular plasticity, such as glycogen synthase K-3 (GSK-3)—a major regulator of apoptosis—and cAMP response element-binding protein (CREB), which is a facilitator of the expression of neurotrophic/neuroprotective proteins such as Bcl-2 and BDNF [92, 93].

Information about histopathological abnormalities of neuronal and glial cells from *postmortem* studies in mental illness is scarce and its interpretation may be limited due to employment of different techniques and presence of confounding factors such as illicit-drugs and alcohol abuse [94]. However, particularly in BD patients, these abnormalities seem to be concentrated on frontolimbic regions associated with emotional regulation, including the DLPFC and ACC [95].

Although histopathological findings vary among regions and layers of the prefrontal cortex in BD patients, the majority of *postmortem* studies points up reductions in the neuronal density and size, glial cell density and changes in protein expression (implicated in the regulation of synaptic plasticity), which likely result from a combination of dendritic atrophy and/or cell loss in the DLPFC and ACC [95].

In this context, it is possible to hypothesize a link among the neuropathological, neuroimaging, and clinical findings, which dendritic atrophy and cell loss may result in reductions of volume in prefrontal areas, as abnormal synaptic interactions among cortical and subcortical brain structures may result in structural and functional intra- and interhemispheric disconnections and culminate in more vulnerability to stressful stimuli from environment, emotional dysregulation, and BD-related affective, cognitive, and behavioral symptoms [96].

However, lithium and valproic acid, respectively, through inhibition of glycogen synthase kinase-3 (GSK-3) and the histone deacetylases (HDACs), regulate the transcription and expression of neurotrophic, angiogenic, and neuroprotective proteins, such as BDNF, glial cell line-derived neurotrophic factor (GDNF), and angiogenic vascular endothelial growth factor (VEGF). Also, lithium in particular acts on factors that affect apoptotic signaling, such as Bcl-2, p53, Bax, caspase, and heat shock proteins (HSP); both lithium and valproate activate ERK/MAPK pathway. Finally, lithium contributes to induction of the ubiquitin-proteasome system and autophagy, two major intracellular quality control mechanisms for protein clearance that prevent abnormal protein accumulation. Overall, these findings highlight the properties of lithium and probably other mood stabilizers to suppress cell death, attenuate neuroinflammation, and promote angiogenesis and cellular plasticity in BD patients, which contribute to the reduction of neuronal loss [5].

However, not all neuroimaging studies show benefits from long-term use of mood stabilizers. For instance, in a study that evaluated both medicated with antipsychotics or lithium manic (most hospitalized) and outpatient euthymic patients and healthy controls using fMRI demonstrated loss of functional connectivity between amygdala and ACC in manic, but not in euthymic patients;

according to its authors, these findings may suggest a state-dependent neuronal dysfunction [97], but these results may be a marker of treatment non-response, since all patients were medicated.

This latter hypothesis has been brought up a longitudinal study in which bipolar I patients were assigned to euthymic, responders, and non-responders to lithium therapy. When baseline and after treatment volumes of the hippocampus, amygdala, PFC, DLPFC, and ACC volumes were compared, there was a significant enlargement in the left PFC and DLPF in bipolar I patients who responded to treatment, and the left hippocampus and right ACC volumes were decreased in non-responders [98].

5. Conclusion

In summary, the main findings in structural and functional neuroimaging studies suggest that there is frontolimbic circuitry dysregulation in BD, characterized by impairment of control of subcortical regions by cortical ones; changes in specific brain areas have been replicated in several studies, which may reflect impairments in physiological neuroplastic phenomena in the central nervous system. However, growing body of evidence from neuroimaging studies also shows that long-term treatment with mood stabilizers may be associated with metabolic/functional compensation or structural restoration, at least in bipolar responders, and neuroimaging techniques may be considered as a potential tool for establishing prognostic, predictive, or pharmacodynamic biomarkers in BD in the future.

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