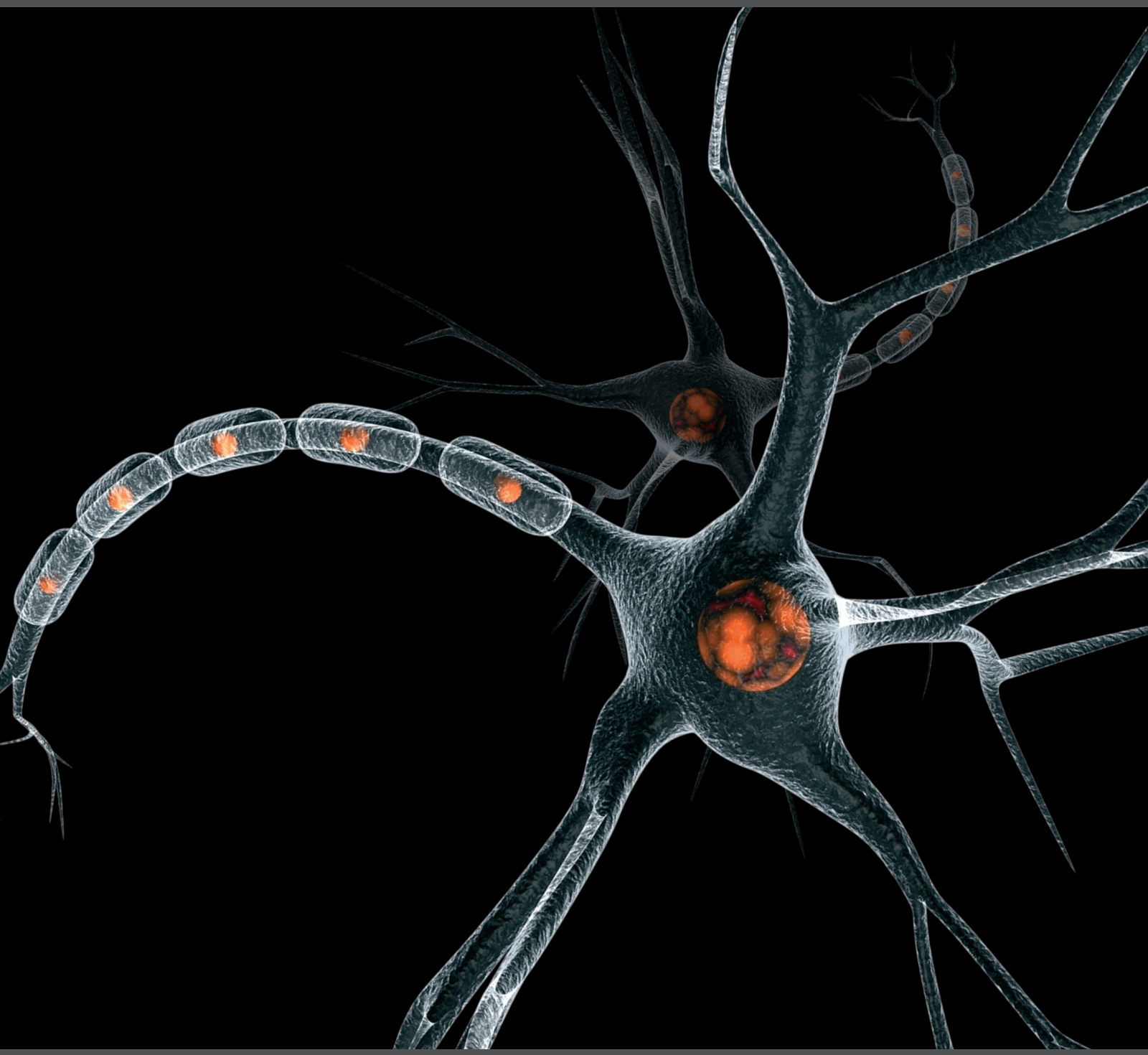
Neural Plasticity

Dendritic Spine Plasticity and Cognition

Guest Editors: Ignacio González Burgos, Irina Nikonenko, and Volker Korz



Dendritic Spine Plasticity and Cognition

Neural Plasticity

Dendritic Spine Plasticity and Cognition

Guest Editors: Ignacio González Burgos, Irina Nikonenko, and Volker Korz

Editorial Board

Contents

Dendritic Spine Plasticity and Cognition, Ignacio González Burgos, Irina Nikonenko, and Volker Korz
Volume 2012, Article ID 875156, 2 pages

Synaptic Functions of Invertebrate Varicosities: What Molecular Mechanisms Lie Beneath, Carlo Natale Giuseppe Giachello, Pier Giorgio Montarolo, and Mirella Ghirardi
Volume 2012, Article ID 670821, 14 pages

Examining Form and Function of Dendritic Spines, Kevin F. H. Lee, Cary Soares, and Jean-Claude Béïque
Volume 2012, Article ID 704103, 9 pages

Immune System in the Brain: A Modulatory Role on Dendritic Spine Morphophysiology?, Oscar Kurt Bitzer-Quintero and Ignacio González-Burgos
Volume 2012, Article ID 348642, 7 pages

Kalirin, a Key Player in Synapse Formation, Is Implicated in Human Diseases, Prashant Mandela and Xin-Ming Ma
Volume 2012, Article ID 728161, 9 pages

Divergent Roles of $p75^{NTR}$ and Trk Receptors in BDNF's Effects on Dendritic Spine Density and Morphology, Christopher A. Chapleau and Lucas Pozzo-Miller
Volume 2012, Article ID 578057, 9 pages

Selective Estrogen Receptor Modulators Regulate Dendritic Spine Plasticity in the Hippocampus of Male Rats, Ignacio González-Burgos, Martha C. Rivera-Cervantes, Dulce A. Velázquez-Zamora, Alfredo Feria-Velasco, and Luis Miguel Garcia-Segura
Volume 2012, Article ID 309494, 6 pages

Hindawi Publishing Corporation
Neural Plasticity
Volume 2012, Article ID 875156, 2 pages
doi:10.1155/2012/875156

Editorial

Dendritic Spine Plasticity and Cognition

Ignacio González Burgos,[1,2] Irina Nikonenko,[3] and Volker Korz[4]

[1] *Laboratorio de Psicobiología, División de Neurociencias, CIBO, IMSS, Guadalajara, JAL 44340, Mexico*
[2] *Departamento de Biología Celular y Molecular, CUCBA, Universidad de Guadalajara, Guadalajara, JAL 45110, Mexico*
[3] *Department of Neuroscience, Geneva University School of Medicine, 1211 Geneva, Switzerland*
[4] *Otto von Guericke University Magdeburg, Institute of Biology, Leipziger Straße 44, Building. 91, 39120 Magdeburg, Germany*

Correspondence should be addressed to Ignacio González Burgos, igonbur@hotmail.com

Received 25 March 2012; Accepted 25 March 2012

The scientific studies of dendritic spines have experienced an overwhelming growth in the last three decades. Since the primary concept that dendritic spines are sites of excitatory synaptic contact, the most recent studies have led to theoretical proposals that relate spines' morphophysiology with the information processing that underlies cognition. Furthermore, some specific spine shapes have been related to specific components of cognitive process, such as acquisition of information (learning) or long-term information retention (memory).

Dendritic spines are highly dynamic structures. In fact, their structural modifications occurring under specific conditions are strongly related to synaptic plasticity which underlies cognitive flexibility. This provides the ability to create a mnemonic trace that defines the individual experience and allows making useful decisions under different circumstances.

Contributions to this special issue provide some theoretical and experimental evidence related to the dendritic spine and synapse plasticity underlying mnemonic activity, as well as modulatory mechanisms involved in it. Although the data discussed in this special issue deal mainly with the findings derived from the animal research, it is clear, however, that these experimental results can lead to the clinical approaches concerning a wide variety of neuropsychopathological conditions in humans.

The mechanisms that regulate spine and synapse morphology and function are characterized by the remarkable complexity. An example of such complex mechanism is described in the study by Chapleau and Pozzo-Miller that reveals the opposing effects of p75NTR and Trk receptors signaling in BDNF actions on spine density and morphology. The functional interplay between neuronal activity and different signaling pathways can induce new spine growth and affect spine maturation and stabilization/pruning, the processes that are essential for learning and memory. Another pathway regulating dendritic spine structural plasticity was studied by González-Burgos et al. They provided the evidence that estrogen receptor modulators raloxifene and tamoxifen can rapidly modify spine density and morphology and discussed the possible repercussion of these spine plastic changes on learning and memory processes. These data, obtained in experimental animals, are also of interest for the practical medicine that uses both drugs in the human treatment, and they once again highlight the importance of the fundamental knowledge for the clinical applications.

Based on the fact that causal relationships between cellular changes underlying synaptic plasticity and their functional significance are difficult to establish from experimental studies in the mammalian brain, Giachello et al. review some of the actual evidence regarding the cellular and molecular mechanisms underlying synaptogenesis and synaptic plasticity in the invertebrate nervous system and their possible validation in vertebrates. According to this view, Lee et al. discuss some of the actual trends in the conceptualization of dendritic spines as discrete functional compartments regulating synaptic plasticity, as well as the spine's structural changes associated with synaptic function.

In other review papers presented in this issue, the mechanisms regulating normal brain plasticity and their implications in the pathological processes are discussed. Mandela and Ma focus on Kalirin-7, a Rho guanine nucleotide

exchange factor that plays important role in signaling pathways regulating formation of dendritic spines and synapses. Recent data demonstrate that Kalirin-7 not only affects spine and synapse morphology but, consequently, modulates plasticity properties of a synapse. More importantly, human analogue of this rodent protein is implicated in a wide range of human diseases related to the cognitive disability. The paper by Bitzer-Quintero and Gonzalez-Burgos reviews the role of the immune system in regulation of synaptogenesis, especially under conditions of brain injury or inflammation. Increasing data also suggest implication of the complex interactions between immune and nervous systems in the normal neuronal structural plasticity underlying learning and memory mechanisms.

We hope that papers published in this special issue will serve to increase the scientific knowledge on the cellular and molecular mechanisms involved in dendritic spine's plastic changes underlying cerebral organization of learning and memory.

Ignacio González Burgos
Irina Nikonenko
Volker Korz

Hindawi Publishing Corporation
Neural Plasticity
Volume 2012, Article ID 670821, 14 pages
doi:10.1155/2012/670821

Review Article

Synaptic Functions of Invertebrate Varicosities: What Molecular Mechanisms Lie Beneath

Carlo Natale Giuseppe Giachello,[1] Pier Giorgio Montarolo,[1,2] and Mirella Ghirardi[1,2]

[1] *Department of Neuroscience, University of Torino, Corso Raffaello 30, 10125 Torino, Italy*
[2] *Istituto Nazionale di Neuroscienze, Corso Raffaello 30, 10125 Torino, Italy*

Correspondence should be addressed to Carlo Natale Giuseppe Giachello, carlo.giachello@unito.it

Received 30 November 2011; Accepted 27 February 2012

Academic Editor: Volker Korz

In mammalian brain, the cellular and molecular events occurring in both synapse formation and plasticity are difficult to study due to the large number of factors involved in these processes and because the contribution of each component is not well defined. Invertebrates, such as *Drosophila, Aplysia, Helix, Lymnaea,* and *Helisoma*, have proven to be useful models for studying synaptic assembly and elementary forms of learning. Simple nervous system, cellular accessibility, and genetic simplicity are some examples of the invertebrate advantages that allowed to improve our knowledge about evolutionary neuronal conserved mechanisms. In this paper, we present an overview of progresses that elucidates cellular and molecular mechanisms underlying synaptogenesis and synapse plasticity in invertebrate varicosities and their validation in vertebrates. In particular, the role of invertebrate synapsin in the formation of presynaptic terminals and the cell-to-cell interactions that induce specific structural and functional changes in their respective targets will be analyzed.

1. Introduction

Interneuronal communication is essential for all nervous system functions. Neurons transmit their signals to one another at specialized structures termed synapses in which a presynaptic and a postsynaptic compartment are both morphologically and functionally distinguished.

Cellular accessibility and the relative simplicity of their nervous system have made invertebrate models, such as *Aplysia, Lymnaea*, *Hirudo*, *Helix,* and *Helisoma* [1–9], a particularly suitable solution for investigating the formation of synapse and the specificity of neuronal connectivity. A large number of invertebrate neurons can be individually identified and isolated in cell culture, since they share similar size, position, biophysical properties, synaptic connections, and physiological functions among animals of the same species [10]. Therefore, it is even possible to reconstruct in dissociated cell culture synapses between individually identified invertebrate neurons that recapitulate *in vitro* their *in vivo* features [6, 7, 11–13]. Culture conditions, specific to each system, promote the regeneration of new neuritic arbors and the establishment of synaptic connections with remarkable accuracy. Thus, cell culture approaches combined with imaging and electrophysiological techniques have allowed neuroscientists to monitor cellular and molecular events underlying neurite outgrowth, synaptogenesis, and synaptic plasticity.

Commonly, vertebrate neurons display a stereotyped polarity in which it is possible to identify well-distinguished areas deputed for receiving and integrating synaptic inputs (dendrites, soma, and proximal axon), for action potential initiation (axon initial segment), and signal propagation (axonal arborization). On the other hand, invertebrate neurons normally lack myelinated axons, and their afferent and efferent processes often branch from the same offshoot of the soma. Although the presence of spine-like processes along dendrites of *Drosophila* visual interneurons [14] and honeybee calycal interneurons has been observed [15, 16], there is no evidence that neurons of other invertebrate models bear dendritic spines with a well-defined morphology as described in vertebrates. Invertebrate synapses are clustered onto varicose-like structures that appear as irregular small

swellings distributed along neurites. Varicosities have been described in both invertebrate and vertebrate models, such as *Helix* [7, 17–19], *Aplysia* [20–24], *Lymnaea* [25], *Helisoma* [26], rat cortical neurons [27], pyramidal neurons [28], and hippocampal neurons [29, 30].

2. Initial Steps in the Formation of Varicosities

Studies in culture have revealed that varicosities can result from the transformation of growth cone into synaptic terminal after the contact of a postsynaptic cell [31, 32], as well as along axons even in the absence of a postsynaptic target [27, 29, 33–42] (Figure 1). The formation of functional active zones lacking postsynaptic partners may be attributed to substances used for coating culture surfaces (such as polylysine, polyornithine, and basic growth factor) [37, 43–46], nevertheless this configuration is observed *in vivo* in many invertebrate [47] and mammalian central nervous system, that is, climbing fibers in cerebellum [48], mossy fibers of the dentate gyrus [49], and primary visual cortex of adult macaque [50].

In this way, the presence of multiple presynaptic regions that are dispersed along the length of the axon allows a single neuron to form *en passant* synaptic connections with many partners. Thus, we can infer that synapse formation is not a simple result of a physical contact among neurons. Interestingly, it has been demonstrated that *Helisoma* buccal neuron B5 can form an efficacious chemical synapse with B19 neuron [13, 51] within minutes after contact [3]. This mechanism does not imply a target-dependent induction of secretory capabilities. In fact, in neuron B5 the release machinery is assembled through intrinsic developmental mechanisms prior to contact [52]. Moreover, cultured *Xenopus* spinal neurons, rat hippocampal neurons, and *Drosophila* neurons show some ability for evoked synaptic vesicle recycling along entire axonal segments, even in the absence of their targets [29, 33, 35–37, 39–41]. Morphological studies performed on *Aplysia* sensory neurons cultured in contact with postsynaptic neurons as well as in isolated configuration suggest that varicosities are formed either at the tips of advancing growth cones, or along neurites after their advancement, or by splitting of pre-existing varicosities [23, 24, 53, 54].

Actually, the model proposed in the literature [55] includes a series of hierarchical steps that occur through a combination of vesicle trafficking and local recruitment of synaptic proteins. Firstly, a huge accumulation of organelles leads to vesicle cluster formation at the palm of advancing growth cone. During the assembly of presynaptic boutons, clusters of pleiomorphic vesicles have been observed at newly forming synapses [56]. Synaptic vesicle clustering to actin cytoskeleton and the following reorganization in synaptic pools may cause the sequestration of G-actin and other proteins with the consequent slowing of neuritic extension and the swelling of the central area of growth cone. Afterwards, the supply or resources are resumed, and the growth cone may carry on its advance, leaving behind a new varicosity. Finally, the newly formed varicosity is further supplemented with organelles delivered along the axons by anterograde transport. Varicosities host a heterogeneous population of subcellular organelles that include clear and dense core vesicles, mitochondria, and endoplasmic reticulum [54]. Electron microscope studies revealed that the content of varicosities formed by neurons grown in the absence of postsynaptic partners ranges from organelle high-density varicosity to those that are almost free of organelles [57, 58].

3. Molecular Mechanisms at Presynaptic Level: Role of Synapsin

At presynaptic level, synapsins have a prominent role in regulating the formation and the maturation of new varicosities. Synapsins are a family of synaptic vesicle-associated phosphoproteins identified in a wide range of vertebrate and invertebrate organisms [59–63]. These proteins are predominantly localized at the surface of synaptic vesicles [64–66] and maintain vesicle pool organization tethering synaptic vesicles to actin cytoskeleton. Thus, vesicle mobilization may be regulated by synapsins in a phosphorylation-dependent manner. Real-time imaging in hippocampal cultures has demonstrated that phosphorylated synapsin dissociates from vesicle clusters during tetanic stimulation [67], delivering vesicles from reserve pool to replenish the readily releasable pool, which has been depleted upon activity [68]. Synapsins are multidomain proteins sharing a common N-terminal region composed of three domains (domains A, B, and C) that are highly conserved across isoforms and species with the exception of domain B. The C-terminal domain composition (D-I) is more variable and derives from alternative splicing events [61, 69].

While in mammals the different isoforms of synapsin proteins are coded by three distinct genes, invertebrates and lower vertebrates contain only one single gene. It may be plausible that synapsin family originates from one ancestral precursor, which was subjected to duplication events when vertebrates diverged from invertebrates [61]. The hypothesis of an ancestral single synapsin gene has been validated after cloning and sequencing of synapsin in some invertebrate species such as two ecdysozoans, the fly *Drosophila melanogaster* [70] and the nematode *Caenorhabditis elegans* [61], and three lophotrochozoans, the mollusks *Loligo pealei* [71], *Aplysia californica* [72], and *Helix pomatia* [73]. Therefore, the evolution of these proteins in the different phyla correlates with the development of a progressively more complex nervous system.

There are many pieces of evidence that synapsins play a role in axon elongation and synapse formation. It has been demonstrated that synapsin I and II regulate synaptic functions following the early neurogenesis in mouse brain [74]. Synapsin III is expressed mainly in early phases of neuronal development and is highly concentrated in growth cones [75]. Moreover, the onset of presynaptic maturation at *Xenopus* neuromuscular junctions is causally related to the onset of synapsin expression [76], indeed experimentally elevated levels of synapsin I [77] or synapsin IIa [78] accelerate presynaptic maturation characterized by a precocious assembly of active zone structures, organization of synaptic

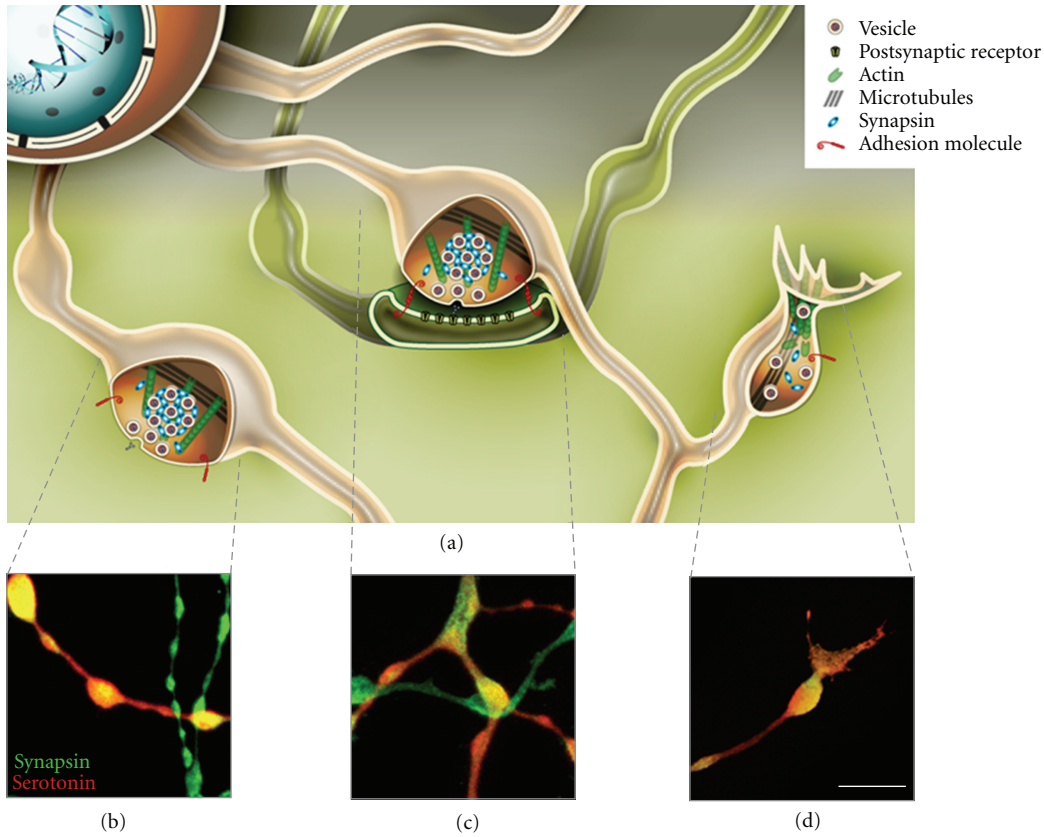


Figure 1: Schematic representation of the three most common types of varicosities observed in invertebrate neuronal cultures (a). The bottom panels show confocal acquisitions of neurites belonging to the serotonergic *Helix* neuron C1 cocultured with its physiological target B2 and immunostained with anti-serotonin (red) and anti-synapsin (green) antibodies. In these sample images it is possible to identify a varicosity without a postsynaptic target in which neurotransmitter release can be detected using functional dyes or electrophysiological techniques (b); a presynaptic varicosity interconnected with its postsynaptic counterpart (c); a newly formed varicosity derived from an advancing growth cone in which well-defined synaptic vesicle pools have not yet organized (d). Scale bar: 10 μm.

vesicle pools, and also a rapid formation of thickenings of postsynaptic muscle cell membrane [79]. Interestingly, even the overexpression of synapsin in non-neuronal cells results in the formation of neurite- and synaptic-like structures similar to varicosities [80].

As in vertebrates, synapsin expression correlates well with the time course of presynaptic terminal maturation and synaptogenesis in mollusks, such as *Helix* and *Aplysia*. In isolated *Helix* neurons, immunostaining for mammalian synapsin I appears uniformly distributed in the cell body, the distal axonal segments and the growth cones. The contact and the formation of a chemical connection with an appropriate juxtaposed target induce a redistribution of the synapsin mainly in varicosity-like structures immunoreactive for the neurotransmitter serotonin along neurites close to the target neuron [19], similar to the changes in synapsin I distribution following synaptic contacts in hippocampal neurons developing in culture [81]. Interestingly, the number of synapsin-positive varicosity-like structures increases progressively parallel to the enhancement in the mean amplitude of the postsynaptic potentials recorded at the same times in *Helix* cocultures.

A distribution of synapsin in distinct puncta along neurites has also been shown in neurons of *Aplysia punctata* [19] and of *Aplysia californica* [72] where synapsin undergoes dispersion following serotonin and TGFβ1 treatments that induce its phosphorylation mediated by PKA [72] and MAPK kinases [82], respectively. Therefore, the phosphorylation of *Aplysia* synapsin may result in its dissociation from synaptic vesicles in processes activated by neuromodulators and growth factors involved both in modulation of neurotransmitter release and in remodelling of growing neurons during development.

To study the synaptic fidelity of neuronal regenerating connections in culture, a multitude of experiments about target recognition during synaptogenesis has been performed in invertebrate neurons [4, 83–87]. Identified motoneurons, isolated from the buccal ganglia of *Helisoma trivolvis*, display selective synapse formation in culture [3, 51]. In particular, the identified B19 neuron forms appropriate cholinergic connections with buccal muscle fibers, but not with other buccal neurons [88].

In *Helix* nervous system the giant metacerebral neuron C1, homologous of the *Aplysia* MGC [89], physiologically forms a serotonergic monosynaptic connection with the giant neuron B2 in the buccal ganglia [90]. *In vitro* studies demonstrated that the presence of a non-physiological target neuron C3 results in a general inhibitory effect on the

maturation of the presynaptic terminals of neuron C1, reducing both the number of presynaptic varicosities and their ability to release neurotransmitter in the presynaptic neuron [91], through mechanisms that involve the down-regulation of both MAPK/Erk and PKA pathways [92]. These pathways are rapidly activated by the contact with the physiological target neuron B2 that can quickly reverse the wrong target-induced inhibition.

In *Helix* C1 neurons cultured in contact with a wrong target C3, injection of bovine synapsin I has been shown to exert an enhancing effect on the efficiency of the neurotransmitter release machinery [93]. Interestingly, the injected synapsin was able to rescue neurotransmitter release strongly depressed by the presence of the non-physiological target bringing it to levels comparable to those observed when the C1 neuron is cocultured with its physiological target B2 [91]. This suggests that exogenous synapsin I may accelerate the maturation or simply disinhibit the quantal release mechanisms by affecting cytoskeleton assembly and/or synaptic vesicle clustering in *Helix* presynaptic terminals, in agreement with the results obtained by injection of synapsin I or II into embryonic *Xenopus* neurons that accelerates both the morphological and functional development of synapses [77–79].

4. Synapsin Phosphorylation and Synapse Formation

Further experiments in *Helix* neurons in culture have analyzed the role of specific domains of synapsin in regulating structure and activity of synapses [73, 94, 95]. A multiple alignment of the primary structure of *Helix* synapsin [73] with *Aplysia* synapsin [72] and other mammalian orthologs reveals the high phylogenetic conservation of the PKA/CaMKI/IV phosphorylation site located in the N-terminal domain A (Ser-9). The phosphorylation of this site by either PKA or CaMKI/IV is necessary for the enhancement of neurotransmitter release from neuron C1 to overcome the inhibitory effect of the wrong target in C1–C3 soma-soma coculture, since the facilitating effect due to the injection of exogenous wild-type synapsin is maintained in the presence of the pseudo-phosphorylated form and virtually lost after the injection of the non-phosphorylatable mutant. Moreover, the functional effects of gastropod synapsin are associated with a phosphorylation-dependent ultrastructural rearrangement of neurons C1. In fact, electron microscopy analysis showed that in the region of contact between C1, overexpressing wild-type synapsin, and neuron C3 there were dense interdigitations of microtubule-packed neurite-like processes with the appearance of dense core synaptic vesicle clusters typical of C1, that were virtually absent in uninjected C1–C3 pairs or after injection of the nonphosphorylatable domain A mutant [94].

Studies on vesicle dynamics in growth cones [96] show a critical role of the PKA phosphorylation site in synapsin I, suggesting that the same molecular mechanisms involved in modulating neurotransmitter release from mature nerve terminals may also underlie the activity of the protein in developing terminals. The increase in cAMP in the presynaptic terminal following the contact with the postsynaptic target [97] may regulate synapsins activity in the control of synaptic vesicle distribution and recycling leading to the transformation of the growth cone into a mature presynaptic bouton. Recently, the overexpression of synapsin domain A phosphomutants in mice lacking endogenous synapsins has restated that this phosphorylation site plays an important role in controlling synapses formation. While the presence of the pseudo-phosphorylated form can accelerate synapse formation, the overexpression of the non-phosphorylatable mutant may cause a significant decrease in the total amount of both glutamatergic and GABAergic synapses during development [98].

In addition to PKA pathway, the phosphorylation of synapsin by MAPK/Erk kinase has also a critical role in the formation of synapses between *Helix* neurons in culture [95], consistent with a lot of evidence supporting the role of MAPK/Erk kinase in neurotrophic regulation of synapse formation and plasticity in vertebrate and invertebrate models [99–104]. *Helix* synapsin bears two putative MAPK/Erk consensus sites in domain B, Ser36 and Ser42, that are highly conserved among the known invertebrate synapsin proteins and might represent homologous MAPK/Erk phosphorylation sites to sites 4 and 5 of mammalian synapsin [105].

Overexpression of both MAPK/Erk phosphomutants induce a significant reduction of the presynaptic differentiation of the injected neuron and of the number of synaptic connections between the paired cells. In addition, the basal amplitude of the postsynaptic potentials recorded in *Helix* B2-B2 neurons is markedly reduced following injection of the non-phosphorylatable MAPK/Erk mutant while it is slightly decreased by the injection of the pseudo-phosphorylated MAPK/Erk mutant. Both mutants have no effect on the rising time of the postsynaptic potentials and do not induce any changes in the neurite outgrowth, suggesting that the reduction in synaptic strength occurs in the absence of changes in neurotransmitter release kinetics and ruling out the possibility that the altered connectivity depends on impairment in neurite growth. These observations suggest that MAPK-dependent synapsin phosphorylation regulates the occurrence of chemical synapses through a growth-independent mechanism [95]. The similar negative effect of both non-phosphorylatable and pseudo-phosphorylated synapsin mutants on synaptic formation suggests that cycles of MAPK/Erk phosphorylation may play a fundamental role in regulating synapsin activity during synaptogenesis, perhaps acting on cytoskeletal assembly and vesicle clustering at synaptic terminals, as suggested by the role of MAPK/Erk phosphorylation in modulating synapsin affinity for actin [105].

All the effects observed with mutagenesis experiments described so far cannot be ascribed to mistargeting of synapsin localization. Confocal acquisitions of soma-soma *Helix* neurons cocultures overexpressing GFP-conjugated synapsin phosphomutants for either PKA/CaMKI/IV or MAPK/Erk in the presynaptic compartment display a deeper clusterization pattern but the same localization observed for the wild-type form. In particular, ectopic synapsins are

present in presynaptic neurons with a preferential localization in the contact area with the postsynaptic target and along presynaptic neurites projecting onto the postsynaptic cell, in the areas of the soma-soma pairs containing the majority of the synaptic vesicle clusters and synaptic structures. Therefore, the phosphorylation of the synapsin N-terminus seems not to be implicated in the correct targeting of *Helix* synapsin, consistent with the observation in cultured hippocampal neurons where the deletion of synapsin domain A does not significantly impair the synaptic targeting of mammalian synapsins [106]. The higher degrees of clustering of overexpressed GFP-tagged synapsin mutants compared with the wild-type protein are possibly due to a stronger association of synapsin mutants with synaptic vesicles and/or to a lower rate of its dispersion and reclustering cycles [67, 107]. Conversely, the pseudo-phosphorylated mutants show a very low degree of clustering and appear uniformly diffuse along neurites. These observations are consistent with previous morphological studies showing that serotonin-induced dispersion of synapsin clusters in *Aplysia* neurons depends on both PKA and MAPK/Erk activity [72], and that PKA and MAPK/Erk phosphorylation regulates the mobility of synapsin as well as the trafficking of synaptic vesicles in nerve terminals upon stimulation [108].

5. Synapsin Phosphorylation and Plasticity

For many years the studies of synapsin functions have been focalized on synaptic plasticity rather than synaptogenesis. Synapsin proteins are implicated in maintenance of presynaptic vesicular pools and in the regulation of vesicle mobility among them during short-term plasticity [109–111]. In particular, synapsins appear to have a fundamental role in the expression of post-tetanic potentiation (PTP) since both genetically altered mice and *Aplysia* synapses exhibit a marked impairment of PTP after genetic deletion or neutralization of synapsin I and/or synapsin II [109, 112]. Interestingly, synaptic vesicle mobilization from the reserve pool during PTP in *Drosophila* is strongly dependent on PKA activation [113, 114]. Phosphorylation of synapsin domain A might also modulate PTP by altering the presynaptic release probability as shown in PTP at the calyx of Held synapse [115] that may be mediated by the activation of CaMKs [116]. However, microinjection of domain A peptide into the squid giant synapse had no effect on vesicle pool size, synaptic depression, or transmitter release kinetics, indicating that this domain may be predominantly involved in regulating synaptic vesicle trafficking at pre-docking stages [117]. Presynaptic overexpression of the *Helix* synapsin non-phosphorylatable mutant in domain A specifically impairs PTP, while the overexpression of the wild-type form has no effect on peak amplitude or time course of PTP [73]. Similarly, these results have been confirmed at *Aplysia* synapses [118]. In addition, PTP expression in *Helix* neurons critically depends on MAPK/Erk activation [95], which might occur upon intracellular calcium build-up during the tetanus [119, 120] or via crosstalk with other calcium-dependent pathways [121–124]. Although MAPK/Erk activity does not appear to be required for short-term heterosynaptic facilitation induced by serotonin at *Aplysia* sensory-motor synapses [8, 125, 126], other studies in invertebrates show that modulation of short- and long-term synaptic plasticity paradigms is mediated by MAPK/Erk [82, 127–129]. An involvement of MAPK/Erk in short-term plasticity is also supported by studies in transgenic mice that express a constitutively active form of H-Ras, which exhibit an enhancement of paired-pulse facilitation and long-term potentiation that is dependent on MAPK/Erk activation [130].

As demonstrated by the studies described above, the same molecular pathways and effectors that regulate the formation of functional synaptic contacts are also involved in synaptic transmission and plasticity. Even if these mechanisms acting at presynaptic level seem to be intrinsically regulated, the presence of a target cell during synaptogenesis has a prominent role in triggering the formation and maturation of specialized structures in both pre- and postsynaptic neurons.

6. Synapse Formation: Crosstalk between Pre- and Postsynaptic Sites

At postsynaptic level, synapse formation requires a coordinated assembly of synaptic structures conferring the competence to translate the presynaptic signal into a postsynaptic response. In vertebrate neurons, two important steps are the formation of a protrusion that differentiates into a dendritic spine and the formation of a postsynaptic density facing the active zone. These events require the involvement of a multitude of different proteins, which have been partially identified and characterized. Actually, several models have been proposed in spinogenesis: spines may derive either from the stabilization of an initial filopodium after the contact with the axon [131–133], or from filopodium-independent sprouting [134, 135], or, alternatively, they might initially grow without synaptic contact [136–142].

Considering the requirement of a synaptic contact, several protein families have been proposed to trigger spinogenesis mediating cell-to-cell communication, such as cadherins, neuroligin-β-neurexin cell adhesion complexes, and ephrins/Eph receptors [143–145]. Although these molecules have been shown to play a role in the various aspects of synaptogenesis, matching pre- and postsynaptic components, no single protein factor has been found to be essential for all these processes, from initial synapse specification to the formation of functional connections.

Cadherins are a large family of Ca^{2+}-dependent, homophilic, cell-surface adhesion molecules [146–152]. Both E-cadherins and N-cadherins are present in synapses, and they are symmetrically localized in the adhesive junctions that surround the active zone in the presynaptic terminal and the postsynaptic density [146]. In cultured hippocampal neurons, N-cadherin is ubiquitary expressed in all synapses only at early stages of development, then becomes restricted to a subpopulation of excitatory synapses during maturation [153]. Recent studies have linked these proteins to dendritic

spine morphogenesis. A delay in spine formation has been observed in cultured hippocampal neurons overexpressing a dominant-negative form of N-cadherin, lacking part of the extracellular domain. Although the loss of N-cadherin activity promotes the appearance of immature filopodia with irregular shapes, synaptic contacts are retained. Moreover, the presence of dominant-negative N-cadherin impairs the localization of both presynaptic and postsynaptic protein markers, that is, synapsin and PSD-95, respectively [154]. This effect seems to be more pronounced at early stages of synaptogenesis, suggesting that cadherins may be more involved in synapse formation rather than stabilization and maturation.

Nevertheless the role of classical cadherins in triggering synapse formation is still debated. Indeed genetic studies in *Drosophila* has greatly contributed to determining the function of N-cadherins *in vivo*. Loss of N-cadherin in *Drosophila* embryos affects the trajectories of longitudinal CNS axons and the guidance of growth cones [155]. It has been demonstrated that N-cadherin is important for coordinating the targeting of multiple neuronal types, such as R7 photoreceptor axons and L1–L5 lamina neurons, to the right target layer in the medulla neuropil of the visual system [156–159]. *Drosophila* contains 12 isoforms of N-cadherin, but the expression of a single isoform is sufficient to rescue null mutations, suggesting functional redundancy [159]. Thus, these observations indicate that cadherins may be involved in target recognition and perhaps stabilization of early synaptic contact sites but not in the induction of synapse formation.

Another protein, neuronal-cell adhesion molecule (N-CAM), belonging to the Ca^{2+}-independent cell adhesion molecules of the immunoglobulin superfamily, is also present in synapses [160–164]. This protein bears fibronectin type III repeats in the extracellular domain and a short cytoplasmic domain, anchored to the cytoskeleton, which interact with intracellular signaling pathways [165, 166]. *In vitro* studies showed that several identified CAM members regulate the number of synaptic contacts, their morphology and functions; however a strong evidence that any of these molecules is necessary for synapse formation *in vivo* is lacking, probably suggesting a redundancy in their functions. In cell cultures, N-CAMs accumulate quickly at sites of contact formation during the initial assembly of synaptic components [167]. Through interaction with spectrin-coated trans-Golgi-derived organelles, N-CAM may promote the accumulation of those postsynaptic proteins that are necessary to form the synaptic contact [168]. In fact, a reduction in size of postsynaptic densities and an impaired recruitment of spectrin, NMDA receptors, and CaMKIIa to the synapse is observable in neurons lacking N-CAM [169]. Furthermore, studies on mixed cultures of hippocampal neurons from N-CAM knockout and wild-type mice have revealed that postsynaptic N-CAM promotes the formation and increases the strength of excitatory synapses in concert with NMDA receptor activity [170].

In literature a large amount of evidence that suggest the involvement of N-CAM not only in neuronal development, but also in synapse plasticity, results from invertebrate models [171–179]. In *Drosophila,* the concentration of fasciclin II, homologue of vertebrate N-CAM, regulates sprouting and the capability of neurons to form new synaptic contacts [177, 178]. In nerve-muscle cocultures from *Xenopus* embryos, the percentage of functional neuromuscular contacts is decreased by means of antibody against N-CAM [180].

In *Aplysia*, apCAM is predominantly expressed at synaptic contacts [172, 181] and modulates synapse formation and long-term plasticity at sensory-motor synapses [172, 179, 182–186]. The ability of sensory neuron to form *in vitro* chemical connections with motoneuron L7, detected as number of branches and varicosities, correlates with the expression level of apCAM on different regions of the postsynaptic cell L7 [184]. Moreover, a reduction in fasciculation of growth cones has been observed with the preincubation of isolated sensory or motoneurons with a monoclonal antibody against apCAM [172, 181, 187]. While the addition of the antibody on preformed sensory-motor cocultures results in a failure of serotonin-induced long-term changes in synaptic efficacy and the concomitant morphological changes of sensory neuron, such as formation of new varicosities, without altering the transmission of pre-existing synapses and their short-term modulation [185]. Interestingly, the same anti-apCAM antibody recognizes apCAM-like proteins of the *Helix* nervous system. The neurotransmitter releasing ability of *Helix* neuron C1 is detectable when it is cultured alone or in presence of its physiological target B2, whereas it is inhibited by the presence of the wrong target C3 [19, 91]. In C1–C3 cocultures, the buildup of neurotransmitter release triggered by the appropriate target B2 is prevented by preincubation of this neuron with anti-apCAM antibody [188], confirming that N-CAM orthologs may play an important role during the contact of two synaptic partners in modulate the efficiency of excitation-secretion coupling.

One potential signaling cascade implicated in this phenomenon is PKC [189, 190], since the presence of apCAM on membrane of motoneuron L7 and the activation of *Aplysia* PKC isoforms PKC *Ap*l II are both necessary events for the initial synapse formation and the increase of sensorin expression by sensory neurons [191]. Therefore, apCAM exposed on L7 membrane surface may activate signaling cascades not only in the motor neuron itself, but also in the coupled sensory neuron via the heterophilic receptor to regulate both pre- and postsynaptically the expression of effectors necessary for the assembly of functional synapses [169, 192–197].

Based on invertebrate *Drosophila* Fasciclin II and *Aplysia* apCAM sequences, a database-search analysis resulted in an identification of a similar protein in vertebrates, called SynCAM [198]. SynCAM is a transmembrane member of the Ig superfamily that mediates Ca^{2+}-independent homophilic interactions and displays a structure similar to the nectins [199]: 3 Ig-domains followed by an intercellular C-terminal PDZ-binding motif able to bind the synaptic scaffolding proteins CASK and syntenin. High level of SynCAM expression has been detected in young rat brain in the first few weeks after birth, corresponding with the main period of synaptogenesis. Overexpression studies in cultured hippocampal neurons confirmed that SynCAM promotes

synapse formation and increases spontaneous synaptic activity while its isolated cytoplasmic tail inhibits synaptic function, perhaps by acting as a dominant negative [198]. Remarkably, this protein has the ability to promote the formation of active presynaptic terminals in non-neuronal cells, when cocultured with hippocampal neurons [198]. Hence, SynCAM may act at multiple stages of synaptogenesis from the initial synaptic contact to the modulation of neurotransmitter release. However, its effects on dendritic spine morphology remain to be determined.

7. Synapse Modulation and Plasticity: Role of Adhesion Molecules

Once a functional contact is established, the new synapse goes through a series of maturation processes that is likely to be regulated by neural activity. For example, hippocampal synapses undergo structural changes after long-term potentiation (LTP) *in vitro* and experience *in vivo* [200, 201]. Generally, at postsynaptic level, newly formed spines acquire a postsynaptic density and increase their volume which closely correlates with the exposure in membrane of additional AMPA receptors [202] and the reorganization of the actin cytoskeleton [203]. These processes are strictly associated with the induction of LTP [139, 200, 204–206].

Before the large number of data collected from hippocampal neurons about the involvement of adhesion molecules, such as N-CAM, in long-term potentiation [160, 161, 166, 176, 207–211], early studies about long-term modifications were performed on invertebrate models. In particular, an important step in our understanding of N-CAM functions comes from studies on long-term functional and structural plasticity of the *Aplysia* sensory-motor synapse. ApCAM is expressed at the highest levels at sites of synaptic contact between sensory and motor neurons in culture, consistent with its *in vivo* distribution [181]. Long-term facilitation induced by serotonin application is accompanied by the formation of new branches and varicosities in sensory neuron [21, 212]. On the other hand, long-term depression of the same synapse by the neuropeptide Phe-Met-Arg-Phe-amide (FMRFamide) is correlated with the loss of presynaptic sensory neurites and varicosities [213, 214]. Both modifications of synaptic efficiency involve a rapid and cell-specific change in the distribution of apCAM. The treatment with 5-HT causes a downregulation of apCAM from the surface of the sensory neuron via a cAMP-dependent increase in endocytosis of clathrin-coated vesicles [172, 182, 215], while application of FMRFamide induces a downregulation of apCAM from the surface of the target motor neuron by a similar cAMP-dependent mechanism [183, 187]. Consistent with these observations, transgenic mice in which N-CAM has been depleted showed deficits in learning and memory [216]. Furthermore, the interference with N-CAM levels through specific antibodies or suppression of NCAM results in a reduced or even abolished LTP in the CA1 region of the hippocampus [161, 176, 217].

8. Concluding Remarks

Synaptogenesis is a complex process that results in the assembly of a functional release machinery in the presynaptic terminals and the formation of specialized structures at the corresponding postsynaptic level. In recent years, considerable progress has been reached in understanding the cellular and molecular mechanisms of vertebrate synaptogenesis. New techniques and approaches have allowed scientists to characterize several molecules that regulate not only when and where synapses are formed but also their continuous plastic modifications. Beside this, it is important to mention the contribution of pioneering experimental studies performed on invertebrate models that permitted the identification of the basic mechanisms of neuronal functions implicated in behavioral responses that are phylogenetically conserved in vertebrate animals.

Acknowledgments

The authors would like to thank Dr. Ferdinando Fiumara for his comments and support. This work was supported by Grants from Italian Ministry of the University and Research Grant (PRIN 2006 and 2009 grants to P. G. Montarolo) and Compagnia di San Paolo (to P. G. Montarolo and M. Ghirardi).

References

[1] P. G. Haydon, "The formation of chemical synapses between cell-cultured neuronal somata," *Journal of Neuroscience*, vol. 8, no. 3, pp. 1032–1038, 1988.

[2] D. L. Glanzman, E. R. Kandel, and S. Schacher, "Identified target motor neuron regulates neurite outgrowth and synapse formation of *Aplysia* sensory neurons *in vitro*," *Neuron*, vol. 3, no. 4, pp. 441–450, 1989.

[3] P. G. Haydon and M. J. Zoran, "Formation and modulation of chemical connections: evoked acetylcholine release from growth cones and neurites of specific identified neurons," *Neuron*, vol. 2, no. 5, pp. 1483–1490, 1989.

[4] N. I. Syed, A. G. M. Bulloch, and K. Lukowiak, "*In vitro* reconstruction of the respiratory central pattern generator of the mollusk *Lymnaea*," *Science*, vol. 250, no. 4978, pp. 282–285, 1990.

[5] M. Klein, "Synaptic augmentation by 5-HT at rested *Aplysia* sensorimotor synapses: independence of action potential prolongation," *Neuron*, vol. 13, no. 1, pp. 159–166, 1994.

[6] F. Fernandez-de-Miguel and P. Drapeau, "Synapse formation and function: insights from identified leech neurons in culture," *Journal of Neurobiology*, vol. 27, no. 3, pp. 367–379, 1995.

[7] M. Ghirardi, A. Casadio, L. Santarelli, and P. G. Montarolo, "*Aplysia* hemolymph promotes neurite outgrowth and synaptogenesis of identified *Helix* neurons in cell culture," *Invertebrate Neuroscience*, vol. 2, no. 1, pp. 41–49, 1996.

[8] K. C. Martin, D. Michael, J. C. Rose et al., "MAP kinase translocates into the nucleus of the presynaptic cell and is required for long-term facilitation in *Aplysia*," *Neuron*, vol. 18, no. 6, pp. 899–912, 1997.

[9] S. Schacher and F. Wu, "Synapse formation in the absence of cell bodies requires protein synthesis," *Journal of Neuroscience*, vol. 22, no. 5, pp. 1831–1839, 2002.

[10] E. R. Kandel, *Cellular Basis of Behavior: an Introduction to Behavioral Neurobiology*, W. H. Freeman and Company, 1976.

[11] S. Schacher, S. G. Rayport, and R. T. Ambron, "Giant *Aplysia* neuron R2 reliably forms strong chemical connections *in vitro*," *Journal of Neuroscience*, vol. 5, no. 11, pp. 2851–2856, 1985.

[12] D. L. Glanzman, "Postsynaptic regulation of the development and long-term plasticity of *Aplysia* sensorimotor synapses in cell culture," *Journal of Neurobiology*, vol. 25, no. 6, pp. 666–693, 1994.

[13] P. G. Haydon and M. J. Zoran, "Retrograde regulation of presynaptic development during synaptogenesis," *Journal of Neurobiology*, vol. 25, no. 6, pp. 694–706, 1994.

[14] F. Leiss, E. Koper, I. Hein et al., "Characterization of dendritic spines in the *Drosophila* central nervous system," *Developmental Neurobiology*, vol. 69, no. 4, pp. 221–234, 2009.

[15] R. G. Coss, J. G. Brandon, and A. Globus, "Changes in morphology of dendritic spines on honeybee calycal interneurons associated with cumulative nursing and foraging experiences," *Brain Research*, vol. 192, no. 1, pp. 49–59, 1980.

[16] J. G. Brandon and R. G. Coss, "Rapid dendritic spine stem shortening during one-trial learning: the Honeybee's first orientation flight," *Brain Research*, vol. 252, no. 1, pp. 51–61, 1982.

[17] K. Elekes and J. Ude, "An immunogold electron microscopic analysis of FMRFamide-like immunoreactive neurons in the CNS of *Helix pomatia*: ultrastructure and synaptic connections," *Journal of Neurocytology*, vol. 22, no. 1, pp. 1–13, 1993.

[18] S. Ratte and R. Chase, "Synapse distribution of olfactory interneurons in the procerebrum of the snail *Helix aspersa*," *The Journal of Comparative Neurology*, vol. 417, pp. 366–384, 2000.

[19] G. Cibelli, M. Ghirardi, F. Onofri et al., "Synapsin-like molecules in *Aplysia punctata* and *Helix pomatia*: identification and distribution in the nervous system and during the formation of synaptic contacts *in vitro*," *European Journal of Neuroscience*, vol. 8, no. 12, pp. 2530–2543, 1996.

[20] C. H. Bailey and M. Chen, "Morphological basis of long-term habituation and sensitization in *Aplysia*," *Science*, vol. 220, no. 4592, pp. 91–93, 1983.

[21] P. G. Montarolo, P. Goelet, V. F. Castellucci, J. Morgan, E. R. Kandel, and S. Schacher, "A critical period for macromolecular synthesis in long-term heterosynaptic facilitation in *Aplysia*," *Science*, vol. 234, pp. 1249–1254, 1986.

[22] C. H. Bailey and M. Chen, "Long-term memory in *Aplysia* modulates the total number of varicosities of single identified sensory neurons," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 7, pp. 2373–2377, 1988.

[23] Y. Hatada, F. Wu, Z. Y. Sun, S. Schacher, and D. J. Goldberg, "Presynaptic morphological changes associated with long-term synaptic facilitation are triggered by actin polymerization at preexisting varicositis," *Journal of Neuroscience*, vol. 20, no. 13, Article ID RC82, 2000.

[24] P. W. Grabham, F. Wu, S. Schacher, and D. J. Goldberg, "Initiating morphological changes associated with long-term facilitation in *Aplysia* is independent of transcription or translation in the cell body," *Journal of Neurobiology*, vol. 64, no. 2, pp. 202–212, 2005.

[25] P. Buma, "Synaptic and nonsynaptic release of neuromediators in the central nervous system," *Acta Morphologica Neerlando-Scandinavica*, vol. 26, no. 2-3, pp. 81–113, 1988.

[26] H. R. Khan, D. A. Price, K. E. Doble, M. J. Greenberg, and A. S. M. Saleuddin, "FMRFamide-related peptides, partial serotonin depletion, and osmoregulation in *Helisoma duryi* (Mollusca: Pulmonata)," *The Journal of Comparative Neurology*, vol. 393, pp. 25–33, 1998.

[27] F. D. Morgenthaler, G. W. Knott, J. C. F. Sarria et al., "Morphological and molecular heterogeneity in release sites of single neurons," *European Journal of Neuroscience*, vol. 17, no. 7, pp. 1365–1374, 2003.

[28] T. Umeda, T. Ebihara, and S. Okabe, "Simultaneous observation of stably associated presynaptic varicosities and postsynaptic spines: morphological alterations of CA3-CA1 synapses in hippocampal slice cultures," *Molecular and Cellular Neuroscience*, vol. 28, no. 2, pp. 264–274, 2005.

[29] S. R. Krueger, A. Kolar, and R. M. Fitzsimonds, "The presynaptic release apparatus is functional in the absence of dendritic contact and highly mobile within isolated axons," *Neuron*, vol. 40, no. 5, pp. 945–957, 2003.

[30] S. Sankaranarayanan, P. P. Atluri, and T. A. Ryan, "Actin has a molecular scaffolding, not propulsive, role in presynaptic function," *Nature Neuroscience*, vol. 6, no. 2, pp. 127–135, 2003.

[31] R. P. Rees, M. B. Bunge, and R. P. Bunge, "Morphological changes in the neuritic growth cone and target neuron during synaptic junction development in culture," *The Journal of Cell Biology*, vol. 68, no. 2, pp. 240–263, 1976.

[32] J. Buchanan, Y. A. Sun, and M. M. Poo, "Studies of nerve-muscle interactions in *Xenopus* cell culture: fine structure of early functional contacts," *Journal of Neuroscience*, vol. 9, no. 5, pp. 1540–1554, 1989.

[33] Y. A. Sun and M. M. Poo, "Evoked release of acetylcholine from the growing embryonic neuron," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 8, pp. 2540–2544, 1987.

[34] J. Christenson, S. Cullheim, S. Grillner, and T. Hokfelt, "5-Hydroxytryptamine immunoreactive varicosities in the lamprey spinal cord have no synaptic specializations—an ultrastructural study," *Brain Research*, vol. 512, no. 2, pp. 201–209, 1990.

[35] M. Matteoli, K. Takei, M. S. Perin, T. C. Sudhof, and P. De Camilli, "Exo-endocytotic recycling of synaptic vesicles in developing processes of cultured hippocampal neurons," *The Journal of Cell Biology*, vol. 117, no. 4, pp. 849–861, 1992.

[36] K. Kraszewski, O. Mundigl, L. Daniell, C. Verderio, M. Matteolli, and P. De Camilli, "Synaptic vesicle dynamics in living cultured hippocampal neurons visualized with CY3-conjugated antibodies directed against the lumenal domain of synaptotagmin," *Journal of Neuroscience*, vol. 15, no. 6, pp. 4328–4342, 1995.

[37] Z. S. Dai and H. B. Peng, "Dynamics of synaptic vesicles in cultured spinal cord neurons in relationship to synaptogenesis," *Molecular and Cellular Neurosciences*, vol. 7, no. 6, pp. 443–452, 1996.

[38] A. Prokop, M. Landgraf, E. Rushton, K. Broadie, and M. Bate, "Presynaptic development at the *Drosophila* neuromuscular junction: assembly and localization of presynaptic active zones," *Neuron*, vol. 17, no. 4, pp. 617–626, 1996.

[39] I. Antonov, S. Chang, S. Zakharenko, and S. V. Popov, "Distribution of neurotransmitter secretion in growing axons," *Neuroscience*, vol. 90, no. 3, pp. 975–984, 1999.

[40] S. Zakharenko, S. H. Chang, M. O'Donoghue, and S. V. Popov, "Neurotransmitter secretion along growing nerve processes: comparison with synaptic vesicle exocytosis," *The Journal of Cell Biology*, vol. 144, no. 3, pp. 507–518, 1999.

[41] W. D. Yao, J. Rusch, M. M. Poo, and C. F. Wu, "Spontaneous acetylcholine secretion from developing growth cones of *Drosophila* central neurons in culture: effects of cAMP-pathway mutations," *Journal of Neuroscience*, vol. 20, no. 7, pp. 2626–2637, 2000.

[42] E. Takao-Rikitsu, S. Mochida, E. Inoue et al., "Physical and functional interaction of the active zone proteins, CAST, RIM1, and Bassoon, in neurotransmitter release," *The Journal of Cell Biology*, vol. 164, no. 2, pp. 301–311, 2004.

[43] R. W. Burry, "Presynaptic elements on artificial surfaces. A model for the study of development and regeneration of synapses," *Neurochemical Pathology*, vol. 5, no. 3, pp. 345–360, 1986.

[44] M. J. Anderson, S. Champaneria, and L. E. Swenarchuk, "Synaptic differentiation can be evoked by polymer microbeads that mimic localized pericellular proteolysis by removing proteins from adjacent surfaces," *Developmental Biology*, vol. 147, no. 2, pp. 464–479, 1991.

[45] Z. S. Dai and H. B. Peng, "Presynaptic differentiation induced in cultured neurons by local application of basic fibroblast growth factor," *Journal of Neuroscience*, vol. 15, no. 8, pp. 5466–5475, 1995.

[46] D. A. DiGregorio, O. Negrete, A. Jeromin, H. B. Peng, and J. L. Vergara, "Contact-dependent aggregation of functional Ca^{2+} channels, synaptic vesicles and postsynaptic receptors in active zones of a neuromuscular junction," *European Journal of Neuroscience*, vol. 14, no. 3, pp. 533–546, 2001.

[47] K. Shen and C. I. Bargmann, "The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in C. *elegans*," *Cell*, vol. 112, no. 5, pp. 619–630, 2003.

[48] H. Nishiyama, M. Fukaya, M. Watanabe, and D. J. Linden, "Axonal motility and its modulation by activity are branch-type specific in the intact adult cerebellum," *Neuron*, vol. 56, no. 3, pp. 472–487, 2007.

[49] D. G. Amaral and J. A. Dent, "Development of the mossy fibers of the dentate gyrus: I. A light and electron microscopic study of the mossy fibers and their expansions," *The Journal of Comparative Neurology*, vol. 195, no. 1, pp. 51–86, 1981.

[50] D. D. Stettler, H. Yamahachi, W. Li, W. Denk, and C. D. Gilbert, "Axons and synaptic boutons are highly dynamic in adult *visual cortex*," *Neuron*, vol. 49, no. 6, pp. 877–887, 2006.

[51] P. G. Haydon and S. B. Kater, "The differential regulation of formation of chemical and electrical connections in *Helisoma*," *Journal of Neurobiology*, vol. 19, no. 7, pp. 636–655, 1988.

[52] P. G. Haydon and P. Drapeau, "From contact to connection: early events during synaptogenesis," *Trends in Neurosciences*, vol. 18, no. 4, pp. 196–201, 1995.

[53] Y. Hatada, F. Wu, R. Silverman, S. Schacher, and D. J. Goldberg, "En passant synaptic varicosities form directly from growth cones by transient cessation of growth cone advance but not of actin-based motility," *Journal of Neurobiology*, vol. 41, pp. 242–251, 1999.

[54] G. Malkinson, Z. M. Fridman, D. Kamber, A. Dormann, E. Shapira, and M. E. Spira, "Calcium-induced exocytosis from actomyosin-driven, motile varicosities formed by dynamic clusters of organelles," *Brain Cell Biology*, vol. 35, no. 1, pp. 57–73, 2006.

[55] G. Malkinson and M. E. Spira, "Clustering of excess growth resources within leading growth cones underlies the recurrent "deposition" of varicosities along developing neurites," *Experimental Neurology*, vol. 225, no. 1, pp. 140–153, 2010.

[56] S. E. Ahmari, J. Buchanan, and S. J. Smith, "Assembly of presynaptic active zones from cytoplasmic transport packets," *Nature Neuroscience*, vol. 3, no. 5, pp. 445–451, 2000.

[57] E. Koenig, S. Kinsman, E. Repasky, and L. Sultz, "Rapid mobility of motile varicosities and inclusions containing α-spectrin, actin, and calmodulin in regeneratig axons *in vitro*," *Journal of Neuroscience*, vol. 5, no. 3, pp. 715–729, 1985.

[58] G. M. G. Shepherd and K. M. Harris, "Three-dimensional structure and composition of CA3 → CA1 axons in rat hippocampal slices: implications for presynaptic connectivity and compartmentalization," *Journal of Neuroscience*, vol. 18, no. 20, pp. 8300–8310, 1998.

[59] T. C. Sudhof, A. J. Czernik, H. T. Kao et al., "Synapsins: mosaics of shared and individual domains in a family of synaptic vesicle phosphoproteins," *Science*, vol. 245, no. 4925, pp. 1474–1480, 1989.

[60] B. Porton, T. T. Kao, and P. Greengard, "Characterization of transcripts from the synapsin III gene locus," *Journal of Neurochemistry*, vol. 73, no. 6, pp. 2266–2271, 1999.

[61] H. T. Kao, B. Porton, S. Hilfiker et al., "Molecular evolution of the synapsin gene family," *Journal of Experimental Zoology*, vol. 285, pp. 360–377, 1999.

[62] F. Cesca, P. Baldelli, F. Valtorta, and F. Benfenati, "The synapsins: key actors of synapse function and plasticity," *Progress in Neurobiology*, vol. 91, no. 4, pp. 313–348, 2010.

[63] Y. Humeau, S. Candiani, M. Ghirardi, B. Poulain, and P. Montarolo, "Functional roles of synapsin: lessons from invertebrates," *Seminars in Cell & Developmental Biology*, vol. 22, pp. 425–433, 2011.

[64] P. De Camilli, S. M. Harris, W. B. Huttner, and P. Greengard, "Synapsin I (Protein I), a nerve terminal-specific phosphoprotein. II. Its specific association with synaptic vesicles demonstrated by immunocytochemistry in agarose-embedded synaptosomes," *The Journal of Cell Biology*, vol. 96, no. 5, pp. 1355–1373, 1983.

[65] T. Ueda and P. Greengard, "Adenosine 3′ : 5′ monophosphate regulated phosphoprotein system of neuronal membranes. II. Solubilization, purification, and some properties of an endogenous phosphoprotein," *Journal of Biological Chemistry*, vol. 252, no. 14, pp. 5155–5163, 1977.

[66] F. Benfenati, P. Neyroz, M. Bahler, L. Masotti, and P. Greengard, "Time-resolved fluorescence study of the neuron-specific phosphoprotein synapsin I. Evidence for phosphorylation-dependent conformational changes," *Journal of Biological Chemistry*, vol. 265, no. 21, pp. 12584–12595, 1990.

[67] P. Chi, P. Greengard, and T. A. Ryan, "Synapsin dispersion and reclustering during synaptic activity," *Nature Neuroscience*, vol. 4, no. 12, pp. 1187–1193, 2001.

[68] P. Greengard, F. Valtorta, A. J. Czernik, and F. Benfenati, "Synaptic vesicle phosphoproteins and regulation of synaptic function," *Science*, vol. 259, no. 5096, pp. 780–785, 1993.

[69] T. C. Sudhof, A. J. Czernik, H. T. Kao et al., "Synapsins: mosaics of shared and individual domains in a family of synaptic vesicle phosphoproteins," *Science*, vol. 245, no. 4925, pp. 1474–1480, 1989.

[70] B. R. E. Klagges, G. Heimbeck, T. A. Godenschwege et al., "Invertebrate synapsins: a single gene codes for several isoforms in *Drosophila*," *Journal of Neuroscience*, vol. 16, no. 10, pp. 3154–3165, 1996.

[71] S. Hilfiker, F. E. Schweizer, H. T. Kao, A. J. Czernik, P. Greengard, and G. J. Augustine, "Two sites of action for synapsin domain E in regulating neurotransmitter release," *Nature Neuroscience*, vol. 1, no. 1, pp. 29–35, 1998.

[72] A. Angers, D. Fioravante, J. Chin, L. J. Cleary, A. J. Bean, and J. H. Byrne, "Serotonin stimulates phosphorylation of *Aplysia* synapsin and alters its subcellular distribution in sensory neurons," *Journal of Neuroscience*, vol. 22, no. 13, pp. 5412–5422, 2002.

[73] F. Fiumara, C. Milanese, A. Corradi et al., "Phosphorylation of synapsin domain A is required for post-tetanic potentiation," *Journal of Cell Science*, vol. 120, no. 18, pp. 3228–3237, 2007.

[74] I. L. Bogen, V. Jensen, O. Hvalby, and S. I. Walaas, "Synapsin-dependent development of glutamatergic synaptic vesicles and presynaptic plasticity in postnatal mouse brain," *Neuroscience*, vol. 158, no. 1, pp. 231–241, 2009.

[75] A. Ferreira, H. T. Kao, J. Feng, M. Rapoport, and P. Greengard, "Synapsin III: developmental expression, subcellular localization, and role in axon formation," *Journal of Neuroscience*, vol. 20, no. 10, pp. 3736–3744, 2000.

[76] B. Lu, A. J. Czernik, S. Popov, T. Wang, M. M. Poo, and P. Greengard, "Expression of synapsin I correlates with maturation of the neuromuscular synapse," *Neuroscience*, vol. 74, no. 4, pp. 1087–1097, 1996.

[77] B. Lu, P. Greengard, and M. M. Poo, "Exogenous synapsin I promotes functional maturation of developing neuromuscular synapses," *Neuron*, vol. 8, no. 3, pp. 521–529, 1992.

[78] E. Schaeffer, J. Alder, P. Greengard, and M. M. Poo, "Synapsin IIa accelerates functional development of neuromuscular synapses," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 9, pp. 3882–3886, 1994.

[79] F. Valtorta, N. Lezzi, F. Benfenati, B. Lu, M. M. Poo, and P. Greengard, "Accelerated structural maturation induced by synapsin I at developing neuromuscular synapses of *Xenopus laevis*," *European Journal of Neuroscience*, vol. 7, no. 2, pp. 261–270, 1995.

[80] H. Q. Han, R. A. Nichols, M. R. Rubin, M. Bahler, and P. Greengard, "Induction of formation of presynaptic terminals in neuroblastoma cells by synapsin IIB," *Nature*, vol. 349, no. 6311, pp. 697–700, 1991.

[81] T. L. Fletcher, P. Cameron, P. De Camilli, and G. Banker, "The distribution of synapsin I and synaptophysin in hippocampal neurons developing in culture," *Journal of Neuroscience*, vol. 11, no. 6, pp. 1617–1626, 1991.

[82] J. Chin, A. Angers, L. J. Cleary, A. Eskin, and J. H. Byrne, "Transforming growth factor beta1 alters synapsin distribution and modulates synaptic depression in *Aplysia*," *Journal of Neuroscience*, vol. 22, no. 9, Article ID RC220, 2002.

[83] J. Camardo, E. Proshansky, and S. Schacher, "Identified *Aplysia* neurons form specific chemical synapses in culture," *Journal of Neuroscience*, vol. 3, no. 12, pp. 2614–2620, 1983.

[84] H. Arechiga, M. Chiquet, D. P. Kuffler, and J. G. Nicholls, "Formation of specific connections in culture by identified leech neurons containing serotonin, acetylcholine and peptide transmitters," *Journal of Experimental Biology*, vol. 126, pp. 15–31, 1986.

[85] L. Vyklicky and J. Nicholls, "Specificity of connections formed by nociceptive cells of the leech in tissue-culture," *Journal of Experimental Biology*, vol. 134, pp. 17–26, 1988.

[86] G. M. Carrow and I. B. Levitan, "Selective formation and modulation of electrical synapses between cultured *Aplysia* neurons," *Journal of Neuroscience*, vol. 9, no. 10, pp. 3657–3664, 1989.

[87] N. I. Syed and G. E. Spencer, "Target-cell selection and specific synapse formation by identified *Lymnaea neurons in vitro*," *Netherlands Journal of Zoology*, vol. 44, pp. 327–338, 1994.

[88] M. J. Zoran, R. T. Doyle, and P. G. Haydon, "Target-dependent induction of secretory capabilities in an identified motoneuron during synaptogenesis," *Developmental Biology*, vol. 138, no. 1, pp. 202–213, 1990.

[89] K. R. Weiss and I. Kupfermann, "Homology of the giant serotonergic neurons (metacerebral cells) in *Aplysia* and pulmonate molluscs," *Brain Research*, vol. 117, no. 1, pp. 33–49, 1976.

[90] G. A. Cottrell and J. B. Macon, "Synaptic connexions of two symmetrically placed giant serotonin containing neurones," *Journal of Physiology*, vol. 236, no. 2, pp. 435–464, 1974.

[91] M. Ghirardi, A. Casadio, G. Naretto, R. Levi, and P. G. Montarolo, "Influence of the target on distribution and functioning of the varicosities of *Helix pomatia* metacerebral cell C1 in dissociated cell culture," *Neuroscience*, vol. 96, no. 4, pp. 843–853, 2000.

[92] M. Ghirardi, F. Benfenati, S. Giovedi, F. Fiumara, C. Milanese, and P. G. Montarolo, "Inhibition of neurotransmitter release by a nonphysiological target requires protein synthesis and involves cAMP-dependent and mitogen-activated protein kinases," *Journal of Neuroscience*, vol. 24, no. 21, pp. 5054–5062, 2004.

[93] F. Fiumara, F. Onofri, F. Benfenati, P. G. Montarolo, and M. Ghirardi, "Intracellular injection of synapsin I induces neurotransmitter release in C1 neurons of *Helix pomatia* contacting a wrong target," *Neuroscience*, vol. 104, no. 1, pp. 271–280, 2001.

[94] F. Fiumara, S. Giovedi, A. Menegon et al., "Phosphorylation by cAMP-dependent protein kinase is essential for synapsin-induced enhancement of neurotransmitter release in invertebrate neurons," *Journal of Cell Science*, vol. 117, no. 21, pp. 5145–5154, 2004.

[95] C. N. G. Giachello, F. Fiumara, C. Giacomini et al., "MAPK/Erk-dependent phosphorylation of synapsin mediates formation of functional synapses and short-term homosynaptic plasticity," *Journal of Cell Science*, vol. 123, no. 6, pp. 881–893, 2010.

[96] D. Bonanomi, A. Menegon, A. Miccio et al., "Phosphorylation of synapsin I by cAMP-dependent protein kinase controls synaptic vesicle dynamics in developing neurons," *Journal of Neuroscience*, vol. 25, no. 32, pp. 7299–7308, 2005.

[97] L. R. Funte and P. G. Haydon, "Synaptic target contact enhances presynaptic calcium influx by activating cAMP-dependent protein kinase during synaptogenesis," *Neuron*, vol. 10, no. 6, pp. 1069–1078, 1993.

[98] L. E. Perlini, F. Botti, E. F. Fornasiero et al., "Effects of phosphorylation and neuronal activity on the control of synapse formation by synapsin I," *Journal of Cell Science*, vol. 124, pp. 3643–3653, 2011.

[99] A. Martinez, S. Alcantara, V. Borrell et al., "TrkB and TrkC signaling are required for maturation and synaptogenesis of hippocampal connections," *Journal of Neuroscience*, vol. 18, no. 18, pp. 7336–7350, 1998.

[100] C. Vicario-Abejon, C. Collin, R. D. G. McKay, and M. Segal, "Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons," *Journal of Neuroscience*, vol. 18, no. 18, pp. 7256–7271, 1998.

[101] C. Vicario-Abejon, D. Owens, R. McKay, and M. Segal, "Role of neurotrophins in central synapse formation and stabilization," *Nature Reviews Neuroscience*, vol. 3, no. 12, pp. 965–974, 2002.

[102] C. Collin, C. Vicario-Abejon, M. E. Rubio, R. J. Wenthold, R. D. G. McKay, and M. Segal, "Neurotrophins act at presynaptic terminals to activate synapses among cultured hippocampal neurons," *European Journal of Neuroscience*, vol. 13, no. 7, pp. 1273–1282, 2001.

[103] W. J. Tyler and L. D. Pozzo-Miller, "BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses," *Journal of Neuroscience*, vol. 21, no. 12, pp. 4249–4258, 2001.

[104] M. Alonso, J. H. Medina, and L. Pozzo-Miller, "ERK1/2 activation is necessary for BDNF to increase dendritic spine density in hippocampal CA1 pyramidal neurons," *Learning and Memory*, vol. 11, no. 2, pp. 172–178, 2004.

[105] J. N. Jovanovic, F. Benfenati, Y. L. Siow et al., "Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I-actin interactions," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 8, pp. 3679–3683, 1996.

[106] D. Gitler, Y. Xu, H. T. Kao et al., "Molecular determinants of synapsin targeting to presynaptic terminals," *Journal of Neuroscience*, vol. 24, no. 14, pp. 3711–3720, 2004.

[107] M. Hosaka, R. E. Hammer, and T. C. Sudhof, "A phospho-switch controls the dynamic association of synapsins with synaptic vesicles," *Neuron*, vol. 24, no. 2, pp. 377–387, 1999.

[108] P. Chi, P. Greengard, and T. A. Ryan, "Synaptic vesicle mobilization is regulated by distinct synapsin I phosphorylation pathways at different frequencies," *Neuron*, vol. 38, no. 1, pp. 69–78, 2003.

[109] Y. Humeau, F. Doussau, F. Vitiello, P. Greengard, F. Benfenati, and B. Poulain, "Synapsin controls both reserve and releasable synaptic vesicle pools during neuronal activity and short-term plasticity in *Aplysia*," *Journal of Neuroscience*, vol. 21, no. 12, pp. 4195–4206, 2001.

[110] M. A. Cousin, C. S. Malladi, T. C. Tan, C. R. Raymond, K. J. Smillie, and P. J. Robinson, "Synapsin I-associated phosphatidylinositol 3-kinase mediates synaptic vesicle delivery to the readily releasable pool," *Journal of Biological Chemistry*, vol. 278, no. 31, pp. 29065–29071, 2003.

[111] S. Giovedi, P. Vaccaro, F. Valtorta et al., "Synapsin is a novel Rab3 effector protein on small synaptic vesicles: I. Identification and characterization of the synapsin I-Rab3 interactions *in vitro* and in intact nerve terminals," *Journal of Biological Chemistry*, vol. 279, no. 42, pp. 43760–43768, 2004.

[112] T. W. Rosahl, D. Spillane, M. Missler et al., "Essential functions of synapsins I and II in synaptic vesicle regulation," *Nature*, vol. 375, no. 6531, pp. 488–493, 1995.

[113] H. Kuromi and Y. Kidokoro, "Selective replenishment of two vesicle pools depends on the source of Ca^{2+} at the *Drosophila* synapse," *Neuron*, vol. 35, no. 2, pp. 333–343, 2002.

[114] Y. Kidokoro, H. Kuromi, R. Delgado, C. Maureira, C. Oliva, and P. Labarca, "Synaptic vesicle pools and plasticity of synaptic transmission at the *Drosophila* synapse," *Brain Research Reviews*, vol. 47, no. 1–3, pp. 18–32, 2004.

[115] R. L. P. Habets and J. G. G. Borst, "Post-tetanic potentiation in the rat calyx of Held synapse," *Journal of Physiology*, vol. 564, no. 1, pp. 173–187, 2005.

[116] J. Y. Sun, P. Bronk, X. Liu, W. Han, and T. C. Sudhof, "Synapsins regulate use-dependent synaptic plasticity in the calyx of Held by a Ca^{2+}/calmodulin-dependent pathway," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 8, pp. 2880–2885, 2006.

[117] S. Hilfiker, F. D. R. Benfenati, F. Doussau et al., "Structural domains involved in the regulation of transmitter release by synapsins," *Journal of Neuroscience*, vol. 25, no. 10, pp. 2658–2669, 2005.

[118] F. Doussau, Y. Humeau, F. Benfenati, and B. Poulain, "A novel form of presynaptic plasticity based on the fast reactivation of release sites switched off during low-frequency depression," *Journal of Neuroscience*, vol. 30, no. 49, pp. 16679–16691, 2010.

[119] S. Impey, K. Obrietan, and D. R. Storm, "Making new connections: role of ERK/MAP kinase signaling in neuronal plastic0ity," *Neuron*, vol. 23, no. 1, pp. 11–14, 1999.

[120] N. Agell, O. Bachs, N. Rocamora, and P. Villalonga, "Modulation of the Ras/Raf/MEK/ERK pathway by Ca^{2+}, and Calmodulin," *Cellular Signalling*, vol. 14, no. 8, pp. 649–654, 2002.

[121] T. R. Soderling, "The Ca^{2+}-calmodulin-dependent protein kinase cascade," *Trends in Biochemical Sciences*, vol. 24, no. 6, pp. 232–236, 1999.

[122] G. D. Ferguson and D. R. Storm, "Why calcium-stimulated adenylyl cyclases?" *Physiology*, vol. 19, no. 5, pp. 271–276, 2004.

[123] Y. D. Shaul and R. Seger, "The MEK/ERK cascade: from signaling specificity to diverse functions," *Biochimica et Biophysica Acta*, vol. 1773, no. 8, pp. 1213–1226, 2007.

[124] N. Gerits, S. Kostenko, A. Shiryaev, M. Johannessen, and U. Moens, "Relations between the mitogen-activated protein kinase and the cAMP-dependent protein kinase pathways: comradeship and hostility," *Cellular Signalling*, vol. 20, no. 9, pp. 1592–1607, 2008.

[125] A. L. Purcell, S. K. Sharma, M. W. Bagnall, M. A. Sutton, and T. J. Carew, "Activation of a tyrosine kinase-MAPK cascade enhances the induction of long-term synaptic facilitation and long-term memory in *Aplysia*," *Neuron*, vol. 37, no. 3, pp. 473–484, 2003.

[126] G. A. Phares and J. H. Byrne, "Analysis of 5-HT-induced short-term facilitation at *Aplysia* sensorimotor synapse during bursts: increased synaptic gain that does not require ERK activation," *Journal of Neurophysiology*, vol. 94, no. 1, pp. 871–877, 2005.

[127] V. Tancredi, M. D'Antuono, C. Cafe et al., "The inhibitory effects of interleukin-6 on synaptic plasticity in the rat hippocampus are associated with an inhibition of mitogen-activated protein kinase ERK," *Journal of Neurochemistry*, vol. 75, no. 2, pp. 634–643, 2000.

[128] M. Roberto, T. E. Nelson, C. L. Ur, M. Brunelli, P. P. Sanna, and D. L. Gruol, "The transient depression of hippocampal CA1 LTP induced by chronic intermittent ethanol exposure is associated with an inhibition of the MAP kinase pathway," *European Journal of Neuroscience*, vol. 17, no. 8, pp. 1646–1654, 2003.

[129] A. Khoutorsky and M. E. Spira, "Activity-dependent calpain activation plays a critical role in synaptic facilitation and post-tetanic potentiation," *Learning and Memory*, vol. 16, no. 2, pp. 129–141, 2009.

[130] S. A. Kushner, Y. Elgersma, G. G. Murphy et al., "Modulation of presynaptic plasticity and learning by the H-ras/extracellular signal-regulated kinase/synapsin I signaling pathway," *Journal of Neuroscience*, vol. 25, no. 42, pp. 9721–9734, 2005.

[131] G. S. Marrs, S. H. Green, and M. E. Dailey, "Rapid formation and remodeling of postsynaptic densities in developing dendrites," *Nature Neuroscience*, vol. 4, no. 10, pp. 1006–1013, 2001.

[132] J. E. Vaughn, "Fine structure of synaptogenesis in the vertebrate central nervous system," *Synapse*, vol. 3, no. 3, pp. 255–285, 1989.

[133] N. E. Ziv and S. J. Smith, "Evidence for a role of dendritic filopodia in synaptogenesis and spine formation," *Neuron*, vol. 17, no. 1, pp. 91–102, 1996.

[134] F. Engert and T. Bonhoeffer, "Dendritic spine changes associated with hippocampal long-term synaptic plasticity," *Nature*, vol. 399, no. 6731, pp. 66–70, 1999.

[135] P. Jourdain, K. Fukunaga, and D. Muller, "Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation," *Journal of Neuroscience*, vol. 23, no. 33, pp. 10645–10649, 2003.

[136] C. Sotelo, "Purkinje cell ontogeny: formation and maintenance of spines," *Progress in Brain Research*, vol. 48, pp. 149–170, 1978.

[137] C. Sotelo, "Cerebellar synaptogenesis: what we can learn from mutant mice," *Journal of Experimental Biology*, vol. 153, pp. 225–249, 1990.

[138] R. Yuste and T. Bonhoeffer, "Genesis of dendritic spines: insights from ultrastructural and imaging studies," *Nature Reviews Neuroscience*, vol. 5, no. 1, pp. 24–34, 2004.

[139] M. De Roo, P. Klauser, P. Mendez, L. Poglia, and D. Muller, "Activity-dependent PSD formation and stabilization of newly formed spines in hippocampal slice cultures," *Cerebral Cortex*, vol. 18, no. 1, pp. 151–161, 2008.

[140] G. W. Knott, A. Holtmaat, L. Wilbrecht, E. Welker, and K. Svoboda, "Spine growth precedes synapse formation in the adult neocortex *in vivo*," *Nature Neuroscience*, vol. 9, no. 9, pp. 1117–1124, 2006.

[141] R. Cesa and P. Strata, "Activity-dependent axonal and synaptic plasticity in the cerebellum," *Psychoneuroendocrinology*, vol. 32, no. 1, pp. S31–S35, 2007.

[142] R. Cesa and P. Strata, "Axonal competition in the synaptic wiring of the cerebellar cortex during development and in the mature cerebellum," *Neuroscience*, vol. 162, no. 3, pp. 624–632, 2009.

[143] J. R. Sanes and J. W. Lichtman, "Development of the vertebrate neuromuscular junction," *Annual Review of Neuroscience*, vol. 22, pp. 389–442, 1999.

[144] T. Nguyen and T. C. Sudhof, "Binding properties of neuroligin 1 and neurexin 1β reveal function as heterophilic cell adhesion molecules," *Journal of Biological Chemistry*, vol. 272, no. 41, pp. 26032–26039, 1997.

[145] M. A. Takasu, M. B. Dalva, R. E. Zigmond, and M. E. Greenberg, "Modulation of NMDA receptor-dependent calcium influx and gene expression through EphB receptors," *Science*, vol. 295, no. 5554, pp. 491–495, 2002.

[146] J. L. Bruses, "Cadherin-mediated adhesion at the interneuronal synapse," *Current Opinion in Cell Biology*, vol. 12, no. 5, pp. 593–597, 2000.

[147] A. M. Fannon and D. R. Colman, "A model for central synaptic junctional complex formation based on the differential adhesive specificities of the cadherins," *Neuron*, vol. 17, no. 3, pp. 423–434, 1996.

[148] L. Shapiro and D. R. Colman, "The diversity of cadherins and implications for a synaptic adhesive code in the CNS," *Neuron*, vol. 23, no. 3, pp. 427–430, 1999.

[149] Y. Takai, K. Shimizu, and T. Ohtsuka, "The roles of cadherins and nectins in interneuronal synapse formation," *Current Opinion in Neurobiology*, vol. 13, no. 5, pp. 520–526, 2003.

[150] N. Uchida, Y. Honjo, K. R. Johnson, M. J. Wheelock, and M. Takeichi, "The catenin/cadherin adhesion system is localized in synaptic junctions bordering transmitter release zones," *The Journal of Cell Biology*, vol. 135, no. 3, pp. 767–779, 1996.

[151] T. Uemura, "The cadherin superfamily at the synapse: more members, more missions," *Cell*, vol. 93, no. 7, pp. 1095–1098, 1998.

[152] T. Yagi and M. Takeichi, "Cadherin superfamily genes: functions, genomic organization, and neurologic diversity," *Genes and Development*, vol. 14, no. 10, pp. 1169–1180, 2000.

[153] D. L. Benson and H. Tanaka, "N-cadherin redistribution during synaptogenesis in hippocampal neurons," *Journal of Neuroscience*, vol. 18, no. 17, pp. 6892–6904, 1998.

[154] H. Togashi, K. Abe, A. Mizoguchi, K. Takaoka, O. Chisaka, and M. Takeichi, "Cadherin regulates dendritic spine morphogenesis," *Neuron*, vol. 35, no. 1, pp. 77–89, 2002.

[155] Y. Iwai, T. Usui, S. Hirano, R. Steward, M. Takeichi, and T. Uemura, "Axon patterning requires DN-cadherin, a novel neuronal adhesion receptor, in the *Drosophila* embryonic CNS," *Neuron*, vol. 19, no. 1, pp. 77–89, 1997.

[156] C. H. Lee, T. Herman, T. R. Clandinin, R. Lee, and S. L. Zipursky, "N-cadherin regulates target specificity in the *Drosophila* visual system," *Neuron*, vol. 30, no. 2, pp. 437–450, 2001.

[157] A. Nern, L. V. T. Nguyen, T. Herman, S. Prakash, T. R. Clandinin, and S. L. Zipursky, "An isoform-specific allele of *Drosophila* N-cadherin disrupts a late step of R7 targeting," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 36, pp. 12944–12949, 2005.

[158] A. Nern, Y. Zhu, and S. L. Zipursky, "Local N-cadherin interactions mediate distinct steps in the targeting of lamina neurons," *Neuron*, vol. 58, no. 1, pp. 34–41, 2008.

[159] C. Y. Ting, S. Yonekura, P. Chung et al., "*Drosophila* N-cadherin functions in the first stage of the two-stage layer-selection process of R7 photoreceptor afferents," *Development*, vol. 132, no. 5, pp. 953–963, 2005.

[160] R. D. Fields and K. Itoh, "Neural cell adhesion molecules in activity-dependent development and synaptic plasticity," *Trends in Neurosciences*, vol. 19, no. 11, pp. 473–480, 1996.

[161] A. Luthl, J. P. Laurent, A. Figurov, D. Muller, and M. Schachner, "Hippocampal long-term potentiation and pleural cell adhesion molecules L1 and NCAM," *Nature*, vol. 372, pp. 777–779, 1994.

[162] E. Persohn, G. E. Pollerberg, and M. Schachner, "Immunoelectron-microscopic localization of the 180 kD component of the neural cell adhesion molecule N-CAM in postsynaptic membranes," *The Journal of Comparative Neurology*, vol. 288, no. 1, pp. 92–100, 1989.

[163] E. Persohn and M. Schachner, "Immunohistological localization of the neural adhesion molecules L1 and N-CAM in the developing hippocampus of the mouse," *Journal of Neurocytology*, vol. 19, no. 6, pp. 807–819, 1990.

[164] T. Schuster, M. Krug, M. Stalder, N. Hackel, R. Gerardy-Schahn, and M. Schachner, "Immunoelectron microscopic localization of the neural recognition molecules L1, NCAM, and its isoform NCAM180, the NCAM-associated polysialic acid, beta1 integrin and the extracellular matrix molecule tenascin-R in synapses of the adult rat hippocampus," *Journal of Neurobiology*, vol. 49, no. 2, pp. 142–158, 2001.

[165] G. Rougon and O. Hobert, "New insights into the diversity and function of neuronal immunoglobulin superfamily molecules," *Annual Review of Neuroscience*, vol. 26, pp. 207–238, 2003.

[166] M. Schachner, "Neural recognition molecules and synaptic plasticity," *Current Opinion in Cell Biology*, vol. 9, no. 5, pp. 627–634, 1997.

[167] V. Sytnyk, I. Leshchyns'ka, M. Delling, G. Dityateva, A. Dityatev, and M. Schachner, "Neural cell adhesion molecule promotes accumulation of TGN organelles at sites of neuron-to-neuron contacts.," *The Journal of Cell Biology*, vol. 159, no. 4, pp. 649–661, 2002.

[168] V. Sytnyk, I. Leshchyns'ka, A. Dityatev, and M. Schachner, "Trans-Golgi network delivery of synaptic proteins in synaptogenesis," *Journal of Cell Science*, vol. 117, no. 3, pp. 381–388, 2004.

[169] V. Sytnyk, I. Leshchyns'Ka, A. G. Nikonenko, and M. Schachner, "NCAM promotes assembly and activity-dependent remodeling of the postsynaptic signaling complex," *The Journal of Cell Biology*, vol. 174, no. 7, pp. 1071–1085, 2006.

[170] A. Dityatev, G. Dityateva, and M. Schachner, "Synaptic strength as a function of post-versus presynaptic expression of the neural cell adhesion molecule NCAM," *Neuron*, vol. 26, no. 1, pp. 207–217, 2000.

[171] V. Budnik, Y. Zhong, and C. F. Wu, "Morphological plasticity of motor axons in *Drosophila* mutants with altered excitability," *Journal of Neuroscience*, vol. 10, no. 11, pp. 3754–3768, 1990.

[172] M. Mayford, A. Barzilai, F. Keller, S. Schacher, and E. R. Kandel, "Modulation of an NCAM-related adhesion molecule with long-term synaptic plasticity in *Aplysia*," *Science*, vol. 256, no. 5057, pp. 638–644, 1992.

[173] Y. Zhong, V. Budnik, and C. F. Wu, "Synaptic plasticity in *Drosophila* memory and hyperexcitable mutants: role of cAMP cascade," *Journal of Neuroscience*, vol. 12, no. 2, pp. 644–651, 1992.

[174] C. H. Bailey and E. R. Kandel, "Structural changes accompanying memory storage," *Annual Review of Physiology*, vol. 55, pp. 397–426, 1993.

[175] J. Q. Davis, S. Lambert, and V. Bennett, "Molecular composition of the node of Ranvier: identification of ankyrin-binding cell adhesion molecules neurofascin (Mucin+/third FNIII domain-) and NrCAM at nodal axon segments," *The Journal of Cell Biology*, vol. 135, no. 5, pp. 1355–1367, 1996.

[176] D. Muller, C. Wang, G. Skibo et al., "PSA-NCAM is required for activity-induced synaptic plasticity," *Neuron*, vol. 17, no. 3, pp. 413–422, 1996.

[177] C. M. Schuster, G. W. Davis, R. D. Fetter, and C. S. Goodman, "Genetic dissection of structural and functional components of synaptic plasticity. I. Fasciclin II controls synaptic stabilization and growth," *Neuron*, vol. 17, no. 4, pp. 641–654, 1996.

[178] C. M. Schuster, G. W. Davis, R. D. Fetter, and C. S. Goodman, "Genetic dissection of structural and functional components of synaptic plasticity. II. Fasciclin II controls presynaptic structural plasticity," *Neuron*, vol. 17, no. 4, pp. 655–667, 1996.

[179] C. H. Bailey, B. K. Kaang, M. Chen et al., "Mutation in the phosphorylation sites of MAP kinase blocks learning- related internalization of apCAM in *Aplysia* sensory neurons," *Neuron*, vol. 18, no. 6, pp. 913–924, 1997.

[180] I. Chow, "Cell-cell interaction during synaptogenesis," *Journal De Physiologie*, vol. 84, no. 1, pp. 121–127, 1990.

[181] F. Keller and S. Schacher, "Neuron-specific membrane glycoproteins promoting neurite fasciculation in *Aplysia californica*," *The Journal of Cell Biology*, vol. 111, no. 6, pp. 2637–2650, 1990.

[182] C. H. Bailey, M. Chen, F. Keller, and E. R. Kandel, "Serotonin-mediated endocytosis of apCAM: an early step of learning-related synaptic growth in *Aplysia*," *Science*, vol. 256, no. 5057, pp. 645–649, 1992.

[183] F. Wu and S. Schacher, "Presynaptic and postsynaptic changes mediated by two second messengers contribute to expression of *Aplysia* long-term heterosynaptic inhibition," *Neuron*, vol. 12, no. 2, pp. 407–421, 1994.

[184] H. Zhu, F. Wu, and S. Schacher, "*Aplysia* cell adhesion molecules and serotonin regulate sensory cell-motor cell interactions during early stages of synapse formation *in vitro*," *Journal of Neuroscience*, vol. 14, no. 11, pp. 6886–6900, 1994.

[185] H. Zhu, F. Wu, and S. Schacher, "Changes in expression and distribution of *Aplysia* cell adhesion molecules can influence synapse formation and elimination *in vitro*," *Journal of Neuroscience*, vol. 15, no. 6, pp. 4173–4183, 1995.

[186] S. Schacher, F. Wu, Z. Y. Sun, and D. N. Wang, "Cell-specific changes in expression of mRNAs encoding splice variants of *Aplysia* cell adhesion molecule accompany long-term synaptic plasticity," *Journal of Neurobiology*, vol. 45, pp. 152–161, 2000.

[187] N. Peter, B. Aronoff, F. Wu, and S. Schacher, "Decrease in growth cone-neurite fasciculation by sensory or motor cells *in vitro* accompanies downregulation of *Aplysia* cell adhesion molecules by neurotransmitters," *Journal of Neuroscience*, vol. 14, no. 3, pp. 1413–1421, 1994.

[188] M. Ghirardi, G. Naretto, F. Fiumara, F. Vitiello, and P. G. Montarolo, "Target-dependent modulation of neurotransmitter release in cultured *Helix* neurons involves adhesion molecules," *Journal of Neuroscience Research*, vol. 65, no. 2, pp. 111–120, 2001.

[189] P. F. Maness and M. Schachner, "Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration," *Nature Neuroscience*, vol. 10, no. 1, pp. 19–26, 2007.

[190] D. K. Ditlevsen, G. K. Povlsen, V. Berezin, and E. Bock, "NCAM-induced intracellular signaling revisited," *Journal of Neuroscience Research*, vol. 86, no. 4, pp. 727–743, 2008.

[191] J. Y. Hu, Y. Chen, J. K. Bougie, W. S. Sossin, and S. Schacher, "*Aplysia* cell adhesion molecule and a novel protein kinase C activity in the postsynaptic neuron are required for presynaptic growth and initial formation of specific synapses," *Journal of Neuroscience*, vol. 30, no. 25, pp. 8353–8366, 2010.

[192] P. Conrad, F. Wu, and S. Schacher, "Changes in functional glutamate receptors on a postsynaptic neuron accompany formation and maturation of an identified synapse," *Journal of Neurobiology*, vol. 39, pp. 237–248, 1999.

[193] P. Washbourne, A. Dityatev, P. Scheiffele et al., "Cell adhesion molecules in synapse formation," *Journal of Neuroscience*, vol. 24, no. 42, pp. 9244–9249, 2004.

[194] H. Kohsaka, E. Takasu, and A. Nose, "*In vivo* induction of postsynaptic molecular assembly by the cell adhesion molecule Fasciclin2," *The Journal of Cell Biology*, vol. 179, no. 6, pp. 1289–1300, 2007.

[195] D. Cai, S. Chen, and D. L. Glanzman, "Postsynaptic regulation of long-term facilitation in *Aplysia*," *Current Biology*, vol. 18, no. 12, pp. 920–925, 2008.

[196] H.-L. Li, B. S. Huang, H. Vishwasrao et al., "Dscam mediates remodeling of glutamate receptors in *Aplysia* during de novo and learning-related synapse formation," *Neuron*, vol. 61, no. 4, pp. 527–540, 2009.

[197] G. Villareal, Q. Li, D. Cai et al., "Role of protein kinase C in the induction and maintenance of serotonin-dependent enhancement of the glutamate response in isolated siphon motor neurons of *Aplysia* californica," *Journal of Neuroscience*, vol. 29, no. 16, pp. 5100–5107, 2009.

[198] T. Biederer, Y. Sara, M. Mozhayeva et al., "SynCAM, a synaptic adhesion molecule that drives synapse assembly," *Science*, vol. 297, no. 5586, pp. 1525–1531, 2002.

[199] T. Shingai, W. Ikeda, S. Kakunaga et al., "Implications of nectin-like molecule-2/IGSF4/RA175/SgIGSF/TSLC1/SynCAM1 in cell-cell adhesion and transmembrane protein localization in epithelial cells," *Journal of Biological Chemistry*, vol. 278, no. 37, pp. 35421–35427, 2003.

[200] M. Matsuzaki, N. Honkura, G. C. R. Ellis-Davies, and H. Kasai, "Structural basis of long-term potentiation in single dendritic spines," *Nature*, vol. 429, no. 6993, pp. 761–766, 2004.

[201] A. Holtmaat, L. Wilbrecht, G. W. Knott, E. Welker, and K. Svoboda, "Experience-dependent and cell-type-specific spine growth in the neocortex," *Nature*, vol. 441, no. 7096, pp. 979–983, 2006.

[202] K. Zito, V. Scheuss, G. Knott, T. Hill, and K. Svoboda, "Rapid functional maturation of nascent dendritic spines," *Neuron*, vol. 61, no. 2, pp. 247–258, 2009.

[203] N. Honkura, M. Matsuzaki, J. Noguchi, G. C. R. Ellis-Davies, and H. Kasai, "The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines," *Neuron*, vol. 57, no. 5, pp. 719–729, 2008.

[204] C. D. Harvey and K. Svoboda, "Locally dynamic synaptic learning rules in pyramidal neuron dendrites," *Nature*, vol. 450, no. 7173, pp. 1195–1200, 2007.

[205] I. Ehrlich, M. Klein, S. Rumpel, and R. Malinow, "PSD-95 is required for activity-driven synapse stabilization," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 10, pp. 4176–4181, 2007.

[206] C. D. Kopec, E. Real, H. W. Kessels, and R. Malinow, "GluR1 links structural and functional plasticity at excitatory synapses," *Journal of Neuroscience*, vol. 27, no. 50, pp. 13706–13718, 2007.

[207] C. W. Cotman, N. P. Hailer, K. K. Pfister, I. Soltesz, and M. Schachner, "Cell adhesion molecules in neural plasticity and pathology: similar mechanisms, distinct organizations?" *Progress in Neurobiology*, vol. 55, no. 6, pp. 659–669, 1998.

[208] H. Cremer, G. Chazal, A. Carleton, C. Goridis, J. D. Vincent, and P. M. Lledo, "Long-term but not short-term plasticity at mossy fiber synapses is impaired in neural cell adhesion molecule-deficient mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 22, pp. 13242–13247, 1998.

[209] J. Z. Kiss and D. Muller, "Contribution of the neural cell adhesion molecule to neuronal and synaptic plasticity," *Reviews in the Neurosciences*, vol. 12, no. 4, pp. 297–310, 2001.

[210] S. Murase and E. M. Schuman, "The role of cell adhesion molecules in synaptic plasticity and memory," *Current Opinion in Cell Biology*, vol. 11, no. 5, pp. 549–553, 1999.

[211] L. C. B. Ronn, E. Bock, D. Linnemann, and H. Jahnsen, "NCAM-antibodies modulate induction of long-term potentiation in rat hippocampal CA1," *Brain Research*, vol. 677, no. 1, pp. 145–151, 1995.

[212] D. L. Glanzman, E. R. Kandel, and S. Schacher, "Target-dependent structural changes accompanying long-term synaptic facilitation in *Aplysia* neurons," *Science*, vol. 249, no. 4970, pp. 799–802, 1990.

[213] P. G. Montarolo, E. R. Kandel, and S. Schacher, "Long-term heterosynaptic inhibition in *Aplysia*," *Nature*, vol. 333, no. 6169, pp. 171–174, 1988.

[214] S. Schacher and P. G. Montarolo, "Target-dependent structural changes in sensory neurons of *Aplysia* accompany long-term heterosynaptic inhibition," *Neuron*, vol. 6, no. 5, pp. 679–690, 1991.

[215] Y. Hu, A. Barzilai, M. Chen, C. H. Bailey, and E. R. Kandel, "5-HT and cAMP induce the formation of coated pits and vesicles and increase the expression of clathrin light chain in sensory neurons of *Aplysia*," *Neuron*, vol. 10, no. 5, pp. 921–929, 1993.

[216] H. Cremer, R. Lange, A. Christoph et al., "Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning," *Nature*, vol. 367, no. 6462, pp. 455–459, 1994.

[217] C. G. Becker, A. Artola, R. GerardySchahn, T. Decker, H. Welzl, and M. Schachner, "The polysialic acid modification of the neural cell adhesion molecule is involved in spatial learning and hippocampal long-term potentiation," *Journal of Neuroscience Research*, vol. 45, pp. 143–152, 1996.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2012, Article ID 704103, 9 pages
doi:10.1155/2012/704103

Review Article

Examining Form and Function of Dendritic Spines

Kevin F. H. Lee, Cary Soares, and Jean-Claude Béïque

Heart and Stroke Foundation Centre for Stroke Recovery, Centre for Neural Dynamics, and, Department of Cellular and Molecular Medicine, University of Ottawa, 451 Smyth Road, Rm 3501N, Ottawa, ON, Canada K1H 8M5

Correspondence should be addressed to Jean-Claude Béïque, jbeique@uottawa.ca

Received 13 December 2011; Accepted 10 January 2012

Academic Editor: Volker Korz

The majority of fast excitatory synaptic transmission in the central nervous system takes place at protrusions along dendrites called spines. Dendritic spines are highly heterogeneous, both morphologically and functionally. Not surprisingly, there has been much speculation and debate on the relationship between spine structure and function. The advent of multi-photon laser-scanning microscopy has greatly improved our ability to investigate the dynamic interplay between spine form and function. Regulated structural changes occur at spines undergoing plasticity, offering a mechanism to account for the well-described correlation between spine size and synapse strength. In turn, spine structure can influence the degree of biochemical and perhaps electrical compartmentalization at individual synapses. Here, we review the relationship between dendritic spine morphology, features of spine compartmentalization and synaptic plasticity. We highlight emerging molecular mechanisms that link structural and functional changes in spines during plasticity, and also consider circumstances that underscore some divergence from a tight structure-function coupling. Because of the intricate influence of spine structure on biochemical and electrical signalling, activity-dependent changes in spine morphology alone may thus contribute to the metaplastic potential of synapses. This possibility asserts a role for structural dynamics in neuronal information storage and aligns well with current computational models.

1. Introduction

Ever since the first description of *espinas* on Purkinje cells by Cajal more than 100 years ago [1, 2], these tiny, femtolitre-sized, structures have been found on dendrites of a wide variety of neuronal cell types and the role of these minute structures in neuronal function has been the subject of considerable attention, speculation, and debate. These discrete dendritic protrusions form a rich structural scaffold for the majority of excitatory synapses in the brain, harbouring a complement of biochemical signalling machinery as well as a proteinaceous postsynaptic density (PSD) containing, amongst others, ionotropic glutamate receptors of the AMPA and NMDA subtypes [3]. These receptors mediate the bulk of fast excitatory neurotransmission in the brain. During postnatal development, dendritic spines acquire AMPARs and undergo structural enlargement, resulting in a positive correlation between spine size and AMPAR function. Interestingly, the high degree of heterogeneity in dendritic spine structure and function at maturity suggests that spine growth is regulated in a synapse-specific manner and not simply a consequence of *en masse* spine development.

In the past decade or so, a number of technological developments in fluorescence microscopy and molecular techniques have greatly accelerated our understanding of the relationship between structure and function at dendritic spines. For one, the induction of synaptic plasticity at single synapses was found to result in changes in spine structure, providing a plausible mechanism to explain the concurrent developmental changes in spine size and function [4]. Furthermore, recent studies have elaborated a mechanistic and molecular framework to suggest that spines function as discrete compartments, offering a basis for computationally relevant synaptic autonomy. Based on the robust concordance between structural and functional plasticity, and on the similarities in the molecular underpinnings that drive these two processes, there is a growing trend in synaptic physiology to infer synaptic strength based on characteristics of spine morphology. However, the dissociation of spine structure and function under some experimental conditions suggests an

important mechanistic divergence in the regulation of spine form and function. In this paper, we will provide an outline of the dendritic spine as a discrete functional compartment, discuss new developments in structural and functional plasticity at single spines and highlight key aspects of our understanding of the relationship between spine structure and function.

2. Two-Photon Microscopy and the Investigation of Individual Dendritic Spines

Despite unsheathing fundamental properties of various forms of synaptic plasticity [5], investigations based solely upon electrically evoked synaptic events left a number of open questions. Although minimum stimulation methods allow the functional study of single synapses in isolation [6–9], the inherent technical challenges of these experiments hinder the ability to efficiently amass data and resolve spatial parameters such as the morphology and location of the activated synapses (relative to each other and to the soma). The advent of two-photon (2P) laser scanning microscopy circumvented a number of these experimental limitations and has contributed considerable depth to our understanding of spine function and plasticity.

The longer wavelengths and lower excitation energy used in 2P imaging increase imaging depth in scattering tissue (such as brain) while also reducing photodamage/toxicity compared to 1P imaging [10–12]. Furthermore, the 2P excitation event is highly restricted in physical space with an excitation volume that roughly approximates the diffraction limits of the optical system [10]. This small excitation volume thus confers the ability to photoactivate molecules with high spatial precision, thereby providing novel opportunities for the study of synaptic physiology. For instance, 2P "uncaging" of caged forms of neurotransmitters (for, e.g., MNI-Glutamate) provides the ability to selectively activate spatially discrete glutamate receptors in a number of experimental preparations *in vitro* [4, 13–24] and *in vivo* [25]. Pioneering work by Matsuzaki and colleagues used 2P imaging and glutamate uncaging to probe AMPAR content and induce LTP at individual dendritic spines on hippocampal CA1 pyramidal neurons, generating key insight into single synapse plasticity [4, 22]. For instance, the induction of LTP at single dendritic spines via 2P glutamate uncaging circumvented the presynaptic component of synaptic transmission and provided unequivocal support to the notion that, at least under certain conditions, synaptic plasticity can be mediated by solely postsynaptic mechanisms [4, 26].

In addition to providing important information regarding plasticity at single synapses, advances in 2P imaging and related optical techniques have been instrumental in generating novel understanding of other neuronal mechanisms and properties such as the spatial distribution of synaptic weights, the autonomy of the spine as a functional compartment, the integrative behaviour of dendritic branches, and the recurrent connectivity of cortical circuits [14, 16, 21, 27].

3. A Compartmental Model of Dendritic Spines

Dendritic spines are specialized structures exhibiting a high degree of molecular organization and exist in a wide range of morphologies. Although a number of nomenclatures have been proposed to describe the breadth of morphologies that individual spines can adopt, they can be broadly summarized as follows: "Mushroom-like," identified by a round dendritic spine head connected to the parent dendrite by a spine neck; "Stubby" spines, which are short, stout protrusions or bumps with no definitive spine neck; filipodial/long spines, which appear as thin, finger-like protrusions [28, 29]. There has been considerable speculation on the specific role imparted by these varying morphologies on aspects of spine function. For one, substantial attention has been given to the role of the spine neck and accumulating experimental evidence suggests that it serves to compartmentalize the dendritic spine head. This compartmental model is particularly attractive in light of the synapse specificity of the structural and functional changes that take place over development and during plasticity. The compartmentalization of dendritic spines can be broadly divided into two functional domains: (i) the biochemical compartment, which describes the spatial confinement of biochemical signalling due to diffusional restriction and physical segregation of proteins and signalling molecules; (ii) the electrical compartment, where spine neck morphology can impact the kinetics and propagation of synaptic potentials in a spine-specific manner. Here, we will sequentially review these two functional domains.

3.1. The Biochemical Compartment. Postsynaptic induction and expression of several forms of synaptic plasticity requires calcium influx through NMDARs and the initiation of calcium-dependent biochemical signalling in the dendritic spine. The development of calcium-sensitive fluorescent indicators and imaging techniques has greatly facilitated the study of calcium dynamics during synaptic activity. Specifically, calcium imaging experiments demonstrate that NMDAR-mediated calcium influx elicited during synaptic transmission is tightly restricted to the spine head, with minimal calcium diffusion into the parent dendrite [18, 30–33]. Given the key role of calcium as a second messenger in the regulation of synaptic plasticity, highly compartmentalized calcium signalling at dendritic spines is likely critical for providing the synapse specificity of synaptic plasticity. As a result, it has been proposed that the primary function of the dendritic spine structure is to compartmentalize signalling molecules such as calcium [31, 34]. Many factors can influence the intracellular diffusion of calcium. For instance, the presence of a spine neck has been suggested to restrict calcium diffusion and also appears to limit the diffusion of other molecules such as GFP and fluorescein dextran [31, 33, 35–37]. In addition, calcium pumps such as PMCAs and SERCA, calcium-binding molecules such as calmodulin (CaM) or calbindin, and differential cytosolic viscocities at individual spines can all contribute to regulate free-calcium concentrations (and its dynamics) and influence intracellular diffusion [38–40]. Together, these diverse mechanisms indicate that

dendritic spines utilize multiple strategies to compartmentalize biochemical signals and promote autonomous synaptic function (see Figure 1).

Dendritic spines must also communicate with protein synthesis machinery located in the parent dendrite to sustain late phases of LTP [23, 41–43]. Thus, the movement of signalling molecules to and from the dendritic spine must not be fully compartmentalized but conforms to some degree of regulation. An illustration of such regulation is provided by recent experiments showing that calcium/calmodulin-activated kinase II (CaMKII) and Ras, two important molecules for synaptic plasticity, exhibit differential displacements from activated spines into the parent dendrites during synaptic plasticity [19, 44]. Recent work by Murakoshi et al. (2011) extended these findings using a FRET-based approach [45]. The authors assessed the spatial spread of two synaptically activated Rho-GTPases, RhoA and Cdc42. Whereas single-spine LTP induced by 2P glutamate uncaging leads to sustained activation (up to 30 min) of both RhoA and Cdc42, only activated RhoA readily traversed the spine neck into the parent dendrite, with activated Cdc42 restricted to the stimulated spine [45]. Since the measured diffusional properties of these proteins were similar, it was proposed that mechanisms localized to the spine head were likely required to spatially restrict Cdc42 activation, thereby enforcing the notion that spines are highly regulated biochemical compartments. Taken together, the spatial compartmentalization of key regulatory molecules (e.g., protein kinases) may also offer powerful constraints that impact the spread of intracellular signals from the spine to the parent dendrite.

Surface (plasma membrane-bound) AMPARs and NMDARs exist at both synaptic and extrasynaptic locations. These surface receptor populations are not rigidly fixed, but in perpetual diffusional flux laterally through the membrane [46–48]. Similar to intracellular diffusion, the lateral mobility of proteins in the plasma membrane can also be influenced by morphological parameters of spines [49–51]. For instance, FRAP analysis demonstrated that spine necks restrict the lateral diffusion of surface AMPARs. Specifically, AMPARs at spines connected to the parent dendrite by a spine neck exhibit a twofold slower rate of lateral mobility compared to those at spines without a distinguishable spine neck (Figure 1). Similar results were obtained using membrane-bound GFP, indicating that the impedance of lateral mobility was dictated by morphological parameters of the spine, and not by intrinsic properties of AMPAR trafficking *per se* [49]. Furthermore, AMPARs also undergo constitutive vesicular cycling via endo- and exocytosis. Evidence from both electron microscopy and fluorescence imaging indicates the presence of endocytic and exocytic zones within dendritic spines [52–55]. Interestingly, the dynamic balance of endo- and exocytosis modulates synaptic strength and underlie certain forms of plasticity. Indeed, LTP induction results in an enhancement of AMPAR exocytosis [56–60]. Taken together, the strategic clustering of signalling proteins, the development of narrow spine necks, and the organization of vesicular cycling machinery can all contribute to biochemical compartmentalization of spines. This compartmentalization provides individual spines with the autonomous capacity to dynamically regulate the surface expression of distinct pools of AMPARs to promote the synapse specificity of synaptic plasticity.

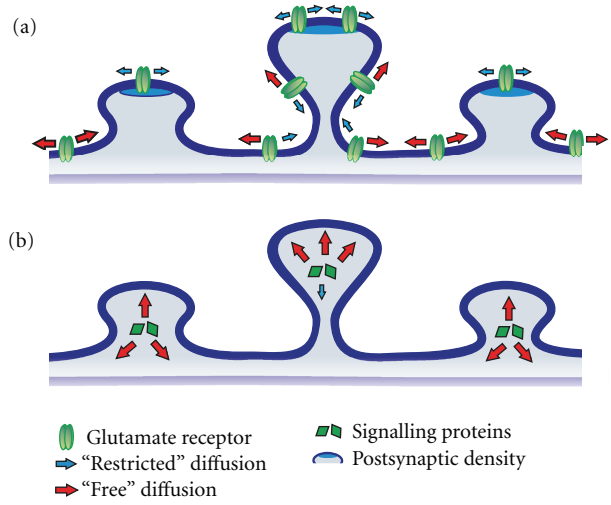


Figure 1: *Biochemical compartmentalization in dendritic spines.* The spine neck may offer enhanced compartmentalization of biochemical signalling at synapses. (a) The lateral mobility of surface glutamate receptors is attenuated across longer spine necks and at the postsynaptic density. (b) The spine neck imposes diffusional constraints on cytosolic signalling proteins. These mobility restraints imposed by the spine neck create spine-specific compartmentalization of cytosolic signalling and surface receptor dynamics.

3.2. The Electrical Compartment. In addition to providing the morphological substrate for bestowing features of biochemical compartmentalization, spines may also function as electrical compartments capable of modulating the amplitude, kinetics, and integration of synaptic potentials. Early estimations based on Rallian cable theory and measurements of molecular diffusion indicated that only modest ohmic resistances would be provided by spine necks and therefore largely dismissed the notion that spines behave as electrical compartments [37, 61]. However, recent experimental evidence suggests that electrical compartmentalization can take place in at least a subset of dendritic spines [13, 17, 62]. A combination of current-clamp recordings, 2P uncaging, and second harmonic membrane potential measurements provided evidence that long spine necks attenuate synaptic potentials between spine head and the parent dendrite [13]. In this line, it is interesting to note that calcium transients induced by activation of NMDARs can be readily detected by 2P calcium imaging in physiological extracellular magnesium concentrations (~1.0 mM) in slices [63–65] and *in vivo* [66–68], despite the presence of the voltage-dependent magnesium block of NMDARs near resting membrane potentials. Furthermore, calcium transients mediated by voltage-sensitive calcium channels (VSCCs) can also be visualized upon synaptic activation, indicating unexpectedly large depolarizations at the spine head [17]. Together, these data suggest that spine necks may impart an appreciable

degree of electrical resistance—and charge accumulation in spine heads—and thus electrically compartmentalize dendritic spines [62]. An intriguing and functionally powerful idea is that the degree of both electrical and biochemical compartmentalization might be dictated by active modifications in spine morphology. This possibility is becoming increasingly prominent given the dynamic structural changes which accompany the expression of synaptic plasticity (see below).

4. Structural and Functional Plasticity at Spines

The development of 2P glutamate uncaging to stimulate and induce LTP at single dendritic spines has been instrumental in providing key insights on the structural and functional changes that take place during plasticity. In 2004, Matsuzaki and colleagues induced LTP at individual dendritic spines by 2P glutamate uncaging and showed that the expression of LTP is associated with spine enlargement [4]. Furthermore, smaller spines carried an inherently higher propensity for LTP expression compared to larger spines, which were functionally and structurally more stable. Interestingly, some of the molecular mechanisms underlying structural plasticity have been found to closely parallel those for synaptic plasticity. For instance, LTP induction stimuli involving strong synaptic input and large postsynaptic rises in calcium facilitate actin branching and polymerization, providing a protrusive force to mediate spine enlargement [4, 69–72]. Conversely, LTD-inducing stimuli lead to actin depolymerization, spine shrinkage, and retraction [70]. Moreover, similar to the expression of long-lasting phases of LTP, the temporal stability of structural plasticity requires the synthesis of new proteins [23, 42]. These fundamental similarities in the induction of both structural and functional plasticity indicate an intimate relationship between these two processes.

One critical molecular link is CaMKII, a highly abundant protein in spines with an established role in synaptic plasticity [4, 44, 73–75]. At rest, CaMKII directly bundles and stabilizes actin filaments and is involved in the structural stability of spines [76]. CaMKII is activated by LTP-inducing stimuli, remaining persistently active and spatially compartmentalized to the stimulated spine properties that correlate well with the spatiotemporal characteristics of structural and functional plasticity [44]. Moreover, active CaMKII dissociates from the actin cytoskeleton causing structural destabilization, thus permitting structural modifications of the spine to take place [76]. Downstream, CaMKII activates a number of signalling molecules such as members of the Rho-GTPase family (RhoA, Cdc42, Rac1, and Rnd1) to mediate changes in spine structure [45, 72, 77]. For instance, Cdc42 becomes activated during LTP induction and interacts with p21-activated kinase (PAK) proteins to stabilize structural modifications [45]. Mice expressing a dominant-negative PAK (dnPAK) transgene in the forebrain show abnormal dendritic spine morphology, defects in both LTP and LTD, and impairments in the consolidation of spatial and fear memory [78]. Whereas it is difficult to attribute the behavioural deficits exhibited by dnPAK mice to synaptic impairments alone, these experimental strategies help to elucidate the interplay between structural and functional plasticity.

Although structural and functional changes rely on common signalling molecules, is it possible for these changes to occur independently of one another? Some evidence suggests that structural and functional plasticity are mutually stabilizing processes. For instance, in CA1 pyramidal neurons, the temporal stability of LTP expression is dependent on actin polymerization [79]. Subsequent investigations have expanded on these findings, underscoring a critical role for cytoskeletal actin dynamics in the directed trafficking of AMPARs [69, 70, 78, 80, 81]. Conversely, the insertion of AMPARs during LTP not only acts to increase synaptic strength, but has also been suggested to stabilize structural changes of the spine [82]. These data suggest that the molecular components that drive structural changes in dendritic spines during plasticity also act to stabilize AMPAR insertion and vice versa. This dynamic interplay thus provides a basis for the tight association between changes in spine volume and AMPAR content during LTP.

5. Structure versus Function

Dendritic spines on pyramidal cell dendrites number in the thousands and exhibit a high degree of morphological heterogeneity. High-resolution electron microscopy studies provided the first indication that spine form and function were related by demonstrating that the size of the PSD and number of AMPARs positively correlate with the size of spines [83–86]. A number of recent studies provided functional support to these ultrastructural findings by showing a strong positive correlation between dendritic spine size and AMPAR function (i.e., size of AMPAR-mediated current), as determined by 2P glutamate uncaging [14, 15, 22, 25, 35]. Considering the parallel changes observed in both structure (i.e., spine volume) and function (i.e., AMPAR content) during activity-dependent plasticity, it is perhaps not at all surprising that such a correlation exists. However, a more detailed and in-depth look at the literature, as outlined below, reveals that spines, at least in certain conditions, have the ability to significantly depart from such a tight structure/function coupling.

One of the first indications pointing to a divergence of spine form and function was provided by Smith et al. (2003) while describing the distance-dependent scaling of synaptic AMPARs in hippocampal CA1 pyramidal neurons [27]. Using a combination of whole-cell and dendritic recordings with 2P glutamate uncaging, they showed the synaptic weights of spines were progressively larger with increasing distances from the soma. However, this apparent increase in spine function was not accompanied with measurable changes in spine volume. Nonstationary fluctuation analysis on 2P glutamate uncaging currents further revealed that this increase in function with dendritic distance reflected a higher density of spine AMPARs, and not an enhanced sin-

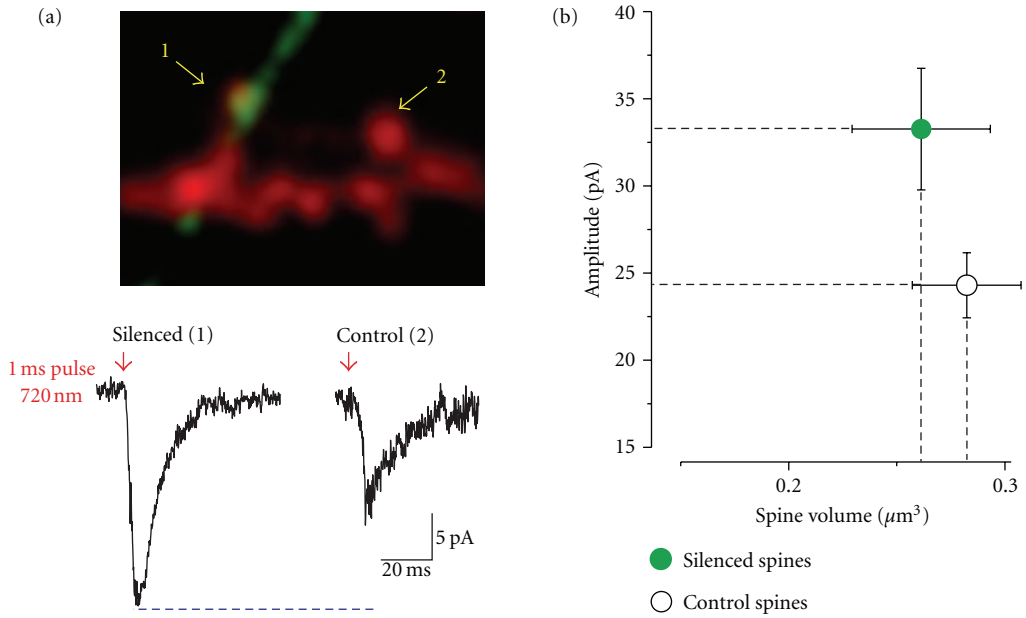


FIGURE 2: *Dissociation of spine size and synaptic strength.* (a) The release of glutamate was reduced for 48 hours specifically on the spine marked (1). This leads to a homeostatic enhancement of synaptic strength, as assessed by 2P-uncaging of MNI-Glutamate. The size of the synaptic currents induced by 2P-uncaging is shown in the bottom panel. (b) The significant enhancement of the amplitude of synaptic currents onto "silenced" spines was not accompanied by any measurable changes in spine volume. Adapted from [15].

gle-channel conductance. Together, these data provide a convincing illustration that spines of similar size can express a strikingly wide range of AMPAR density.

A disconnect between dendritic spine structure and function is further evidenced in studies of "silent" synapses. Silent synapses contain detectable NMDARs but are devoid of AMPARs and are therefore largely "silent" at rest (ought to the magnesium-dependent blockade NMDARs at resting membrane potential). They are thought to represent immature glutamateric synapses in part because their expression drastically diminishes during postnatal development [26, 87, 88]. Not surprisingly, early 2P-glutamate uncaging investigations described the presence of silent synapses on thin, filipodial-like spines in developing CA1 pyramidal neurons [15]. Interestingly, however, subsequent investigations in the rodent somatosensory cortex reported that silent synapses can be detected at spines spanning a broad range of morphologies [14, 18]. Although providing indirect support to this notion, work in PSD-95 KO mice also documented the presence of a structure/function uncoupling for spines. At an age where silent synapses were no longer detected in WT mice, PSD-95 KO mice displayed a high proportion of silent synapses onto large spines that otherwise appeared mature [15]. Collectively, these data indicate that although there is a clear correlation between spine size and function, there is also room for a significant departure from this tight structure/function coupling.

Studies on the dynamical nature of spine structure during LTD also indicate a divergence in the signalling pathways that regulate spine size and function. For instance, Zhou et al. (2004) reported that LTD and spine shrinkage at hippocampal synapses show differential requirements for protein phosphatase signalling via PP1 and calcineurin, despite sharing a similar requirement for NMDAR activation [89]. Furthermore, while the actin-binding protein cofilin was involved in spine shrinkage, it seemed to play no role in the expression of LTD. More recent investigations have also indicated that clathrin-mediated endocytosis is not required for spine shrinkage, despite being essential for LTD expression [90]. Finally, LTD studies in cerebellar Purkinje cells also reported dissociation between spine structure and function. Indeed, Sdrulla and Linden reported that LTD at cerebellar parallel fiber-Purkinje cell synapses was not associated with changes in either spine number or volume. In an interesting twist, the authors further identified a manipulation that led to a dramatic and global retraction of spines on Purkinje neurons that, surprisingly, was not associated with significant changes in synaptic strength [91]. Thus, evidence obtained from LTD experiments in two distinct brain regions underscores a mechanistic divergence of spine structure and function.

Lastly, this divergence is further exemplified in a model of single-synapse homeostatic plasticity in dissociated cortical neuronal cultures. Homeostatic plasticity refers to the ability of a neuron to bidirectionally tune synaptic AMPAR content in response to changes in overall network activity [92]. Recent experiments have expanded these findings by showing that homeostatic regulation of synaptic strength can be achieved at the level of individual dendritic spines [16, 93]. In one experimental paradigm, chronic suppression of presynaptic glutamatergic input onto single spines leads to an enhancement of postsynaptic AMPAR function, as determined by 2P glutamate uncaging [16]. Interestingly, despite a marked increase in AMPAR currents, there were no discernable changes in spine volume (see Figure 2). By comparing the current-voltage (I-V) relationship of AMPARs at

these two populations of dendritic spines, activity-deprived synapses were found to express AMPARs with a distinct subunit composition (AMPARs lacking the GluA2 subunit). Because this AMPAR subtype has an inherently higher conductance, this switch in subunit composition provides a mechanistically plausible model to account for the increased synaptic strength onto spines of similar volume.

6. Conclusion

As a major component of excitatory synapses, spines are strategically poised to support important modulatory roles in synaptic transmission and neuronal function. Although still subject to debate, an emerging notion posits that spines provide a structural scaffold to act as biochemical and electrical compartments. Interestingly, discrete differences in dendritic spine morphology may directly influence the degree of functional compartmentalization (Figure 1).

In addition, the dynamic nature of spine structure [94, 95] may generate parallel changes in the compartmentalization features of individual spines. One can speculate that these morphologically dependent degrees of compartmentalization lead to distinct states of metaplasticity at individual synapses. This notion aligns well with emerging theoretical models of synaptic learning that demonstrate that synapses exhibiting a gradation of states, each bridged by distinct metaplastic transitions, bestow neural networks with enhanced information storage capacity [96, 97]. Altogether, these considerations highlight the rich computational potential afforded by the yet to be completely understood relationship between form and function of dendritic spines.

References

[1] S. R. Cajal, "Estructura de los centros nerviosos de las aves," *Revista Trimestral de Histología Normal y Patológica*, vol. 1, pp. 1–10, 1888.

[2] R. Yuste, *Dendritic Spines*, The MIT Press, Cambridge, Mass, USA, 2010.

[3] S. F. Traynelis, L. P. Wollmuth, C. J. McBain et al., "Glutamate receptor ion channels: structure, regulation, and function," *Pharmacological Reviews*, vol. 62, no. 3, pp. 405–496, 2010.

[4] M. Matsuzaki, N. Honkura, G. C. R. Ellis-Davies, and H. Kasai, "Structural basis of long-term potentiation in single dendritic spines," *Nature*, vol. 429, no. 6993, pp. 761–766, 2004.

[5] R. C. Malenka and M. F. Bear, "LTP and LTD: an embarrassment of riches," *Neuron*, vol. 44, no. 1, pp. 5–21, 2004.

[6] J. T. R. Isaac, G. O. Hjelmstad, R. A. Nicoll, and R. C. Malenka, "Long-term potentiation at single fiber inputs to hippocampal CA1 pyramidal cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 16, pp. 8710–8715, 1996.

[7] C. Allen and C. F. Stevens, "An evaluation of causes for unreliability of synaptic transmission," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 22, pp. 10380–10383, 1994.

[8] C. F. Stevens and Y. Wang, "Facilitation and depression at single central synapses," *Neuron*, vol. 14, no. 4, pp. 795–802, 1995.

[9] L. E. Dobrunz and C. F. Stevens, "Heterogeneity of release probability, facilitation, and depletion at central synapses," *Neuron*, vol. 18, no. 6, pp. 995–1008, 1997.

[10] K. Svoboda and R. Yasuda, "Principles of two-photon excitation microscopy and its applications to neuroscience," *Neuron*, vol. 50, no. 6, pp. 823–839, 2006.

[11] W. Denk, K. R. Delaney, A. Gelperin et al., "Anatomical and functional imaging of neurons using 2-photon laser scanning microscopy," *Journal of Neuroscience Methods*, vol. 54, no. 2, pp. 151–162, 1994.

[12] F. Helmchen and W. Denk, "Deep tissue two-photon microscopy," *Nature Methods*, vol. 2, no. 12, pp. 932–940, 2005.

[13] R. Araya, J. Jiang, K. B. Eisenthal, and R. Yuste, "The spine neck filters membrane potentials," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 47, pp. 17961–17966, 2006.

[14] M. Ashby and J. Isaac, "Maturation of a recurrent excitatory neocortical circuit by experience-dependent unsilencing of newly formed dendritic spines," *Neuron*, vol. 70, no. 3, pp. 510–521, 2011.

[15] J. C. Béïque, D. T. Lin, M. G. Kang, H. Aizawa, K. Takamiya, and R. L. Huganir, "Synapse-specific regulation of AMPA receptor function by PSD-95," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 51, pp. 19535–19540, 2006.

[16] J. C. Béïque, Y. Na, D. Kuhl, P. F. Worley, and R. L. Huganir, "Arc-dependent synapse-specific homeostatic plasticity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 2, pp. 816–821, 2011.

[17] B. L. Bloodgood, A. J. Giessel, and B. L. Sabatini, "Biphasic synaptic Ca influx arising from compartmentalized electrical signals in dendritic spines," *PLoS Biology*, vol. 7, no. 9, Article ID e1000190, 2009.

[18] G. Busetto, M. J. Higley, and B. L. Sabatini, "Developmental presence and disappearance of postsynaptically silent synapses on dendritic spines of rat layer 2/3 pyramidal neurons," *Journal of Physiology*, vol. 586, no. 6, pp. 1519–1527, 2008.

[19] C. D. Harvey, R. Yasuda, H. Zhong, and K. Svoboda, "The spread of Ras activity triggered by activation of a single dendritic spine," *Science*, vol. 321, no. 5885, pp. 136–140, 2008.

[20] C. D. Harvey and K. Svoboda, "Locally dynamic synaptic learning rules in pyramidal neuron dendrites," *Nature*, vol. 450, no. 7173, pp. 1195–1200, 2007.

[21] A. Losonczy and J. C. Magee, "Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons," *Neuron*, vol. 50, no. 2, pp. 291–307, 2006.

[22] M. Matsuzaki, G. C. R. Ellis-Davies, T. Nemoto, Y. Miyashita, M. Iino, and H. Kasai, "Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons," *Nature Neuroscience*, vol. 4, no. 11, pp. 1086–1092, 2001.

[23] J. I. Tanaka, Y. Horiike, M. Matsuzaki, T. Miyazaki, G. C. R. Ellis-Davies, and H. Kasai, "Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines," *Science*, vol. 319, no. 5870, pp. 1683–1687, 2008.

[24] M. Matsuzaki and H. Kasai, "Two-photon uncaging microscopy," *Cold Spring Harbor Protocols*, vol. 6, no. 5, 2011.

[25] J. Noguchi, A. Nagaoka, S. Watanabe et al., "In vivo two-photon uncaging of glutamate revealing the structure-function relationships of dendritic spines in the neocortex of adult mice," *Journal of Physiology*, vol. 589, no. 10, pp. 2447–2457, 2011.

[26] G. A. Kerchner and R. A. Nicoll, "Silent synapses and the emergence of a postsynaptic mechanism for LTP," *Nature Reviews Neuroscience*, vol. 9, no. 11, pp. 813–825, 2008.

[27] M. A. Smith, G. C. R. Ellis-Davies, and J. C. Magee, "Mechanism of the distance-dependent scaling of Schaffer collateral synapses in rat CA1 pyramidal neurons," *Journal of Physiology*, vol. 548, no. 1, pp. 245–258, 2003.

[28] K. M. Harris, F. E. Jensen, and B. Tsao, "Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation," *Journal of Neuroscience*, vol. 12, no. 7, pp. 2685–2705, 1992.

[29] J. I. Arellano, R. Benavides-Piccione, J. Defelipe, and R. Yuste, "Ultrastructure of dendritic spines: correlation between synaptic and spine morphologies," *Frontiers in Neuroscience*, vol. 1, pp. 131–143, 2007.

[30] J. A. Connor, L. D. P. Miller, J. Petrozzino, and W. Muller, "Calcium signaling in dendritic spines of hippocampal neurons," *Journal of Neurobiology*, vol. 25, no. 3, pp. 234–242, 1994.

[31] J. Noguchi, M. Matsuzaki, G. C. R. Ellis-Davies, and H. Kasai, "Spine-neck geometry determines NMDA receptor-dependent Ca^{2+} signaling in dendrites," *Neuron*, vol. 46, no. 4, pp. 609–622, 2005.

[32] T. G. Oertner, B. L. Sabatini, E. A. Nimchinsky, and K. Svoboda, "Facilitation at single synapses probed with optical quantal analysis," *Nature Neuroscience*, vol. 5, no. 7, pp. 657–664, 2002.

[33] W. Muller and J. A. Connor, "Ca2+ signalling in postsynaptic dendrites and spines of mammalian neurons in brain slice," *Journal of Physiology Paris*, vol. 86, no. 1-3, pp. 57–66, 1992.

[34] R. Yuste, A. Majewska, and K. Holthoff, "From form to function: calcium compartmentalization in dendritic spines," *Nature Neuroscience*, vol. 3, no. 7, pp. 653–659, 2000.

[35] K. Zito, V. Scheuss, G. Knott, T. Hill, and K. Svoboda, "Rapid functional maturation of nascent dendritic spines," *Neuron*, vol. 61, no. 2, pp. 247–258, 2009.

[36] B. L. Bloodgood and B. L. Sabatini, "Neuronal activity regulates diffusion across the neck of dendritic spines," *Science*, vol. 310, no. 5749, pp. 866–869, 2005.

[37] K. Svoboda, D. W. Tank, and W. Denk, "Direct measurement of coupling between dendritic spines and shafts," *Science*, vol. 272, no. 5262, pp. 716–719, 1996.

[38] B. A. Smith, H. Roy, P. De Koninck, P. Grütter, and Y. De Koninck, "Dendritic spine viscoelasticity and soft-glassy nature: balancing dynamic remodeling with structural stability," *Biophysical Journal*, vol. 92, no. 4, pp. 1419–1430, 2007.

[39] A. Majewska, E. Brown, J. Ross, and R. Yuste, "Mechanisms of calcium decay kinetics in hippocampal spines: role of spine calcium pumps and calcium diffusion through the spine neck in biochemical compartmentalization," *Journal of Neuroscience*, vol. 20, no. 5, pp. 1722–1734, 2000.

[40] K. A. Kenyon, E. A. Bushong, A. S. Mauer, E. E. Strehler, R. J. Weinberg, and A. C. Burette, "Cellular and subcellular localization of the neuron-specific plasma membrane calcium ATPase PMCA1a in the rat brain," *Journal of Comparative Neurology*, vol. 518, no. 16, pp. 3169–3183, 2010.

[41] R. L. Redondo and R. G. M. Morris, "Making memories last: the synaptic tagging and capture hypothesis," *Nature Reviews Neuroscience*, vol. 12, no. 1, pp. 17–30, 2011.

[42] A. Govindarajan, I. Israely, S. Y. Huang, and S. Tonegawa, "The dendritic branch is the preferred integrative unit for protein synthesis-dependent LTP," *Neuron*, vol. 69, no. 1, pp. 132–146, 2011.

[43] W. C. Abraham and J. M. Williams, "LTP maintenance and its protein synthesis-dependence," *Neurobiology of Learning and Memory*, vol. 89, no. 3, pp. 260–268, 2008.

[44] S. J. R. Lee, Y. Escobedo-Lozoya, E. M. Szatmari, and R. Yasuda, "Activation of CaMKII in single dendritic spines during long-term potentiation," *Nature*, vol. 458, no. 7236, pp. 299–304, 2009.

[45] H. Murakoshi, H. Wang, and R. Yasuda, "Local, persistent activation of Rho GTPases during plasticity of single dendritic spines," *Nature*, vol. 472, no. 7341, pp. 100–106, 2011.

[46] M. Heine, L. Groc, R. Frischknecht et al., "Surface mobility of postsynaptic AMPARs tunes synaptic transmission," *Science*, vol. 320, no. 5873, pp. 201–205, 2008.

[47] A. J. Borgdorff and D. Choquet, "Regulation of AMPA receptor lateral movements," *Nature*, vol. 417, no. 6889, pp. 649–653, 2002.

[48] L. Groc, M. Heine, L. Cognet et al., "Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors," *Nature Neuroscience*, vol. 7, no. 7, pp. 695–696, 2004.

[49] M. C. Ashby, S. R. Maier, A. Nishimune, and J. M. Henley, "Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology," *Journal of Neuroscience*, vol. 26, no. 26, pp. 7046–7055, 2006.

[50] D. Holcman and A. Triller, "Modeling synaptic dynamics driven by receptor lateral diffusion," *Biophysical Journal*, vol. 91, no. 7, pp. 2405–2415, 2006.

[51] D. Choquet, "Fast AMPAR trafficking for a high-frequency synaptic transmission," *The European Journal of Neuroscience*, vol. 32, no. 2, pp. 250–260, 2010.

[52] B. Rácz, T. A. Blanpied, M. D. Ehlers, and R. J. Weinberg, "Lateral organization of endocytic machinery in dendritic spines," *Nature Neuroscience*, vol. 7, no. 9, pp. 917–918, 2004.

[53] J. Lu, T. D. Helton, T. A. Blanpied et al., "Postsynaptic positioning of endocytic zones and AMPA receptor cycling by physical coupling of dynamin-3 to Homer," *Neuron*, vol. 55, no. 6, pp. 874–889, 2007.

[54] N. Z. Gerges, D. S. Backos, C. N. Rupasinghe, M. R. Spaller, and J. A. Esteban, "Dual role of the exocyst in AMPA receptor targeting and insertion into the postsynaptic membrane," *EMBO Journal*, vol. 25, no. 8, pp. 1623–1634, 2006.

[55] T. A. Blanpied, D. B. Scott, and M. D. Ehlers, "Dynamics and regulation of clathrin coats at specialized endocytic zones of dendrites and spines," *Neuron*, vol. 36, no. 3, pp. 435–449, 2002.

[56] E. M. Petrini, J. Lu, L. Cognet, B. Lounis, M. D. Ehlers, and D. Choquet, "Endocytic trafficking and recycling maintain a pool of mobile surface AMPA receptors required for synaptic potentiation," *Neuron*, vol. 63, no. 1, pp. 92–105, 2009.

[57] M. Park, E. C. Penick, J. G. Edwards, J. A. Kauer, and M. D. Ehlers, "Recycling endosomes supply AMPA receptors for LTP," *Science*, vol. 305, no. 5692, pp. 1972–1975, 2004.

[58] G. A. Yudowski, M. A. Puthenveedu, D. Leonoudakis et al., "Real-time imaging of discrete exocytic events mediating surface delivery of AMPA receptors," *Journal of Neuroscience*, vol. 27, no. 41, pp. 11112–11121, 2007.

[59] D. T. Lin, Y. Makino, K. Sharma et al., "Regulation of AMPA receptor extrasynaptic insertion by 4.1N, phosphorylation and palmitoylation," *Nature Neuroscience*, vol. 12, no. 7, pp. 879–887, 2009.

[60] C. Lüscher, H. Xia, E. C. Beattie et al., "Role of AMPA receptor cycling in synaptic transmission and plasticity," *Neuron*, vol. 24, no. 3, pp. 649–658, 1999.

[61] C. Koch and A. Zador, "The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization," *Journal of Neuroscience*, vol. 13, no. 2, pp. 413–422, 1993.

[62] D. Tsay and R. Yuste, "On the electrical function of dendritic spines," *Trends in Neurosciences*, vol. 27, no. 2, pp. 77–83, 2004.

[63] Y. Kovalchuk, E. Hanse, K. W. Kafitz, and A. Konnerth, "Postsynaptic induction of BDNF-mediated long-term potentiation," *Science*, vol. 295, no. 5560, pp. 1729–1734, 2002.

[64] A. Grunditz, N. Holbro, L. Tian, Y. Zuo, and T. G. Oertner, "Spine neck plasticity controls postsynaptic calcium signals through electrical compartmentalization," *Journal of Neuroscience*, vol. 28, no. 50, pp. 13457–13466, 2008.

[65] J. R. Chalifoux and A. G. Carter, "Glutamate spillover promotes the generation of NMDA spikes," *Journal of Neuroscience*, vol. 31, no. 45, pp. 16435–16446, 2011.

[66] H. Jia, N. L. Rochefort, X. Chen, and A. Konnerth, "In vivo two-photon imaging of sensory-evoked dendritic calcium signals in cortical neurons," *Nature Protocols*, vol. 6, no. 1, pp. 28–35, 2011.

[67] X. Chen, U. Leischner, N. L. Rochefort, I. Nelken, and A. Konnerth, "Functional mapping of single spines in cortical neurons in vivo," *Nature*, vol. 475, no. 7357, pp. 501–505, 2011.

[68] Z. Varga, H. Jia, B. Sakmann, and A. Konnerth, "Dendritic coding of multiple sensory inputs in single cortical neurons in vivo," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 37, pp. 15420–15425, 2011.

[69] Y. Fukazawa, Y. Saitoh, F. Ozawa, Y. Ohta, K. Mizuno, and K. Inokuchi, "Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance in vivo," *Neuron*, vol. 38, no. 3, pp. 447–460, 2003.

[70] K. I. Okamoto, T. Nagai, A. Miyawaki, and Y. Hayashi, "Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity," *Nature Neuroscience*, vol. 7, no. 10, pp. 1104–1112, 2004.

[71] H. Kasai, T. Hayama, M. Ishikawa, S. Watanabe, S. Yagishita, and J. Noguchi, "Learning rules and persistence of dendritic spines," *The European journal of neuroscience*, vol. 32, no. 2, pp. 241–249, 2010.

[72] M. Patterson and R. Yasuda, "Signalling pathways underlying structural plasticity of dendritic spines," *British Journal of Pharmacology*, vol. 163, no. 8, pp. 1626–1638, 2011.

[73] A. Barria, D. Muller, V. Derkach, L. C. Griffith, and T. R. Soderling, "Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation," *Science*, vol. 276, no. 5321, pp. 2042–2045, 1997.

[74] R. Malinow, H. Schulman, and R. W. Tsien, "Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP," *Science*, vol. 245, no. 4920, pp. 862–866, 1989.

[75] J. Lisman, H. Schulman, and H. Cline, "The molecular basis of CaMKII function in synaptic and behavioural memory," *Nature Reviews Neuroscience*, vol. 3, no. 3, pp. 175–190, 2002.

[76] K. I. Okamoto, R. Narayanan, S. H. Lee, K. Murata, and Y. Hayashi, "The role of CaMKII as an F-actin-bundling protein crucial for maintenance of dendritic spine structure," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 15, pp. 6418–6423, 2007.

[77] L. A. Cingolani and Y. Goda, "Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy," *Nature Reviews Neuroscience*, vol. 9, no. 5, pp. 344–356, 2008.

[78] M. L. Hayashi, S. Y. Choi, B. S. Shankaranarayana Rao et al., "Altered cortical synaptic morphology and impaired memory consolidation in forebrain- specific dominant-negative PAK transgenic mice," *Neuron*, vol. 42, no. 5, pp. 773–787, 2004.

[79] T. Krucker, G. R. Siggins, and S. Halpain, "Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 12, pp. 6856–6861, 2000.

[80] L. Y. Chen, C. S. Rex, M. S. Casale, C. M. Gall, and G. Lynch, "Changes in synaptic morphology accompany actin signaling during LTP," *Journal of Neuroscience*, vol. 27, no. 20, pp. 5363–5372, 2007.

[81] B. Ramachandran and J. U. Frey, "Interfering with the actin network and its effect on long-term potentiation and synaptic tagging in hippocampal CA1 neurons in slices in vitro," *Journal of Neuroscience*, vol. 29, no. 39, pp. 12167–12173, 2009.

[82] C. D. Kopec, E. Real, H. W. Kessels, and R. Malinow, "GluR1 links structural and functional plasticity at excitatory synapses," *Journal of Neuroscience*, vol. 27, no. 50, pp. 13706–13718, 2007.

[83] A. Baude, Z. Nusser, E. Molnar, R. A. J. McIlhinney, and P. Somogyi, "High-resolution immunogold localization of AMPA type glutamate receptor subunits at synaptic and non-synaptic sites in rat hippocampus," *Neuroscience*, vol. 69, no. 4, pp. 1031–1055, 1995.

[84] V. N. Kharazia and R. J. Weinberg, "Immunogold localization of AMPA and NMDA receptors in somatic sensory cortex of albino rat," *Journal of Comparative Neurology*, vol. 412, no. 2, pp. 292–302, 1999.

[85] Y. Katz, V. Menon, D. A. Nicholson, Y. Geinisman, W. L. Kath, and N. Spruston, "Synapse distribution suggests a two-stage model of dendritic integration in CA1 pyramidal neurons," *Neuron*, vol. 63, no. 2, pp. 171–177, 2009.

[86] Y. Takumi, A. Matsubara, E. Rinvik, and O. P. Ottersen, "The arrangement of glutamate receptors in excitatory synapses," *Annals of the New York Academy of Sciences*, vol. 868, pp. 474–482, 1999.

[87] J. T. R. Isaac, M. C. Crair, R. A. Nicoll, and R. C. Malenka, "Silent synapses during development of thalamocortical inputs," *Neuron*, vol. 18, no. 2, pp. 269–280, 1997.

[88] J. J. Zhu and R. Malinow, "Acute versus chronic NMDA receptor blockade and synaptic AMPA receptor delivery," *Nature Neuroscience*, vol. 5, no. 6, pp. 513–514, 2002.

[89] Q. Zhou, K. J. Homma, and M. M. Poo, "Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses," *Neuron*, vol. 44, no. 5, pp. 749–757, 2004.

[90] X. B. Wang, Y. Yang, and Q. Zhou, "Independent expression of synaptic and morphological plasticity associated with long-term depression," *Journal of Neuroscience*, vol. 27, no. 45, pp. 12419–12429, 2007.

[91] A. D. Sdrulla and D. J. Linden, "Double dissociation between long-term depression and dendritic spine morphology in cerebellar Purkinje cells," *Nature Neuroscience*, vol. 10, no. 5, pp. 546–548, 2007.

[92] G. Turrigiano, "Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement," *Annual Review of Neuroscience*, vol. 34, pp. 89–103, 2011.

[93] M. C. Lee, R. Yasuda, and M. D. Ehlers, "Metaplasticity at Single Glutamatergic Synapses," *Neuron*, vol. 66, no. 6, pp. 859–870, 2010.

[94] T. A. Blanpied and M. D. Ehlers, "Membrane trafficking and cytoskeletal dynamics in neuronal function," *Molecular and Cellular Neuroscience*, vol. 48, no. 4, pp. 267–268, 2011.

[95] T. A. Blanpied, J. M. Kerr, and M. D. Ehlers, "Structural plasticity with preserved topology in the postsynaptic protein network," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 34, pp. 12587–12592, 2008.

[96] S. Fusi, P. J. Drew, and L. F. Abbott, "Cascade models of synaptically stored memories," *Neuron*, vol. 45, no. 4, pp. 599–611, 2005.

[97] S. Fusi and L. F. Abbott, "Limits on the memory storage capacity of bounded synapses," *Nature Neuroscience*, vol. 10, no. 4, pp. 485–493, 2007.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2012, Article ID 348642, 7 pages
doi:10.1155/2012/348642

Review Article

Immune System in the Brain: A Modulatory Role on Dendritic Spine Morphophysiology?

Oscar Kurt Bitzer-Quintero[1] and Ignacio González-Burgos[2, 3]

[1] *Laboratorio de Neuroinmunomodulación, División de Neurociencias, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Sierra Mojada No. 800 Col. Independencia, 44340 Guadalajara, JAL, Mexico*
[2] *Laboratorio de Psicobiología, División de Neurociencias, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Sierra Mojada No. 800 Col. Independencia, 44340 Guadalajara, JAL, Mexico*
[3] *Departamento de Biología Celular y Molecular, CUCBA, Universidad de Guadalajara, 45110 Zapopan, JAL, Mexico*

Correspondence should be addressed to Ignacio González-Burgos, igonbur@hotmail.com

Received 30 November 2011; Revised 10 January 2012; Accepted 26 January 2012

Academic Editor: Irina Nikonenko

The central nervous system is closely linked to the immune system at several levels. The brain parenchyma is separated from the periphery by the blood brain barrier, which under normal conditions prevents the entry of mediators such as activated leukocytes, antibodies, complement factors, and cytokines. The myeloid cell lineage plays a crucial role in the development of immune responses at the central level, and it comprises two main subtypes: (1) resident microglia, distributed throughout the brain parenchyma; (2) perivascular macrophages located in the brain capillaries of the basal lamina and the choroid plexus. In addition, astrocytes, oligodendrocytes, endothelial cells, and, to a lesser extent, neurons are implicated in the immune response in the central nervous system. By modulating synaptogenesis, microglia are most specifically involved in restoring neuronal connectivity following injury. These cells release immune mediators, such as cytokines, that modulate synaptic transmission and that alter the morphology of dendritic spines during the inflammatory process following injury. Thus, the expression and release of immune mediators in the brain parenchyma are closely linked to plastic morphophysiological changes in neuronal dendritic spines. Based on these observations, it has been proposed that these immune mediators are also implicated in learning and memory processes.

1. Introduction

Cell survival in the central nervous system (CNS) has been closely associated with activation of the immune system, a tightly controlled process [1]. The CNS parenchyma is separated from the bloodstream by the blood brain barrier (BBB), the integrity of which is maintained by endothelial tight junctions. Under normal conditions, the BBB prevents the entry into the brain parenchyma of leukocytes, antibodies, complement factors, and cytokines, as well as antigen presenting cells (APCs) such as dendritic cells, B cells, and macrophages [1, 2].

In the CNS, the immune system mediates several anatomical and physiological adaptations to protect its vital functions from the damage caused by inflammation [3] (Figure 1). In addition to the BBB and the cerebrospinal fluid (CSF), which also prevents the entry of plasma protein and immune cells into the parenchyma [3, 4], other mechanisms appear to sustain the "immune privilege" of the CNS, including local production of anti-inflammatory molecules such as transforming growth factor (TGF-β), melanocyte-stimulating hormone-alpha (α-MSH), and vasoactive intestinal peptide (VIP), as well as the constitutive expression of the Fas ligand (FasL or CD95L) that mediates the infiltrating immune cells death in the CNS [3, 4].

Studies of CNS damage have predominantly focused on neurons, as these cells determine the survival and function of the CNS [5–7]. However, in recent decades, additional cell subtypes have been seen to be involved in maintaining CNS homeostasis [7]. Microglial cells can generate an immune response after damage and alter nerve cell and dendritic spine morphophysiology. Similarly, dendritic spines may play a role in such events, these spines specializing in translating

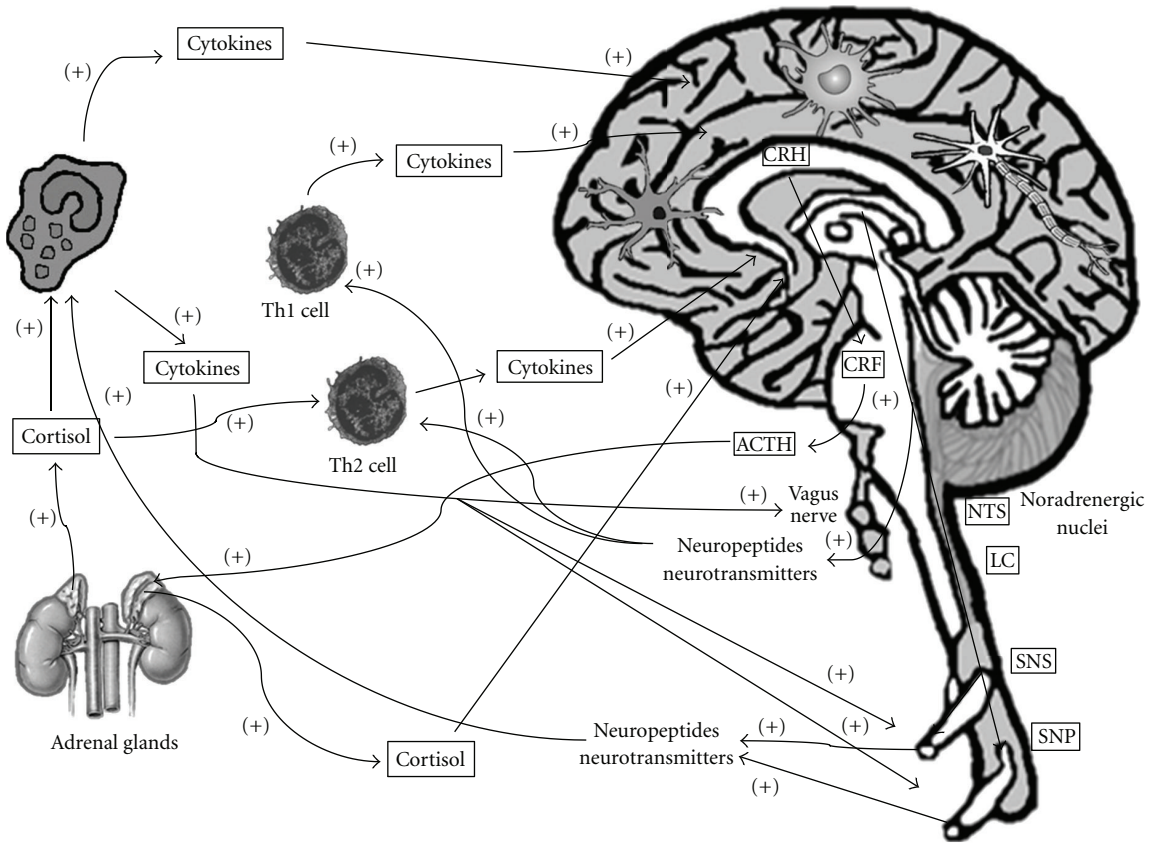


Figure 1: A bidirectional relationship links the immune and nervous systems, which communicate through the synthesis and the release of mediators such as cytokines, prostanoids, neuropeptides, and neurotransmitters. CRH: corticotropin-releasing hormone. CRF: corticotropin-releasing factor. ACTH: adrenocorticotropic hormone. NTS: nuclei of the solitary tract. LC: locus coeruleus. SNS: sympathetic nervous system. PNS: parasympathetic nervous system.

afferent excitatory synaptic inputs to postsynaptic neurons. Plastic changes in these structures are closely associated with the processing of cognitive-related afferent information. Accordingly, alterations in dendritic spine morphophysiology induced by microglia-associated immune responses exert deleterious effects on the transmission of mnemonic information, although the underlying mechanisms remain poorly understood.

2. Immune Cells in the Nervous System

The monocyte/macrophage cell lineage plays a key role in the development of innate and adaptive immune responses. Furthermore, some cell subpopulations associated with this lineage exhibit highly specialized phagocytic activity following cell degradation [8]. Two major subtypes of $CD45^+$ cells have been described in the myeloid lineage: the microglia resident population that is distributed throughout the brain parenchyma; perivascular macrophages located in the brain capillaries of the basal lamina and the choroid plexus. Parenchymal microglia cells are precursor cells derived from $CD45^+$ bone marrow cells that migrate into the CNS in the early stages of development, and they are capable of self-renewal within the brain parenchyma [7, 8]. By contrast, perivascular macrophages replace bone marrow elements at a more rapid rate and they have more features of monocytes/macrophages than parenchymal microglia [7, 8]. To date, no specific markers for these cell types have been described, although perivascular macrophages express higher levels of CD45, major histocompatibility complex (MHC-II), costimulatory molecules, and receptor recognition patterns than CD14 cells. The levels of these mediators are slightly lower in parenchymal microglial cells [8] (Figure 2).

Glial cells are the main source of many of the resident cytokines in the CNS. In addition to being "target" cells for cytokines, glial cells release important amounts of neuroactive substances that promote neuronal survival, such as neurotrophins and growth factors. By contrast, microglia release potentially neurotoxic substances, including nitric oxide (NO), reactive oxygen species (ROS), proteases, excitatory amino acids, and cytokines [9].

Activated macrophages (MQs) and microglia represent a major source of proinflammatory cytokines and chemokines within the CNS. These cells release reactive nitrogen species (RNS) and ROS and produce several factors that exert cytotoxic effects on oligodendrocytes and neurons [7]. Activated microglia can provoke excess MHC-II expression, facilitating

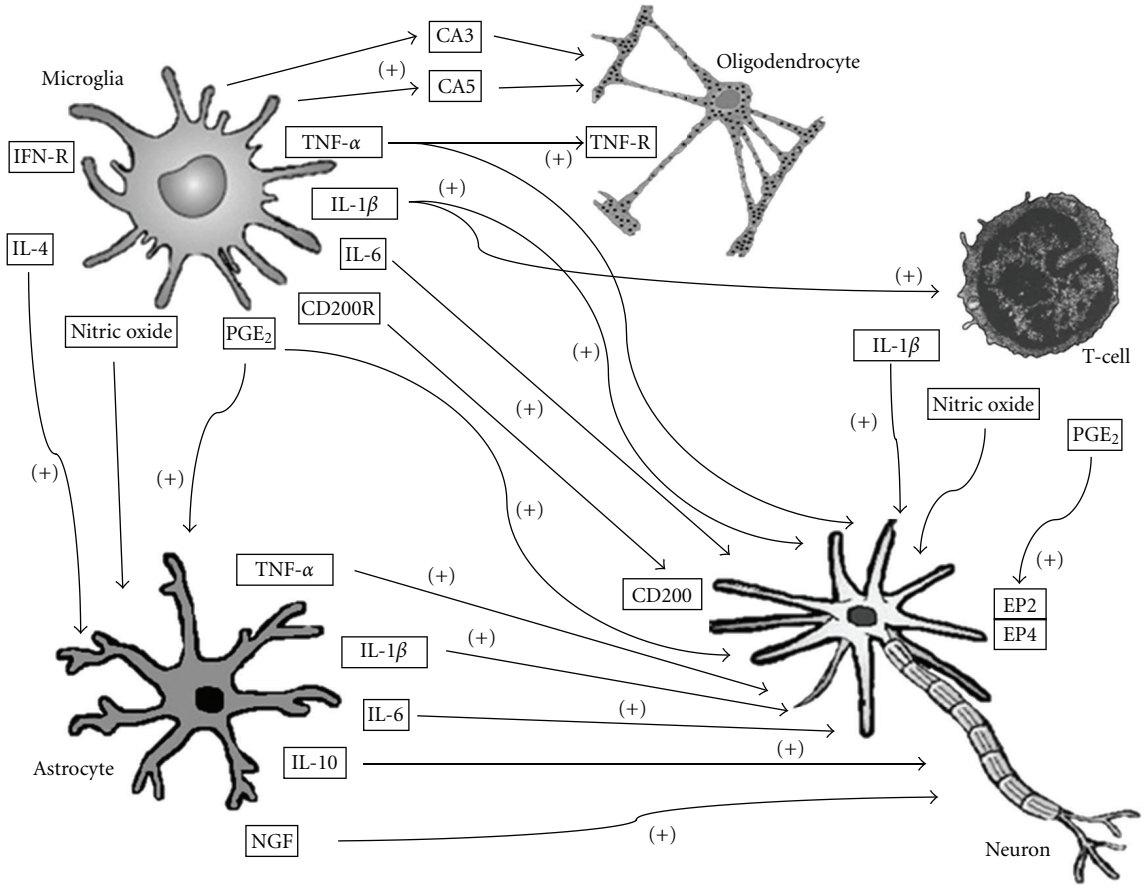


Figure 2: Microglia are activated in the brain parenchyma and mediate the synthesis and release of proinflammatory and anti-inflammatory cytokines, prostanoids, complement system molecules, and free radicals such as nitric oxide, affecting other cells including astrocytes, oligodendrocytes, and neurons. The signal established between microglial cells and neurons through the expression of CD200-CD200R maintains the microglia in a nonactivated state, and, when this signal is interrupted, microglia are immediately activated and initiate the generation and release of inflammatory mediators. PGE2: prostaglandin-E2; IFN-R: interferon-receptor; TNF-α: tumor necrosis factor-alpha; TNFR: tumor necrosis factor receptor; IL-1β: interleukin-1 beta; IL-6: interleukin-6; IL-10: interleukin-10; IL-4: interleukin-4; NGF: nerve growth factor.

its involvement in events mediated by the immune system. Microglial activation strongly influences the profile of cytokines released by these cells through two distinct mechanisms: pattern recognition receptors (PRRs; molecular traces remaining after a pathogenic insult) and immune response activation [6]. Activation of the monocyte/macrophage cell lineage through PRRs represents an important component of the innate immune response, which induces the production of proinflammatory cytokines and chemokines like IL-8, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1α), and MIP-1β. Moreover, other forms of PRR can activate regulatory cytokines within the CNS, such as interleukin-4 (IL-4) and interleukin-10 (IL-10) [6, 7].

Microglia located in perivascular areas can act as antigen presenting cells (APCs.) These cells evolve from their resting state and release significant quantities of Th1 cytokines, such as tumor necrosis factor-alpha (TNF-α) and IL-12 [7]. Such perivascular microglia alerts the parenchymal microglia to the signals of "damage," indicating the presence of inflammatory cells in the perivascular space. This provokes the activation of the parenchymal microglia, which proliferate and produce effector cells that eliminate these damage signals and that restore the tissue integrity [7]. Microglia can exhibit diverse activation states, including migratory, phagocytic, and regenerative. These different functional states are regulated by positive and negative feedback loops and by microenvironment damage signals [7]. Activated microglia in turn activate several proinflammatory cytokines, anti-inflammatory cytokines (IL-10, TGF-β, IL-1Ra), prostanoids (prostaglandin-E_2 or PGE_2, prostaglandin-D_2 or PGD_2 and thromboxane-A_2 or TXA_2), cytotoxic molecules (ROS, RNS, inducible nitric oxide synthase or iNOS), and chemokines (IL-8, IL-10, MCP-1, MIP-1α, MIP-1β, MIP-3β, MDC, RANTES) [5, 10, 11].

The contribution of astrocytes to the neuroimmune system is even more complex than that of microglia. This population plays an essential role in neuronal life-support, and it is essentially divided into 2 main subtypes: fibrous astrocytes located in the white matter; protoplasmic

astrocytes located in the gray matter, and they contribute to the BBB [9, 12, 13]. Some of the most important functions of astrocytes are related to the regulation of ion transport and neurotransmitter concentrations and the transmission of electric impulses. Astrocytes also act as neuroprotectors by secreting neurotrophins and releasing potentially toxic inflammatory molecules [9] (Figure 2). The third type of glial cells is the oligodendrocyte, which participates actively in myelination and is also involved in the secretion of specific inflammatory molecules [7] (Figure 2).

The microvasculature is a key element in cerebral damage. Endothelial cells represent an important source of immune mediators such as prostanoids and nitric oxide, both of which are implicated in the immune cell adhesion process [9]. Damage to the BBB can result in increased permeability, facilitating leukocyte access to the cerebral parenchyma, inducing the release of neurotoxins and endogenous inflammatory mediators and promoting phagocytosis of cell debris by perivascular MQs [9].

Diseases of the nervous system result in the upregulation of MHC-II and adhesion-costimulation molecules (CD11a, CD40, CD54, CD80, CD86) in activated microglia, indicating that these cells may acquire APC activity and actively participate in T-cell restimulation [14, 15]. This suggests the existence of a complex interaction network linking microglia, astrocytes, and invader T cells and involving a balance between Th1/Th2 signals, which defines the immune response of the CNS [14, 16].

In vivo microglial activation is regulated by several neuron-derived molecules, such as the CD200 marker, whilst microglia expresses CD200R. When CD200 expression is suppressed as a result of cell damage or after pathogenic stimulus, microglia switch to an activated phenotype, inducing the expression of the CD45 receptor, MHC-II, and the complement receptor 3 [17]. Resident microglia perform a wide variety of highly specialized functions in the cerebral parenchyma. These include the modulation of synaptic strength and efficacy through the remodeling of synaptic architecture [10] and induction of new synapses through regulation of synaptogenesis at the early stages of brain development. Microglia have also been suggested to regulate secretion of thrombospondin by astrocytes and secretion of the T-cell-derived serine protease (TSP-1). TSP-1 interacts with integrins that are associated with the CD47 protein and with the regulatory protein SRIPa, a transmembrane protein expressed in neurons and macrophages. The SRIPa-CD47 system regulates migration, phagocytosis, immune homeostasis, and the maintenance of the neuronal network [10].

Microglia play a key role in restoring neuronal connectivity following damage, by controlling reactive synaptogenesis [10]. Microglial cells may influence the homeostatic process of "synaptic scaling" that provides the adjustment in the strength of all synapses through distinct mechanisms in response to long-lasting changes in neuronal activity [10]. In addition, microglia are intimately involved in the development of the nervous system and in controlling the balance between neurogenesis and neuronal death. As mentioned previously, these cells play a dual role being implicated both in neurogenesis and neuronal death. While the mechanisms underlying this dichotomy remain unclear, differences in the activation state of microglia [10, 18] and the differential release of cytotoxic or cyto protective factors [18] have been reported. Thus, we conclude that microglia have the potential to regulate the development and function of neuronal networks by constantly monitoring the "status" of synaptic contacts and integrating new information [10].

3. The Relationship between the Immune System and Dendritic Spines

The inflammatory process observed in the CNS during either sepsis or endotoxic shock involves a wide variety of molecules that regulate the synthesis and release of inflammatory mediators, such as proinflammatory cytokines, prostanoids, and cytotoxic molecules. Such events are triggered both in the peripheral and the central nervous systems. These mediators are also generated and released during excitotoxicity induced by excessive glutamate release, ischemia reperfusion, brain trauma and neurodegenerative processes such as those associated with multiple sclerosis, Alzheimer's and Parkinson's diseases, leading to the activation of common inflammatory pathways [15–17, 19]. One such mediator is TNF-α, which is released by nerve cells and modifies the firing rate and excitability of neurons, thereby affecting processes such as long-term potentiation (LTP) and long-term depression (LTD) [15–17, 19].

The complex neural networks in the mammalian forebrain are regulated by interactions with glia cells (Figure 3), but the underlying mechanisms are poorly understood. Microglia may induce synaptic remodeling though direct contact or via the release of soluble factors (cytokines) that destabilize nearby synapses [19]. Microglia are thought to directly modulate dendritic spine dynamics in response to lipopolysaccharide (LPS) administration [19], which in mice results in a significant long-term upregulation of the expression of genes involved in the release and metabolism of arachidonic acid (AA) in the hippocampus [20, 21]. AA is a precursor of prostaglandins, thromboxanes, and leukotrienes, biochemical mediators of the inflammatory process responsible for endotoxic shock [20]. At a dose of 1 mg/kg body weight, intraperitoneal administered LPS to rats and mice induces "sickness behavior," changes in temperature and oxidative stress in the brain [20, 22, 23]. Likewise, acute peripheral administration of LPS is sufficient to trigger microglial activation, inducing the release of proinflammatory cytokines at both the peripheral and central levels [19, 24].

A recent study reported altered cognitive processes in a group of patients recovering from sepsis and endotoxic shock, including deficits in memory and attention [19]. LPS interacts with toll-like receptors (TLRs) at the MQs level, triggering the generation and release of a variety of important proinflammatory cytokines, including TNF-α, IL-1β, and IL-6. These proteins can cross the BBB to activate both microglia and astrocytes near brain capillaries. In addition, TNF-α, IL-1β, and IL-6 promote information

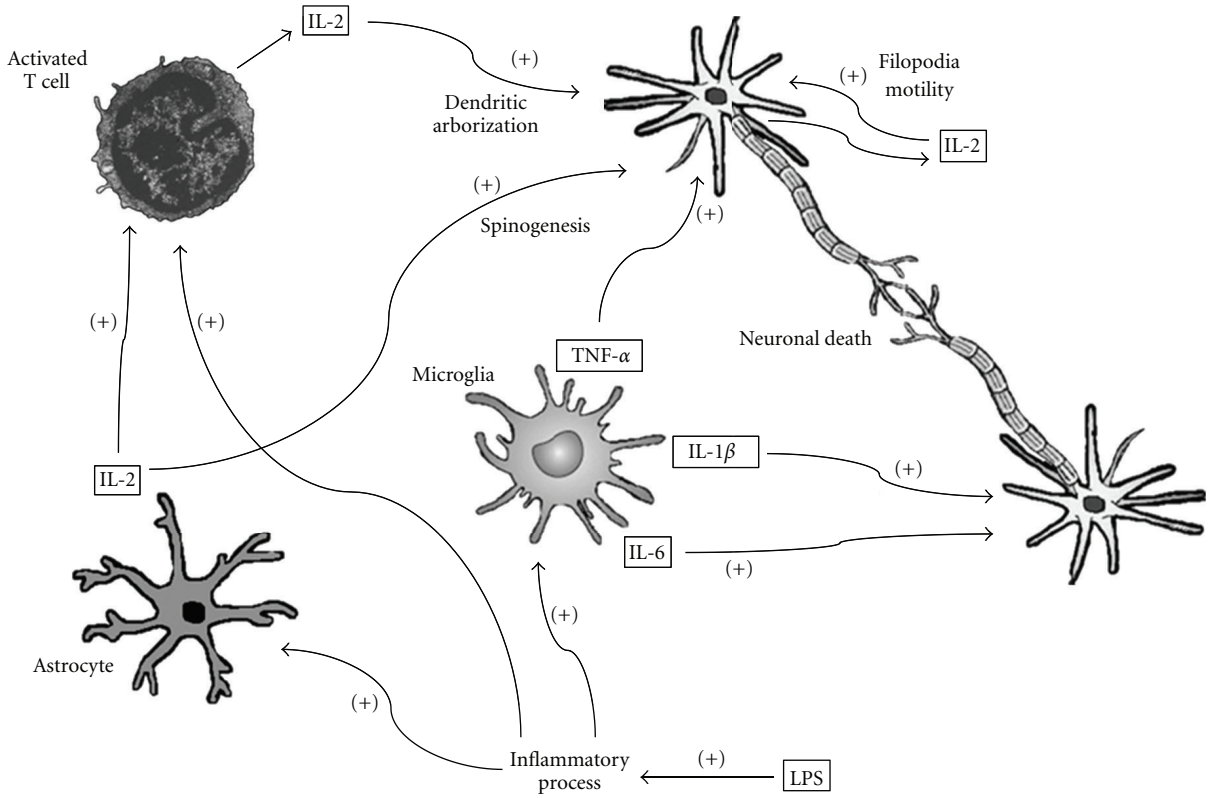


Figure 3: Interleukin-2 is involved in developmental processes such as neuronal dendritic arborization, and it induces a significant increase in spinogenesis and dendritic filopodia motility. Activated microglia release significant amounts of inflammatory mediators that induce neuronal death, synapse remodeling, and destabilization of synaptic contacts. IL-2: interleukin-2; TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin-1 beta; IL-6: interleukin-6; LPS: lipopolysaccharide.

flow via the vagus nerve, through the so-called inflammatory reflex. These responses promote the synthesis of PGs and cytokines in the brain parenchyma, which induce a fever state and sickness behavior [19]. Peripheral administration of LPS in rodents can affect certain cognitive functions by impairing LTP, a key physiological process in learning and spatial memory. LPS administration also induces peripheral inflammation, affecting the function of neural networks and glial cells. In addition, resident microglial cells in the brain parenchyma are activated, altering their phenotype and in turn affecting synaptic function [19].

One week after LPS administration, the dynamics of dendritic spines are comparable to those of control animals, indicating a minimal effect of acute systemic LPS treatment, at least in the short term. However, by 4 weeks after LPS administration, dendritic spine density increases to twice that of control animals. Moreover, this increase in spine density is associated with microglial activity, suggesting long-term effects on the CNS triggered by a transient peripheral immune response [19].

Acute induction of sickness behavior, which is accompanied by adverse effects on both emotional and cognitive processes, is at least partially responsible for the altered morphophysiological dynamics of dendritic spines [19, 24]. Cytokines released from glial cells can affect synaptic function, leading to alterations in spine morphology. Microglial cell proliferation has been reported in animal models of neurodegenerative disorders, accompanied by increased levels of proinflammatory cytokines and alterations in dendritic spine morphology and density [19]. Recent studies also suggest that the creation of an "inflammatory environment" may influence the generation of new synaptic connections. Indeed, microglia activated by inflammation may release cytokines and growth factors that "modulate" synaptic transmission, possibly by altering dendritic spine morphology. The inflammatory process also regulates functional synaptic connectivity, generating new connections in the adult brain [24].

Interestingly, new hippocampal neurons grown under conditions of chronic inflammation are more responsive to inhibitory afferent signals than those grown in normal conditions prior to the onset of inflammation. In the latter case, inflammation has no effect on the cell migration or polarity, on dendritic arborization or development, or on spine density, although dendritic spines in the new granule cells of the dentate gyrus exhibit very high mobility [24–26].

IL-2 is an immunoregulatory cytokine released from neurons and astrocytes in the CNS upon T-cell activation. This cytokine binds to receptors that are widely distributed throughout the brain. Following its release, IL-2 is thought to exert neuroregulatory effects and is implicated in various CNS disorders. In the hippocampus of experimental animals,

IL-2 influences several cognitive processes, such as LTP, modifying the molecular substrates underlying learning and memory. In addition, IL-2 provides trophic support to rat hippocampal neurons in primary culture, with positive effects on morphology and neurite branching [25]. In cultured hippocampal neurons, IL-2 also affects dendritic development and spinogenesis by day 14 *in vitro*, increasing dendritic arborization and neuronal growth. These effects are more pronounced on day 7, when the cytokine significantly augments the motility of dendritic filopodia. Together, these findings suggest that IL-2 exerts promoter-like effects on dendritic development and spinogenesis in cultured hippocampal neurons at early developmental stages [25].

The hippocampal region is a very plastic structure, displaying fluctuations in dendritic arborization, as well as changes in dendritic spine density and neuronal cell body size. These and other plastic modifications of neuronal cytoarchitecture are heavily implicated in hippocampal-dependent learning and memory processes. Many regulatory and stress factors can affect neuronal circuitry and interfere with the development of dendritic trees. In this context, IL-2 increases the number and length of dendritic branches in neuronal primary culture on days 7, 10, and 14 *in vitro* [25]. The length, diameter, and arborization of dendritic trees significantly influence neuronal function, and, hence, by increasing dendritic arborization and length, the signal transmission between neurons and the function of local neuronal networks are modulated. Accordingly, such alterations are associated with the development of behavioral disorders [25].

The formation of filopodia is a highly dynamic process that occurs early in CNS development in mammals. These structures may be involved in the formation of new dendritic spines and synapses at later stages of development. The ability of IL-2 to increase filopodia motility is likely involved in the development of new dendrites [25, 27].

4. Conclusion

In conclusion, we propose a close relationship between cytokine expression and the regulation of dendritic spine dynamics within a microuniverse in which the immune system and nervous system are closely linked. These internal interdependent systems mediate nonspecific behavioral responses, as well as learning and memory processes. Further studies are required to fully determine the full nature and physiological outcome of the relationship between immune system activity and dendritic spine dynamics, as well as the potential implications for learning and memory processes.

Abbreviations

α-MSH: Melanocyte stimulating hormone-alpha
AA: Arachidonic acid
APC: Antigen presenting cells
BBB: Blood brain barrier
CD: Cluster of differentiation
CNS: Central nervous system
CSF: Cerebrospinal fluid
iNOS: Inducible nitric oxide synthase
IL-1β: Interleukin-1 beta
IL-2: Interleukin-2
IL-4: Interleukin-4
IL-6: Interleukin-6
IL-10: Interleukin-10
LPS: Lipopolysaccharide
LTD: Long-term depression
LTP: Long-term potentiation
MQs: Macrophages
MHC: Major histocompatibility complex
MCP-1: Monocyte chemoattractant protein-1
MIP-1: Macrophage inflammatory protein-1
NO: Nitric oxide
PGs: Prostaglandins
PRRs: Pattern recognition receptors
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
TGFβ: Transforming growth factor
TNF-α: Tumor necrosis factor-alpha
TLRs: Toll-like receptors
TSP-1: T-cell-derived serine protease
VIP: Vasoactive intestinal peptide.

References

[1] H. Neumann, "Control of glial immune function by neurons," *GLIA*, vol. 36, no. 2, pp. 191–199, 2001.

[2] M. Schultzberg, C. Lindberg, A. F. Aronsson, E. Hjorth, S. D. Spulber, and M. Oprica, "Inflammation in the nervous system—physiological and pathophysiological aspects," *Physiology and Behavior*, vol. 92, no. 1-2, pp. 121–128, 2007.

[3] F. Aloisi, "Immune function of microglia," *GLIA*, vol. 36, no. 2, pp. 165–179, 2001.

[4] A. R. Simard and S. Rivest, "Neuroprotective effects of resident microglia following acute brain injury," *Journal of Comparative Neurology*, vol. 504, no. 6, pp. 716–729, 2007.

[5] D. Wronna, "Neural immune interactions: an integrative view of the directional relationship between the brain and the immune system," *Journal of Neuroimmunology*, vol. 172, no. 1, pp. 38–58, 2006.

[6] G. R. John, S. C. Lee, and C. F. Brosnan, "Cytokines: powerful regulators of glial cell activation," *The Neuroscientist*, vol. 9, no. 1, pp. 10–22, 2003.

[7] A. Chavarria and J. Alcocer-Varela, "Is damage in central nervous system due to inflammation," *Autoimmunity Reviews*, vol. 3, no. 4, pp. 251–260, 2004.

[8] C. Lambert, A. R. Ase, P. Séguéla, and J. P. Antel, "Distinct Migratory and cytokine responses of human microglia and macrophages to ATP," *Brain, Behavior and Immunity*, vol. 24, pp. 1241–1248, 2010.

[9] E. M. Sternberg, "Neural regulation of innate immunity: a coordinated non-specific host response to pathogens," *Nature Reviews Immunology*, vol. 6, pp. 318–328, 2006.

[10] H. Kettenmann, U. K. Hanish, M. Noda, and A. Verkhratsky, "Physiology of microglia," *Physiology Reviews*, vol. 91, no. 2, pp. 461–553, 2011.

[11] U.-K. Hanisch and H. Ketenmann, "Microglia: active sensor and versatile effector cells in the normal and pathological brain," *Nature Neuroscience*, vol. 10, pp. 1387–1394, 2007.

[12] M. E. Truckenmiller, M. F. Princiotta, C. C. Norbury, and R. H. Bonnean, "Corticosterone impairs MHC class I antigen presentation by dendritic cells via reduction of peptide generation," *Journal of Neuroimmunology*, vol. 160, no. 1, pp. 48–60, 2005.

[13] M. Kerschensteiner, E. Meinl, and R. Hohlfeld, "Neuro-immune crosstalk in CNS diseases," *Neuroscience*, vol. 158, no. 3, pp. 1122–1132, 2009.

[14] T. Benn, C. Halfpenny, and N. Sading, "Glial cells as targets for cytotoxic immune mediators," *GLIA*, vol. 36, no. 2, pp. 200–211, 2001.

[15] J. Goverman, "Autoimmune T cells responses in the central nervous system," *Nature Review Immunology*, vol. 9, pp. 393–407, 2009.

[16] E. G. De Araujo, G. M. Da Silva, and A. A. Dos Santos, "Neuronal cell survival. The role of interleukins," *Annals of the New York Academy of Sciences*, vol. 1153, pp. 57–64, 2009.

[17] M. Pickering and J. J. O'Connor, "Pro-inflammatory cytokines and their effects in the dentate gyrus," in *Progress in Brain Research*, H. E. Scharfaman, Ed., vol. 163, pp. 339–354, 2007.

[18] L. Muzio, G. Martino, and R. Furlan, "Multifaceted aspects of inflammation in multiple sclerosis: the role of microglia," *Journal of Neuroimmunology*, vol. 191, no. 1, pp. 39–44, 2007.

[19] S. Kondo, S. Kohsaka, and S. Okabe, "Long-term changes of spine dynamics and microglia after transient peripheral immune response triggered by LPS in vivo," *Molecular Brain*, vol. 4, no. 1, article 27, 2011.

[20] G. A. Czapski, B. Gajkowska, and J. B. Storsznajder, "Systemic administration of lipopolysaccharide induces molecular and morphological alterations in the hippocampus," *Brain Research*, vol. 1356, pp. 85–94, 2010.

[21] V. Eggers, A. Schilling, W. J. Kox, and C. Spies, "Septic encephalopathy. Diagnosis and therapy," *Anaesthesist*, vol. 52, no. 4, pp. 294–303, 2003.

[22] M. Boogaard, B. P. Ramakers, N. Alfen et al., "Endotoxemia-induced inflammation and the effect on the human brain," *Critical Care*, vol. 14, article R81, 2010.

[23] A. Suzumura, H. Takeuchi, G. Zhang, R. Kuno, and T. Mizuno, "Roles of glia-derived cytokines on neuronal degeneration and regeneration," *Annals of the New York Academy of Sciences*, vol. 1088, pp. 219–229, 2006.

[24] K. Jakubs, S. Bonde, R. E. Iosif et al., "Inflammation regulates functional integration of neurons born in adult brain," *Journal of Neuroscience*, vol. 28, no. 47, pp. 12477–12488, 2008.

[25] O. Bloom, J. J. Unternaehrer, A. Jiang et al., "Spinophilin participates in information transfer at immunological synapses," *Journal of Cell Biology*, vol. 181, no. 2, pp. 203–211, 2008.

[26] J. M. Mateos, A. Luthi, N. Savic et al., "Synaptic modifications at CA3-CA1 synapse after chronic AMPA receptor blockade in rat hippocampal slices," *Journal of Physiology*, vol. 581, pp. 129–138, 2007.

[27] Y. Shen, S. S. Liu, M. Y. Zhan, J. H. Luo, and J. L. Zhu, "Interleukin-2 enhances dendritic development and spinogenesis in cultured hippocampal neurons," *Anatomical Records*, vol. 293, no. 6, pp. 1017–1023, 2010.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2012, Article ID 728161, 9 pages
doi:10.1155/2012/728161

Review Article

Kalirin, a Key Player in Synapse Formation, Is Implicated in Human Diseases

Prashant Mandela and Xin-Ming Ma

Department of Neuroscience, University of Connecticut Health Center, Farmington, CT 06030, USA

Correspondence should be addressed to Xin-Ming Ma, ma@nso.uchc.edu

Received 30 November 2011; Accepted 13 January 2012

Academic Editor: Irina Nikonenko

Synapse formation is considered to be crucial for learning and memory. Understanding the underlying molecular mechanisms of synapse formation is a key to understanding learning and memory. Kalirin-7, a major isoform of Kalirin in adult rodent brain, is an essential component of mature excitatory synapses. Kalirin-7 interacts with multiple PDZ-domain-containing proteins including PSD95, spinophilin, and GluR1 through its PDZ-binding motif. In cultured hippocampal/cortical neurons, overexpression of Kalirin-7 increases spine density and spine size whereas reduction of endogenous Kalirin-7 expression decreases synapse number, and spine density. In Kalirin-7 knockout mice, spine length, synapse number, and postsynaptic density (PSD) size are decreased in hippocampal CA1 pyramidal neurons; these morphological alterations are accompanied by a deficiency in long-term potentiation (LTP) and a decreased spontaneous excitatory postsynaptic current (sEPSC) frequency. Human Kalirin-7, also known as Duo or Huntingtin-associated protein-interacting protein (HAPIP), is equivalent to rat Kalirin-7. Recent studies show that Kalirin is relevant to many human diseases such as Huntington's Disease, Alzheimer's Disease, ischemic stroke, schizophrenia, depression, and cocaine addiction. This paper summarizes our recent understanding of Kalirin function.

1. Kalirin Is a Rho Guanine Nucleotide Exchange Factor (GEF)

Kalirin was discovered 15 years ago as a novel protein that interacts with the cytosolic carboxyl-terminal of peptidylglycine α-amidating monooxygenase (PAM), an integral membrane peptide processing enzyme [1]. We have made significant progress in understanding the functions of Kalirin; like the many other Rho-GEFs encoded in mammalian genomes, Kalirin promotes the exchange of GDP for GTP and thus stimulates the activity of specific Rho GTPases [2, 3]. Rho GTPases that regulate multiple cellular processes play a key role in transducing signals from extracellular stimuli to the intracellular pathways that play a pivotal role in the formation of dendritic spines and synaptic development [4–6].

2. Multiple Kalirin Isoforms

The mouse Kalirin gene (*Kalrn*) consists of 65 exons spanning >650 kb of the genome; the presence of multiple promoters and transcriptional start sites enables the production of multiple functional isoforms of Kalirin [7–9]. Each Kalirin isoform is composed of a unique collection of domains (Figure 1). Major Kalirin isoforms including Kalirin-7, -9, and -12 are generated through the use of alternative 3′ exons [8]. The major isoforms share some common features including nine spectrin-like repeats, the GEF1 domain and the Sec14p domain. Sec14p domains facilitate lipid interactions and cellular localization. The nine spectrin-like repeat regions that follow the Sec14p domain have been shown to interact with many proteins including disrupted in schizophrenia 1 (DISC1) [10], peptidylglycine α-amidating monooxygenase (PAM) [1], inducible nitric oxide synthase (iNOS) [11], Huntingtin-associated protein 1 (HAP1) [12], and Arf6 (ADP-ribosylation factor 6) [13]. Kalirin-12 is the longest isoform and contains additional domains that include GEF2, an immunoglobulin-like (Ig) domain, a fibronectin III (FnIII) domain, and a serine/threonine protein kinase domain that is followed by a short, unique carboxyl-terminus [7, 14]. Kalirin-12 is found

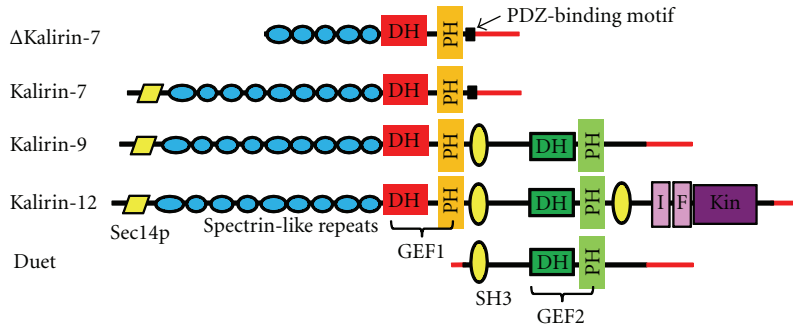


Figure 1: Major Kalirin isoforms. Alternative splicing generates different isoforms of Kalirin. DH: Dbl homology; PH: pleckstrin homology; GEF1: guanine nucleotide exchange factor 1; SH3: Src homology domain; GEF2: guanine nucleotide exchange factor 2; I: immunoglobulin-like; F: fibronectin III-like; Kin: kinase domain; red lines, unique 5′- and 3′-untranslated regions.

in the growth cones of immature neurons (Figure 2) and dendritic spines of mature cultured hippocampal neurons, suggesting a role for Kalirin-12 in axon outgrowth and synaptic plasticity. Interaction of the Ig-FnIII region unique to Kalirin-12 with the GTPase domain of dynamin may facilitate the coordination of endocytic trafficking and changes in the actin cytoskeleton [15]. Endogenous Kalirin-9 is localized to neurites and growth cones, and expression of exogenous Kalirin-9 induces longer neurites and altered neuronal morphology in cultured cortical neurons [16]. The functions of Kalirin-9 and Kalirin-12 in neurons remain to be elucidated. The ΔKalirin-7 (also referred as Kalirin-5) isoform is generated using a different promoter, and translation initiation begins at the start of spectrin-like repeat 5, producing an isoform with only 5 spectrin-like repeats. Overexpression of ΔKalirin-7 results in an increase in spine size, but not spine density, in cultured cortical neurons [17]. Duet, an isoform of Kalirin that begins just before the second GEF domain and continues through the unique C-terminal of Kalirin-12, uses a third promoter [7]. Duet was discovered as a protein homologous to the catalytic domain of death associated protein kinase and is localized to actin-associated cytoskeletal elements, suggesting the involvement of Duet in cytoskeleton-dependent functions [18].

3. Tissue Expression

Kalirin expression is detectable in a wide array of adult tissues including neurons, endocrine cells, liver, muscle, and heart [2, 20, 21]. In addition, developmentally regulated, tissue specific-Kalirin isoform expression is evident. Kalirin-9 and -12 are highly expressed in neuronal tissue during embryonic development, while in the adult brain expression of each is drastically decreased; a concomitant increase in Kalirin-7 expression occurs [8, 20, 22]. Kalirin-7 expression is largely limited to neurons of the central nervous system; its levels are extremely low at birth (postnatal days 1–7) and begin to increase markedly at postnatal day 14, which coincides with the onset of maximum synaptogenesis [22, 23].

4. Kalirin-7 Is the Major Kalirin Isoform in Adult Brain

Kalirin-7 is the most abundant Kalirin isoform in the adult rodent brain and is exclusively localized to the postsynaptic side of excitatory synapses [19, 22, 39, 40]. Kalirin-7 is a multifaceted molecule containing domains that interact with a wide array of molecular machinery. The Sec14p domain located at the N-terminus interacts with phosphatidylinositol-3-phosphate and plays a key role in Kalirin-7-mediated spine morphogenesis (Ma et al., unpublished). The C-terminus of Kalirin-7 contains a unique PDZ-binding motif through which Kalirin-7 interacts with PDZ-domain-containing proteins including PSD-95, AF-6, and spinophilin [39]. Binding of the NMDA receptor subunit NR2B to the PH domain of Kalirin-7 is important for normal synaptic plasticity [41]. The spectrin-like domains of Kalirin-7 through which it interacts with DISC1, iNOS, PAM, HAP1, and Arf6 play a key role in Kalirin-7-induced synapse formation (Ma et al., unpublished). The nucleotide sequences of human Kalirin-7 (Duo) and rat Kalirin-7 are 91% identical, and their amino acid sequences are 98% identical; human Kalirin-7 contains a 27-nucleotide insert not found in rat Kalirin-7, located at the end of the region encoding the seventh spectrin repeat [8, 12].

5. Kalirin-7 Contains Multiple Phosphorylation Sites

Phosphorylation of Kalirin-7 has recently been shown to be a pivotal mechanism mediating Kalirin-7-induced spine formation and synaptic plasticity [42–44]. Purified PKA, PKC, CaMKII, Cdk5, and Fyn each phosphorylate purified Kalirin-7 [45]. Kalirin-7 is extensively phosphorylated *in vivo*. The phosphorylation sites identified *in vitro* using purified CaMKII, PKA, PKC, or CKII, identified only 5 of the 22 sites that undergo phosphorylation in cells or tissue. These findings emphasize a critical role for additional protein kinases and the importance of cellular localization in the phosphorylation of Kalirin-7 [45]. Densely distributed

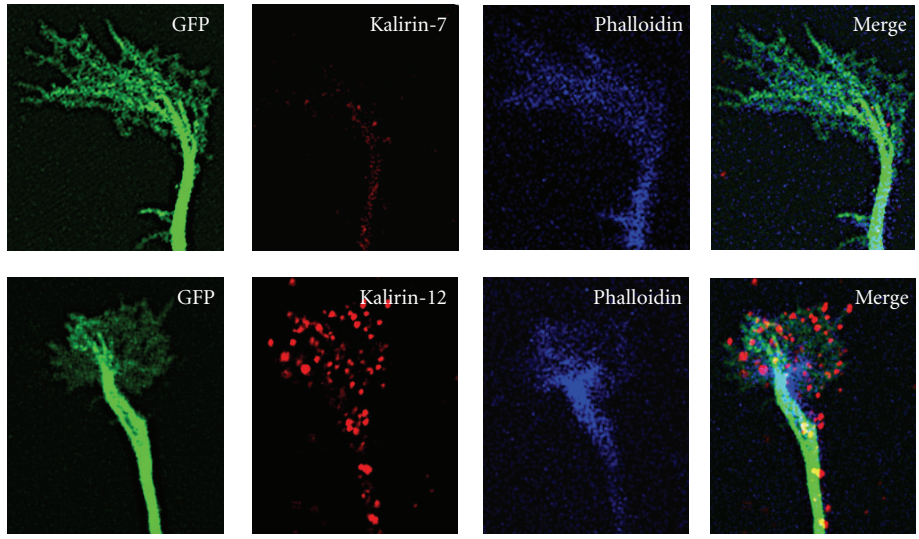


Figure 2: Kalirin-12, not Kalirin-7, is localized to the growth cone of hippocampal neurons. Primary cultures of hippocampal neurons prepared at embryonic day 20 (E20) were transfected with a vector encoding GFP on the day of culture preparation as described [19]. On day 3 after transfection, cultures were fixed for staining of filamentous actin with Alexa Fluor 633 Phalloidin (Life Technologies) and endogenous Kalirin-7 or Kalirin-12 with isoform-specific rabbit antibodies. Rabbit antibodies were visualized using Cy3 donkey anti-rabbit IgG (The Jackson Laboratory). Images were collected with a Zeiss confocal microscope LSM510.

phosphorylation sites have been identified in the spectrin-like repeat region, many lying on the fourth and fifth repeats. Their phosphorylation state could influence on a wide array of protein-protein interactions that involve this region. Since the first four spectrin-like repeats are absent in ΔKalirin-7 (Figure 1), the phosphorylation sites in the missing region could play a key role in the differences in spine size and density associated with expression of full length Kalirin-7 versus ΔKalirin-7. Overexpression of exogenous Kalirin-7 results in an increase in both spine density and spine size while overexpression of ΔKalirin-7 only increases spine size without altering spine density [17]. Although phosphorylation sites that regulate GEF activity as assessed in intact cells have been identified, a relationship between phosphorylation state and GEF activity has not yet been demonstrated using purified proteins.

6. Kalirin-7 Plays a Key Role in Spine/Synapse Formation *In Vitro*

Kalirin-7 expression is limited to neurons in the CNS [22, 30, 46]. Immunostaining in cultured hippocampal and cortical neurons demonstrates that Kalirin-7 clusters are apposed to glutamate-transporter-1-(Vglut1-) positive clusters, a marker for excitatory presynaptic terminals [19, 47, 48]. When cultured neurons are fixed with cold methanol, it can be seen that the Kalirin-7 positive clusters consistently overlap clusters positive for PSD95, NMDA receptor subunits NR1 and NR2B, or AMPA receptor subunits GluR1 and GluR2; overlap is apparent in both dendritic spines and in the dendritic shaft [19, 40]. In contrast, Kalirin-7 clusters neither align with GAD65-positive clusters, a marker for inhibitory presynaptic terminals, nor with $GABA_A$ receptor positive clusters, a marker for inhibitory postsynaptic endings [19]. These findings lead to the conclusion that Kalirin-7 is localized almost entirely to postsynaptic excitatory terminals.

Regulation of Kalirin-7 expression by synaptic activity in hippocampal neurons suggests that Kalirin-7 plays a pivotal role in the regulation of excitatory synapse formation and signaling [40, 42]. Overexpression of Kalirin-7 causes an increase in dendritic spine density, spine size, and synapse number, while reduction of endogenous Kalirin-7 levels leads to a reduction in spine density, spine size, and synapse number in cultured hippocampal and cortical neurons [19, 22, 39] (Figure 3). Interestingly, overexpression of Kalirin-7 induces spine formation in spine-free hippocampal interneurons [19] and a recent study reports an important role of Kalirin-7 in regulating dendrite growth in cortical interneurons [44]. A delicate balance between synaptic excitation and inhibition is critical for maintaining normal circuits in the CNS, and interneurons play an essential role in regulating local circuit excitability [49]. Understanding Kalirin-7 function in interneurons may increase our understanding of neurological diseases such as epilepsy, bipolar disorder, schizophrenia, autism, and Alzheimer's Disease, which are related to disruption of GABAergic interneuron development [50–53].

7. Kalirin-7 Plays a Key Role in Spine/Synapse Formation *In Vivo*

Two *Kalrn* knockout mouse models have been developed: the Kalirin-7 knockout mouse ($Kalirin\text{-}7^{KO}$) [54] and the GEF1 Kalirin knockout mouse ($Kalirin^{GEF1-KO}$) [55]. $Kalirin\text{-}7^{KO}$ mice, in which the terminal exon unique to Kalirin-7 was deleted, grow and reproduce normally. Hippocampal CA1

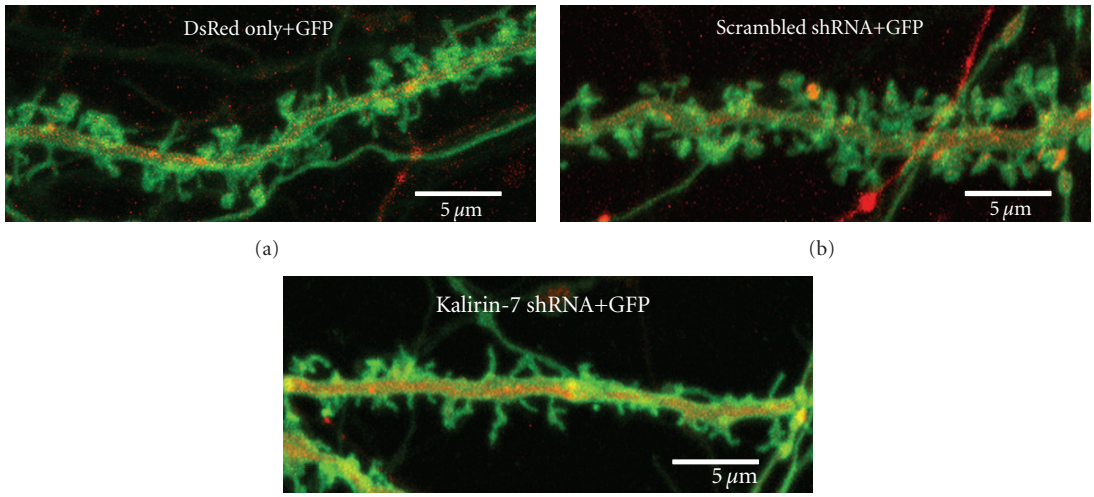


Figure 3: Expression of Kalirin-7 shRNA causes a reduction in spine density and spine size in cultured hippocampal neurons. Cultured hippocampal neurons prepared at E20 were transfected with vector encoding a membrane-tethered version of GFP (pmGFP) alone [(a), spine density 8.9 ± 0.8/10 μm], pmGFP plus a scrambled shRNA [(b), spine density 9.2 ± 0.9/10 μm], or pmGFP plus pSIREN-Kalirin-7 shRNA [(c), spine density 5.2 ± 0.6/10 μm] on the day of culture preparation. The specificity of pSIREN-Kalirin-7 shRNA was determined previously [19]. DsRed marks transfected neurons expressing the shRNA. Images were collected on day 20 with an LSM510 confocal microscope. Expression of Kalirin-7 shRNA, not scrambled shRNA, caused a 80% decrease in Kalirin-7 staining (not shown). Spine density was determined using Metamorph (Molecular Devices, Downington, PA) as described [54]. Scale bar = 5 μm.

pyramidal neurons of Kalirin-7^{KO} mice show a 15% decrease in spine density and deficits in long-term potentiation (LTP). Morphological alterations in Kalirin-7^{KO} mice are accompanied by behavioral alterations including decreased anxiety-like behavior in the elevated zero maze and impaired acquisition of a passive avoidance task. Kalirin-7^{KO} mice exhibit normal behavior in the open field, object recognition, and radial arm maze tasks [54]. PSDs purified from the cortices of Kalirin-7^{KO} mice show a deficit in Cdk5, a kinase known to phosphorylate Kalirin-7 and play an essential role in Kalirin-7-mediated spine formation and synaptic function [43, 54, 56]. Furthermore, NR2B levels are modestly decreased in PSD preparations from the cortices of Kalirin-7^{KO} animals [54]. This decrease is accompanied by decreased levels of NR2B-dependent NMDA receptor currents in cortical pyramidal neurons [41]. NR2B plays a critical role in LTP induction, dendritic spine formation, different forms of synaptic plasticity, learning, and memory [57–60]. Decreased NR2B levels may partially contribute to decreased spine density and the deficit in LTP induction observed in Kalirin-7^{KO} mice. Importantly, expression of exogenous Kalirin-7 in cultured Kalirin-7^{KO} neurons rescues decreased spine density. These findings show that Kalirin-7 plays an essential role in synaptic structure and function.

The Kalirin$^{GEF1-KO}$ mice were generated by replacing exons 27-28 in the first GEF domain by a neomycin resistance cassette, thus eliminating production of the major Kalirin isoforms; in addition to Kalirin-7, these mice are unable to produce Kalirin-9 and Kalirin-12 [55]. Kalirin$^{GEF1-KO}$ mice show significant morphological deficits including reduced size of both cortex and decreased spine density in pyramidal neurons of the cortex [55]. Interestingly, in Kalirin$^{GEF1-KO}$ mice the hippocampus is reduced in size but spine density in hippocampal neurons is normal. This reduction in hippocampal size in Kalirin$^{GEF1-KO}$ mice results from neuronal loss since Kalirin is exclusively expressed in pyramidal neurons, granule cells of the dentate gyrus, and interneurons scattered throughout the hippocampus [19, 22, 46, 54]. Both Kalirin-7^{KO} and Kalirin$^{GEF1-KO}$ mice show impaired hippocampal LTP induction, impaired contextual fear conditioning, and reduced spine density in cultured cortical neurons and normal long-term memory [54, 61]. Kalirin$^{GEF1-KO}$ mice show deficits in working memory and an intact reference memory. However, Kalirin-7^{KO} and Kalirin$^{GEF1-KO}$ mice exhibit distinct differences in their behavioral phenotype. Kalirin$^{GEF1-KO}$ mice show very high locomotor activity in the open field and a deficit in spatial memory, which are not affected in Kalirin-7^{KO} mice [54, 55]. Further comparisons of the phenotypes of the Kalirin$^{GEF1-KO}$ and Kalirin-7^{KO} mice are needed, along with the generation of additional *Kalrn* knockout mouse models generated using different knockout strategies.

8. Kalirin-7 Is Implicated in Cocaine Addiction

Cocaine addiction is a chronic relapsing neurological disorder associated with severe medical and psychosocial complications [62–66]. The long-lasting nature of cocaine addiction leads to relapse and makes it especially difficult to treat [21]. Repeated cocaine treatments increase dendritic spine density/spine head size and neurite complexity in the brain's reward circuitry such as the medium spiny neurons (MSNs) of the nucleus accumbens (NAc). Our study

Table 1: Kalirin and human diseases.

Kalirin isoform	Disease	Physiological relevance	References
Kalirin-7 (Duo)	Schizophrenia	Decreased spine density, decreased Kalirin-7 mRNA levels in the prefrontal cortex	[24]
Kalirin (unknown isoforms)	Schizophrenia	Kalirin mRNA increases 2-fold in the prefrontal cortex	[25]
Kalirin-7	Schizophrenia	DISC1 regulates spine formation via Kal7-Rac1	[10]
Kalirin	Schizophrenia	Kalirin is a risk factor for schizophrenia	[26]
Kalirin-7	Alzheimer's Disease	Decreased levels of Kalirin-7 mRNA and protein in hippocampus	[27, 28]
Kalirin (unknown isoforms)	Animal model of depression	Decreased Kalirin expression in the prefrontal cortex and hippocampus. ECT increases Kalirin expression in hippocampus	[29, 30]
Kalirin-7	Animal model of depression	Decreased Kalirin-7 expression in hippocampus	[31]
Kalirin (unknown isoforms)	Animal model of epilepsy	Decreased Kalirin expression in hippocampus	[32]
Kalirin (unknown isoforms)	ADHD	Unknown	[33]
Kalirin (unknown isoforms)	Stroke	Unknown	[34, 35]
Kalirin (unknown isoforms)	Coronary Heart disease	Unknown	[36]
Kalirin (unknown isoforms)	Huntington's disease	Spectrin-like domains of Kalirin interact with HAP1	[12]
Kalirin-7	Animal model of cocaine addiction	An essential determinant of dendritic spine formation following cocaine treatment	[37, 38]

shows that Kalirin-7 is an essential determinant of dendritic spine formation following cocaine treatment [38]. Kalirin-7 is expressed in the MSNs of the NAc, a key area in the brain involved in drug addiction and reward pathways [37]. Chronic cocaine treatment of wild-type mice results in an increase in Kalirin-7 expression in the NAc which is accompanied by an increase in spine density in the MSNs of NAc core [37, 67]. This cocaine-induced increase in dendritic spine density in the NAc MSNs in wild-type mice is abolished in Kalirin-7^{KO} mice. Both wild-type and Kalirin-7^{KO} mice have identical spine densities in the MSNs of the NAc prior to cocaine treatment. These morphological changes could underlie the behavioral variations seen in these mice following cocaine treatment. Chronic cocaine treatment leads to increased locomotor sensitization in Kalirin-7^{KO} mice compared to wild-type controls [38]. These data suggest Kalirin-7 plays an important role in the mechanism of cocaine addiction, which needs to be addressed in the future.

9. Kalirin Is Implicated in Human Diseases

Altered Kalirin expression has been reported in several neuropsychiatric, neurological and cardiovascular diseases as well as animal models of depression, epilepsy and cocaine addiction (Table 1). Genetic analyses have identified *KALRN* as a major risk factor in stroke and early onset of coronary artery disease [34–36]. Similarly in schizophrenia, decreased dendritic spine density in the prefrontal cortex is reported to correlate with decreased Kalirin mRNA levels [24]. A rare missense mutation in the *KALRN* gene has been shown to be a genetic risk factor for schizophrenia [26]. The spectrin-like repeat region of Kalirin has also been shown to interact with DISC1, a genetic risk factor for schizophrenia which plays an important role in activity-dependent spine elongation by promoting Kalirin-7/Rac-1 interactions [10, 24, 68, 69]. Attention-deficit/hyperactivity disorder (ADHD) is the most common neurobehavioral disorder and the underlying molecular mechanisms of ADHD are largely unknown. Animal models of ADHD are associated with spine loss in striatal MSNs [70] and functional impairments in glutamatergic synaptic transmission in the hippocampus [71]. A genomewide association study of ADHD patients has also implicated alterations in Kalirin expression in ADHD [33]. Dendritic pathology and decreased dendritic spine density are prominent phenomena in early cases of Alzheimer's Disease, which correlate significantly with the progressive decline of mental faculties [72–75]. Alzheimer's Disease patients also show a significant decrease in Kalirin mRNA and protein expression in the hippocampus without significant changes in other brain regions [28]. This decrease in Kalirin expression has been associated with increased iNOS activity both in hippocampus from Alzheimer's patients and in cultured neuroblastoma cells [27]. These data lead to the hypothesis that lack of Kalirin is associated with the dendritic alterations and substantial decrease in spine density observed in Alzheimer's Disease.

The cause underlying major depression and the neurobiological basis of antidepressant therapy are not clear. Altered synaptic plasticity may play a key role in the pathogenesis

and treatment of depression [76]. Major depression is associated with spine reductions in the hippocampus [77]. Kalirin expression and spine density in the hippocampus are decreased in the animal models of depression [29, 31, 78]. Kalirin levels and spine density in the hippocampal CA1 pyramidal neurons increase after repeated electroconvulsive treatment (ECT) [30, 79], one of the most effective therapies for depression [80, 81]. These observations suggest a role for Kalirin in the development of depression. In epileptic patients and in experimental models of epilepsy, there is a marked spine loss, abnormal spine shape, and alteration in dendritic morphology in hippocampal CA1 pyramidal neurons [82, 83]. Animal models of epilepsy show a marked increase in Kalirin expression in the hippocampus [32], suggesting a role for Kalirin in the neuropathology of human epilepsy. Kalirin interacts with Huntingtin-associated protein 1 (HAP1) [12] and HAP1 dysfunction could contribute to the selective neuropathology in Huntingtin's disease [84]. Huntingtin's disease is characterized by loss of medium-sized spiny neurons in the striatum [85], alterations in spine density, and abnormalities in the size and shape of dendritic spines in striatal MSNs [86–88]. Overexpression of Kalirin-7 caused an increase in spine density while reduced expression of Kalirin-7 resulted in loss of dendritic spines and a decrease in dendritic complexity in the MSNs of striatal slice cultures which mimic *in vivo* conditions (Ma et al., unpublished). These data raise the possibility that Kalirin-7 may play a role in the neuropathology of Huntington's disease [87]. Finally, Kalirn-7 plays an important role in estrogen-mediated spine/synapse formation in the hippocampus [31, 40]. Regulation of Kalirin-7 by estrogen suggests a role for Kalirin in ovarian hormone-associated cognitive function and menopause-associated disorders [89–91]. Taken together, altered dendritic spine morphology and spine density remain the hallmarks of many human neurological and psychiatric disorders. There is a correlation between the levels of Kalirin expression and the pathology of dendritic spines in some psychiatric and neurological disorders. It is important to understand whether Kalirin is a trigger or one of many factors mediating the dendritic spine pathology of these diseases, which may be rescued by altering Kalirin expression/function or targeting downstream signals of Kalirin.

10. Conclusion/Future Studies

Kalirin-7, the major isoform of Kalirin in the adult brain, plays a critical role in spine formation/synaptic plasticity. Estrogen-mediated spine formation in hippocampal neurons and cocaine-induced increases in spine density in striatal MSNs require Kalirin-7. Kalirin has also been implicated in many neuropsychiatric and neurological diseases. Future studies will focus on investigating the underlying molecular mechanisms of Kalirin-7-mediated spine formation/synaptic plasticity and its role in neuropsychiatric and neurological diseases.

Acknowledgments

This work was supported by grants DK32948, DA22876, MH086552, DA032973 from NIH and 11SCA28 from Connecticut Innovations. Thanks are due to Drs. Dick Mains and Betty Eipper for their comments.

References

[1] M. Rashidul Alam, B. D. Caldwell, R. G. Johnson, D. N. Darlington, R. E. Mains, and B. A. Eipper, "Novel proteins that interact with the COOH-terminal cytosolic routing determinants of an integral membrane peptide-processing enzyme," *Journal of Biological Chemistry*, vol. 271, no. 45, pp. 28636–28640, 1996.

[2] M. R. Alam, R. C. Johnson, D. N. Darlington, T. A. Hand, R. E. Mains, and B. A. Eipper, "Kalirin, a cytosolic protein with spectrin-like and GDP/GTP exchange factor-like domains that interacts with peptidylglycine α-amidating monooxygenase, an integral membrane peptide-processing enzyme," *Journal of Biological Chemistry*, vol. 272, no. 19, pp. 12667–12675, 1997.

[3] K. L. Rossman, C. J. Der, and J. Sondek, "GEF means go: turning on Rho GTPases with guanine nucleotide-exchange factors," *Nature Reviews Molecular Cell Biology*, vol. 6, no. 2, pp. 167–180, 2005.

[4] M. Negishi and H. Katoh, "Rho family GTPases and dendrite plasticity," *Neuroscientist*, vol. 11, no. 3, pp. 187–191, 2005.

[5] K. F. Tolias, J. G. Duman, and K. Um, "Control of synapse development and plasticity by Rho GTPase regulatory proteins," *Progress in Neurobiology*, vol. 94, no. 2, pp. 133–148, 2011.

[6] P. Penzes, K. M. Woolfrey, and D. P. Srivastava, "Epac2-mediated dendritic spine remodeling: implications for disease," *Molecular and Cellular Neuroscience*, vol. 46, no. 2, pp. 368–380, 2011.

[7] R. C. Johnson, P. Penzes, B. A. Eipper, and R. E. Mains, "Isoforms of Kalirin, a neuronal Dbl family member, generated through use of different 5′- and 3′-ends along with an internal translational initiation site," *Journal of Biological Chemistry*, vol. 275, no. 25, pp. 19324–19333, 2000.

[8] C. E. McPherson, B. A. Eipper, and R. E. Mains, "Genomic organization and differential expression of Kalirin isoforms," *Gene*, vol. 284, no. 1-2, pp. 41–51, 2002.

[9] C. E. McPherson, B. A. Eipper, and R. E. Mains, "Kalirin expression is regulated by multiple promoters," *Journal of Molecular Neuroscience*, vol. 22, no. 1-2, pp. 51–62, 2004.

[10] A. Hayashi-Takagi, M. Takaki, N. Graziane et al., "Disrupted-in-Schizophrenia 1 (DISC1) regulates spines of the glutamate synapse via Rac1," *Nature Neuroscience*, vol. 13, no. 3, pp. 327–332, 2010.

[11] E. A. Ratovitski, M. R. Alam, R. A. Quick et al., "Kalirin inhibition of inducible nitric-oxide synthase," *Journal of Biological Chemistry*, vol. 274, no. 2, pp. 993–999, 1999.

[12] V. Colomer, S. Engelender, A. M. Sharp et al., "Huntingtin-associated protein 1 (HAP1) binds to a Trio-like polypeptide, with a rac1 guanine nucleotide exchange factor domain," *Human Molecular Genetics*, vol. 6, no. 9, pp. 1519–1525, 1997.

[13] T. H. Koo, B. A. Eipper, and J. G. Donaldson, "Arf6 recruits the Rac GEF Kalirin to the plasma membrane facilitating Rac activation," *BMC Cell Biology*, vol. 8, article no. 29, 2007.

[14] C. A. Rabiner, R. E. Mains, and B. A. Eipper, "Kalirin: a dual Rho guanine nucleotide exchange factor that is so much more than the sum of its many parts," *Neuroscientist*, vol. 11, no. 2, pp. 148–160, 2005.

[15] X. Xin, C. A. Rabiner, R. E. Mains, and B. A. Eipper, "Kalirin12 interacts with dynamin," *BMC Neuroscience*, vol. 10, article no. 61, 2009.

[16] P. Penzes, R. C. Johnson, V. Kambampati, R. E. Mains, and B. A. Eipper, "Distinct roles for the two Rho GDP/GTP exchange factor domains of Kalirin in regulation of neurite growth and neuronal morphology," *Journal of Neuroscience*, vol. 21, no. 21, pp. 8426–8434, 2001.

[17] M. R. Schiller, A. Blangy, J. Huang, R. E. Mains, and B. A. Eipper, "Induction of lamellipodia by Kalirin does not require its guanine nucleotide exchange factor activity," *Experimental Cell Research*, vol. 307, no. 2, pp. 402–417, 2005.

[18] T. Kawai, H. Sanjo, and S. Akira, "Duet is a novel serine/threonine kinase with Dbl-Homology (DH) and Pleckstrin-Homology (PH) domains," *Gene*, vol. 227, no. 2, pp. 249–255, 1999.

[19] X. M. Ma, Y. Wang, F. Ferraro, R. E. Mains, and B. A. Eipper, "Kalirin-7 is an essential component of both shaft and spine excitatory synapses in hippocampal interneurons," *Journal of Neuroscience*, vol. 28, no. 3, pp. 711–724, 2008.

[20] D. E. Hansel, M. E. Quiñones, G. V. Ronnett, and B. A. Eipper, "Kalirin, a GDP/GTP exchange factor of the Dbl family, is localized to nerve, muscle, and endocrine tissue during embryonic rat development," *Journal of Histochemistry and Cytochemistry*, vol. 49, no. 7, pp. 833–844, 2001.

[21] F. Ferraro, X. M. Ma, J. A. Sobota, B. A. Eipper, and R. E. Mains, "Kalirin/Trio Rho guanine nucleotide exchange factors regulate a novel step in secretory granule maturation," *Molecular Biology of the Cell*, vol. 18, no. 12, pp. 4813–4825, 2007.

[22] X. M. Ma, J. Huang, Y. Wang, B. A. Eipper, and R. E. Mains, "Kalirin, a multifunctional rho guanine nucleotide exchange factor, is necessary for maintenance of hippocampal pyramidal neuron dendrites and dendritic spines," *Journal of Neuroscience*, vol. 23, no. 33, pp. 10593–10603, 2003.

[23] D. Muller, P. A. Buchs, and L. Stoppini, "Time course of synaptic development in hippocampal organotypic cultures," *Developmental Brain Research*, vol. 71, no. 1, pp. 93–100, 1993.

[24] J. J. Hill, T. Hashimoto, and D. A. Lewis, "Molecular mechanisms contributing to dendritic spine alterations in the prefrontal cortex of subjects with schizophrenia," *Molecular Psychiatry*, vol. 11, no. 6, pp. 557–566, 2006.

[25] S. Narayan, B. Tang, S. R. Head et al., "Molecular profiles of schizophrenia in the CNS at different stages of illness," *Brain Research*, vol. 1239, pp. 235–248, 2008.

[26] I. Kushima, Y. Nakamura, B. Aleksic et al., "Resequencing and association analysis of the KALRN and EPHB1 genes and their contribution to Schizophrenia susceptibility," *Schizophrenia Bulletin*. In press.

[27] H. Youn, I. Ji, H. P. Ji, W. R. Markesbery, and T. H. Ji, "Under-expression of Kalirin-7 increases iNOS activity in cultured cells and correlates to elevated iNOS activity in Alzheimer's disease hippocampus," *Journal of Alzheimer's Disease*, vol. 12, no. 3, pp. 271–281, 2007.

[28] H. Youn, M. Jeoung, Y. Koo et al., "Kalirin is under-expressed in Alzheimer's disease hippocampus," *Journal of Alzheimer's Disease*, vol. 11, no. 3, pp. 385–397, 2007.

[29] A. Alttoa, K. Kõiv, T. A. Hinsley, A. Brass, and J. Harro, "Differential gene expression in a rat model of depression based on persistent differences in exploratory activity," *European Neuropsychopharmacology*, vol. 20, no. 5, pp. 288–300, 2010.

[30] X. M. Ma, R. E. Mains, and B. A. Eipper, "Plasticity in hippocampal peptidergic systems induced by repeated electroconvulsive shock," *Neuropsychopharmacology*, vol. 27, no. 1, pp. 55–71, 2002.

[31] W. Li, Q. J. Li, and S. C. An, "Preventive effect of estrogen on depression-like behavior induced by chronic restraint stress," *Neuroscience Bulletin*, vol. 26, no. 2, pp. 140–146, 2010.

[32] A. K. Sharma, G. H. Searfoss, R. Y. Reams et al., "Kainic acid-induced f-344 rat model of Mesial temporal lobe epilepsy: gene expression and canonical Pathways," *Toxicologic Pathology*, vol. 37, no. 6, pp. 776–789, 2009.

[33] K.-P. Lesch, N. Timmesfeld, T. J. Renner et al., "Molecular genetics of adult ADHD: converging evidence from genome-wide association and extended pedigree linkage studies," *Journal of Neural Transmission*, vol. 115, no. 11, pp. 1573–1585, 2008.

[34] M. Beręsewicz, J. E. Kowalczyk, and B. Zabłocka, "Kalirin-7, a protein enriched in postsynaptic density, is involved in ischemic signal transduction," *Neurochemical Research*, vol. 33, no. 9, pp. 1789–1794, 2008.

[35] T. Krug, H. Manso, L. Gouveia et al., "Kalirin: a novel genetic risk factor for ischemic stroke," *Human Genetics*, vol. 127, no. 5, pp. 513–523, 2010.

[36] L. Wang, E. R. Hauser, S. H. Shah et al., "Peakwide mapping on chromosome 3q13 identifies the kalirin gene as a novel candidate gene for coronary artery disease," *American Journal of Human Genetics*, vol. 80, no. 4, pp. 650–663, 2007.

[37] R. E. Mains, D. D. Kiraly, J. E. Eipper-Mains, X. Ma, and B. A. Eipper, "Kalrn promoter usage and isoform expression respond to chronic cocaine exposure," *BMC Neuroscience*, vol. 12, article 20, 2011.

[38] D. D. Kiraly, X. M. Ma, C. M. Mazzone, X. Xin, R. E. Mains, and B. A. Eipper, "Behavioral and morphological responses to cocaine require Kalirin7," *Biological Psychiatry*, vol. 68, no. 3, pp. 249–255, 2010.

[39] P. Penzes, R. C. Johnson, R. Sattler et al., "The neuronal Rho-GEF Kalirin-7 interacts with PDZ domain-containing proteins and regulates dendritic morphogenesis," *Neuron*, vol. 29, no. 1, pp. 229–242, 2001.

[40] X.-M. Ma, J.-P. Huang, E.-J. Kim et al., "Kalirin-7, an important component of excitatory synapses, is regulated by estradiol in hippocampal neurons," *Hippocampus*, vol. 21, no. 6, pp. 661–677, 2011.

[41] D. D. Kiraly, F. Lemtiri-Chlieh, E. S. Levine, R. E. Mains, and B. A. Eipper, "Kalirin binds the NR2B subunit of the NMDA receptor, altering its synaptic localization and function," *Journal of Neuroscience*, vol. 31, no. 35, pp. 12554–12565, 2011.

[42] Z. Xie, D. P. Srivastava, H. Photowala et al., "Kalirin-7 controls activity-dependent structural and functional plasticity of dendritic spines," *Neuron*, vol. 56, no. 4, pp. 640–656, 2007.

[43] X. Xin, Y. Wang, X. M. Ma et al., "Regulation of Kalirin by Cdk5," *Journal of Cell Science*, vol. 121, no. 15, pp. 2601–2611, 2008.

[44] M. E. Cahill, K. A. Jones, I. Rafalovich et al., "Control of interneuron dendritic growth through NRG1/erbB4-mediated kalirin-7 disinhibition," *Molecular Psychiatry*, vol. 17, no. 1, pp. 99–107, 2012.

[45] D. D. Kiraly, K. L. Stone, C. M. Colangelo et al., "Identification of kalirin-7 as a potential post-synaptic density signaling hub," *Journal of Proteome Research*, vol. 10, no. 6, pp. 2828–2841, 2011.

[46] X. M. Ma, R. C. Johnson, R. E. Mains, and B. A. Eipper, "Expression of Kalirin, a neuronal GDP/GTP exchange factor of the trio family, in the central nervous system of the adult rat," *Journal of Comparative Neurology*, vol. 429, no. 3, pp. 388–402, 2001.

[47] R. T. Fremeau, M. D. Troyer, I. Pahner et al., "The expression of vesicular glutamate transporters defines two classes of excitatory synapse," *Neuron*, vol. 31, no. 2, pp. 247–260, 2001.

[48] R. T. Fremeau, K. Kam, Y. Qureshi et al., "Vesicular glutamate transporters 1 and 2 target to functionally distinct synaptic release sites," *Science*, vol. 304, no. 5678, pp. 1815–1819, 2004.

[49] G. Maccaferri and J. C. Lacaille, "Interneuron Diversity series: hippocampal interneuron classifications—making things as simple as possible, not simpler," *Trends in Neurosciences*, vol. 26, no. 10, pp. 564–571, 2003.

[50] F. M. Benes and S. Berretta, "GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder," *Neuropsychopharmacology*, vol. 25, no. 1, pp. 1–27, 2001.

[51] E. M. Powell, D. B. Campbell, G. D. Stanwood, C. Davis, J. L. Noebels, and P. Levitt, "Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy, and behavioral dysfunction," *Journal of Neuroscience*, vol. 23, no. 2, pp. 622–631, 2003.

[52] D. A. Lewis, T. Hashimoto, and D. W. Volk, "Cortical inhibitory neurons and schizophrenia," *Nature Reviews Neuroscience*, vol. 6, no. 4, pp. 312–324, 2005.

[53] B. Ramos, D. Baglietto-Vargas, J. C. D. Rio et al., "Early neuropathology of somatostatin/NPY GABAergic cells in the hippocampus of a PS1 × APP transgenic model of Alzheimer's disease," *Neurobiology of Aging*, vol. 27, no. 11, pp. 1658–1672, 2006.

[54] X. M. Ma, D. D. Kiraly, E. D. Gaier et al., "Kalirin-7 is required for synaptic structure and function," *Journal of Neuroscience*, vol. 28, no. 47, pp. 12368–12382, 2008.

[55] M. E. Cahill, Z. Xie, M. Day et al., "Kalirin regulates cortical spine morphogenesis and disease-related behavioral phenotypes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 31, pp. 13058–13063, 2009.

[56] K. O. Lai and N. Y. Ip, "Recent advances in understanding the roles of Cdk5 in synaptic plasticity," *Biochimica et Biophysica Acta*, vol. 1792, no. 8, pp. 741–745, 2009.

[57] K. Akashi, T. Kakizaki, H. Kamiya et al., "NMDA receptor GluN2B (GluRε2/NR2B) subunit is crucial for channel function, postsynaptic macromolecular organization, and actin cytoskeleton at hippocampal CA3 synapses," *Journal of Neuroscience*, vol. 29, no. 35, pp. 10869–10882, 2009.

[58] J. L. Brigman, T. Wright, G. Talani et al., "Loss of GluN2B-containing NMD A receptors in CA1 hippocampus and cortex impairs long-term depression, reduces dendritic spine density, and disrupts learning," *Journal of Neuroscience*, vol. 30, no. 13, pp. 4590–4600, 2010.

[59] T. Ohno, H. Maeda, N. Murabe et al., "Specific involvement of postsynaptic GluN2B-containing NMDA receptors in the developmental elimination of corticospinal synapses," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 34, pp. 15252–15257, 2010.

[60] A. C. Gambrill and A. Barria, "NMDA receptor subunit composition controls synaptogenesis and synapse stabilization," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 14, pp. 5855–5860, 2011.

[61] Z. Xie, M. E. Cahill, J. Radulovic et al., "Hippocampal phenotypes in kalirin-deficient mice," *Molecular and Cellular Neuroscience*, vol. 46, no. 1, pp. 45–54, 2011.

[62] J. Martínez-Raga, C. Knecht, and S. Cepeda, "Modafinil: a useful medication for cocaine addiction? Review of the evidence from neuropharmacological, experimental and clinical studies," *Current drug abuse reviews*, vol. 1, no. 2, pp. 213–221, 2008.

[63] M. J. Smith, J. Thirthalli, A. B. Abdallah, R. M. Murray, and L. B. Cottler, "Prevalence of psychotic symptoms in substance users: a comparison across substances," *Comprehensive Psychiatry*, vol. 50, no. 3, pp. 245–250, 2009.

[64] M. D. Majewska, "Cocaine addiction as a neurological disorder: implications for treatment," *NIDA research monograph*, vol. 163, pp. 1–26, 1996.

[65] J. H. Mendelson and N. K. Mello, "Drug therapy: management of cocaine abuse and dependence," *New England Journal of Medicine*, vol. 334, no. 15, pp. 965–972, 1996.

[66] A. Büttner, "Review: the neuropathology of drug abuse," *Neuropathology and Applied Neurobiology*, vol. 37, no. 2, pp. 118–134, 2011.

[67] D. D. Kiraly, X. M. Ma, C. M. Mazzone, X. Xin, R. E. Mains, and B. A. Eipper, "Behavioral and morphological responses to cocaine require Kalirin7," *Biological Psychiatry*, vol. 68, no. 3, pp. 249–255, 2010.

[68] J. K. Millar, S. Christie, and D. J. Porteous, "Yeast two-hybrid screens implicate DISC1 in brain development and function," *Biochemical and Biophysical Research Communications*, vol. 311, no. 4, pp. 1019–1025, 2003.

[69] N. J. Bradshaw and D. J. Porteous, "DISC1-binding proteins in neural development, signalling and schizophrenia," *Neuropharmacology*, vol. 62, no. 3, pp. 1230–1241, 2012.

[70] M. L. Berlanga, D. L. Price, B. S. Phung et al., "Multiscale imaging characterization of dopamine transporter knockout mice reveals regional alterations in spine density of medium spiny neurons," *Brain Research*, vol. 1390, pp. 41–49, 2011.

[71] V. Jensen, J. E. Rinholm, T. J. Johansen et al., "N-methyl-d-aspartate receptor subunit dysfunction at hippocampal glutamatergic synapses in an animal model of attention-deficit/hyperactivity disorder," *Neuroscience*, vol. 158, no. 1, pp. 353–364, 2009.

[72] C. Tackenberg, A. Ghori, and R. Brandt, "Thin, stubby or mushroom: spine pathology in alzheimer's disease," *Current Alzheimer Research*, vol. 6, no. 3, pp. 261–268, 2009.

[73] S. J. Baloyannis, "Dendritic pathology in Alzheimer's disease," *Journal of the Neurological Sciences*, vol. 283, no. 1-2, pp. 153–157, 2009.

[74] I. A. Mavroudis, D. F. Fotiou, M. G. Manani et al., "Dendritic pathology and spinal loss in the visual cortex in Alzheimer's disease: a Golgi study in pathology," *International Journal of Neuroscience*, vol. 121, no. 7, pp. 347–354, 2011.

[75] C. Perez-Cruz, M. W. Nolte, M. M. Van Gaalen et al., "Reduced spine density in specific regions of CA1 pyramidal neurons in two transgenic mouse models of Alzheimer's disease," *Journal of Neuroscience*, vol. 31, no. 10, pp. 3926–3934, 2011.

[76] O. von Bohlen und Halbach, "Structure and function of dendritic spines within the hippocampus," *Annals of Anatomy*, vol. 191, no. 6, pp. 518–531, 2009.

[77] A. J. Law, C. S. Weickert, T. M. Hyde, J. E. Kleinman, and P. J. Harrison, "Reduced spinophilin but not microtubule-associated protein 2 expression in the hippocampal formation in schizophrenia and mood disorders: molecular evidence for a pathology of dendritic spines," *American Journal of Psychiatry*, vol. 161, no. 10, pp. 1848–1855, 2004.

[78] S. D. Norrholm and C. C. Ouimet, "Altered dendritic spine density in animal models of depression and in response to antidepressant treatment," *Synapse*, vol. 42, no. 3, pp. 151–163, 2001.

[79] F. Chen, T. M. Madsen, G. Wegener, and J. R. Nyengaard, "Repeated electroconvulsive seizures increase the total number of synapses in adult male rat hippocampus," *European Neuropsychopharmacology*, vol. 19, no. 5, pp. 329–338, 2009.

[80] M. Fink, "Convulsive therapy: a review of the first 55 years," *Journal of Affective Disorders*, vol. 63, no. 1–3, pp. 1–15, 2001.

[81] S. M. McClintock, A. R. Brandon, M. M. Husain, and R. B. Jarrett, "A systematic review of the combined use of electroconvulsive therapy and psychotherapy for depression," *The Journal of ECT*, vol. 27, pp. 236–243, 2011.

[82] J. C. Fiala, J. Spacek, and K. M. Harris, "Dendritic spine pathology: cause or consequence of neurological disorders?" *Brain Research Reviews*, vol. 39, no. 1, pp. 29–54, 2002.

[83] C. Sgobio, V. Ghiglieri, C. Costa et al., "Hippocampal synaptic plasticity, memory, and epilepsy: effects of long-term valproic acid treatment," *Biological Psychiatry*, vol. 67, no. 6, pp. 567–574, 2010.

[84] L. L. Y. Wu and X. F. Zhou, "Huntingtin associated protein 1 and its functions," *Cell Adhesion and Migration*, vol. 3, no. 1, pp. 71–76, 2009.

[85] J.-P. Vonsattel, R. H. Myers, and T. J. Stevens, "Neuropathological classification of Huntington's disease," *Journal of Neuropathology and Experimental Neurology*, vol. 44, no. 6, pp. 559–577, 1985.

[86] G. A. Graveland, R. S. Williams, and M. DiFiglia, "Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease," *Science*, vol. 227, no. 4688, pp. 770–773, 1985.

[87] R. J. Ferrante, N. W. Kowall, and E. P. Richardson, "Proliferative and degenerative changes in striatal spiny neurons in Huntington's disease: a combined study using the section-Golgi method and calbindin D28k immunocytochemistry," *Journal of Neuroscience*, vol. 11, no. 12, pp. 3877–3887, 1991.

[88] T. L. Spires, H. E. Grote, S. Garry et al., "Dendritic spine pathology and deficits in experience-dependent dendritic plasticity in R6/1 Huntington's disease transgenic mice," *European Journal of Neuroscience*, vol. 19, no. 10, pp. 2799–2807, 2004.

[89] M. I. Boulware, B. A. Kent, and K. M. Frick, "The impact of age-related ovarian hormone loss on cognitive and neural function," *Current Topics in Behavioral Neurosciences*. In press.

[90] E. Scott, Q.-G. Zhang, R. Wang, R. Vadlamudi, and D. Brann, "Estrogen neuroprotection and the critical period hypothesis," *Frontiers in Neuroendocrinology*, vol. 33, no. 1, pp. 85–104, 2012.

[91] A. Riecher-Rössler and J. Kulkarni, "Estrogens and gonadal function in schizophrenia and related psychoses," *Current topics in behavioral neurosciences*, vol. 8, pp. 155–171, 2011.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2012, Article ID 578057, 9 pages
doi:10.1155/2012/578057

Research Article

Divergent Roles of p75^{NTR} and Trk Receptors in BDNF's Effects on Dendritic Spine Density and Morphology

Christopher A. Chapleau and Lucas Pozzo-Miller

Department of Neurobiology, SHEL-1002, Civitan International Research Center, The University of Alabama at Birmingham, 1825 University Boulevard, Birmingham, AL 35294-2182, USA

Correspondence should be addressed to Lucas Pozzo-Miller, lucaspm@uab.edu

Received 29 November 2011; Revised 13 January 2012; Accepted 13 January 2012

Academic Editor: Irina Nikonenko

Activation of TrkB receptors by brain-derived neurotrophic factor (BDNF) followed by MAPK/ERK signaling increases dendritic spine density and the proportion of mature spines in hippocampal CA1 pyramidal neurons. Considering the opposing actions of p75^{NTR} and Trk receptors in several BDNF actions on CNS neurons, we tested whether these receptors also have divergent actions on dendritic spine density and morphology. A function-blocking anti-p75^{NTR} antibody (REX) did not affect spine density by itself but it prevented BDNF's effect on spine density. Intriguingly, REX by itself increased the proportion of immature spines and prevented BDNF's effect on spine morphology. In contrast, the Trk receptor inhibitor k-252a increased spine density by itself, and prevented BDNF from further increasing spine density. However, most of the spines in k-252a-treated slices were of the immature type. These effects of k-252a on spine density and morphology required neuronal activity because they were prevented by TTX. These divergent BDNF actions on spine density and morphology are reminiscent of opposing functional signaling by p75^{NTR} and Trk receptors and reveal an unexpected level of complexity in the consequences of BDNF signaling on dendritic morphology.

1. Introduction

The mammalian neurotrophins, a family of growth factors that include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin 4/5 (NT-4/5), have essential roles in neuronal survival and differentiation [1, 2]. In addition to these classical functions, BDNF in particular has been shown to be one of the most potent modulators of synaptic transmission and plasticity, as well as neuronal and synaptic morphology [3–5]. Each neurotrophin exerts its actions through binding and activation of specific, membrane-bound tropomyosin-related kinase (Trk) receptors or a single pan-neurotrophin receptor, the so-called p75^{NTR} [6]. Individual Trk receptors have high affinity for specific neurotrophins: TrkA for NGF, TrkB for BDNF and NT-4, and TrkC for NT-3; on the other hand, all neurotrophins bind to p75^{NTR} with equal affinity and no apparent selectivity [7].

Neurotrophin binding to the aforementioned receptors, in addition to interactions between p75^{NTR} and Trk receptors, organizes complex signaling cascades that control various neuronal actions such as survival, differentiation, neurite and axonal outgrowth, and synaptic function during nervous system development [8–12]. Current work to examine neurotrophin receptors has added an intriguing level of complexity, specifically the opposing functional actions of p75^{NTR} and Trk receptors. Opposing receptor actions have been implicated in several neurotrophin functions, such as neuronal survival (Trk activates prosurvival signals, while p75^{NTR} leads to cell death), axonal outgrowth (Trk is a promoting signal, while p75^{NTR} inhibits axonal growth), and hippocampal synaptic plasticity (TrkB is necessary for long-term potentiation, LTP, while p75^{NTR} receptors are required for long-term depression, LTD) (reviewed by [13]). With respect to dendritic development, TrkB activation enhances dendritic growth [14, 15], while p75^{NTR} negatively regulates dendritic complexity in hippocampal neurons from adult mice [16].

Studies comparing the level of function of TrkB and p75^{NTR} during postnatal spinogenesis has not been extensively examined presumably because of the developmental

deficits that exist in TrkB knockout mice [17]. Reports demonstrate that $p75^{NTR}$ knockout mice display an increase in spine density and a significant reduction in the proportion of stubby spines in CA1 pyramidal neurons from hippocampal slice cultures [16]. While postnatal TrkB knockout mice (P13-14) demonstrate a reduction in synapse number in the hippocampus [18, 19], it should be noted that these results might be a consequence contributed to increased neuronal death also observed in this region [20]. Therefore, it remains to be determined if a functional antagonism exists between $p75^{NTR}$ and Trk receptors in regards to BDNF-induced changes in spine density and form.

2. Material and Methods

2.1. Organotypic Slice Cultures. Hippocampal slice cultures were prepared from postnatal-day 7 to 10 (P7–P10) Sprague-Dawley rats and maintained *in vitro* as previously described [21, 22]. Briefly, rats were quickly decapitated and their brains aseptically dissected and immersed in ice-cold dissecting solution, consisting of Hanks' Balanced Salt Solution (HBSS), supplemented with glucose (36 mM) and antibiotics/antimycotics (1 : 100; penicillin/streptomycin/amphotericin B). Hippocampi were then dissected and transversely sectioned into ~500 μm slices using a custom-made tissue slicer [23] strung with 20 μm-thick tungsten wire (California Fine Wire Company; Grover Beach, CA). Slices were incubated at 4°C for ~30 min and then plated on tissue culture inserts (0.4 μm pore size, Millicell-CM, Millipore Corporation; Billerica, MA). Culture media contained minimum essential media (MEM; 50%), HBSS (25%), heat-inactivated equine serum (20%), L-glutamine (1 mM), and D-glucose (36 mM). Slices were maintained in incubators set at 36°C, 5% CO_2, and 98% relative humidity (Thermo-Forma, Waltham, MA). Culture medium was first changed at 4 days *in vitro* (div) and every 2 days afterwards.

2.2. Particle-Mediated Gene Transfer. After 7 days *in vitro*, slices were transfected as previously described [24]. Briefly, plasmid cDNA for enhanced yellow fluorescent protein (eYFP; Clontech; Mountain View, CA) was introduced by biolistic transfection using a Helios gene gun (Bio-Rad; Hercules, CA). Plasmid cDNA was precipitated onto 1.6 μm-diameter colloidal gold at a ratio of 2 μg DNA/1 mg gold and then coated onto Tefzel tubing. Slices on tissue culture inserts were bombarded with gold particles accelerated by ~100 psi He from a distance of 2 cm using a modified gene gun nozzle. Prior to transfection, an antibiotic/antimycotic mixture (1 : 100; penicillin/streptomycin/amphotericin B) was added to culture media to prevent contamination during biolistic transfection. The antibiotic/antimycotic mixture was only used during biolistic transfection and was removed after 24 hrs to avoid the consequences of network desinhibition from their known actions on $GABA_A$ receptor channels [25].

2.3. Treatment Conditions. Slices were kept in a serum containing media throughout the course of the experiments and were randomly assigned to the following groups: (1) control, serum-containing media; (2) BDNF (250 ng/mL); (3) anti-$p75^{NTR}$ antibody REX (50 μg/mL), known to block $p75^{NTR}$ function [26] (provided by L. Reichardt, UCSF); (4) BDNF in the presence of REX antibody; (5) k-252a (200 nM, in DMSO; Calbiochem; San Diego, CA) to block autophosphorylation and activation of tyrosine kinase domains of plasma membrane neurotrophin receptors [27]; (6) BDNF in the presence of k-252a; (7) TTX (1 μM; Alomone Labs; Jerusalem, Israel); (8) TTX in the presence of k-252a. The DMSO concentration never exceeded 0.01%, which did not affect any of the parameters under study. In experiments where BDNF was added in the presence of k-252a or REX, these compounds were added 30 min before application of BDNF. A droplet (50 μL) of medium was gently applied onto each slice to facilitate penetration, followed by full medium exchange (1 mL per tissue culture well). In each slice culture preparation (which came from the same litter of P7 rat pups), slices were randomly assigned to 1 of the 8 experimental groups (including controls). The 8 different experimental treatments (including controls) were applied to at least 2 different culture plates from at least 2 different culture preparations from 2 different litters of P7 rat pups (sometimes weeks apart). Each culture preparation had its own control group, and we have at least 3 different sets of control cultures coming from 3 different culture preparations from 3 different litters of P7 rat pups. All treatments lasted 48 hrs, beginning 48 hrs after biolistic transfection, and slices were coded for subsequent blind quantitative analyses of dendritic spine density and morphology by an investigator unaware of treatment groups.

2.4. Laser Scanning Confocal Microscopy. After 48 hrs in each of the treatment conditions, slices were fixed by immersion in 4% paraformaldehyde in 100 mM phosphate buffer (overnight at 4°C) and washed in phosphate buffer saline (PBS). Filter membranes around each slice were trimmed, and each slice was individually mounted on glass slides and coverslipped using Vectashield (Vector Laboratories; Burlingame, CA). Transfected pyramidal neurons located in the CA1 region displaying eYFP fluorescence throughout the entire dendritic tree and lacking signs of degeneration (e.g., dendritic blebbing) were selected for confocal imaging. High-resolution images of secondary and tertiary branches of apical dendrites were acquired with a Fluoview FV-300 laser scanning confocal microscope (Olympus; Center Valley, PA) using an oil immersion 100x (NA 1.4) objective lens (PlanApo). eYFP was excited using the 488 nm line of an Argon laser and detected using standard FITC filters. Series of optical sections in the z-axis were acquired at 0.1 μm intervals through each dendritic branch.

2.5. Analysis of Spine Density. Dendritic spines of CA1 pyramidal neurons were identified as small protrusions that extended $\leq 3\,\mu$m from the parent dendrite and counted offline in maximum-intensity projections of the z-stacks using ImageJ software (National Institutes of Health), as described [28]. Protrusions longer than 3 μm were rarely observed in CA1 pyramidal neurons in slice cultures at this developmental age (P7–P10 harvesting, 11 days *in vitro*) and since likely represent dendritic filopodia, they were not

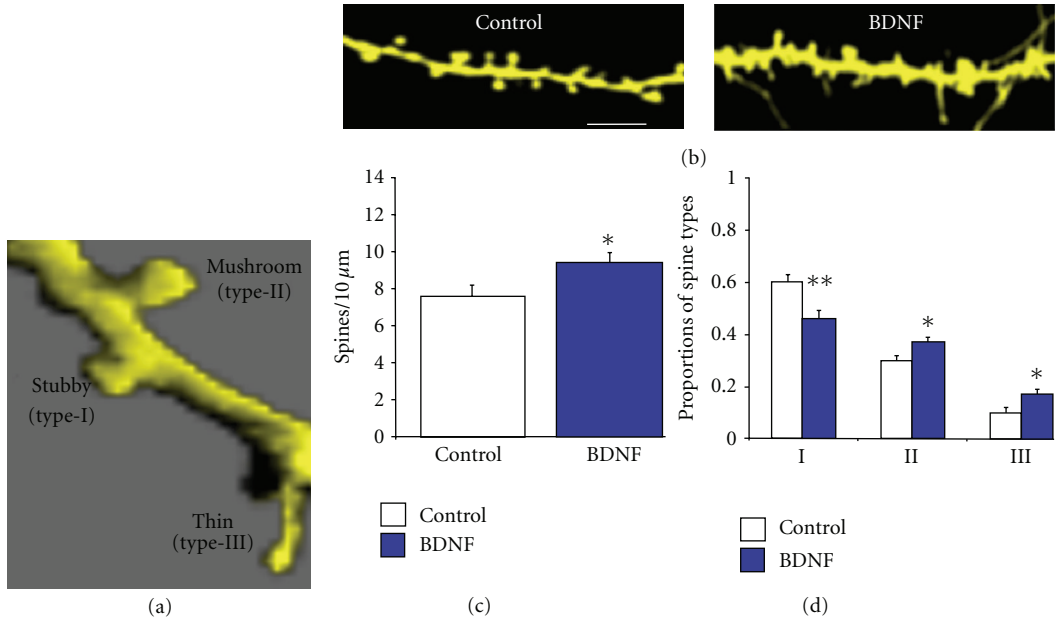


Figure 1: BDNF increases dendritic spine density and affects the proportion of morphological spine types. (a) Dendritic segment of a CA1 pyramidal neuron that was volume-rendered to illustrate individual spine geometrical dimensions and examples of different spine types. (b) Representative examples of dendritic segments of CA1 pyramidal neurons maintained in serum-containing media (SM) and treated with BDNF (250 ng/mL) for 48 hrs (scale bar represents 2 μm). (c) Dendritic spine density expressed per 10 μm of apical dendrite. (d) Proportion of each morphological type of dendritic spine, expressed as a fraction of the total spine population. $^{*}P < 0.05$ and $^{**}P < 0.01$, after an unpaired Student's t-test.

considered in the following analyses. Care was taken to ensure that each spine was counted only once by following its projection course through the stack of z-sections. Spines were counted only if they appeared continuous with the parent dendrite. Spine density was calculated by quantifying the number of spines per dendritic segment and normalized to 10 μm of dendrite length. Microscope calibrations were performed using 1.07 μm fluorescent microspheres (Polysciences Inc.; Warrington, PA), which yielded a lateral resolution of 10.8 pixels per μm (i.e., 92 nm per pixel).

2.6. Measurements of Spine Dimensions for Spine-Type Classification. The categorization of different morphological spine types was performed as described [29]. Briefly, spines were classified into three classical subjective categories [30, 31], but based on objective geometric measurements of their dimensions. Spines were classified as stubby (type I), mushroom (type II), or thin (type III) types based on the L/N and H/N ratios, where L is spine length, H is the maximum head width, and N is the maximum neck width [32, 33]. Following these criteria, stubby spines have a length that is similar to the diameter of the neck and is similar to the diameter of the head ($L \approx N \approx H$), mushroom spines have a greater H/N ratio ($H > N$), and the length of thin spines is much greater than their neck diameters ($L \gg N$) (Figure 1(a)). The majority (~65%) of these dendritic spines have presynaptic partners as assessed by synaptobrevin staining [28], and despite morphological differences, all the three spine types make synaptic contacts *in vitro* [34]. Spine dimensions were measured in maximum-intensity projections of the z-stacks using ImageJ by an investigator unaware of treatment groups.

2.7. Statistical Analyses. Data were analyzed statistically using unpaired Student's t-test or analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons using Prism (GraphPad; San Diego, CA). $P < 0.05$ was considered significant. Data are presented as mean ± standard error of the mean (SEM).

3. Results

Organotypic cultures from P7–10 rat hippocampal slices were biolistically transfected with eYFP and fixed 96 hrs after transfection. Confocal images of secondary and tertiary apical dendrites of CA1 pyramidal neurons were collected (Figures 1(b), 2(a), 2(d), and 3(a)), and the density and dimensions of individual dendritic spines were measured as previously described [21]. Table 1 has the results of the quantitative analyses of spine density and morphology, and Table 2 has the number of slices, neurons, and spines counted and measured in each treatment group, as well as the total dendritic length analyzed. Because serum removal for 48 hrs reduced the expression of TrkB and $p75^{NTR}$ receptors in cultured slices [21], and BDNF (250 ng/mL) increased spine density in the presence of serum (Control = 7.60 ± 0.57 spines/10 μm versus BDNF = 9.39 ± 0.56 spines/10 μm, 10 cells from 7 slices; $P < 0.05$; Figure 1(c)) [21], for the current studies we used serum-containing media. In addition, BDNF affected spine morphology by decreasing the proportion of stubby spines (Control = 0.60 ± 0.03, 13 cells/9 slices versus BDNF = 0.46 ± 0.01, 10 cells/7 slices; $P < 0.01$) and increasing the proportion of mushroom spines (SM = 0.30 ± 0.02 versus SM + BDNF = 0.37 ± 0.01;

Table 1: Quantitative results of dendritic spine analyses.

	Spine density (per 10 μm)	Spine length (μm)	Head width (μm)	Neck width (μm)	Proportion of type I (stubby)	Proportion of type II (mushroom)	Proportion of type III (thin)
Serum media (SM)	7.60 ± 0.57	0.57 ± 0.02	0.37 ± 0.01	0.30 ± 0.01	0.60 ± 0.03	0.30 ± 0.02	0.10 ± 0.02
SM + BDNF	9.39 ± 0.56	0.62 ± 0.01	0.37 ± 0.01	0.26 ± 0.01	0.46 ± 0.01	0.37 ± 0.01	0.17 ± 0.02
SM + REX	8.04 ± 1.32	0.67 ± 0.04	0.39 ± 0.02	0.29 ± 0.02	0.48 ± 0.03	0.32 ± 0.02	0.20 ± 0.02
SM + REX + BDNF	7.82 ± 0.39	0.61 ± 0.03	0.37 ± 0.01	0.28 ± 0.01	0.54 ± 0.03	0.28 ± 0.01	0.18 ± 0.02
SM + k-252a	11.16 ± 1.51	0.65 ± 0.07	0.34 ± 0.01	0.24 ± 0.01	0.42 ± 0.07	0.37 ± 0.04	0.22 ± 0.06
SM + k-252a + BDNF	10.72 ± 0.77	0.63 ± 0.02	0.37 ± 0.01	0.29 ± 0.01	0.53 ± 0.02	0.31 ± 0.01	0.16 ± 0.01
SM + TTX + k-252a	5.05 ± 0.51	0.71 ± 0.02	0.37 ± 0.02	0.28 ± 0.01	0.47 ± 0.03	0.29 ± 0.02	0.23 ± 0.02
SM + TTX	8.60 ± 1.03	0.59 ± 0.02	0.34 ± 0.01	0.27 ± 0.01	0.56 ± 0.02	0.27 ± 0.02	0.17 ± 0.02

Table 2: Summary of total dendritic length, cells, slices, and individual spines sampled for the quantitative dendritic spine analyses.

Condition	Total dendritic length (μm)	Number of cells	Number of slices	Total spines counted and measured
Serum Media (SM)	1,706.34	13	9	1,309
SM + BDNF	2,131.13	10	7	2,056
SM + REX	1,482.14	6	3	1,238
SM + REX + BDNF	2,513.30	10	4	2,021
SM + k-252a	1,196.25	5	5	1,468
SM + k-252a + BDNF	5,221.77	15	12	5,965
SM + TTX + k-252a	2,225.84	14	7	1,077
SM + TTX	1,431.73	7	5	1,307

$P < 0.05$), as well as thin spines (SM = 0.10 ± 0.02 versus SM + BDNF = 0.17 ± 0.02; $P < 0.05$; Figure 1(d)) [21].

4. Role of p75^{NTR} on BDNF's Actions on Dendritic Spine Density and Morphology

To test the role of p75^{NTR} in BDNF's effects on dendritic spines, we used the function-blocking anti-p75^{NTR} antibody REX [26] at a concentration (50 μg/mL) that blocked p75^{NTR}-dependent LTD induction in acute hippocampal slices [35]. Blocking p75^{NTR} function for 48 hrs had no effect on spine density by itself (REX: 8.04 ± 1.32 spines/10 μm, 6 cells/3 slices versus Control: 7.60 ± 0.57 spines/10 μm, 13 cells/9 slices; $P = 0.907$; Figure 2(b)). However, BDNF failed to increase dendritic spine density in the presence of REX (REX + BDNF: 7.82 ± 0.39 spines/10 μm, 10 cells/4 slices versus REX or Control; $P = 0.907$; Figure 2(b)).

Intriguingly, REX increased the proportion of thin spines (thin type III in REX: 0.20 ± 0.02, 6 cells/3 slices versus Control: 0.10 ± 0.02; 13 cells/9 slices; $P < 0.05$), without affecting the proportion of the other spine types (stubby type I in REX: 0.48 ± 0.03 versus Control 0.60 ± 0.03; $P = 0.07$) (mushroom type II in REX: 0.32 ± 0.02 versus Control: 0.30 ± 0.02; $P = 0.444$) (Figure 2(c)). This effect is reminiscent to the reduction in the proportion of stubby spines in p75^{NTR} knockout mice [16]. Furthermore, BDNF failed to change the proportion of spine types in the presence of the anti-p75^{NTR} REX antibody (thin type III in REX + BDNF: 0.18 ± 0.02; 10 cells/4 slices; $P > 0.05$ versus Control) (stubby type I in REX + BDNF: 0.54 ± 0.03; $P = 0.07$ versus Control) (mushroom type II in REX + BDNF: 0.28 ± 0.01; $P = 0.444$ versus Control; Figure 2(c)). Taken altogether, these results demonstrate that BDNF requires functional p75^{NTR} to increase dendritic spine density and modulate dendritic spine morphology.

4.1. Role of Trk Receptors on BDNF's Actions on Dendritic Spine Density and Morphology. We next blocked the kinase activity of Trk receptors with k-252a [27]. We previously reported that k-252a (200 nM) applied for 5–9 days *in vitro* led to a significant reduction in spine density in CA1 pyramidal neurons from hippocampal slice cultures maintained in serum-free media [28]. Surprisingly, exposure to k-252a for a shorter time (48 hs) and in the presence of horse serum in the media significantly increased spine density in CA1 pyramidal neurons (k-252a: 11.2 ± 1.51 spines/10 μm, 5 cells/5 slices; $P < 0.05$ versus Control; Figure 2(e)). In contrast to its blockade of BDNF's effects in serum-free slices [28], k-252a failed to prevent the effects of BDNF to increase spine density (k-252a + BDNF: 10.7 ± 0.8 spines/10 μm, 15 cells/12 slices; $P < 0.05$ versus Control). However, BDNF did not further increase spine density in the presence of k-252a ($P > 0.05$ versus k-252a alone).

The increase in spine density by k-252a was unexpected; however, the majority of these spines were of the thin immature type. Indeed, k-252a increased the fraction of thin type III spines (k-252a: 0.22 ± 0.06, 5 cells/5 slices; $P < 0.05$ versus Control) and decreased the proportion of stubby type I spines (k-252a: 0.42 ± 0.07; $P < 0.01$ versus Control; Figure 2(f)). In addition, k-252a prevented BDNF to change the proportion of morphological spine types (k-252a + BDNF type I: 0.53 ± 0.02; type II: 0.31 ± 0.01; type III: 0.16 ± 0.01; 15 cells/12 slices; all $P > 0.05$ versus Control).

Considering the unexpected increase in dendritic spine density induced by the Trk inhibitor k-252a—albeit mostly of the long and thin type III category—and the role of neuronal activity in spine number and form [36], we tested

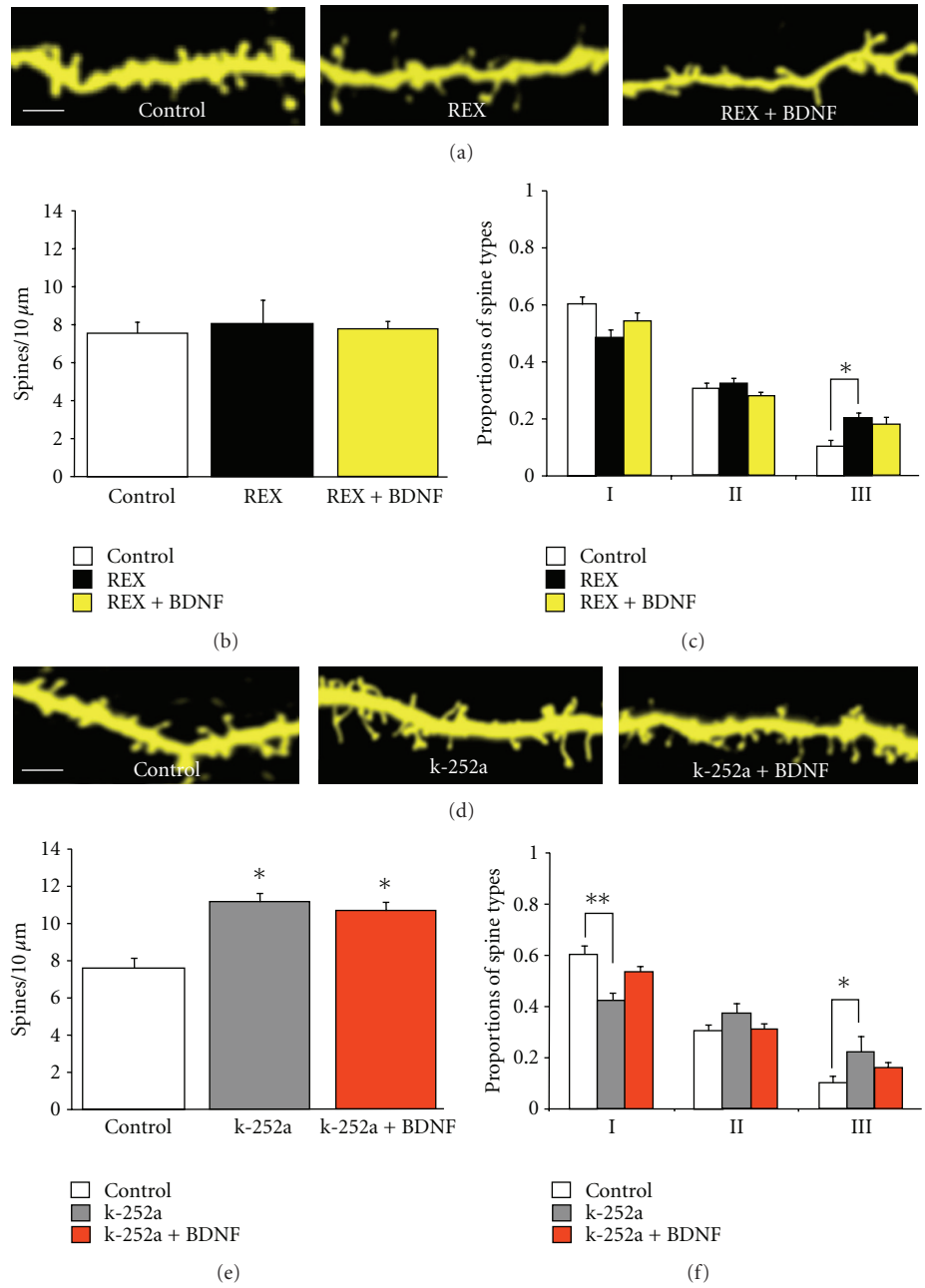


Figure 2: Role of Trk and $p75^{NTR}$ in BDNF's effects on dendritic spine density and morphology. (a) Representative examples of dendritic segments of CA1 pyramidal neurons maintained in serum-containing media (SM) and treated with the function-blocking antibody of $p75^{NTR}$, REX (50 μg/mL), and BDNF (250 ng/mL) for 48 hrs (scale bar represents 2 μm). (b) Dendritic spine density expressed per 10 μm of apical dendrite. (c) Proportion of each morphological type of dendritic spine, expressed as a fraction of the total spine population. (d) Representative examples of dendritic segments of CA1 pyramidal neurons maintained in SM and treated with k-252a (200 nM) and BDNF (250 ng/mL) for 48 hrs. (e) Dendritic spine density expressed per 10 μm of apical dendrite. (f) Proportion of each morphological type of dendritic spines, expressed as a fraction of the total spine population. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, after a one-way ANOVA.

whether the effect of k-252a required neuronal activity in the form of Na^+-dependent action potentials. Indeed, TTX (1 μM) prevented the effect of k-252a (200 nM) (TTX + k-252a: 5.05 ± 0.51 spines/10 μm, 14 cells/7 slices; $P < 0.001$ versus k-252a; Figure 3(b)). Furthermore, exposure to both TTX and k-252a caused a loss of dendritic spines compared to slices maintained in the control serum media conditions (TTX + k-252a: 5.05 ± 0.51 spines/10 μm, 14 cells/7 slices versus Control: 7.60 ± 0.57 spines/10 μm, 13 cells/9 slices; $P < 0.05$; Figure 3(b)). It should be noted that this short exposure to TTX (48 hs) did not affect spine density (TTX: 8.60 ± 1.03 spines/10 μm, 7 cells/5 slices; $P > 0.05$ versus Control; Figure 3(b)). On the other hand, TTX did not prevent the morphological spine changes induced by k-252a,

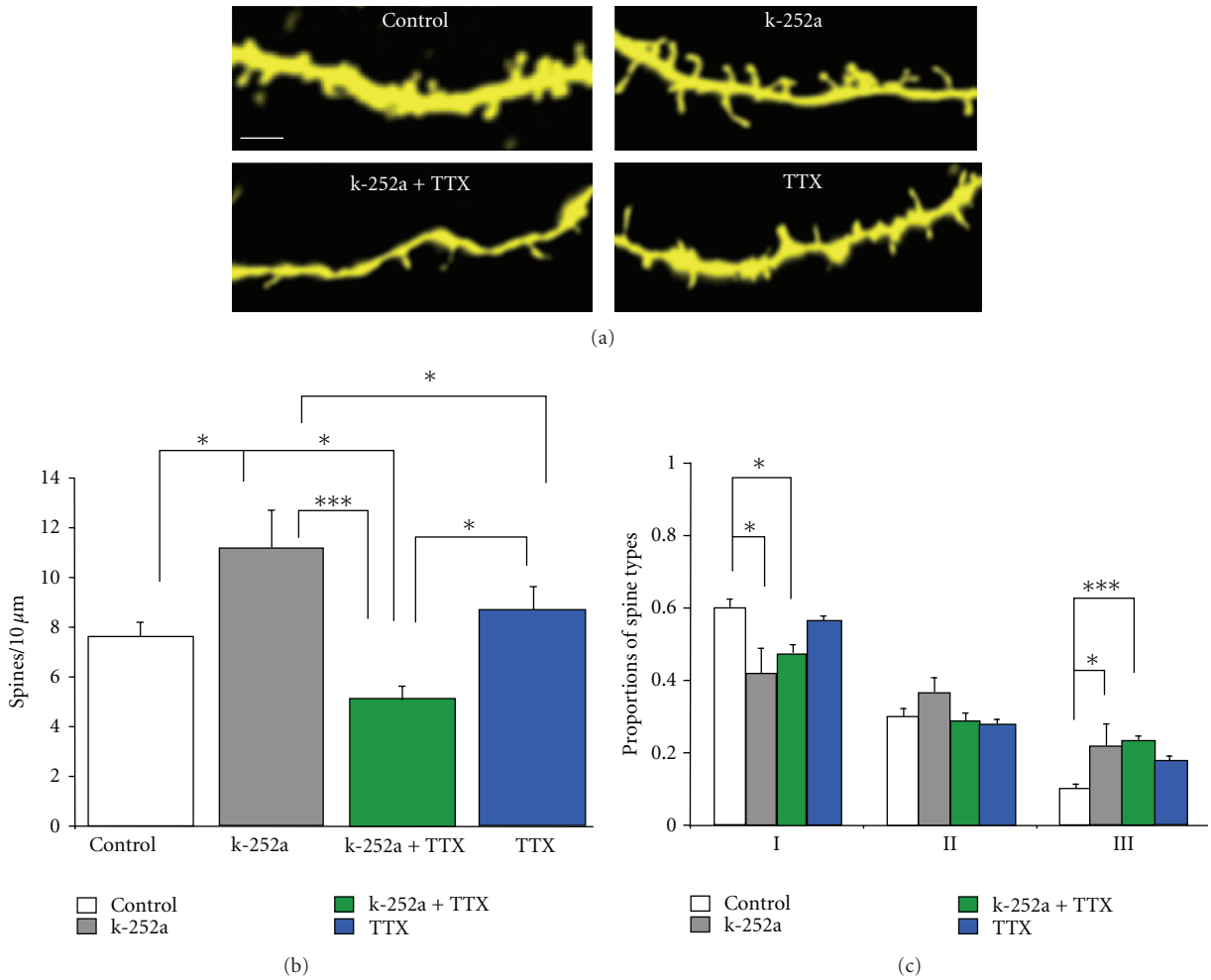


Figure 3: Role of neuronal activity in k-252a's effects on dendritic spine density and morphology. (a) Representative examples of dendritic segments of CA1 pyramidal neurons maintained in serum-containing media (SM) and treated with k-252a (200 nM), TTX (1 μM), or both k-252a and TTX for 48 hrs (scale bar represents 2 μm). (b) Dendritic spine density expressed per 10 μm of apical dendrite. (c) Proportion of each morphological type of dendritic spines, expressed as a fraction of the total spine population. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, after a one-way ANOVA.

including the increase in the proportion of thin type III spines induced by k-252a (TTX + k-252a: 0.23 ± 0.02; 14 cells/7 slices $P < 0.001$ versus Control; Figure 3(c)) and the reduction of stubby type I spines (TTX + k-252a: 0.47 ± 0.03; 14 cells/7 slices $P < 0.05$ versus Control; Figure 3(c)). The apparent increase in the proportion of thin spines in the TTX group did not reach statistical significance (TTX: 0.17 ± 0.02, 7 cells/5 slices; $P > 0.05$ versus Control; Figure 3(c)). These results suggest that ongoing BDNF signaling through TrkB receptors and spontaneous neuronal activity are intimately related in dendritic spine maintenance, as well as in the structural maturation of those morphological spine types thought to represent the postsynaptic compartment of mature synapses [37].

5. Discussion

To address the role of each BDNF receptor on dendritic spine density and morphology, we blocked either Trk or $p75^{NTR}$ for 48 hs in the absence or presence of BDNF. We observed that brief exposures to the Trk inhibitor k-252a caused a significant increase in spine density in CA1 pyramidal neurons. However, most of these spines were of the thin category, thought to be highly motile and unstable structures characteristic of immature synapses [38–40]. The fact that longer exposures to k-252a by itself caused spine loss [28] suggests that an initial increase in thin immature spines precedes spine pruning [36]. In contrast, $p75^{NTR}$ blockade with the function-blocking antibody REX prevented BDNF's effect on spine density. The importance of BDNF-induced modifications on neuron structure and physiology are well documented and continue to emerge. Since BDNF binds and activates two different receptors, determining how each receptor influences dendritic remodeling will provide greater understanding into the function of BDNF in synaptic plasticity. The observations reported here reflect a functional antagonism between $p75^{NTR}$ and TrkB receptor signaling in the maintenance of dendritic spines.

Consistent with our previous study [7], BDNF increased spine density and shifted the proportion of spine types towards the thin and mushroom-shaped spines in hippocampal slice cultures maintained in serum-containing media. We also uncovered that antagonism of either $p75^{NTR}$ or Trk receptors increased the proportion of thin (type-III) spines. It has been suggested that thin spines represent "learning spines" due to their highly motile and unstable nature, while mushroom spines are "memory spines" because they are highly stable [41]. Since inhibition of either BDNF receptor increased the proportion of thin spines, we speculate that BDNF participates in the formation/maintenance/pruning of these "learning spines." However, the difference between these two receptor systems is in their ability to differentially modulate spine density. Blocking Trk signaling with the tyrosine kinase inhibitor k-252a, caused a significant increase in spine density, while blocking $p75^{NTR}$ with the function-blocking antibody REX had no effect on spine density. These results strongly suggest the existence of a sustained tone of BDNF signaling that contributes to dendritic spine maintenance in a manner dependent on the activation of Trk receptors (but not $p75^{NTR}$). Taken together, these results suggest that during postnatal development, $p75^{NTR}$ activation is important for initial dendritic spine formation, while Trk receptors participate in dendritic spine maintenance at later developmental stages. Indeed, conditional deletion of TrkB in postnatal forebrain excitatory neurons caused a reduction in spine density and a higher proportion of long and thin spines in hippocampal and primary visual cortex neurons [42–44], suggesting that sequential activation of TrkB receptors followed by $p75^{NTR}$ might be critical for BDNF-mediated modulation of dendritic spine density and morphology. Evidence of such developmental differences has been observed in subventricular zone-derived neurons, where $p75^{NTR}$ activation modulates dendritic growth in early stages of development, while TrkB activation plays a role in later stages [45].

Ongoing neuronal activity was required for the unexpected effect of the Trk inhibitor k-252a : TTX prevented the increase in spine density and proportion of thin immature spines induced by k-252a. Intriguingly, there was a dramatic loss of spines in slice cultures exposed to both TTX and k-252a compared to control serum media conditions. While the specific mechanisms of dendritic spine maintenance and pruning remain somewhat unknown, it is well accepted that ongoing levels of synaptic transmission and the ensuing intracellular Ca^{2+} levels contribute in a significant manner [36]. Consistent with this view, silencing neuronal activity for 7 days *in vitro* with TTX reduced spine density in CA1 pyramidal neurons in slice cultures [46]. The spine loss in those week-long silencing experiments likely results from prolonged absence of excitatory synaptic input [47]. It should be noted that a shorter period of neuronal inactivity (TTX for 2 days *in vitro*) did not cause spine loss, but rather altered the proportion of morphological spine types favoring the thin and immature spine type [29]. Our present results suggest that ongoing Trk signaling is required for spine maintenance in TTX-silenced slice cultures, revealing a novel aspect of activity-dependent maintenance and pruning of dendritic spines in hippocampal pyramidal neurons.

The observations on dendritic spine density and morphology reported here may reflect the functional antagonism between $p75^{NTR}$ and Trk receptor signaling [13]. Even though current reports indicate that $p75^{NTR}$ and Trk do not directly interact, it has been proposed that these receptor complexes share similar downstream signaling pathways to create more complex actions [48]. On the other hand, $p75^{NTR}$ can act as a coreceptor for Trk receptors, creating high affinity sites for Trk receptor activation [49]. Furthermore, the interaction of truncated TrkB receptors (TrkB.T1) with $p75^{NTR}$ enhanced dendritic filopodia outgrowth in the absence of neurotrophin binding [50]. Thus, signaling through the two BDNF receptors may have different consequences for dendritic spine density and morphology depending on whether they are activated alone, in concert or under different levels of ongoing neuronal activity. Taken altogether, these studies have revealed an unexpected level of complexity in the consequences of BDNF signaling on dendritic morphology.

Acknowledgments

The authors thank Dr. Reichardt (UCSF) for the generous gift of anti- $p75^{NTR}$ REX antibody. This paper was supported by NIH-NINDS Grants NS40593, NS057780, and NS065027 (LP-M). They thank the Alabama Neuroscience Blueprint Core Center (P30-NS57098), the UAB Intellectual and Developmental Disabilities Research Center (P30-HD38985), and the UAB Neuroscience Core (P30-NS47466) for instrumentation.

References

[1] E. J. Huang and L. F. Reichardt, "Neurotrophins: roles in neuronal development and function," *Annual Review of Neuroscience*, vol. 24, pp. 677–736, 2001.

[2] G. R. Lewin and Y. A. Barde, "Physiology of the neurotrophins," *Annual Review of Neuroscience*, vol. 19, pp. 289–317, 1996.

[3] C. R. Bramham and E. Messaoudi, "BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis," *Progress in Neurobiology*, vol. 76, no. 2, pp. 99–125, 2005.

[4] M. M. Poo, "Neurotrophins as synaptic modulators," *Nature Reviews Neuroscience*, vol. 2, no. 1, pp. 24–32, 2001.

[5] W. J. Tyler, M. Alonso, C. R. Bramham, and L. D. Pozzo-Miller, "From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning," *Learning and Memory*, vol. 9, no. 5, pp. 224–237, 2002.

[6] M. Barbacid, "Nerve growth factor: a tale of two receptors," *Oncogene*, vol. 8, no. 8, pp. 2033–2042, 1993.

[7] M. V. Chao and B. L. Hempstead, "p75 and Trk: a two-receptor system," *Trends in Neurosciences*, vol. 18, no. 7, pp. 321–326, 1995.

[8] B. L. Hempstead, "The many faces of $p75^{NTR}$," *Current Opinion in Neurobiology*, vol. 12, no. 3, pp. 260–267, 2002.

[9] E. J. Huang and L. F. Reichardt, "Trk receptors: roles in neuronal signal transduction," *Annual Review of Biochemistry*, vol. 72, pp. 609–642, 2003.

[10] A. Nykjaer, T. E. Willnow, and C. M. Petersen, "p75^{NTR}—live or let die," *Current Opinion in Neurobiology*, vol. 15, no. 1, pp. 49–57, 2005.

[11] A. Patapoutian and L. F. Reichardt, "Trk receptors: mediators of neurotrophin action," *Current Opinion in Neurobiology*, vol. 11, no. 3, pp. 272–280, 2001.

[12] P. P. Roux and P. A. Barker, "Neurotrophin signaling through the p75 neurotrophin receptor," *Progress in Neurobiology*, vol. 67, no. 3, pp. 203–233, 2002.

[13] B. Lu, P. T. Pang, and N. H. Woo, "The yin and yang of neurotrophin action," *Nature Reviews Neuroscience*, vol. 6, no. 8, pp. 603–614, 2005.

[14] A. K. McAllister, D. C. Lo, and L. C. Katz, "Neurotrophins regulate dendritic growth in developing visual cortex," *Neuron*, vol. 15, no. 4, pp. 791–803, 1995.

[15] T. A. Yacoubian and D. C. Lo, "Truncated and full-length TrkB receptors regulate distinct modes of dendritic growth," *Nature Neuroscience*, vol. 3, no. 4, pp. 342–349, 2000.

[16] M. Zagrebelsky, A. Holz, G. Dechant, Y. A. Barde, T. Bonhoeffer, and M. Korte, "The p75 neurotrophin receptor negatively modulates dendrite complexity and spine density in hippocampal neurons," *Journal of Neuroscience*, vol. 25, no. 43, pp. 9989–9999, 2005.

[17] R. Klein, R. J. Smeyne, W. Wurst et al., "Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death," *Cell*, vol. 75, no. 1, pp. 113–122, 1993.

[18] A. Martínez, S. Alcántara, V. Borrell et al., "TrkB and TrkC signaling are required for maturation and synaptogenesis of hippocampal connections," *Journal of Neuroscience*, vol. 18, no. 18, pp. 7336–7350, 1998.

[19] R. Otal, A. Martínez, and E. Soriano, "Lack of TrkB and TrkC signaling alters the synaptogenesis and maturation of mossy fiber terminals in the hippocampus," *Cell and Tissue Research*, vol. 319, no. 3, pp. 349–358, 2005.

[20] S. Alcántara, J. Frisén, J. A. Del Río, E. Soriano, M. Barbacid, and I. Silos-Santiago, "TrkB signaling is required for postnatal survival of CNS neurons and protects hippocampal and motor neurons from axotomy-induced cell death," *Journal of Neuroscience*, vol. 17, no. 10, pp. 3623–3633, 1997.

[21] C. A. Chapleau, M. E. Carlo, J. L. Larimore, and L. Pozzo-Miller, "The actions of BDNF on dendritic spine density and morphology in organotypic slice cultures depend on the presence of serum in culture media," *Journal of Neuroscience Methods*, vol. 169, no. 1, pp. 182–190, 2008.

[22] L. D. Pozzo Miller, J. J. Petrozzino, N. K. Mahanty, and J. A. Connor, "Optical imaging of cytosolic calcium, electrophysiology, and ultrastructure in pyramidal neurons of organotypic slice cultures from rat hippocampus," *NeuroImage*, vol. 1, no. 2, pp. 109–120, 1993.

[23] L. C. Katz, "Local circuitry of identified projection neurons in cat visual cortex brain slices," *Journal of Neuroscience*, vol. 7, no. 4, pp. 1223–1249, 1987.

[24] M. Alonso, J. H. Medina, and L. Pozzo-Miller, "ERK1/2 activation is necessary for BDNF to increase dendritic spine density in hippocampal CA1 pyramidal neurons," *Learning and Memory*, vol. 11, no. 2, pp. 172–178, 2004.

[25] R. E. Twyman, R. M. Green, and R. L. Macdonald, "Kinetics of open channel block by penicillin of single GABA(A) receptor channels from mouse spinal cord neurones in culture," *Journal of Physiology*, vol. 445, pp. 97–127, 1992.

[26] P. S. Mischel, S. G. Smith, E. R. Vining, J. S. Valletta, W. C. Mobley, and L. F. Reichard, "The extracellular domain of p75^{NTR} is necessary to inhibit neurotrophin-3 signaling through TrkA," *Journal of Biological Chemistry*, vol. 276, no. 14, pp. 11294–11301, 2001.

[27] P. Tapley, F. Lamballe, and M. Barbacid, "K252a is a selective inhibitor of the tyrosine protein kinase activity of the trk family of oncogenes and neurotrophin receptors," *Oncogene*, vol. 7, no. 2, pp. 371–381, 1992.

[28] W. J. Tyler and L. D. Pozzo-Miller, "BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses," *Journal of Neuroscience*, vol. 21, no. 12, pp. 4249–4258, 2001.

[29] W. J. Tyler and L. Pozzo-Miller, "Miniature synaptic transmission and BDNF modulate dendritic spine growth and form in rat CA1 neurones," *Journal of Physiology*, vol. 553, no. 2, pp. 497–509, 2003.

[30] A. Peters and I. R. Kaiserman-Abramof, "The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines," *American Journal of Anatomy*, vol. 127, no. 4, pp. 321–355, 1970.

[31] A. Peters and I. R. Kaiserman-Abramof, "The small pyramidal neuron of the rat cerebral cortex—the synapses upon dendritic spines," *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, vol. 100, no. 4, pp. 487–506, 1969.

[32] K. M. Harris, F. E. Jensen, and B. Tsao, "Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation," *Journal of Neuroscience*, vol. 12, no. 7, pp. 2685–2705, 1992.

[33] I. Y. Y. Koh, W. B. Lindquist, K. Zito, E. A. Nimchinsky, and K. Svoboda, "An image analysis algorithm for dendritic spines," *Neural Computation*, vol. 14, no. 6, pp. 1283–1310, 2002.

[34] A. De Simoni and F. A. Edwards, "Pathway specificity of dendritic spine morphology in identified synapses onto rat hippocampal CA1 neurons in oranotypic slices," *Hippocampus*, vol. 16, no. 12, pp. 1111–1124, 2006.

[35] N. H. Woo, H. K. Teng, C. J. Siao et al., "Activation of p75^{NTR} by proBDNF facilitates hippocampal long-term depression," *Nature Neuroscience*, vol. 8, no. 8, pp. 1069–1077, 2005.

[36] M. Segal, E. Korkotian, and D. D. Murphy, "Dendritic spine formation and pruning: common cellular mechanisms?" *Trends in Neurosciences*, vol. 23, no. 2, pp. 53–57, 2000.

[37] H. Kasai, M. Matsuzaki, J. Noguchi, N. Yasumatsu, and H. Nakahara, "Structure-stability-function relationships of dendritic spines," *Trends in Neurosciences*, vol. 26, no. 7, pp. 360–368, 2003.

[38] M. E. Dailey and S. J. Smith, "The dynamics of dendritic structure in developing hippocampal slices," *Journal of Neuroscience*, vol. 16, no. 9, pp. 2983–2994, 1996.

[39] A. Dunaevsky, A. Tashiro, A. Majewska, C. Mason, and R. Yuste, "Developmental regulation of spine motility in the mammalian central nervous system," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 23, pp. 13438–13443, 1999.

[40] K. E. Sorra and K. M. Harris, "Overview on the structure, composition, function, development, and plasticity of hippocampal dendritic spines," *Hippocampus*, vol. 10, no. 5, pp. 501–511, 2000.

[41] J. Bourne and K. M. Harris, "Do thin spines learn to be mushroom spines that remember?" *Current Opinion in Neurobiology*, vol. 17, no. 3, pp. 381–386, 2007.

[42] S. Chakravarthy, M. H. Saiepour, M. Bence et al., "Postsynaptic TrkB signaling has distinct roles in spine maintenance in adult visual cortex and hippocampus," *Proceedings of the*

National Academy of Sciences of the United States of America, vol. 103, no. 4, pp. 1071–1076, 2006.

[43] B. W. Luikart, S. Nef, T. Virmani et al., "TrkB has a cell-autonomous role in the establishment of hippocampal schaffer collateral synapses," *Journal of Neuroscience*, vol. 25, no. 15, pp. 3774–3786, 2005.

[44] O. von Bohlen und Halbach, S. Krause, D. Medina, C. Sciarretta, L. Minichiello, and K. Unsicker, "Regional- and age-dependent reduction in trkB receptor expression in the hippocampus is associated with altered spine morphologies," *Biological Psychiatry*, vol. 59, no. 9, pp. 793–800, 2006.

[45] E. Gascon, L. Vutskits, H. Zhang et al., "Sequential activation of p75 and TrkB is involved in dendritic development of subventricular zone-derived neuronal progenitors in vitro," *European Journal of Neuroscience*, vol. 21, no. 1, pp. 69–80, 2005.

[46] C. Collin, K. Miyaguchi, and M. Segal, "Dendritic spine density and LTP induction in cultured hippocampal slices," *Journal of Neurophysiology*, vol. 77, no. 3, pp. 1614–1623, 1997.

[47] R. A. McKinney, M. Capogna, R. Dürr, B. H. Gähwiler, and S. M. Thompson, "Miniature synaptic events maintain dendritic spines via AMPA receptor activation," *Nature Neuroscience*, vol. 2, no. 1, pp. 44–49, 1999.

[48] T. Wehrman, X. He, B. Raab, A. Dukipatti, H. Blau, and K. C. Garcia, "Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors," *Neuron*, vol. 53, no. 1, pp. 25–38, 2007.

[49] M. Benedetti, A. Levi, and M. V. Chao, "Differential expression of nerve growth factor receptors leads to altered binding affinity and neurotrophin responsiveness," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 16, pp. 7859–7863, 1993.

[50] M. Hartmann, T. Brigadski, K. S. Erdmann et al., "Truncated TrkB receptor-induced outgrowth of dendritic filopodia involves the p75 neurotrophin receptor," *Journal of Cell Science*, vol. 117, no. 24, pp. 5803–5814, 2004.

Hindawi Publishing Corporation
Neural Plasticity
Volume 2012, Article ID 309494, 6 pages
doi:10.1155/2012/309494

Research Article

Selective Estrogen Receptor Modulators Regulate Dendritic Spine Plasticity in the Hippocampus of Male Rats

Ignacio González-Burgos,[1,2] Martha C. Rivera-Cervantes,[2] Dulce A. Velázquez-Zamora,[1,2] Alfredo Feria-Velasco,[2] and Luis Miguel Garcia-Segura[3]

[1] *Centro de Investigación Biomédica de Occidente, Guadalajara, Jalisco 44340, Mexico*
[2] *CUCBA, Universidad de Guadalajara, Guadalajara, Jalisco 45100, Mexico*
[3] *Instituto Cajal, CSIC, 28002 Madrid, Spain*

Correspondence should be addressed to Luis Miguel Garcia-Segura, lmgs@cajal.csic.es

Received 19 July 2011; Accepted 12 August 2011

Academic Editor: Irina Nikonenko

Some selective estrogen receptor modulators, such as raloxifene and tamoxifen, are neuroprotective and reduce brain inflammation in several experimental models of neurodegeneration. In addition, raloxifene and tamoxifen counteract cognitive deficits caused by gonadal hormone deprivation in male rats. In this study, we have explored whether raloxifene and tamoxifen may regulate the number and geometry of dendritic spines in CA1 pyramidal neurons of the rat hippocampus. Young adult male rats were injected with raloxifene (1 mg/kg), tamoxifen (1 mg/kg), or vehicle and killed 24 h after the injection. Animals treated with raloxifene or tamoxifen showed an increased numerical density of dendritic spines in CA1 pyramidal neurons compared to animals treated with vehicle. Raloxifene and tamoxifen had also specific effects in the morphology of spines. These findings suggest that raloxifene and tamoxifen may influence the processing of information by hippocampal pyramidal neurons by affecting the number and shape of dendritic spines.

1. Introduction

Selective estrogen receptor modulators (SERMs) either synthetic or natural, such as phytoestrogens, are candidates for the treatment or the prevention of cognitive and affective disorders in men and women [1–5]. Several studies have shown that some synthetic SERMs, such as tamoxifen, raloxifene, or bazedoxifene [6–29], some nonfeminizing estrogens [30–34], and some natural SERMs, such as genistein [35, 36], are neuroprotective in vitro and in vivo. The neuroprotective effects of SERMs are associated with a decrease in the activation of microglia and astroglia and a reduction in brain inflammation [37–43]. In addition, some SERMs have shown to induce neuritic outgrowth in vitro [44, 45], suggesting that these molecules may also affect synaptic connectivity in vivo. Indeed, ERs are involved in the regulation of dendritic spines in the hippocampus of female animals in vivo [46–51], where tamoxifen regulates synaptophysin expression [52]. SERMs are also able to regulate cholinergic, serotonergic, and dopaminergic neurotransmission in female animals [53–56]. However, the effects of SERMs on synaptic connectivity in males have not been adequately explored. Nevertheless, previous studies have shown that SERMs such as raloxifene and tamoxifen are able to counteract hippocampus-dependent cognitive deficits caused by androgen deprivation in male rats [57]. In addition, raloxifene reduces working memory deficits in male rats after traumatic brain injury [20].

To further characterize the mechanisms of action of SERMs in the male brain, we have assessed in this study the effects of tamoxifen and raloxifene on the number and geometry of dendritic spines in CA1 pyramidal neurons of the rat hippocampus.

2. Material and Methods

2.1. Animals and Treatments. Sprague-Dawley adult male rats were maintained under regular 12 h light-dark cycles (lights on: 07:00–19:00 h) and controlled environmental humidity (45–50%) and temperature (22 ± 2°C). Animals had free access to food and water. All the experimental procedures

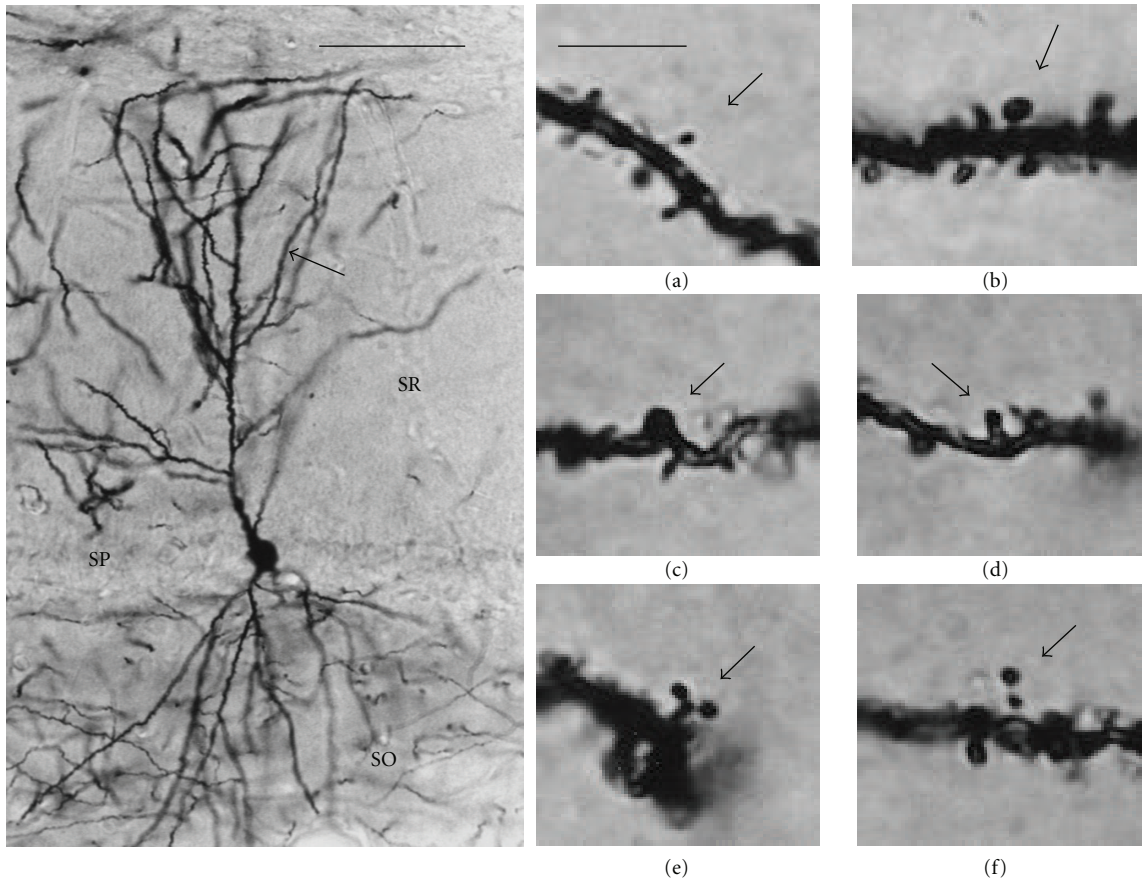


Figure 1: Examples of dendritic spines stained with the Golgi method. Left panel: photomicrograph of a CA1 pyramidal neuron impregnated with a modification of the Golgi method. Spines studied in the present work were counted in a segment 50 μm in length of a secondary dendrite (arrow) protruding from its parent apical dendrite. SO: stratum oriens; SP: stratum pyramidale; SR: stratum radiatum. Scale bar = 100 μm. In the right panels, photomicrographs show representative examples of thin (a), mushroom (b), stubby (c), wide (d), branched (e), and double (f) spines (arrows). Scale bar = 5 μm.

were conducted to minimize pain or discomfort in the animals and performed in accordance with the NIH guide for Care and Use of Laboratory Animals (NIH Publications no. 80-23, 1996 revised). Protocols were approved by our institutional animal care committee.

At the age of three months, animals were injected with raloxifene (1 mg/kg; $n = 6$), tamoxifen (1 mg/kg; $n = 6$), or vehicle (20 mg/mL DMSO diluted 3% in saline solution; $n = 6$). Animals were killed 24 h after the injection.

2.2. Golgi Studies. Animals were anesthetized with 30 mg/kg intramuscular ketamine and 50 mg/kg i.p. sodium pentobarbital. Then, animals were perfused intracardially with 100 mL of a washing phosphate-buffered solution (pH 7.4; 0.01 M) containing 1000 IU/L of sodium heparin and 1 g/L of procaine hydrochloride. Then, 200 mL of a fixing phosphate-buffered 4% formaldehyde solution was perfused. Both solutions flowed at a rate of 11.5 mL/min. Each brain remained for at least 48 h in 100 mL of a fresh fixing solution.

The bilateral dorsal hippocampi were dissected out and impregnated using a modification of the Golgi method [58]. Several coronal slices 100 μm thick were mounted on one slide per animal. Spine numerical density and the proportion of thin, mushroom, stubby, wide, branched, and double spines [59–61] were assessed in CA1 pyramidal neurons. Spines were counted in one 50 μm segment per cell, located in the middle of one of the secondary dendrites that protrude from the apical dendrite (Figure 1). Six CA1 pyramidal neurons were studied per animal. The total number of spines counted was 5,796 in the animals treated with vehicle: 9,295 in the animals treated with raloxifene and 9,180 in the animals treated with tamoxifen.

2.3. Statistical Analysis. The one-way ANOVA and Tukey *post hoc* test were used for statistical comparisons of data from spine numerical density. In addition, one-way ANOVA and Bonferroni correction *post hoc* test were used for statistical comparisons of the proportion of the different types of spines. The n used for statistical analysis was the number of animals ($n = 6$, per experimental group).

3. Results

Raloxifene and tamoxifen increased significantly the total numerical density of dendritic spines compared to control animals (Table 1). Both SERMs increased the numerical

Table 1: Numerical density of dendritic spines in hippocampal CA1 pyramidal neurons of male rats 24 hours after the treatment with vehicle, raloxifene, or tamoxifen.

	Vehicle	Raloxifene	Tamoxifen
Total spines	161.0 ± 5.0	258.2 ± 3.0[a]	255.0 ± 4.0[b]
Thin	74.8 ± 2.8	89.6 ± 3.8[a]	78.0 ± 3.6[c]
Mushroom	50.4 ± 1.8	84.6 ± 3.2[a]	92.8 ± 1.6[bc]
Stubby	28.6 ± 1.4	65.8 ± 1.8[a]	71.0 ± 2.2[b]
Wide	6.2 ± 0.6	15.6 ± 1.0[a]	11.8 ± 1.2[bc]
Branched	0.4 ± 0.1	0.6 ± 0.2	0.6 ± 0.1
Double	0.1 ± 0.04	0.04 ± 0.04	0.4 ± 0.1

Data represent mean ± SEM of the number of dendritic spines per 100 μm dendritic segment from 6 animals in each experimental group. [a–c]Significant differences, $P < 0.05$; [a]raloxifene versus Vehicle; [b]tamoxifen versus. Vehicle; [c]tamoxifen versus Raloxifene.

Table 2: Proportion (%) of the different types of dendritic spines in hippocampal CA1 pyramidal neurons 24 hours after the treatment with vehicle, raloxifene, or tamoxifen.

	Vehicle	Raloxifene	Tamoxifen
Thin	46.4	34.7[a]	30.5[b]
Mushroom	31.3	32.7	36.3[bc]
Stubby	17.7	25.4[a]	27.8[b]
Wide	3.8	6.0[a]	4.6
Branched	0.2	0.2	0.2
Double	0.07	0.01	0.1

Data represent means from 6 animals in each experimental group. [a–c]Significant differences, $P < 0.05$; [a]raloxifene versus Vehicle; [b]tamoxifen versus Vehicle; [c]tamoxifen versus raloxifene.

density of mushroom, stubby, and wide spines (Table 1). In addition, raloxifene increased the numerical density of thin spines (Table 1). Numerical density of mushroom spines was greater in tamoxifen-treated rats than in raloxifene-treated animals. In contrast, thin and wide spines were less numerous in the tamoxifen group than in raloxifene-treated animals (Table 1).

The experimental treatments also resulted in changes in the proportion of different spine morphologies. The proportion of thin spines was reduced in the animals treated with raloxifene. Furthermore, raloxifene increased the proportion of stubby and wide spines and did not significantly affect the proportion of mushroom, branched and double spines (Table 2).

As observed for raloxifene, the proportion of thin spines was also reduced in the animals treated with tamoxifen. In contrast, mushroom and stubby spines were seen in greater proportion in animals treated with tamoxifen than in control animals. Tamoxifen had no significant effects in the proportion of wide, branched, and double spines (Table 2). The animals treated with tamoxifen showed a higher proportion of mushroom spines than those treated with raloxifene (Table 2).

4. Discussion

Our present findings indicate that some SERMs, such as raloxifene and tamoxifen, affect the number of dendritic spines in male rats. This action of SERMs may affect the processing of novel information used in memory formation [62].

In addition to increase the numerical density of spines, raloxifene and tamoxifen also affected spine geometry. Both SERMs increased the numerical density of stubby, mushroom, and wide spines. In addition, raloxifene increased the number of thin spines. However, both SERMs reduced the proportion of thin dendritic spines. Dendritic spine morphology affects the diffusion and compartmentalization of membrane-associated proteins [63] and the expression of AMPA receptors [64–67]. In particular, the length of the spine neck seems to be a key regulator of spinodendritic Ca2+ signaling [68–72] and of the transmission of membrane potentials [73]. In consequence, the geometry of dendritic spines may influence the processing of synaptic impulses [74–79]. Our findings suggest, therefore, that raloxifene and tamoxifen, decreasing the proportion of thin dendritic spines, may influence the processing of information by hippocampal pyramidal neurons. In addition, the action of raloxifene and tamoxifen presents some differences that may have functional significance. Tamoxifen, but not raloxifene, increased the proportion of mushroom spines. Thus, the animals treated with tamoxifen had an increased numerical density and proportion of mushroom spines compared to animals treated with raloxifene. Mushroom spines may be involved in the management of previously acquired information since they have larger postsynaptic densities [80] and express higher levels of AMPA receptors [64–67]. Therefore, the synapses on mushroom spines are functionally stronger [78] and it has been suggested that these spines would sustain memory storage [78, 81, 82].

The induction of plastic changes in dendritic spines by raloxifene and tamoxifen may be linked with the precognitive effects of these molecules in male rats [20, 57]. However, the possible impact of raloxifene and tamoxifen on cognitive decline in men remains to be adequately explored, in particular in association with neurodegenerative diseases. For instance, both SERMs increase the levels of luteinizing hormone (LH) in men [83] and it has been proposed that elevated levels of LH may contribute to Alzheimer's disease pathogenesis [84]. Indeed, leuprolide acetate, a GnRH agonist that lower serum levels of LH, has been shown to improve cognitive performance and decrease amyloid-β deposition in a mouse transgenic model of Alzheimer's disease [85].

5. Conclusions

The findings of this study indicate that raloxifene and tamoxifen, two SERMs currently used in clinical treatments, promote an increase in the numerical density of dendritic spines and changes in spine geometry in the hippocampus of male rats. These findings, together with the regulation exerted by tamoxifen and raloxifene on hippocampus-dependent cognitive function in male rats [57], suggest that

SERMs may influence the processing of information by male hippocampal pyramidal neurons by affecting the number and shape of dendritic spines.

Acknowledgments

This work was supported by the Coordinación de Investigación en Salud, IMSS (FIS/IMSS/PROT/G10/821), Universidad de Guadalajara (U de G, P3e), México, and Ministerio de Ciencia e Innovación, Madrid, Spain (BFU2008-02950-C03-01).

References

[1] R. D. Brinton, "Requirements of a brain selective estrogen: advances and remaining challenges for developing a NeuroSERM," *Journal of Alzheimer's Disease*, vol. 6, supplement, pp. S27–S35, 2004.

[2] F. Bernardi, N. Pluchino, M. Stomati, M. Pieri, and A. R. Genazzani, "CNS: sex steroids and SERMs," *Annals of the New York Academy of Sciences*, vol. 997, pp. 378–388, 2003.

[3] L. Zhao, K. O'Neill, and R. Diaz Brinton, "Selective estrogen receptor modulators (SERMs) for the brain: current status and remaining challenges for developing NeuroSERMs," *Brain Research Reviews*, vol. 49, no. 3, pp. 472–493, 2005.

[4] L. L. DonCarlos, I. Azcoitia, and L. M. Garcia-Segura, "Neuroprotective actions of selective estrogen receptor modulators," *Psychoneuroendocrinology*, vol. 34, supplement 1, pp. S113–S122, 2009.

[5] M. A. Arevalo, M. Santos-Galindo, N. Lagunas, I. Azcoitia, and L. M. Garcia-Segura, "Selective estrogen receptor modulators as brain therapeutic agents," *Journal of Molecular Endocrinology*, vol. 46, no. 1, pp. R1–R9, 2011.

[6] K. M. Dhandapani and D. W. Brann, "Protective effects of estrogen and selective estrogen receptor modulators in the brain," *Biology of Reproduction*, vol. 67, no. 5, pp. 1379–1385, 2002.

[7] I. Ciriza, P. Carrero, I. Azcoitia, S. G. Lundeen, and L. M. Garcia-Segura, "Selective estrogen receptor modulators protect hippocampal neurons from kainic acid excitotoxicity: differences with the effect of estradiol," *Journal of Neurobiology*, vol. 61, no. 2, pp. 209–221, 2004.

[8] B. F. Bebo, B. Dehghani, S. Foster, A. Kurniawan, F. J. Lopez, and L. S. Sherman, "Treatment with selective estrogen receptor modulators regulates myelin specific T-cells and suppresses experimental autoimmune encephalomyelitis," *GLIA*, vol. 57, no. 7, pp. 777–790, 2009.

[9] L. Zhao, K. O'Neill, and R. D. Brinton, "Selective estrogen receptor modulators (SERMs) for the brain: current status and remaining challenges for developing NeuroSERMs," *Brain Research Reviews*, vol. 49, no. 3, pp. 472–493, 2005.

[10] L. Zhao, K. O'Neill, and R. D. Brinton, "Estrogenic agonist activity of ICI 182,780 (Faslodex) in hippocampal neurons: implications for basic science understanding of estrogen signaling and development of estrogen modulators with a dual therapeutic profile," *Journal of Pharmacology and Experimental Therapeutics*, vol. 319, no. 3, pp. 1124–1132, 2006.

[11] S. Benvenuti, P. Luciani, G. B. Vannelli et al., "Estrogen and selective estrogen receptor modulators exert neuroprotective effects and stimulate the expression of Selective Alzheimer's Disease Indicator-1, a recently discovered antiapoptotic gene, in human neuroblast long-term cell cultures," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 3, pp. 1775–1782, 2005.

[12] E. Biewenga, L. Cabell, and T. Audesirk, "Estradiol and raloxifene protect cultured SN4741 neurons against oxidative stress," *Neuroscience Letters*, vol. 373, no. 3, pp. 179–183, 2005.

[13] M. Bourque, B. Liu, D. E. Dluzen, and T. Di Paolo, "Tamoxifen protects male mice nigrostriatal dopamine against methamphetamine-induced toxicity," *Biochemical Pharmacology*, vol. 74, no. 9, pp. 1413–1423, 2007.

[14] S. Callier, M. Morissette, M. Grandbois, D. Pelaprat, and T. Di Paolo, "Neuroprotective properties of 17β-estradiol, progesterone, and raloxifene in MPTP C57Bl/6 mice," *Synapse*, vol. 41, no. 2, pp. 131–138, 2001.

[15] B. Du, M. Ohmichi, K. Takahashi et al., "Both estrogen and raloxifene protect against β-amyloid-induced neurotoxicity in estrogen receptor α-transfected PC12 cells by activation of telomerase activity via Akt cascade," *Journal of Endocrinology*, vol. 183, no. 3, pp. 605–615, 2004.

[16] Y. Feng, J. D. Fratkins, and M. H. LeBlanc, "Treatment with tamoxifen reduces hypoxic-ischemic brain injury in neonatal rats," *European Journal of Pharmacology*, vol. 484, no. 1, pp. 65–74, 2004.

[17] M. Grandbois, M. Morissette, S. Callier, and T. Di Paolo, "Ovarian steroids and raloxifene prevent MPTP-induced dopamine depletion in mice," *NeuroReport*, vol. 11, no. 2, pp. 343–346, 2000.

[18] H. K. Kimelberg, "Tamoxifen as a powerful neuroprotectant in experimental stroke and implications for human stroke therapy," *Recent Patents on CNS Drug Discovery*, vol. 3, no. 2, pp. 104–108, 2008.

[19] H. K. Kimelberg, Y. Jin, C. Charniga, and P. J. Feustel, "Neuroprotective activity of tamoxifen in permanent focal ischemia," *Journal of Neurosurgery*, vol. 99, no. 1, pp. 138–142, 2003.

[20] O. N. Kokiko, A. K. Murashov, and M. R. Hoane, "Administration of raloxifene reduces sensorimotor and working memory deficits following traumatic brain injury," *Behavioural Brain Research*, vol. 170, no. 2, pp. 233–240, 2006.

[21] E. S. Y. Lee, Z. Yin, D. Milatovic, H. Jiang, and M. Aschner, "Estrogen and tamoxifen protect against Mn-induced toxicity in rat cortical primary cultures of neurons and astrocytes," *Toxicological Sciences*, vol. 110, no. 1, pp. 156–167, 2009.

[22] E. S. Y. Lee, M. Sidoryk, H. Jiang, Z. Yin, and M. Aschner, "Estrogen and tamoxifen reverse manganese-induced glutamate transporter impairment in astrocytes," *Journal of Neurochemistry*, vol. 110, no. 2, pp. 530–544, 2009.

[23] D. L. Lei, J. M. Long, J. Hengemihle et al., "Effects of estrogen and raloxifene on neuroglia number and morphology in the hippocampus of aged female mice," *Neuroscience*, vol. 121, no. 3, pp. 659–666, 2003.

[24] R. McMurray, R. Islamov, and A. K. Murashov, "Raloxifene analog LY117018 enhances the regeneration of sciatic nerve in ovariectomized female mice," *Brain Research*, vol. 980, no. 1, pp. 140–145, 2003.

[25] S. H. Mehta, K. M. Dhandapani, L. M. De Sevilla, R. C. Webb, V. B. Mahesh, and D. W. Brann, "Tamoxifen, a selective estrogen receptor modulator, reduces ischemic damage caused by middle cerebral artery occlusion in the ovariectomized female rat," *Neuroendocrinology*, vol. 77, no. 1, pp. 44–50, 2003.

[26] K. R. Mickley and D. E. Dluzen, "Dose-response effects of estrogen and tamoxifen upon methamphetamine- induced behavioral responses and neurotoxicity of the nigrostriatal dopaminergic system in female mice," *Neuroendocrinology*, vol. 79, no. 6, pp. 305–316, 2004.

[27] M. Morissette, S. A. Sweidi, S. Callier, and T. Di Paolo, "Estrogen and SERM neuroprotection in animal models of Parkinson's disease," *Molecular and Cellular Endocrinology*, vol. 290, no. 1-2, pp. 60–69, 2008.

[28] M. I. Rossberg, S. J. Murphy, R. J. Traystman, and P. D. Hurn, "LY353381.HCl, a selective estrogen receptor modulator, and experimental stroke," *Stroke*, vol. 31, no. 12, pp. 3041–3046, 2000.

[29] H. Zhang, M. Xie, G. P. Schools et al., "Tamoxifen mediated estrogen receptor activation protects against early impairment of hippocampal neuron excitability in an oxygen/glucose deprivation brain slice ischemia model," *Brain Research*, vol. 1247, pp. 196–211, 2009.

[30] P. S. Green, S. H. Yang, K. R. Nilsson, A. S. Kumar, D. F. Covey, and J. W. Simpkins, "The nonfeminizing enantiomer of 17β-estradiol exerts protective effects in neuronal cultures and a rat model of cerebral ischemia," *Endocrinology*, vol. 142, no. 1, pp. 400–406, 2001.

[31] M. E. Jung, A. M. Wilson, and J. W. Simpkins, "A nonfeminizing estrogen analog protects against ethanol withdrawal toxicity in immortalized hippocampal cells," *Journal of Pharmacology and Experimental Therapeutics*, vol. 319, no. 2, pp. 543–550, 2006.

[32] R. Liu, S. H. Yang, E. Perez et al., "Neuroprotective effects of a novel non-receptor-binding estrogen analogue: in vitro and in vivo analysis," *Stroke*, vol. 33, no. 10, pp. 2485–2491, 2002.

[33] J. W. Simpkins, Y. Wen, E. Perez, S. Yang, and X. Wang, "Role of nonfeminizing estrogens in brain protection from cerebral ischemia: an animal model of Alzheimer's disease neuropathology," *Annals of the New York Academy of Sciences*, vol. 1052, pp. 233–242, 2005.

[34] X. Wang, J. A. Dykens, E. Perez et al., "Neuroprotective effects of 17β-estradiol and nonfeminizing estrogens against H2O2 toxicity in human neuroblastoma SK-N-SH cells," *Molecular Pharmacology*, vol. 70, no. 1, pp. 395–404, 2006.

[35] I. Azcoitia, A. Moreno, P. Carrero, S. Palacios, and L. M. Garcia-Segura, "Neuroprotective effects of soy phytoestrogens in the rat brain," *Gynecological Endocrinology*, vol. 22, no. 2, pp. 63–69, 2006.

[36] D. A. Schreihofer and L. Redmond, "Soy phytoestrogens are neuroprotective against stroke-like injury in vitro," *Neuroscience*, vol. 158, no. 2, pp. 602–609, 2009.

[37] D. L. Lei, J. M. Long, J. Hengemihle et al., "Effects of estrogen and raloxifene on neuroglia number and morphology in the hippocampus of aged female mice," *Neuroscience*, vol. 121, no. 3, pp. 659–666, 2003.

[38] S. Tapia-Gonzalez, P. Carrero, O. Pernia, L. M. Garcia-Segura, and Y. Diz-Chaves, "Selective oestrogen receptor (ER) modulators reduce microglia reactivity in vivo after peripheral inflammation: potential role of microglial ERs," *Journal of Endocrinology*, vol. 198, no. 1, pp. 219–230, 2008.

[39] M. Cerciat, M. Unkila, L. M. Garcia-Segura, and M. A. Arevalo, "Selective estrogen receptor modulators decrease the production of interleukin-6 and interferon-γ-inducible protein-10 by astrocytes exposed to inflammatory challenge in vitro," *GLIA*, vol. 58, no. 1, pp. 93–102, 2010.

[40] T. Suuronen, T. Nuutinen, J. Huuskonen, J. Ojala, A. Thornell, and A. Salminen, "Anti-inflammatory effect of selective estrogen receptor modulators (SERMs) in microglial cells," *Inflammation Research*, vol. 54, no. 5, pp. 194–203, 2005.

[41] G. Barreto, M. Santos-Galindo, Y. Diz-Chaves et al., "Selective estrogen receptor modulators decrease reactive astrogliosis in the injured brain: effects of aging and prolonged depletion of ovarian hormones," *Endocrinology*, vol. 150, no. 11, pp. 5010–5015, 2009.

[42] J. L. Liu, D. S. Tian, Z. W. Li et al., "Tamoxifen alleviates irradiation-induced brain injury by attenuating microglial inflammatory response in vitro and in vivo," *Brain Research*, vol. 1316, pp. 101–111, 2010.

[43] D. S. Tian, J. L. Liu, M. J. Xie et al., "Tamoxifen attenuates inflammatory-mediated damage and improves functional outcome after spinal cord injury in rats," *Journal of Neurochemistry*, vol. 109, no. 6, pp. 1658–1667, 2009.

[44] J. Nilsen, G. Mor, and F. Naftolin, "Raloxifene induces neurite outgrowth in estrogen receptor positive PC12 cells," *Menopause*, vol. 5, no. 4, pp. 211–216, 1998.

[45] K. O'Neill, S. Chen, and R. D. Brinton, "Impact of the selective estrogen receptor modulator, tamoxifen, on neuronal outgrowth and survival following toxic insults associated with aging and Alzheimer's disease," *Experimental Neurology*, vol. 188, no. 2, pp. 268–278, 2004.

[46] E. Gould, C. S. Woolley, M. Frankfurt, and B. S. McEwen, "Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood," *Journal of Neuroscience*, vol. 10, no. 4, pp. 1286–1291, 1990.

[47] C. S. Woolley and B. S. McEwen, "Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat," *Journal of Comparative Neurology*, vol. 336, no. 2, pp. 293–306, 1993.

[48] B. S. McEwen and C. S. Woolley, "Estradiol and progesterone regulate neuronal structure and synaptic connectivity in adult as well as developing brain," *Experimental Gerontology*, vol. 29, no. 3-4, pp. 431–436, 1994.

[49] D. D. Murphy and M. Segal, "Regulation of dendritic spine density in cultured rat hippocampal neurons by steroid hormones," *Journal of Neuroscience*, vol. 16, no. 13, pp. 4059–4068, 1996.

[50] C. S. Woolley, "Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus," *Hormones and Behavior*, vol. 34, no. 2, pp. 140–148, 1998.

[51] F. Liu, M. Day, L. C. Muñiz et al., "Activation of estrogen receptor-β regulates hippocampal synaptic plasticity and improves memory," *Nature Neuroscience*, vol. 11, no. 3, pp. 334–343, 2008.

[52] K. Sharma, R. D. Mehra, P. Dhar, and U. Vij, "Chronic exposure to estrogen and tamoxifen regulates synaptophysin and phosphorylated cAMP response element-binding (CREB) protein expression in CA1 of ovariectomized rat hippocampus," *Brain Research*, vol. 1132, no. 1, pp. 10–19, 2007.

[53] X. Wu, M. A. Glinn, N. L. Ostrowski et al., "Raloxifene and estradiol benzoate both fully restore hippocampal choline acetyltransferase activity in ovariectomized rats," *Brain Research*, vol. 847, no. 1, pp. 98–104, 1999.

[54] M. Cyr, M. Landry, and T. Di Paolo, "Modulation by estrogen-receptor directed drugs of 5-hydroxytryptamine-2A receptors in rat brain," *Neuropsychopharmacology*, vol. 23, no. 1, pp. 69–78, 2000.

[55] M. G. Sánchez, M. Bourque, M. Morissette, and T. Di Paolo, "Steroids-dopamine interactions in the pathophysiology and treatment of cns disorders," *CNS Neuroscience and Therapeutics*, vol. 16, no. 3, pp. e43–e71, 2010.

[56] L. J. Smith, J. A. Henderson, C. W. Abell, and C. L. Bethea, "Effects of ovarian steroids and raloxifene on proteins that synthesize, transport, and degrade serotonin in the raphe region of macaques," *Neuropsychopharmacology*, vol. 29, no. 11, pp. 2035–2045, 2004.

[57] N. Lagunas, I. Calmarza-Font, D. Grassi, and L. M. Garcia-Segura, "Estrogen receptor ligands counteract cognitive deficits caused by androgen deprivation in male rats," *Hormones and Behavior*, vol. 59, no. 4, pp. 581–584, 2011.

[58] I. Gonzalez-Burgos, G. Tapia-Arizmendi, and A. Feria-Velasco, "Golgi method without osmium tetroxide for the study of the central nervous system," *Biotechnic and Histochemistry*, vol. 67, no. 5, pp. 288–296, 1992.

[59] A. Peters and I. R. Kaiserman-Abramof, "The small pyramidal neuron of the rat cerebral cortex—the synapses upon dendritic spines," *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, vol. 100, no. 4, pp. 487–506, 1969.

[60] L. Tarelo-Acuña, E. Olvera-Cortés, and I. González-Burgos, "Prenatal and postnatal exposure to ethanol induces changes in the shape of the dendritic spines from hippocampal CA1 pyramidal neurons of the rat," *Neuroscience Letters*, vol. 286, no. 1, pp. 13–16, 2000.

[61] I. González-Burgos, M. Alejandre-Gómez, and M. Cervantes, "Spine-type densities of hippocampal CA1 neurons vary in proestrus and estrus rats," *Neuroscience Letters*, vol. 379, no. 1, pp. 52–54, 2005.

[62] B. Leuner and T. J. Shors, "New spines, new memories," *Molecular Neurobiology*, vol. 29, no. 2, pp. 117–130, 2004.

[63] S. Hugel, M. Abegg, V. De Paola, P. Caroni, B. H. Gähwiler, and R. A. McKinney, "Dendritic spine morphology determines membrane-associated protein exchange between dendritic shafts and spine heads," *Cerebral Cortex*, vol. 19, no. 3, pp. 697–702, 2009.

[64] M. Matsuzaki, G. C. R. Ellis-Davies, T. Nemoto, Y. Miyashita, M. Iino, and H. Kasai, "Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons," *Nature Neuroscience*, vol. 4, no. 11, pp. 1086–1092, 2001.

[65] O. Ganeshina, R. W. Berry, R. S. Petralia, D. A. Nicholson, and Y. Geinisman, "Synapses with a segmented, completely partitioned postsynaptic density express more AMPA receptors than other axospinous synaptic junctions," *Neuroscience*, vol. 125, no. 3, pp. 615–623, 2004.

[66] M. C. Ashby, S. R. Maier, A. Nishimune, and J. M. Henley, "Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology," *Journal of Neuroscience*, vol. 26, no. 26, pp. 7046–7055, 2006.

[67] E. A. Nimchinsky, R. Yasuda, T. G. Oertner, and K. Svoboda, "The number of glutamate receptors opened by synaptic stimulation in single hippocampal spines," *Journal of Neuroscience*, vol. 24, no. 8, pp. 2054–2064, 2004.

[68] A. Majewska, A. Tashiro, and R. Yuste, "Regulation of spine calcium dynamics by rapid spine motility," *Journal of Neuroscience*, vol. 20, no. 22, pp. 8262–8268, 2000.

[69] R. Yuste, A. Majewska, and K. Holthoff, "From form to function: calcium compartmentalization in dendritic spines," *Nature Neuroscience*, vol. 3, no. 7, pp. 653–659, 2000.

[70] Y. Hayashi and A. K. Majewska, "Dendritic spine geometry: functional implication and regulation," *Neuron*, vol. 46, no. 4, pp. 529–532, 2005.

[71] J. Noguchi, M. Matsuzaki, G. C. R. Ellis-Davies, and H. Kasai, "Spine-neck geometry determines NMDA receptor-dependent Ca^{2+} signaling in dendrites," *Neuron*, vol. 46, no. 4, pp. 609–622, 2005.

[72] H. Schmidt and J. Eilers, "Spine neck geometry determines spino-dendritic cross-talk in the presence of mobile endogenous calcium binding proteins," *Journal of Computational Neuroscience*, vol. 27, no. 2, pp. 229–243, 2009.

[73] R. Araya, J. Jiang, K. B. Eisenthal, and R. Yuste, "The spine neck filters membrane potentials," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 47, pp. 17961–17966, 2006.

[74] C. Koch, A. Zador, and T. H. Brown, "Dendritic spines: convergence of theory and experiment," *Science*, vol. 256, no. 5059, pp. 973–974, 1992.

[75] E. Korkotian and M. Segal, "Structure-function relations in dendritic spines: is size important?" *Hippocampus*, vol. 10, no. 5, pp. 587–595, 2000.

[76] M. I. Pérez-Vega, A. Feria-Velasco, and I. González-Burgos, "Prefrontocortical serotonin depletion results in plastic changes of prefrontocortical pyramidal neurons, underlying a greater efficiency of short-term memory," *Brain Research Bulletin*, vol. 53, no. 3, pp. 291–300, 2000.

[77] K. M. Harris, J. C. Fiala, and L. Ostroff, "Structural changes at dendritic spine synapses during long-term potentiation," *Philosophical Transactions of the Royal Society B*, vol. 358, no. 1432, pp. 745–748, 2003.

[78] J. Bourne and K. M. Harris, "Do thin spines learn to be mushroom spines that remember?" *Current Opinion in Neurobiology*, vol. 17, no. 3, pp. 381–386, 2007.

[79] I. González-Burgos, "Dendritic spine plasticity and learning/memory processes: theory, evidence and perspectives," in *Dendritic Spines: Biochemistry, Modeling and Properties*, L. R. Baylog, Ed., pp. 163–186, Nova Science Publishers, Huntington, NY, USA, 2009.

[80] K. M. Harris, F. E. Jensen, and B. Tsao, "Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA 1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation," *Journal of Neuroscience*, vol. 12, no. 7, pp. 2685–2705, 1992.

[81] H. Kasai, M. Matsuzaki, J. Noguchi, N. Yasumatsu, and H. Nakahara, "Structure-stability-function relationships of dendritic spines," *Trends in Neurosciences*, vol. 26, no. 7, pp. 360–368, 2003.

[82] M. Matsuzaki, N. Honkura, G. C. R. Ellis-Davies, and H. Kasai, "Structural basis of long-term potentiation in single dendritic spines," *Nature*, vol. 429, no. 6993, pp. 761–766, 2004.

[83] V. Birzniece, A. Sata, S. Sutanto, and K. K. Y. Ho, "Neuroendocrine regulation of growth hormone and androgen axes by selective estrogen receptor modulators in healthy men," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 12, pp. 5443–5448, 2010.

[84] K. M. Webber, G. Perry, M. A. Smith, and G. Casadesus, "The contribution of luteinizing hormone to Alzheimer Disease pathogenesis," *Clinical Medicine and Research*, vol. 5, no. 3, pp. 177–183, 2007.

[85] G. Casadesus, K. M. Webber, C. S. Atwood et al., "Luteinizing hormone modulates cognition and amyloid-β deposition in Alzheimer APP transgenic mice," *Biochimica et Biophysica Acta*, vol. 1762, no. 4, pp. 447–452, 2006.