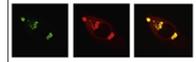
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Review

The structure and function of actin cytoskeleton in mature glutamatergic dendritic spines



Alba Bellot, Biuse Guivernau, Marta Tajés, Mònica Bosch-Morató,
Victòria Valls-Comamala, Francisco J. Muñoz*

Laboratory of Molecular Physiology and Channelopathies, Department of Experimental and Health Sciences, Universitat Pompeu Fabra (UPF), Barcelona, Catalonia, Spain

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ABSTRACT

Dendritic spines are actin-rich protrusions from the dendritic shaft, considered to be the locus where most synapses occur, as they receive the vast majority of excitatory connections in the central nervous system (CNS). Interestingly, hippocampal spines are plastic structures that contain a dense array of molecules involved in postsynaptic signaling and synaptic plasticity. Since changes in spine shape and size are correlated with the strength of excitatory synapses, spine morphology directly reflects spine function. Therefore several neuropathologies are associated with defects in proteins located at the spines. The present work is focused on the spine actin cytoskeleton attending to its structure and function mainly in glutamatergic neurons. It addresses the study of the structural plasticity of dendritic spines associated with long-term potentiation (LTP) and the mechanisms that underlie learning and memory formation. We have integrated the current knowledge on synaptic proteins to relate this plethora of molecules with actin and actin-binding proteins. We further included recent findings that outline key uncharacterized proteins that would be useful to unveil the real ultrastructure and function of dendritic spines. Furthermore, this review is directed to understand how such spine diversity and interplay contributes to the regulation of spine morphogenesis and

Abbreviations: ABPs, actin-binding proteins; A β , amyloid-beta peptide; AD, Alzheimer's disease; AMPARs, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors; APP, amyloid precursor protein; ASD, autistic spectrum disorders; AVC, amorphous vesicular clumps; BDNF, brain-derived neurotrophic factor, BDNF; CAMs, cell adhesion molecules; CA1-4, cornu ammonis; CaMKII, Ca²⁺/calmodulin-dependent protein kinase II; chemLTP, chemical LTP protocol; CNS, central nervous system; Eph, ephrins; EM, electron microscopy; FRAP, fluorescence recovery after photobleaching; FXS, Fragile X syndrome; GABA, gamma-aminobutyric acid; GEF, guanine nucleotide exchange factor; GPCRs, G-protein coupled receptors; HD, Huntington's disease; KO, knock-out; LTD, long-term depression; LTP, long-term potentiation; MAGUKs, membrane-associated guanylate kinase homologs; MAPs, microtubule-associated proteins; mGluRs, metabotropic glutamate receptors; mRNA, messenger ribonucleic acid; mRNP, messenger ribonucleoprotein; MTT, multiple trace theory; NMDARs, N-methyl-D-aspartate receptors; NGLs, neuroligins; NTD, N-terminal domain; NXNs, neurexins; PD, Parkinson's disease; PSD, postsynaptic density; RNAi, ribonucleic acid interference; SA, spine apparatus; SER, smooth endoplasmic reticulum; shRNA, short hairpin ribonucleic acid; siRNA, small interfering ribonucleic acid; SINE, short interspersed repetitive element; SNARE, Nethylmaleimide-sensitive factor attachment protein receptor complex; SYNPO, synaptopodin; UPR, unfolded protein response

*Correspondence to: Laboratori de Fisiologia Molecular i Canalopaties, Universitat Pompeu Fabra, C/Dr. Aiguader, 88, Barcelona 08003, Spain. Fax: +34 93 316 09 01.

E-mail address: paco.munoz@upf.edu (F.J. Muñoz).

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dynamics. It highlights their physiological relevance in the brain function, as well as it provides insights for pathological processes affecting dramatically dendritic spines, such as Alzheimer's disease.

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1. Introduction

The brain function relies on the organization of the neuronal circuitry, which is a vastly interconnected network of synapses. Synapses mediate neuronal communication primarily via neurotransmitters, which are endogenous chemical compounds that can be released from the pre- to the postsynaptic compartment. In general terms, they are composed of a varicosity or bouton from a presynaptic neuron that contains coated vesicles filled with neurotransmitters and communicates with the postsynaptic neuron, usually through a dendritic spine.

Throughout evolution, the vertebrate brain has acquired differential morphological modifications to achieve more complex functions. Thus, vertebrates developed spiny neurons to produce higher levels of cortical processing (Sala et al., 2008). Dendritic spines are membranous protrusions arising from the dendritic shaft, which are considered to be the locus of the vast majority of excitatory synapses in the central nervous system (CNS), accounting for almost the 90%. They are preferentially located on peripheral dendrites of neocortical and hippocampal pyramidal neurons, as well as in the striatum and in cerebellar Purkinje cells. Nevertheless, they can be also found on proximal dendrites or even on the

soma. Each spine receives inputs typically from one excitatory synapse, although spine-type synapses with inhibitory axons have also been described. In addition, there are smooth or aspiny neurons with dendrites carrying few or no spines that are immunopositive for gamma-aminobutyric acid (GABA) (Roussignol et al., 2005). However, for the purposes of the present work we focus on hippocampal spiny neurons, since they are key structures in learning and memory formation and they provide biochemical compartments that locally control and integrate signaling inputs into complex neural networks (Bourne and Harris, 2008).

During the last decades, different speculative hypothesis have been developed in order to grasp why excitatory axons choose to contact spines, since there are also aspiny neurons that form synapses directly on the dendritic shaft. Initially, three principal hypotheses were postulated to explain the function of spines (Lee et al., 2012).

The first one implies that spines connect axons to enhance synaptic connectivity and provide proper synaptic transmission, making the neuronal matrix more distributed.

The second one proposes that spines are electrically favorable, since spine neck morphology can impact the kinetics and propagation of synaptic potentials, allowing input-specific plasticity. In the third place, it is postulated

Table 1 – Spine abnormalities in brain-related disorders.

Neurological disorder	Spine abnormalities	Spine proteins	Animal models	References
ASD Prevalence Autism (0.13%); Asperger (0.03%)	Exaggerated spine formation or pruning; synaptic dysfunction; increased spine numbers	NRXN1, NLGN3/4; Shank3 (maintenance), Shank2 (remodeling); Epac2 (RapGEF4)	NLGN3-R451C knock-in mice: enhanced spatial learning and impaired social interaction; Shank3(+/ Δ C) mice: reduced NMDAR function; 4EBP2 KO mice: increased NGL1-4 translation	Penzes et al. (2011), Bangash et al. (2011)
FXS Prevalence Males (1/ 2500–4000) Females (1/ 7000–8000)	Perturbed spine maturation (filopodia-like spines; synaptic dysfunction; increased spine density)	FMRP mRNA in dendrites binds to PSD95; SAPAP3/4; CamKII; GluR1/2; Caveolin1—mGluR1/5; dysregulation of Arc mRNA translation	Fmr1 KO mouse: enhanced mGluR-LTD and AMPAR endocytosis (ERK1/2—Rac1)	Waug et al. (2008) , Penzes et al. (2011), Thomas et al. (2012)
Down's syndrome Prevalence: 1/ 691 (USA)	Synaptic dysfunction; decreased spine density; impaired arborization, shorter basal dendrites	Debrin, Arp2/3 complex and coronin-like protein p57	Ts65Dn mouse: spine enlargement and deficits in LTP	Nimchinsky et al. (2002), Belichenko et al. (2004)
Schizophrenia Prevalence: 0.5–1%	Exaggerated spine pruning and reduced spine density	NRG1 (L1)—ErbB4 (Rc); DISC1 regulates access of kalirin7 to Rac1; ErbB4 and DISC1: low PSD95	NRG1—ErbB2 and ErbB4 KO mice: reduced spine density	Penzes et al. (2011)
Epilepsy Prevalence: 50 million patients worldwide	Perturbed synaptic plasticity (F-actin and glutamatergic transmission); reduced spine density and spine degeneration	Synaptopodin, DebrinA, acidic calponin	Kindled mouse model; pilocarpine-induced TLE model	Zhang et al. (2009), Ferhat (2012)
AD Prevalence: 26.6 million patients worldwide	Hallmarks: neuronal death, senile plaques (A β), neurofibrillary tangles (Tau). Spine loss (perturbed spine maintenance)	Synaptophysin, Debrin, cofilin, kalirin-7-Rac1-PAK pathway; Activation of PP2B and GSK-3 β	Tg2576 mouse: mutant hAPP and decreased spine density 3xTg-AD mice: mutant APP, PSEN1 and Tau (A β and Tau pathology)	Penzes et al. (2011), Spire-Jones and Knafo (2012)

ASD: Autism spectrum disorder; FXS: Fragile X syndrome; AD: Alzheimer's disease.

that spines play a biochemical role, due to the spatial confinement of biochemical signaling achieved by diffusional restriction and physical segregation of proteins.

More recently, an integrated and not mutually exclusive view of these three hypothetical roles of spines has aroused grouping them into a common goal: achieving the distributed circuit with widespread connectivity of our brain ([Yuste, 2011](#)).

Taking all together, since dendritic spines are the major sites of information processing in the CNS, insults to synapses can lead to alterations in spine morphology and, consequently, to the cognitive impairment characteristic of aging and even to neurodegenerative disorders ([Table 1](#)).

2. Structure and composition of dendritic spines

The variety and heterogeneity in protein composition of spines, even within the same dendrite, makes them worth-studying structures. Indeed spine size and density influence the functioning of neuronal circuits ([Ultanir et al., 2007](#)).

2.1. Types of dendritic spines

The knowledge of dendritic spines has risen during the past decades, mainly thanks to the development of novel imaging

techniques such as electron microscopy (EM), confocal microscopy and two-photon imaging or three-dimensional (3D) reconstructions of serial ultrathin sections ([García-López et al., 2010](#)), which have led to the visualization of the dendritic spine ultrastructure.

Spines are typically categorized into different groups ([Fig. 1](#)). Based on anatomical studies of fixed brain tissue, [Peters and Kaiserman-Abramof \(1970\)](#) established three main categories: the most common are thin spines (long neck and smaller bulbous heads, <0.6 μ m in diameter); mushroom spines (constricted neck and large bulbous head, >0.6 μ m in diameter); and stubby spines (similar head and neck widths) ([Bourne and Harris, 2008](#)). However, since live imaging studies have revealed that spines can be quite dynamic structures ([Honkura et al., 2008](#); [Hotulainen and Hoogenraad, 2010](#)), changing size and shape over timescales of seconds to minutes, this static view in fixed structures does not reflect the real *in vivo* situation and thus the reliability of these categories remains unclear.

Other approaches using confocal microscopy ([Fig. 2](#)) have enabled the distinction of additional spine categories. Filopodia-like spines are transient protrusions mostly found on developing spines and characterized by the absence of the spine head. Spines can also be cup-shaped, branched (containing two or more heads and postsynaptic density; PSDs), or single protrusions with multiple synapses ([Sala et al., 2008](#)).



Fig. 1 – Classification of dendritic spines. Dendritic spines can be classified based on anatomical studies in three major types: stubby, mushroom and thin.

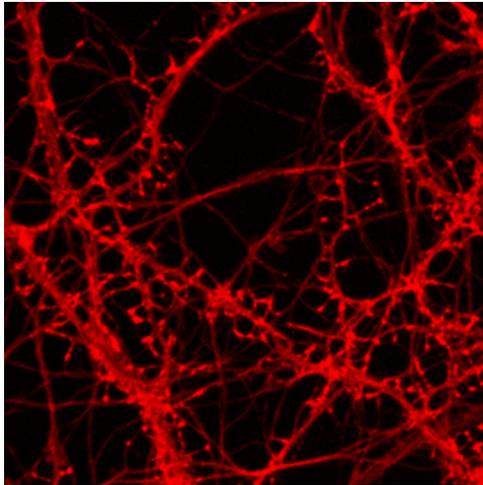


Fig. 2 – Image of hippocampal dendritic spines. Dendritic spines in neurites from primary cultures of mouse embryonic hippocampal neurons after ten days in culture (staining with phalloidin and visualized by confocal microscopy).

Moreover, they can show differences according to the region of the hippocampus where they are found. For instance, in CA3 there are irregular or “thorny” spines, whereas in CA1, the spine’s neck can range from 0.04 to 0.5 μm .

Ultimately spinules, without PSD, are seen adjacent to the active zone and invaginate into the presynaptic terminal (Nimchinsky et al., 2002).

2.2. Composition of dendritic spines

Despite having a small volume (~ 0.1 fl), spines are actin-rich protrusions with thousands of proteins, among other molecular components involved in different functions (Murakoshi and Yasuda, 2012). They are characterized by the absence of free ribosomes and mitochondria as well as microtubules or intermediate filaments, which are present only as bipolar arrays within the dendrites (Calabrese et al., 2006), although some components of the PSD have been shown to interact biochemically with tubulin and microtubule-associated proteins (MAPs) (Fuhrmann et al., 2002). In fact, the microtubule-associated protein 1B (MAP1B) seems to regulate dendritic spine development and synaptic function (Tortosa et al., 2011).

2.2.1. Postsynaptic density

The PSD is a thickening of the postsynaptic membrane that is found at the synaptic junction, exactly aligned with the

presynaptic active zone. It is usually located at the head of the largest spines, occupying roughly 10% of the surface area (Sheng and Hoogenraad, 2007). It was first identified with transmission EM, as an electron-dense specialization of the postsynaptic neuron. The PSD comprises hundreds of proteins involved in synaptic plasticity, including neurotransmitter receptors like N-methyl-D-aspartate receptors (NMDARs), α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA), kainate and metabotropic glutamate receptors (mGluRs), along with a myriad of signaling proteins and other scaffolding proteins such as Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) or PSD-95 protein (Bourne and Harris, 2008).

2.2.2. Actin cytoskeleton

The role of actin dynamics in spine structural plasticity as well as the effects of Ca^{2+} signaling on spine ultrastructure and function have been widely reviewed in the literature (Bhatt et al., 2009; Bourne and Harris, 2008; Ethell and Pasquale, 2005; Higley and Sabatini, 2012; Tada and Sheng, 2006). Both monomeric (G-actin) and filamentous (F-actin) actin are present in spines, below the PSD. Whereas spine heads mainly consist of a network of branched and F-actin, in the neck filaments usually form long bundles lengthwise the spine apparatus (Hotulainen and Hoogenraad, 2010; Rochefort and Konnerth, 2012).

Recently, some studies have proposed the existence of different actin pools within the dendritic spine. A dynamic pool is believed to be found below the spine surface, whereas another pool is thought to be more stable to support the overall spine structure. A third pool of stable F-actin has been described upon glutamate uncaging or long term potentiation (LTP) induction, (Honkura et al., 2008) and its confinement to dendritic spines seems to require CaMKII activity (Okamoto et al., 2009).

Actin filaments in the spine head are very dynamic and show a high turnover, with a total protein replacement every 2–3 min (Honkura et al., 2008). Furthermore, previous studies show that the degree of actin polymerization, and hence the G-actin/F-actin ratio, affects the various aspects of dendritic spine morphology (Murakoshi and Yasuda, 2012).

One of the most relevant roles of actin cytoskeleton in mature spines is to modulate spine head structure in response to postsynaptic signaling (Star et al., 2002). In addition it contributes to overall structure of synapses, organizing the PSD (Sheng and Hoogenraad, 2007), anchoring and stabilizing postsynaptic receptors and localizing the translation machinery (Hotulainen and Hoogenraad, 2010).

Several actin-binding proteins (ABPs) as well as other actin-associated proteins are enriched in dendritic spines and cooperate to regulate actin-based cellular events (Fig. 3 and Table S1). Some of them play a major role in actin nucleation like the Arp2/3 complex, which orchestrates *de novo* actin polymerization, and its activators Cortactin, Abp1, N-WASP, WAVE-1 and Abl interactor 2 (Abi2). There are evidences involving the Arp2/3 complex in synaptic plasticity, since after LTP induction it promotes spine head enlargement. More recently Kim et al. (2013) designed a conditional knock-out (KO) of the Arp2/3 complex that lead to a progressive loss of dendritic spines. In accordance, inhibition of

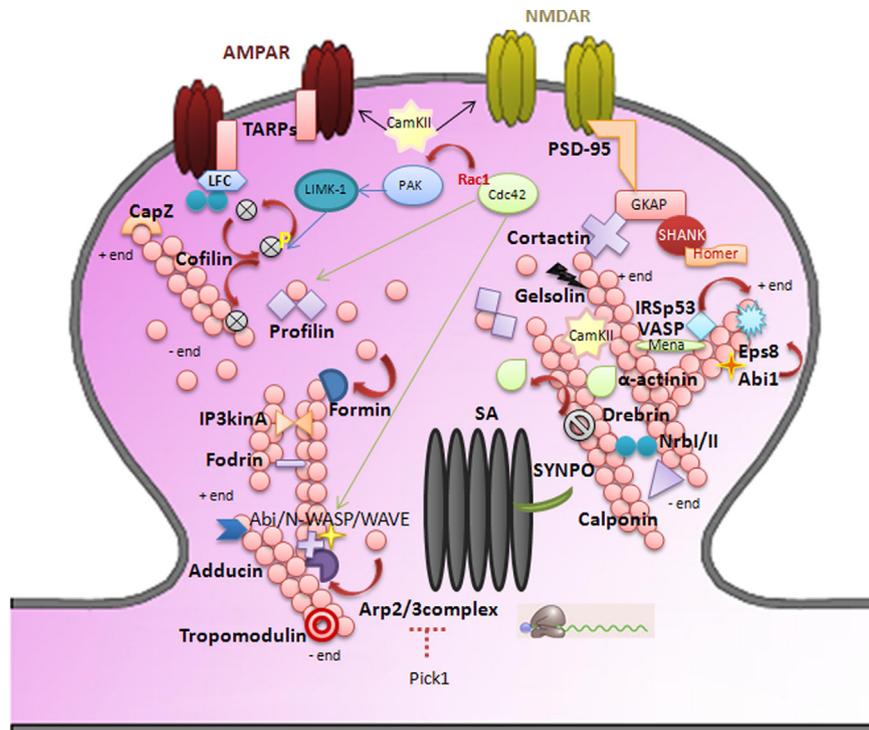


Fig. 3 – Actin-binding proteins (ABPs). ABPs in a dendritic spine involved in actin nucleation (Arp2/3 complex, Formin or DRF3/mDia2, N-WASP, WAVE-1, Abp1, Abi2 and cortactin), actin polymerization (Profilin I/II), actin capping (Capz, Adducin and Tropomodulin), actin severing (ADF/cofilin and gelsolin) and actin bundling (α actinin, α Ncatenin, acidic calponin, Nrb1, NrbII or spinophilin, Derbina, CamKII β , IRSp53, SYNPO, Fodrin or brain spectrin, IP3kinA). Recent findings and interactions are also represented such as Pick1 (inhibits Arp2/3 complex), Eps8 (involved in actin capping) or NESH/Abi3 (involved in actin nucleation).

Arp2/3 complex activators alters the morphology and number of spines. NESH/Abi3 has been described recently as a novel F-actin binding protein that likely plays important roles in the regulation of dendritic spine and synapse formation (Bae et al., 2012). Another recent finding is the interaction between Dock4, a guanine nucleotide exchange factor (GEF) for Rac, and cortactin, which also seems to be important in the regulation of dendritic spine formation (Ueda et al., 2013).

Other actin cytoskeleton-interacting proteins participate in F-actin severing (ADF/cofilin and gelsolin) or actin polymerization (profilin). Upon NMDAR activation, calcineurin/PP2B causes a dephosphorylation of cofilin through slingshot protein phosphatase 1 (SSH1) activity, and active cofilin is translocated into dendritic spines for remodeling, under the control of β -arrestin 2, playing a role in synapse plasticity (Pontrello et al., 2012). Thus, cofilin is essential in controlling the turnover of F-actin at synapses and an overload of F-actin may result in alterations in spine morphology and synapse density, leading to impaired associative learning. Recently it has been shown that upon stress, the rapidly activated cofilin saturates F-actin causing actin filaments to bundle into rod structures, freeing ATP, in a process called cofilin-actin rod stress response (Munsie and Truant, 2012a).

This freed ATP could be used by the protein folding machinery to undergo the unfolded protein response (UPR), as a physiological mechanism needed to address all the neuronal components for the spine growth (Costa-Mattioli et al., 2005).

Overall, the cofilin-actin rod stress response seems to be involved in alleviating a pool of ATP that is normally used for active actin processes, so it can be used elsewhere under pathological conditions (Munsie and Truant, 2012b).

There are also ABPs (α -actinin, α -catenin, Calponin, NeurabinI/NrbI, Spinophilin/NrbII, DebrinA, CaMKII β , insulin receptor substrate p53/IRSp53, Synaptopodin/SYNPO and the vasodilator-stimulated phosphoprotein /VASP) and actin-capping proteins (Eps8, CapZ or Tropomodulin) involved in the bundling of F-actin. Specifically, Eps8 promotes the spinogenesis. Mice lacking Eps8 showed immature spines and a impaired synaptic plasticity (Menna et al., 2013).

More recently, microRNAs have been involved in the actin cytoskeleton regulation. Several evidences also suggest a role for small GTPases (Rac, RhoA, and Cdc42) in the reorganization of F-actin and spine formation (Calabrese et al., 2006).

Altogether, these data provide insights into the pivotal role of actin cytoskeleton in the formation and elimination, motility and stability, size and shape of dendritic spines. It also evidences that the reorganization of F-actin is correlated with synaptic efficacy.

2.2.3. Organelles at the synaptic spine

All dendritic spines in cerebellar Purkinje neurons and roughly half of spines on hippocampal CA1 or cortical cells (Nimchinsky et al., 2002), contain the Smooth Endoplasmic Reticulum (SER), both found in the pre- and postsynaptic compartments. Spine SER is characteristically seen in mature

spines of cortical and hippocampal neurons and can be further specialized to form the so-called spine apparatus (SA) (Segal et al., 2010).

The SA is an organelle that consists of at least two membrane-bound sacs separated by plaques of electron-dense material (Fig. 3) and its formation depends on synaptopodin (SYNPO), an F-actin interacting protein since SYNPO-KO mice lacked SA and showed lower LTP (Deller et al., 2003). This protein has been observed early during the spine development in strategic locations, playing a role in Ca^{2+} handling, in both the spine neck of mature dendritic spines, colocalizing with the SA and axons, where it binds to F-actin. Thus, upon spine maturation SYNPO may be translocated into the SA.

Interestingly, release of Ca^{2+} from stores produces a SYNPO-dependent delivery of GluA1 subunit-containing AMPAR into spines, suggesting a possible role in structural and functional plasticity, as well as in homeostatic plasticity (Vlachos et al., 2013).

In addition, at the base of the dendritic spines, there are clusters of polyribosomes, likely involved in providing newly synthesized gene products locally (Calabrese et al., 2006). Furthermore Buckley et al., (2011) have provided new striking insights on a novel mechanism for mRNA targeting into dendrites. Specifically, they identified a group of mRNAs that retain intronic sequences containing ID elements, a form of SINE (short interspersed repetitive elements) retrotransposon, which could be responsible for targeting the exogenous RNA into dendrites.

On the other hand, maintenance of LTP also relies on proteasomes to degrade proteins (Bourne and Harris, 2008). Endosomes, clathrin-coated vesicles and large amorphous vesicular clumps (AVC), are found occasionally in spines and provide a sufficient membrane pool for spine growth and protein turnover (Bourne and Harris, 2008).

Finally, mitochondria are present in the dendritic shaft but are rarely found in spines. They undergo translocation during active synapse formation or remodeling to provide the energy needed for signal transduction in the spine (Sheng and Hoogenraad, 2007).

3. Formation of dendritic spines and structural plasticity

Since the first observations of spines at the end of the 19th century, many groups have postulated different models for spinogenesis, which suggest that different mechanisms may underlie this plastic process (Ethell and Pasquale, 2005; Segal et al., 2000; Yuste and Bonhoeffer, 2004).

3.1. Formation and maturation of dendritic spines

Early in development, dendritic stubby or filopodia spines are highly abundant and motile structures. Their density reaches its maximum peak during late development, and then decreases to a relatively stable level throughout adulthood (Bhatt et al., 2009).

In general terms, spine development, which directly correlates with synaptogenesis, starts with an adhesive contact

between dynamic filopodia and the presynaptic axon. Some filopodia might receive a synaptic input, allowing them to convert into dendritic spines, whereas others form shaft synapses that can either reemerge as spines or become eliminated later in life (Bourne and Harris, 2008).

Several proteins have been suggested to be involved in early synapse formation, including cadherins, ephrins (Eph) and Eph receptors, integrins, neuroligins (NLGNs) and neuroligins (NGLs), densin-180 and Narp. Telencephalin has been shown to relocate to the dendritic shaft, after spine formation, where it is replaced by N-cadherin and α -catenin, and it is responsible for newly-formed spines stabilization (Matsuno et al., 2006).

Nascent spines are characterized by the presence of postsynaptic NMDARs but not AMPARs (Hanse et al., 2009). The latter involves dynamic reorganization of the actin cytoskeleton that changes spine morphology from thin filopodium-like protrusions into stubby and, ultimately, mushroom-shaped spines. It is also depending on the recruitment of both presynaptic and postsynaptic elements (Washbourne et al., 2004), such as AMPARs and other components of the PSD, like SynGAP, which interacts with PSD-95 and links NMDARs to Ras signaling pathways (Kim et al., 1998).

Stabilization of dendritic spines also relies on the function of different proteins and molecular events. Particularly membrane-associated guanylate kinase homologs (MAGUKs) (Oliva et al., 2012), SAP102 (synaptogenesis) and PSD-95 (maturation) have been shown to play a role in the transition from immature to mature synapses (Elias, 2008). A crucial step is the insertion of AMPARs into the synaptic membrane and its activation in particular decreases spine motility and stabilizes spine shape (Bassani et al., 2013).

Spinogenesis is also regulated by microRNAs and their dysregulation has been recently linked to several neurological pathologies such as epilepsy (Jimenez-Mateos et al., 2012) or autism (Sarachana et al., 2010). Further *in vivo* studies have revealed that mature hippocampal neurons can also produce new dendritic spines, establishing synapses with preexisting axonal boutons forming multiple synapses. This suggests that the mature brain is also able to undergo some kind of remodeling, through the formation of new synapses and spines, a process termed adult neurogenesis. However, it remains to be determined whether this process also involves transient filopodia, which are rare in mature neurons (Sorra and Harris, 2000), or other elements such as astrocytes, which may help newborn neurons to reach their specific targets and not others, probably located closeby (Toni and Sultan, 2011).

Recent insights into the molecular mechanisms that regulate spine morphogenesis unveil potential strategies to manipulate dendritic spines *in vivo* and to explore their physiological roles in the brain, linking alterations in spine morphogenesis to neuropathological conditions (Table 1).

3.2. Synaptic remodeling and turnover of dendritic spines

Growing evidence links dendritic spine morphology not only to synaptic strength (functional plasticity) but also to dynamic remodeling and turnover of synaptic proteins (Kasai et al., 2010). This structural plasticity has been

associated with physiological effectiveness and may be important for memory and cognitive processes (Penzes et al., 2011). Despite the high rate of spine turnover seen in some studies, due to the dynamicity of the actin cytoskeleton, spine density remains stable because only a small proportion of the newly formed spines become finally mature spines, whereas the vast majority are eliminated (De Roo et al., 2008).

Although this relative stability in the adulthood, spines continue to change over time and spine formation, pruning and remodeling can still occur, suggesting that these structures remain plastic in the adult brain (Bhatt et al., 2009).

Imaging methods and other techniques have demonstrated that the stabilization of new protrusions occurs over a critical period of 24 h (Alvarez et al., 2007). This process is associated with a structural enlargement of the spine head (mushroom-like spines), which correlates with PSD expression and the increase in local proteins, mRNA and organelles.

In addition, different experimental or behavioral conditions (Bourne and Harris, 2008) have been shown to influence spine morphology, such as age, neurotrophins (e.g. brain-derived neurotrophic factor; BDNF) or synaptic activity (e.g. LTP).

4. Synaptic plasticity

Synaptic plasticity is intimately associated to LTP and long term depression (LTD). Induction of LTP and LTD has been associated with the enlargement or shrinkage of the spine, respectively (Bourne and Harris, 2008). LTP is a long-lasting enhancement in synaptic transmission between two neurons that results from synchronous or strong stimulation and can be mimicked by brief (≤ 2 s) high frequency stimuli (50–200 Hz, typically 100 Hz for tetanic stimulation), leading to an increase in synaptic strength (Bliss and Lomo, 1973; Kaibara and Leung, 1993). LTD is a long-lasting reduction in synaptic transmission caused by weak, maintained (≥ 30 s) and low-frequency stimulation (< 10 Hz) that occurs via activation of NMDARs and PP2B that also involves ADF/cofilin (Alvarez et al., 2007; Lee et al., 1998). While LTP is in part due to the activation of protein kinases, which subsequently phosphorylate target proteins, LTD arises from activation of calcium-dependent phosphatases such as PP2B and phosphatase 1 (PP1), that can be activated by a weak entry of Ca^{2+} through NMDARs.

4.1. Structural changes

The fact that essential molecules for spine formation are involved in LTP and memory indicates that morphological changes might participate in the maintenance of the structural plasticity in dendritic spines (Fifková and Van Harrevel, 1977; Bosch and Hayashi, 2012) (Fig. 4).

Integrating previous information, Yuste and Bonhoeffer (2004) hypothesized a sequential model of structural events occurring at spines during LTP, in which functional changes occur during the first minutes and morphological changes become detectable after 30 min. Then, perforated synapses are formed and bifurcated spines emanating from the same dendrite might split. However, other authors have reported

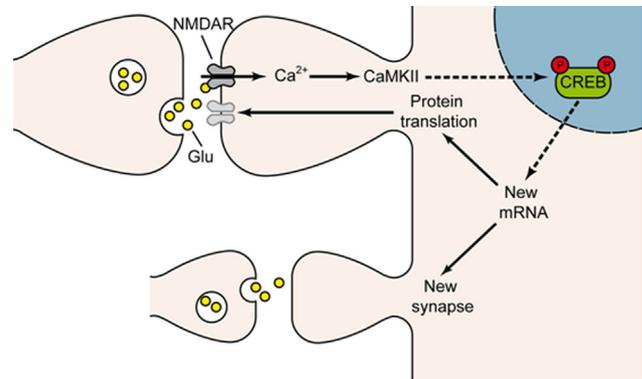


Fig. 4 – LTP molecular mechanisms involved in dendritic spines growth. LTP structural changes require the binding of glutamate to NMDAR, as well as to AMPAR in many cases, to achieve the postsynaptic depolarization. NMDAR activation allows a massive Ca^{2+} influx that activates different downstream kinases such as CamKII. This will induce the nuclear translocation of CREB to initiate the transcription of new proteins to allow the growth of new dendritic spines.

contradictory results (Fiala et al., 2002), claiming for an alternative mechanism, such as the growth of new spines. This controversy could be explained, in part, by the low resolution of light and EM techniques.

It is accepted that there are morphological alterations after LTP and LTD paradigms since they have been largely studied and include changes in spine number, size and shape (Ultanir et al., 2007). For instance, two-photon microscopy experiments have shown that induction of LTP in hippocampal cultures leads to the formation of new spines (Lamprecht, 2011). Thus, increasing spine density could contribute to enhanced transmission, since more connections would be made with the presynaptic neuron. Similar increases in spine head volume have been showed using a chemical LTP protocol (chemLTP) (Alvarez et al., 2007). These morphological changes of spines depend on NMDARs activation and might contribute to the activity-dependent formation and elimination of synaptic connections.

On the other hand, LTD results in reduced spine number and synaptic surface, and this LTD-induced plasticity is thought to be more predominant in presynaptic boutons (Bourne and Harris, 2008). In addition, a widening and shortening of the spine neck have also been reported, which may in turn alter Ca^{2+} influx into the spine.

Experiments using fluorescence recovery after photobleaching (FRAP) have shown that the spread of AMPARs is limited at the spine neck, providing further evidence that alterations in spine shape could modulate synaptic function (Ashby et al., 2006).

4.2. Composition changes

Synaptic plasticity is associated with a rapid reorganization of the spine actin cytoskeleton, which in turn affects spine morphology, showing evidence for a bidirectional regulation (Hotulainen and Hoogenraad, 2010).

4.2.1. Actin cytoskeleton reorganization

The sustained spine enlargement induced by LTP is accompanied by an increase in F-actin levels, suggesting that actin polymerization is required. The actin cytoskeleton becomes stabilized by cofilin inactivation (Chen et al., 2007) and by the bundling action of CaMKII and other actin-binding proteins (Okamoto et al., 2009). On the other hand, LTD results in spine shrinkage associated with actin depolymerization (Honkura et al., 2008). These observations suggest that the reorganization of the actin cytoskeleton is correlated with synaptic plasticity and strengthening of synapses.

4.2.2. Role of CaMKII and other signaling pathways during LTP

CaMKII is an important regulator of dendritic growth and synapse formation (Table S2). CaMKII is a serine/threonine kinase that upon activation through the binding of Ca^{2+} /calmodulin is translocated to the PSD, becoming one of its major constituents (Murakoshi and Yasuda, 2012). The binding of activated CaMKII to GluN2B, as well as to F-actin, may also participate in its targeting (Bayer et al., 2006).

These interactions appear to be required for hippocampal late phase of LTP and some forms of learning and memory, as evidenced by CaMKII β KO mice, which showed deficits in LTP and in synaptic plasticity and learning (Borgesius et al., 2011).

In addition, reduction of endogenous CaMKII β using specific short hairpin RNA (shRNA) significantly affects the shape of mature spines, turning them into immature filopodia-like structures (Okamoto et al., 2009, 2007). Active CaMKII also phosphorylates GluA1, leading to AMPARs exocytosis and increasing synaptic efficacy (Lisman et al., 2012; Lu et al., 2010). A recent study shows that maintenance of reconsolidated memory, together with the balance between protein synthesis and degradation, depends on CaMKII and may enhance activity-dependent localized protein renewal, leading to long-term memory improvement (Da Silva et al., 2013).

Other studies reveal that CaMKII α is also important for the regulation of group I mGluR-induced protein synthesis and LTD (Mockett et al., 2011) in the hippocampus, further supporting crucial roles of this kinase in memory processes.

This kinase phosphorylates PSD-95 leading to its translocation out of the spine together with SHANK2, which has been involved in spine growth, giving rise to PSD disassembly. CaMKII also phosphorylates GluN1, which enhances AMPAR conductance, as well as Stargazing, thereby increasing the density of AMPARs at the synapse and enhancing synaptic transmission (Murakoshi and Yasuda, 2012). Downstream, CaMKII also phosphorylates a number of signaling molecules such as members of the Rho-GTPase family (RhoA, Cdc42, Rac1, and Rnd1), which are potent regulators of F-actin dynamics, directly implicated in spine morphogenesis and plasticity (Okamoto et al., 2009).

Several kinase, phosphatase and protease families have also been implicated in synaptic plasticity and are summarized in Table S2. Kinases include the Src family (Src, Fyn, Yes, Lck, and Lyn), Abl family, (Abl and Arg), as well as IP3 3-kinase A (IP3kinA) or SNK kinases. More recently, NF- κ B inducing IKK kinase complex has been shown to be critically involved in synapse formation and spine maturation in the adult brain (Schmeisser et al., 2012). There are many other

protein kinases present in the PSD, such as PKC, which also plays a critical role in synaptic plasticity and appears to regulate spine formation. PKC activation leads to rapid dispersal of NMDARs and to endocytosis of GluA2-containing AMPARs (Groc et al., 2004). Thus, overexpression of PKC results in spine loss and altered spine morphology. Some studies suggest that PKC acts via phosphorylation of MARCKS to negatively regulate dendritic spine maintenance (Kim et al., 2010).

Furthermore, phosphatases, like PP1 and PP2B, have roles in synaptic transmission and spine plasticity. Also calpain, a Ca^{2+} -dependent protease, found in the PSD, is responsible for the cleaving of spine proteins and for altering the structure of the PSD (Tashiro and Yuste, 2004). Finally, extracellularly secreted molecules such as glial factors (Ethell and Pasquale, 2005), as well as estrogens (Segal et al., 2010) and BDNF (Lisman et al., 2012) can also influence spine development, possibly through a mechanism involving CREB activation.

4.2.3. Glutamate receptors

Most excitatory synapses use glutamate for synaptic transmission and thereby, numerous postsynaptic proteins bind to glutamate receptors, including the PSD-95 and S-SCAM families; α -actinin, spectrin and yotiao for NMDARs; and GRIP, ABP and PICK1 for AMPARs.

NMDARs regulate dendritic spine morphology and stabilization. This is supported by evidences showing that mice lacking GluN3A subunit have increased spine densities in pyramidal neurons (Ethell and Pasquale, 2005). Whereas the loss of GluN2B is sufficient to cause LTD impairment, resulting in reduced spine density and learning deficits (Brigman et al., 2010). Thus, different glutamate receptor subunits confer distinct physiological and molecular properties to the synapse.

NMDARs activation results in an improved synaptic efficiency accompanied by increased number of synapses and structural remodeling of existing spines, which become enlarged and possess perforated PSDs (Toni et al., 1999). Moreover, knocking down NMDARs through RNA interference (RNAi) blocks LTP and increases spine motility and elimination, indicating that NMDAR-dependent actin polymerization is important for the consolidation of the early phase into the late phase of LTP (Foster et al., 2010).

More recently, Sanhueza and Lisman (2013) showed that the CaMKII-NMDAR complex serves as a tag, leading to the sequential binding of several proteins (densin, δ -catenin, N-cadherin), some of which are newly synthesized, playing a crucial role in LTP maintenance.

Another study, intended to unveil the locus and mechanisms underlying the NMDAR-mediated LTP, concluded that both presynaptic and postsynaptic NMDAR expression mechanisms contribute to this type of synaptic plasticity (Bliss and Collingridge, 2013).

The C-terminus of both GluN1 and GluN2 subunits interacts with scaffolding proteins (Fig. 5) and is phosphorylated by PKA and PKC, which increases Ca^{2+} entry via NMDARs (Rebola et al., 2010).

PSD scaffolding proteins can influence synapse development and spine morphogenesis and are differentially associated with either NMDARs or AMPARs. PSD-95 binds

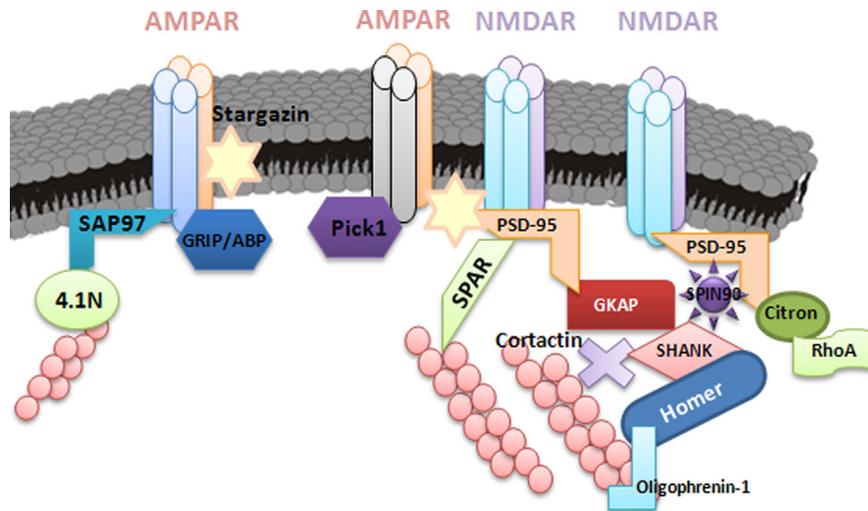


Fig. 5 – Glutamate receptors and PSD proteins in dendritic spines. The different subunits of AMPARs and NMDARs interact with different proteins at the dendritic spines. AMPAR: GluA2 (orange); GluA1 (dark blue); GluA3 (grey). NMDAR: GluN1 (purple); GluN2 (light blue). SPAR (RapGAP) is an attractive candidate for mediating activity-dependent remodeling of synapse. Kim et al. (2009) has also identified a novel Shank binding partner, SPIN90, an actin-binding protein that interacts directly with PSD95, which is involved in the maintenance of spine morphology.

directly to GluN2 subunit of NMDARs and makes a complex which stabilizes nascent spines, presumably by recruiting scaffolding proteins and anchoring receptors at the synapse. Although PSD-95 is not essential for the localization of NMDARs, knockdown of PSD-95 results in spine loss, thereby revealing a role in spine stabilization (Ehrlich et al., 2007).

The NMDAR-PSD-95 complex includes a variety of interacting proteins that may play a role in regulating actin dynamics (Table S3). SynGAP (RasGAP) also appears to regulate the actin cytoskeleton via Ras inhibition. BCR and ABR (RacGAPs) may regulate synaptic Rac1 activity. Mice lacking these proteins exhibit impaired LTP maintenance and memory deficits (Oh et al., 2010).

Furthermore, Kalirin-7 also interacts with PSD-95, in order to regulate spine development and plasticity, being recently implicated in synaptic disorders, such as schizophrenia or AD (Penzes et al., 2011). Recent findings also place the scaffolding protein IQGAP1 as a crucial regulator of spine density and shape, through the N-Wasp-Arp2/3 complex and Cdc42 (Jausoro et al., 2013).

Shank is a multidomain scaffold protein in the PSD that binds to NMDARs and mGluRs. Shank forms a complex with Homer involved in spine morphogenesis and it also associates with F-actin (Fig. 5). As with PSD-95, overexpression of Shank-Homer accelerates spine maturation and promotes the enlargement of mature spines (Sala et al., 2008). AMPARs and NMDARs exist at both synaptic and extrasynaptic locations and have been shown to laterally diffuse through the membrane, as well as some PSD proteins (Bourne and Harris, 2008). However, whereas NMDARs are relatively stable components of the PSD, AMPARs cycle on and off the synaptic membrane. Interestingly, this dynamic balance modulates synaptic strength and underlies certain forms of plasticity such as LTP (Murakoshi and Yasuda, 2012).

Adjacent to the PSD there is the endocytic zone, a stable membrane “hot spot” essential for the recycling of the

synaptic pool of mobile AMPARs, which is required for synaptic potentiation (Hotulainen and Hoogenraad, 2010).

Recent work reveals a mobilization of recycling endosomes and AVCs into spines within minutes after the induction of LTP. Thus, during LTP higher number of AMPARs are present, which potentiate synaptic transmission, whereas LTD involves removal of AMPARs leading to a reduction in synaptic strength. By increasing the efficiency and number of AMPARs, following excitatory stimuli generate larger post-synaptic responses (Bassani et al., 2013). The increase of AMPAR number in the synapse during LTP is the result of three complementary processes: Exocytosis in extra/perisynaptic sites (Kennedy et al., 2010; Yudowski et al., 2006), lateral diffusion into the synapse (Borgdorff and Choquet, 2002) and their subsequent trapping mediated by CaMKII (Makino and Malinow, 2009; Opazo and Choquet, 2011; Opazo et al., 2010; Tomita et al., 2005).

Several proteins are involved in endosome trafficking during LTP, such as SNARE proteins (syntaxin13, syntaxin4) or myosin V (Murakoshi and Yasuda, 2012). The small GTPases Ras and Rap are also crucial regulators of the endosomal membrane system and appear to govern the synaptic insertion and removal of AMPARs, incorporating the different AMPAR subunits through different molecular pathways (MEK-ERK pathway for GluA2 and the PI3 kinase-PKB/AKT pathway for GluA1) (Saneyoshi et al., 2010). Importantly, dysregulation of synaptic trafficking may contribute to various brain disorders by preventing appropriate synaptic signaling and plasticity. Thus, synaptic expression of AMPARs is thought to be dynamically regulated by neuronal activity and, in contrast to NMDARs expression, depends on F-actin. Its expression is proportional to spine-head and PSD size (Kasai et al., 2010). AMPARs bind indirectly to F-actin, via interactions with PSD proteins, thereby forming different complexes: AMPAR-SAP97-protein4.1-F-actin and AMPAR-stargazin-PSD-95-GKAP-shank-cortactin-F-actin (Fig. 5).

In analogous fashion to NMDARs, the C-terminal tails of AMPARs subunits also interact with intracellular proteins (Table S4). In particular, GRIP1 participates in the trafficking of AMPARs, because blocking GluA2/GRIP interactions prevents activation of silent synapses. GRIP/ABP binds to PICK1 (PDZ-containing protein previously shown to bind PKC and to inhibit Arp2/3 complex) which clusters GluA2 subunits. Thereby, PICK1 could recruit PKC to AMPARs, in the same way as PSD-95 recruits Src family to NMDARs, although this remains to be elucidated (Fig. 3).

AMPARs may also play a role in spine growth and maintenance (Tada and Sheng, 2006). Overexpression of GluA2 in mature hippocampal neurons increases spine length, head size and density, and requires the N-terminal domain (NTD) for different interactions (Bassani et al., 2013). Importantly, recent data hint at TARPS, transmembrane AMPAR regulatory proteins (Guzman and Jonas, 2010). Among them, Stargazing (TARPy2) is required for AMPARs delivery to the plasma membrane after LTP induction, which may contribute to spine stability, eventually leading to memory consolidation (Lee et al., 2012). Recently, a type II transmembrane protein (SynDIG1) was identified as a regulator of AMPARs content at developing synapses (Kalashnikova et al., 2010).

On the other hand, silent synapses are characterized by the presence of NMDARs and the lack of AMPARs. They are

usually found in the young brain and disappear during spine development and maturation (Kerchner and Nicoll, 2008). A recent study using an agonist of AMPARs suggested that AMPARs activation *per se* is a sufficient inducing stimulus for generating AMPAR silent synapses (Wasling et al., 2012).

4.2.4. Cell adhesion molecules and synaptic plasticity

Synapse formation is thought to be regulated by bidirectional signaling between pre- and postsynaptic cells and recently a number of adhesion molecules (CAMs) have been identified (Fig. 6 and Table S5). In particular, N-cadherin links pre- and postsynaptic elements through Ca^{2+} -dependent homophilic interactions and indirectly anchors to F-actin via binding to the α/β -catenin complex. Interestingly, overexpression of N-cadherin increases the surface expression of GluA1 (Tai et al., 2007), suggesting a mechanism for coordinating morphological spine growth with functional strengthening of synapses. Thus synaptic activity stabilizes synapse structure via N-cadherin, which recruits AMPARs and maintains synaptic efficacy.

Furthermore, N-cadherin is synthesized and internalized to new assembled synapses during the induction of late-LTP, and genetic ablation of N-cadherins prevents LTP and its associated spine enlargement (Fortin et al., 2012).

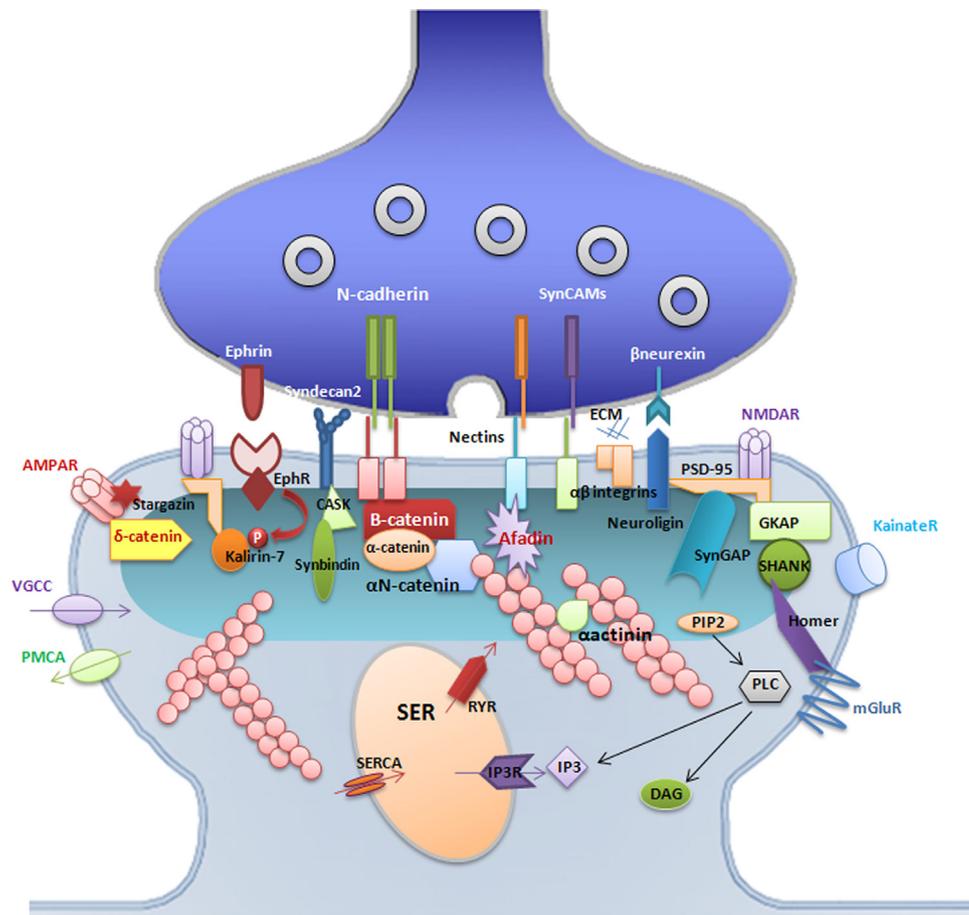


Fig. 6 – Cell adhesion molecules (CAMs) involved in synapse formation and maturation. CAMs include immunoglobulins, integrins, selectins and cadherins. Cadherins comprise a large family of proteins: classical cadherins (N-cadherin) and protocadherins. Neuroligins (postsynaptic compartment) and neurexins (presynaptic compartment) also play a key role in synaptic function.

On the other hand, Neuroligin1 (Nlg1) is essential for intact excitatory synaptic transmission and LTP. It binds to S-SCAM and PSD-95, in order to recruit glutamate receptors and form new dendritic spines. Overexpression of Nlg1 in hippocampal neurons facilitates recruitment of GluA2-containing AMPARs, but not GluA1, raising evidence that AMPARs recruitment at neuroligin–neuroligin contacts is subunit-specific (Bang and Owczarek, 2013).

Barrow et al., (2009) showed that shortly after axodendritic contact, Nlg1 is accumulated at nascent contacts of axonal growth cone filopodia with dendritic shafts, strengthening the adhesion and recruiting both NMDARs and PSD-95 in order to begin the process of synaptogenesis. Nlg1 also forms clusters in existing synapses, providing additional stabilization through regulating the abundance of NMDARs at synapses (Budreck et al., 2013). Also erythrocyte protein band 4.1-like 3 (protein 4.1B) has been identified as an intracellular effector molecule of Synaptic Cell Adhesion Molecule 1 (SynCAM1), directly recruiting NMDARs shortly after synaptic-like contact. Furthermore, postsynaptic protein 4.1B enhances pre-synaptic differentiation through SynCAM1 and proteins 4.1B and 4.1N differentially regulate glutamate receptor recruitment to sites of adhesion (Hoy et al., 2009).

5. Spine degeneration and neuronal loss

During the past decades, different empirical studies have documented that several neurological disorders are characterized by disruptions in dendritic spine shape, size or number. Because spine morphology is linked to synaptic function, perturbed spines are likely to have diverse functional effects such as synapse loss or aberrant signaling and plasticity, resulting in significant clinical manifestations (Calabrese et al., 2006). Thus, a possible common feature that links these pathologies may be abnormalities in dendritic spines and the subsequent disruption of synaptic function (Penzes et al., 2011). Moreover, several hereditary forms of intellectual disabilities exhibit defects in the expression of proteins that regulate spine development and maintenance (Table 1).

For instance, neurodevelopmental disorders such as Down's syndrome or Fragile X syndrome (FXS) show a failure to convert filopodia to dendritic spines, leaving adult dendrites in an immature state, and thereby leading to synaptic dysfunction and learning and memory deficits. Later in life, synapses become dysfunctional, thus contributing to dementia and giving rise to different neurodegenerative disorders such as HD, PD or AD, which often display spine loss, disruption of spine integrity and aberrant spine morphology (Van Spronsen and Hoogenraad, 2010). Spine abnormalities have also been described in stress or drug addiction, where AMPAR trafficking, mGluR signaling, and spine actin dynamics are affected. Finally, aging is a physiological process that causes morphological changes in neurons, including reduced dendritic length and arborization or reduction in spine and synapse density (Tyan et al., 2012).

Animal models have also been developed giving further evidences of alterations in dendritic spines in FXS (Thomas et al., 2012), epilepsy (Zhang et al., 2009), Down's syndrome

(Belichenko et al., 2004), AD, Austistic Spectrum Disorders (ASD) and schizophrenia (Penzes et al., 2011).

Recently, autism has been recently associated with mutations in synaptic molecules and with a hyperactivity of mTORC1–eIF4E pathway, causing an increased E/I balance. Gkogkas et al., (2013) showed that KO mice of the gene encoding the translational repressor eukaryotic translation initiation factor 4E (eIF4E)-binding protein 2 (4EBP2), which inhibits translation by competing with eIF4G for eIF4E binding and is repressed by mtorc1, results in increased translation of NGL1-4. Moreover, pharmacological inhibition of eIF4E activity (4EGI-1) or small interfering RNA (siRNA)-mediated knock-down of NGL-1 rescues E/I balance, thus unveiling novel potential targets for the treatment of ASDs (Wang and Doering, 2013).

In the following section we will give some insights on the recent advances in our understanding of neurodegenerative diseases, in particular AD and its relationship to synaptic and dendritic spine pathology.

5.1. Alzheimer's disease (AD)

AD is the most common neurodegenerative disease and the leading cause of dementia in the elderly, particularly in people over 65 years old, although early-onset AD typically occurs before.

This devastating disease is characterized by cognitive decline and neuronal death, accompanied by the formation of senile plaques, composed primarily of amyloid-beta peptide ($A\beta$), and neurofibrillary tangles, intracellular aggregates of hyperphosphorylated Tau protein, which constitute the hallmarks of AD (Spires-Jones and Knafo, 2012). It can be classified, according to etiology, between type I (autosomal dominant or early-onset AD) that accounts only for 5–10% of all AD cases and is primarily caused by genetic mutations in the parental amyloid precursor protein (APP) gene, as well as in genes encoding for components of the proteolytic- γ -secretase complex, such as presenilin 1 and 2 (PSEN1, PSEN2), which lead to amyloid overproduction; and type II (sporadic or late-onset AD), the most common cause of the disease (90%), related to inflammatory (Eikelenboom et al., 2010) and environmental risk factors such as ApoE variants, clusterin, complement receptor 1 or TREM2 (Jonsson et al., 2013), giving rise to defects in $A\beta$ clearance. Specifically, the $\epsilon 4$ (ApoE $\epsilon 4$) allele has been associated with greater risk of developing AD, whereas ApoE $\epsilon 2$ seems to be neuroprotective (Liu et al., 2013). Both types converge on altered $A\beta$ production/clearance balance that eventually leads to the formation of $A\beta$ deposits, causing impaired synaptic plasticity and memory (Van Spronsen and Hoogenraad, 2010).

$A\beta_{1-40}$ and $A\beta_{1-42}$ constitute the majority of the $A\beta$ peptide found in human brain and it is formed via the sequential cleavage of APP, a single-transmembrane domain protein, which can be processed through either the amyloidogenic or the non-amyloidogenic pathway (Fig. 5). $A\beta$ displays different functions both presynaptically, increasing calcium levels and triggering release of neurotransmitters, and postsynaptically, through the activation of NMDARs and AMPARs (Ferreira et al., 2007). In fact, the remodeling of calcium signaling is thought to be at least partially responsible for the memory

and learning deficits that occur early during the onset of AD (Berridge, 2010).

The amyloid hypothesis of AD points at A β aggregation as the fundamental cause of the disease. Nevertheless, there is a lack of correlation between A β plaques load and the degree of cognitive impairment in AD. This has led to the possibility that mostly soluble and not fibrillar A β may contribute to synaptic dysfunction and spine loss, which precede plaque and tangle formation chronologically, eventually leading to cognitive deficits (Spires-Jones and Knafo, 2012). Indeed, synapse loss is the strongest pathological correlate of dementia in AD (Penzes et al., 2011). However, the molecular mechanisms remain uncertain.

APP is important for promoting spine formation, accompanied by the specific upregulation of GluA2, as evidenced by both *in vitro* and *in vivo* studies where overexpression of APP increased spine density, whereas knockdown of APP reduced spine number (Lee et al., 2010). Interestingly, AD pathology is found prematurely in Down syndrome patients, who possess an extra copy of chromosome 21, where APP is encoded (Castellani et al., 2010). Previous work (Rowan et al., 2003) suggested that misprocessing of APP results in the accumulation of soluble A β , resulting in impaired hippocampal LTP which may be the cause of the cognitive decline seen in AD patients. Since glutamate transmission controls synaptic strength and plasticity, which are the underlying events of learning and memory, some studies have speculated that A β oligomers could affect LTP and LTD by altering these signaling pathways (Li et al., 2010). Calcium plays a dual role as a second messenger, being able to activate either LTP or LTD pathways depending on its intracellular concentration. This is why altering this delicate equilibrium could lead to synaptic functional alterations as it happens in AD. A β -mediated synaptic dysfunction is thought to be based on mildly increased postsynaptic calcium concentrations leading to an excessive synaptic depression and AMPAR removal. This would result in a reduction of spine formation (Toni and Sultan, 2011), facilitating the induction of LTD through both the mGluR and NMDAR pathways (Li et al., 2009).

In addition, recent studies have considered Tau protein as a key mediator of A β -induced synaptic dysfunction and loss (Spires-Jones and Knafo, 2012) and a “Tau hypothesis” has been put forward. Several kinases can be abnormally activated by A β (CDK5, Fyn, GSK3 β and MARK) and lead to Tau hyperphosphorylation, causing Tau to dissociate from the microtubules and accumulate at the dendritic compartments. Phosphorylated Tau facilitates the targeting of Fyn to the PSD of dendritic spines, which in turn phosphorylates GluN2 and stabilizes its interaction with PSD95, enhancing excitotoxicity and potentially altering synaptic structure and function. Thus targeting the Tau-dependent pathway by reducing Tau protein level, inhibiting Tau kinases activity, or increasing phosphatases activity, would represent suitable new ways of treating AD (Hoover et al., 2010). Further studies have implicated different mechanisms, such as PKM ζ , an enzyme that accumulates in neurofibrillary tangles and that is crucial in the maintenance of late LTP (Crary et al., 2006). It has also been suggested that A β could alter dendritic spines via the SNK-SPAR signaling pathway by altering synaptic stability (Gong et al., 2010).

The synapse deterioration that begins early in AD highlights the need to develop better diagnostics and more thoroughly investigate the neurological changes that take place during the onset of the disease (Crimins et al., 2013; Knobloch and Mansuy, 2008; Octave et al., 2013; Yu and Lu, 2012).

6. Conclusion

The present work has focused on hippocampal dendritic spines which contain a dense array of molecules involved in postsynaptic signaling and synaptic plasticity. Thereby they are thought to undergo structural changes associated to learning and memory. Since changes in spine shape and size are correlated with the strength of excitatory synapses, spine morphology directly reflects spine function. Thus, studying spines structure and function and unveiling the specific mechanisms that regulate spine formation and morphology is essential for understanding the cellular changes that underlie learning and memory in normal and pathological conditions.

Spine alterations can be used as indicators of disease severity and progression, helping in the diagnosis and treatment of several neurological disorders, such as AD. Hence, this review also provides further insights to tackle these synaptic alterations that may eventually lead to cognitive deficits and dementia.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.brainres.2014.05.024>.

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