

# Dendritic Spines

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## OUTLINE

6.1 Introduction	95	6.6 Spine Dynamics and Development of Synaptic Networks	102
6.2 General Morphological Characteristics of Dendritic Spines	96	6.7 Spine Alterations and Brain Disease	105
6.3 Variations in Spine Synapse Organization	97	6.8 Conclusion	105
6.4 Molecular Composition and Signaling Mechanisms	99	References	105
6.5 Mechanisms of Spine Formation	101		

## Abbreviations

AMPA	$\alpha$ -Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
AMPAR	$\alpha$ -Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor
Cdc42	Cell division control protein 42
DISC1	Disrupted-in-schizophrenia 1
EM	Electron microscopy
FMRP	Fragile X mental retardation protein
GKAP	Guanilate cyclase associated protein
GluR	Glutamate receptor subunit
LTD	Long-term depression
LTP	Long-term potentiation
NMDA	<i>N</i> -Methyl-D-aspartate
NO	Nitric oxide
NMDAR	<i>N</i> -Methyl-D-aspartate receptor
PAK	P21 activated kinase
PSD	Postsynaptic density
PSD-95	Postsynaptic density 95
SAP97	Synapse associated protein 97
SER	Smooth endoplasmic reticulum
mGluR	Metabotropic glutamate receptor

## 6.1 INTRODUCTION

Dendritic spines are the principal site for excitatory transmission in the brain. Since their description by Ramon y Cajal and Tanzi at the end of the nineteenth

century, dendritic spines have been at the focus of intense research aimed at understanding their function in the processing of neuronal activity. The initial belief that they could play a role in learning and memory processes actually has received strong support from numerous studies that have analyzed their morphological and functional properties. First, dendritic spines exhibit important activity-dependent forms of plasticity that affect both their strength and structural organization, contributing in this way to information processing. Second, recent developments in confocal imaging techniques have provided evidence that spines are dynamic structures that can grow and be eliminated throughout life and thus make possible a continuous adaptation of brain circuits to experience. Third, at the molecular level, dendritic spines, and particularly their postsynaptic density, the region where receptors are located, form a highly complex structure in terms of protein composition, diversity, and implicated signaling mechanisms, illustrating the key role played by dendritic spines in signal integration. Finally, progress in the genetic identification of molecular defects underlying human diseases has provided strong evidence that alterations of a wide array of synaptic proteins lead to important

cognitive and behavioral disorders, establishing a direct link between dendritic spine synapses and higher brain functions.

## 6.2 GENERAL MORPHOLOGICAL CHARACTERISTICS OF DENDRITIC SPINES

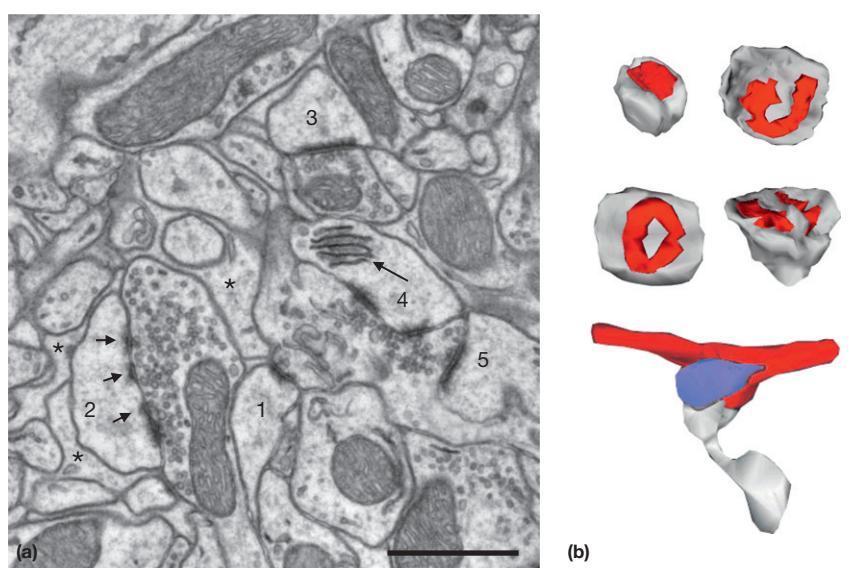
Dendritic spines are mostly found on cortical excitatory neurons. They can however also be found on inhibitory interneurons such as cerebellar Purkinje cells, spiny stellate cells of basal ganglia or olfactory granule cells. Spines are also found in invertebrates, for example in mushroom bodies in flies or arthropods, and many of the properties described here also apply to these other types of spines.

A major characteristic of dendritic spines is their high level of morphological variability (Figure 6.1(a)). To make sense of the functional implications of this variability, researchers have tried to classify them according to various morphological criteria, including size, shape, organization of the spine and/or postsynaptic density (PSD), or presence of specific organelles revealed in electron microscopic (EM) studies. In the rat hippocampus, where most studies of dendritic spines have been performed, the size of the spine head determined by the length of the largest diameter varies between 0.2 and 1.3  $\mu\text{m}$ , which roughly corresponds to volumes of 0.004–1.2  $\mu\text{m}^3$ ; thus, corresponding to variations differed by a factor of about 300. These variations in spine volume usually also are correlated with variations in the size of the PSD, size of the presynaptic terminal, and presence of various organelles

in the spine: large spines are more likely to contain a spine apparatus or ribosomes.

Dendritic spine synapses are composed of several different elements. The PSD is a highly organized structure characterized by a high density of receptors and channels, associated signaling proteins, adhesion molecules, and cytoskeletal elements assembled together by a variety of scaffold proteins. It represents the contact zone, where synaptic transmission occurs. PSD size, measured with three-dimensional (3D) EM reconstruction, can vary widely, between 0.008 and 0.54  $\mu\text{m}^2$  at hippocampal CA1 excitatory synapses (Harris and Stevens, 1989). The shape of the PSD is also quite variable and in most cases, it appears on 3D EM reconstruction as a single, macular area, but more complex shapes are not uncommon and particularly have been associated with increased synaptic remodeling and possibly receptor content and turnover (Ganeshina et al., 2004; Geinisman et al., 1993; Toni et al., 1999, 2001). These include PSDs interrupted in the middle, often referred to as perforated PSDs, or PSDs composed of two or multiple individual parts that may correspond to separate transmission zones, referred to as segmented PSDs (Figure 6.1(b)). Synapses with complex PSDs are mostly present on large mushroom-type spines and may represent, depending on conditions, between 5% and 25% of all PSDs.

The postsynaptic membrane of dendritic spines is separated from the presynaptic terminal by a synaptic cleft (Figure 6.1(a)) that is usually 10–20 nm wide and contains dense material binding the two membranes together. The exact content of the synaptic cleft material is not known, but it likely consists of transsynaptic fibrils that often are regularly spaced (Zuber et al., 2005).



**FIGURE 6.1** EM illustrations of the variability of spine and PSD size and shape. (a) Spine heads of different sizes and shapes (nos 1–5). Spine no. 1 is of a thin type, spine nos. 2–5 are of mushroom type. PSDs in spines 2 and 4 are of a complex shape. Arrow points at spine apparatus in spine no. 4. Spine nos. 1, 4, and 5 contact the same presynaptic bouton (multi-synaptic bouton). Arrows in spine no. 2 point at a complex PSD. Astrocytic processes surrounding this synapse are marked with asterisks. Scale bar 0.5  $\mu\text{m}$ . (b) 3D reconstructions of different types of PSDs (macular, U-shaped, perforated, segmented, and multi-innervated spine with two separate PSDs, shown with contacting presynaptic boutons).

Dendritic spines are almost always in contact with a presynaptic bouton formed by an enlargement of the axonal shaft and filled with synaptic vesicles containing neurotransmitter that is released at the presynaptic active zone facing a PSD (Figure 6.1(a)). In addition, spine synapses are often surrounded by fine astrocytic processes (Figure 6.1(a)) that may contribute to synaptic network functions by controlling the local synaptic environment and coordinating synaptic activity and plasticity (Genoud et al., 2006; Haydon, 2001; Lushnikova et al., 2009; Volterra and Meldolesi, 2005; Witcher et al., 2007).

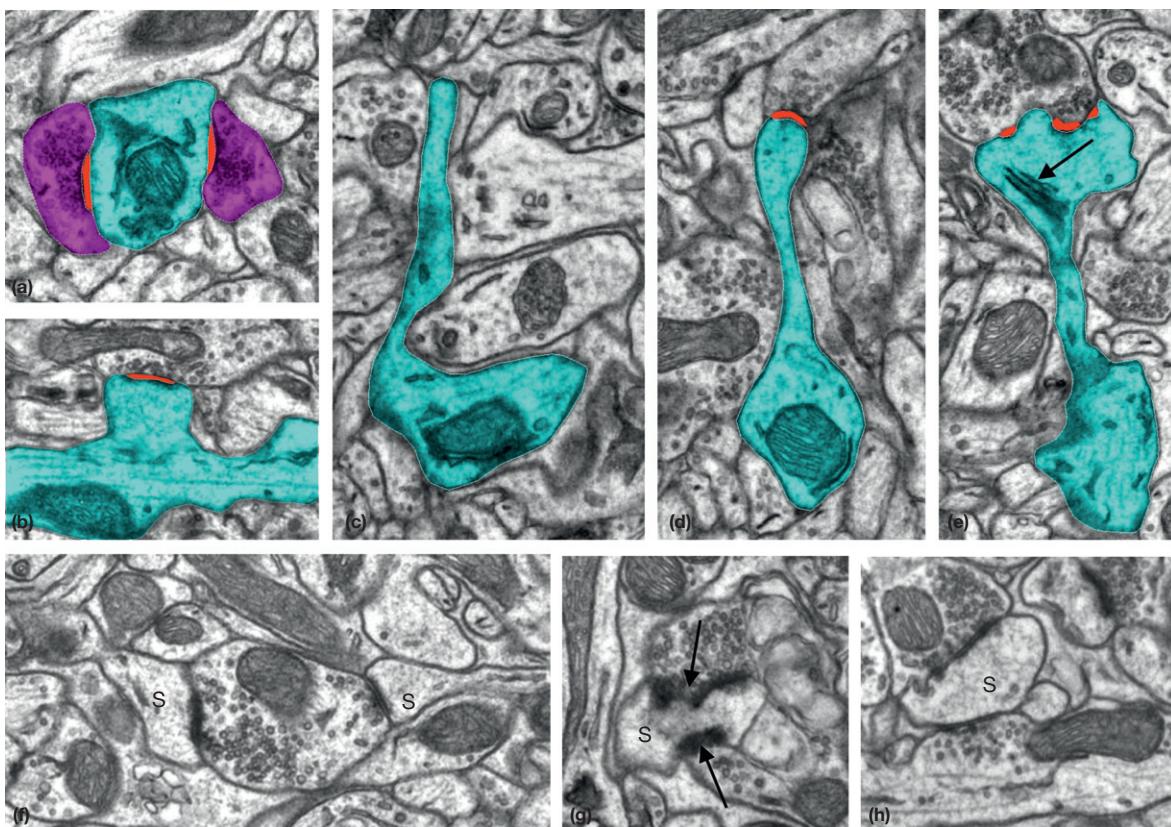
Dendritic spines may also contain several intracellular organelles. About half of them have what is referred to as the spine apparatus composed of smooth endoplasmic reticulum (ER) (Spacek and Harris, 1997). Smooth ER forms a continuous network throughout dendritic segments and spines (Cooney et al., 2002) and is likely to be important for regulating calcium content, local protein synthesis, or both. Confocal imaging has shown a continuity of spine ER with dendritic ER and a surprisingly high degree of turnover in individual spines (Toresson and Grant, 2005). Ribosomes are often found at the base of dendritic spines. They are either associated with ER or free in the cytoplasm and serve local protein synthesis. Free polyribosomes showed redistribution from dendritic shafts into enlarged spines after induction of long-term potentiation (LTP), pointing at the importance of local synthesis for synaptic plasticity (Bourne et al., 2007; Ostroff et al., 2002). Spines may contain endosomal systems consisting of clathrin-coated vesicles and pits, large uncoated vesicles, tubular compartments, multivesicular bodies, and multivesicular bodies–tubule complexes. The recycling endosomes, the part of the system that transports membrane-bound proteins onto and off the cell surface, is of particular importance for activity-related delivery of glutamate receptors to the synapse, as well as for activity-induced structural plasticity of spines (Park et al., 2006; Racz et al., 2004). Another important component of dendritic spines is actin filaments (F-actin). They constitute a major element of dendritic spine cytoskeleton and as such play an essential role in many aspects of spine formation, plasticity and dynamics (Hotulainen and Hoogenraad, 2010). Finally, microtubules, a major cytoskeletal element of dendrites and small unmyelinated axons, are generally absent from dendritic spines. However, recent studies have provided evidence for the presence of microtubules in a small portion of dendritic spines. Notably, growing microtubule ends can transiently enter dendritic spines, interact with F-actin binding protein cortactin, and presumably modulate actin dynamics in the spine in an activity-dependent fashion (Hu et al., 2011; Jaworski et al., 2009). A recent EM study, using a special microtubule-stabilizing fixative, demonstrated the presence of microtubules in CA1

dendritic spines after strong LTP induction and suggested that they could be implicated in AMPA receptors (AMPAR) trafficking to stimulated PSDs (Mitsuyama et al., 2008).

### 6.3 VARIATIONS IN SPINE SYNAPSE ORGANIZATION

Although the significance of the variability in spine size and shape is still largely unclear, one distinction that has emerged as potentially meaningful is between spines with a large head, a constricted neck and a large PSD (usually referred to as mushroom type spines: Figures 6.1(a) and 6.2(e)), and long and thin spines, with a small head and a filopodia-like shape (often referred to as thin spines: Figures 6.1(a) and 6.2(d)). There is now evidence that these two classes of spines may have different functional properties and could correspond to different phases of the life of a spine: thin spines representing immature, newly formed protrusions that are less likely to remain stable, while mushroom type spines would represent mature, stable spines that have undergone a process of activity- or plasticity-mediated enlargement and remodeling (Bourne and Harris, 2007). Along with mushroom spines and thin spines, which represent more than 80% of all excitatory spines in mature tissue, several other protrusion morphologies or spine structures have been reported under particular developmental or activity conditions.

A first type of protrusion, mainly seen during the early phases of synaptic network development, is comprised by filopodia (Figure 6.2(c)). Filopodia are typically referred to as long, very motile protrusions devoid of an enlargement at the tip. They can grow and retract within minutes and have been shown to be able to transform into dendritic spines. They are therefore believed to represent precursors of dendritic spines (Ziv and Smith, 1996). Filopodia are mainly seen during early stages of development, where they can represent up to 20% of all protrusions, but their proportion then decreases with maturation, so that in mature tissue they constitute only a few percent depending upon criteria used to identify them. A clear distinction between filopodia and thin spines is indeed often difficult. At the EM level, filopodia are often defined by the absence of a PSD at the tip, while with confocal imaging it is the absence of enlargement at the tip and their motility that best characterize them. Filopodia may sometimes express a PSD, which is then usually located at the base or along the length of the protrusion. Formation and stability of filopodia appears to be controlled by calcium transients present in the developing dendrite (Lohmann et al., 2005) and their growth seems to be targeted, as they are able to form transient contacts specifically with excitatory axons and not with inhibitory ones (Lohmann and Bonhoeffer, 2008).



**FIGURE 6.2** EM illustrations of different types of excitatory synapses. (a) Two presynaptic terminals (magenta) making shaft synapses (red) on the dendritic shaft (blue), cut transversally, with a mitochondria in the center surrounded with endoplasmic reticulum. (b) Stubby spine on a dendritic segment (blue) with a macular PSD (red). (c) Filopodia. (d) Thin spine with a macular PSD. (e) Mushroom spine with a complex PSD and spine apparatus (arrow). (f) Two spines (s) sharing a common presynaptic bouton (multi-synaptic bouton). (g) Multi-innervated spine (s) with two different PSDs (arrows) contacting two different presynaptic boutons. (h) Cortical spine (s) with excitatory (top) and inhibitory (bottom) synaptic contacts. Scale bars for the top and bottom rows: 0.5 Mm.

While filopodia grow directly from dendrites, thin and motile protrusions extending from dendritic spines can also be observed. These have been referred to as spinules, and they are usually associated with spines having large heads and complex PSDs. Their occurrence appears to be promoted by glutamate application and seems to require AMPAR transmission (Richards et al., 2005). They could therefore result from a reorganization of the spine actin cytoskeleton mediated by synaptic activity and thus be associated with activity- or plasticity-mediated spine remodeling.

Other particular categories of synaptic contacts are made up of shaft and sessile synapses. Shaft synapses are contacts made by an axonal bouton directly on a dendritic shaft (Figure 6.2(a)). In adults, most shaft synapses seen on major apical dendrites are actually formed by inhibitory contacts, but a fraction of them may also be excitatory. Overall, excitatory shaft synapses represent less than 10% of all excitatory synapses in mature tissue. This proportion, however, is higher during development and may also vary as a function of neuronal type and

brain region. It has been shown that shaft synapses can give rise to dendritic spine synapses through the extension of a protrusion (Marrs et al., 2001). Alternatively, shaft synapses could also result from the retraction of dendritic spines. Finally, they might have specific functions, as their contribution to signal integration in dendrites is more important than for spine synapses. Consistent with this interpretation, their formation and number appear to be specifically regulated by a signaling system implicating ephrin B3 (Aoto et al., 2007).

Synaptic contacts can also be made on short dendritic protrusions devoid of a neck; these are referred to as stubby or sessile spines (Figure 6.2(b)). They probably represent an intermediate stage between excitatory shaft synapses and spine synapses with a neck. At the EM level, they usually constitute only a very small proportion (in the range of a few percent) of all protrusions in mature tissue. Reportedly, their number is modulated by activity and induction of LTP (Chang and Greenough, 1984).

While most spine synapses are formed between individual axonal boutons and dendritic spines, there are examples where a single axonal bouton contacts multiple dendritic spines (also referred to as multiple spine bouton, Figure 6.2(f)) and cases where a single spine is contacted by multiple axonal boutons (forming multi-innervated spines, Figures 6.1(b) and 6.2(g)). These cases are usually quite rare, representing only a few percent of all synaptic contacts in mature tissue, but their occurrence has been shown to increase under developmental or plasticity conditions. Notably, an increase in multiple spine boutons has been reported following induction of LTP, with the additional interesting feature that in many cases the two spines contacted by the same axonal boutons arose from the same dendrite (Toni et al., 1999). This observation was therefore interpreted as indicating a process of new spine growth and synapse formation triggered by induction of plasticity. Similar images of multiple spine boutons have also been reported when analyzing the development and integration of new neurons in the hippocampal dentate gyrus (Toni et al., 2007) or under conditions of spine growth promoted by whisker trimming in the somatosensory cortex (Knott et al., 2006). These results therefore suggest that images of multiple spine boutons could reflect a transient competition for the same presynaptic boutons between a pre-existing spine and a newly formed one, and thus translate an ongoing process of synaptogenesis.

Multi-innervated spines are similarly rather infrequent in mature tissue, reaching 2–3% of all protrusions. Recent analysis of the mechanisms underlying this particular synaptic configuration has revealed the role played by the transsynaptic release of nitric oxide (NO) by the growing spine. Under conditions where this release is enhanced, it promotes the differentiation of nearby axonal shafts into presynaptic boutons and the formation of additional synaptic contacts on the same spine. Conversely, blockade of NO production during development is associated with a reduction in the formation of spines and synapses (Nikonenko et al., 2008). As the production of NO at synapses is linked to the level of expression of scaffold proteins such as PSD-95 or SAP97 (Poglia et al., 2010), one might consider the possibility that the formation of a multi-innervated spine occurs as a result of enhanced growth of the PSD. Also, as the strength of a synapse correlates with the size of the PSD, multi-innervated spines could reflect the existence of highly potent synaptic contacts.

Finally, there are also cases of excitatory dendritic spines that bear at the same time an inhibitory synaptic contact (Figure 6.2(h)). This situation is not seen in all tissues and has been mainly reported at spine synapses in different cortical areas. In the somatosensory cortex

for example, the proportion of these spines with excitatory/inhibitory synapses is low under control situations, but can markedly increase as a result of strong, lasting sensory stimulation (Knott et al., 2002). The mechanisms underlying the formation of these particular spine synapses are currently unknown, but could represent a means for controlling the level and specificity of excitation in cortical regions.

## 6.4 MOLECULAR COMPOSITION AND SIGNALING MECHANISMS

Excitatory dendritic spine synapses represent one of the most complex biological structures in terms of their molecular composition and diversity. Despite their small size, dendritic spines contain and express more than 1000 different proteins often organized in multi-protein signaling complexes (Emes et al., 2008). Additionally, synaptic proteins very often exist in different subtypes that confer subtle functional properties.

A central mechanism targeted by many synaptic regulators appears to involve the control of the trafficking of synaptic proteins, among which are receptors and PSD-associated proteins. At excitatory synapses, multiple neurotransmitter receptor subtypes (NMDA, AMPA and metabotropic receptors) are usually expressed and further characterized by various subunit compositions and distinct biophysical properties. These receptors are embedded in a matrix of scaffolding proteins, which together with adhesion and signaling molecules constitute the PSD. Structurally, the PSD is a large network of interconnected protein assemblies, one important function of which might be to regulate the number and subunit composition of the receptors present at the synapse.

Regulation of these mechanisms likely occurs in a number of different ways, but a key role therein is certainly played by synaptic activity. At many excitatory synapses, high-frequency trains of stimulation are able to trigger specific signal transduction pathways that serve as important mechanisms for the regulation of synaptic strength. Changes in synaptic efficacy are currently viewed as the most prevalent molecular model for mechanisms of information processing, learning and memory (Cooke and Bliss, 2006). Numerous forms of synaptic plasticity and changes in synaptic strength have been reported at central synapses depending on brain region, cell type, and age. Some of these are short-lasting such as facilitation or post-tetanic potentiation, but the most interesting forms result in long-lasting changes in synaptic efficacy (Bliss and Collingridge, 1993). These may lead either to increases or to decreases in synaptic strength (Dudek and Bear, 1993) and involve pre- or postsynaptic mechanisms

([Nicoll and Malenka, 1995](#)). The best-known example of synaptic plasticity is probably the form of LTP that is expressed at CA1 hippocampal synapses. In this type of plasticity, the change in synaptic strength is triggered by an activation of postsynaptic NMDA type of glutamate receptors (NMDAR) leading to calcium fluxes in dendritic spines. This in turn activates signal transduction pathways, among which protein kinases – calcium/calmodulin-dependent protein kinase II (CaMKII) in particular – play an important role ([Lisman et al., 2002](#)). While the specific sequence of molecular events involved in the lasting increase in synaptic strength is still a matter of debate, much recent evidence suggests that synaptic potentiation depends heavily upon the number of receptors expressed at synapses and thus upon local trafficking of receptors within individual dendritic spines ([Malenka and Nicoll, 1999](#); [Malinow and Malenka, 2002](#)).

One interesting hypothesis to account for the lasting changes in synaptic strength is the idea that receptors and scaffolding proteins interact to form receptor-binding slots controlling synaptic strength ([Bats et al., 2007](#); [Lisman et al., 2002](#)). Biochemical and imaging studies indicate that the main scaffold proteins (e.g. PSD-95, SAP97, GKAP, Shank or Homer) are more densely expressed at synapses (60–400 molecules of each per synapse) than receptors and thus outnumber glutamate receptors (0–200 receptors per synapse). They have therefore been proposed to provide a structural basis for the formation of a pool of receptor-confining domains likely to account for modifications of synaptic strength. Glutamate receptors, especially AMPARs, exhibit a high level of lateral mobility within membranes and can exchange rapidly at synapses in a way that is highly sensitive to activity ([Triller and Choquet, 2008](#)). Synaptic stimulation or calcium uncaging reduce AMPAR diffusion, so that active synapses tend to trap AMPARs more efficiently than inactive synapses ([Borgdorff and Choquet, 2002](#)). Additionally, large PSDs capture and retain more PSD-95 than smaller PSDs and thus may favor trapping of AMPARs ([Gray et al., 2006](#)). Synaptic activity is therefore able to regulate glutamate receptor and scaffold protein dynamics at synapses, thereby providing a mechanism for activity-dependent targeting and retention of receptors at individual synapses.

Numerous proteins participate to the regulation of receptor expression at synapses. In addition to the major scaffold proteins, auxiliary transmembrane regulatory proteins (TARPs), PDZ proteins and protein kinases also play an important role ([Nicoll et al., 2006](#)). These proteins may affect not only the lateral mobility of receptors at synapses ([Opazo et al., 2010](#)) but also their intracellular trafficking, which is crucial for controlling the abundance of glutamate receptors at synapses. Within dendritic spines, AMPAR internalization is

thought to occur at endocytotic zones, defined as clathrin-coated membrane domains located adjacent to the PSD ([Racz et al., 2004](#)). These internalized receptors are then transferred to recycling endosomes, which may function as a supply mechanism for providing AMPARs to synapses. Upon induction of plasticity, AMPARs are rapidly inserted at synapses, although the precise location at which this exocytic process might take place remains unclear. Evidence suggests that AMPAR incorporation at synapses following LTP induction could occur through lateral diffusion from a pool driven to the surface primarily on dendrites ([Makino and Malinow, 2009](#)).

In addition to activity, several other signaling pathways also contribute to regulate receptor trafficking. Among these, small GTPases play a particularly important role. GTPases form a large family of proteins characterized by their ability to bind and hydrolyze GTP. They generally act as molecular switches affecting various biological activities regulating growth and migration of many cell types. Their action is therefore not restricted to neurons and synapses. GTPases cycle between an active GTP-bound and inactive GDP-bound state and are tightly regulated by a variety of modulators, many of which are either activity-dependent or linked to signaling from synaptic adhesion molecules. At synapses, GTPases appear to control receptor trafficking in different ways, either directly or through their action on the actin cytoskeleton or the local translation machinery ([Boda et al., 2010](#)). Ras and Rap, for example, are two GTPases that exert opposing effects on the regulation of AMPAR delivery to synapses. Ras activation promotes the trafficking of GluA1-containing AMPARs from a deliverable pool located near the PSD to the synapse, while stimulation of Rap signaling promotes spine shrinkage, removal of GluA2/3 containing AMPARs and depression of synaptic transmission ([Kielland et al., 2009](#)).

It is interesting that changes in synaptic strength are also associated with a remodeling of potentiated synapses. Repetitive confocal imaging of identified synapses upon induction of plasticity has shown that the changes in synaptic strength are accompanied by an enlargement of the dendritic spine head which correlates with the increased sensitivity to glutamate ([Matsuzaki et al., 2004](#)). This effect is rapid and can be long-lasting. Although its significance is still unclear, these results indicate that remodeling of the spine architecture through modifications of the actin cytoskeleton is part of the processes that underlie plasticity. Regulation of the actin cytoskeleton is a complex process that involves different signaling pathways, among which are the small GTPases Rac and Cdc42. Much recent evidence suggests that this cascade could participate in the regulation of spine size. By integrating activity and transsynaptic signals through adhesion molecules such as N-cadherin or EphrinB receptors, this pathway could tightly control

the function of the actin cytoskeleton through PAK, LIM kinase, or cofilin-dependent mechanisms and in this way regulate the growth, size, and morphology of spines (Penzes and Jones, 2008; Tolias et al., 2007). Another pathway implicated in the regulation of the spine actin cytoskeleton could integrate signals from the extracellular matrix (Gundelfinger et al., 2010). The importance of this signaling is particularly well illustrated by studies of matrix metalloproteases (MMPs), which degrade the extracellular matrix and markedly affect synaptic structure and function. One of them, MMP9, is associated with enhanced activity, is required for LTP at hippocampal synapses, and mediates through integrin signaling spine enlargement and synaptic potentiation (Wang et al., 2008). The extracellular matrix additionally plays an important role in controlling the lateral diffusion of membrane proteins at synapses by forming compartments that might affect plasticity properties (Frischknecht et al., 2009).

Another mechanism likely to play an important role in the functional maturation and plasticity properties of excitatory spine synapses is the local regulation of protein synthesis. Pharmacological inhibition of protein synthesis interferes with induction of plasticity and much recent evidence indicates that activity is able to promote a local translation of messenger RNAs in dendritic spines (Kelleher et al., 2004). This is associated with a redistribution of polyribosomes into large dendritic spines upon induction of LTP (Ostroff et al., 2002). At the molecular level, evidence points to an activity-mediated translation of proteins such as CaMKII or Arc (Bramham, 2008) that could play a role in mechanisms of synaptic plasticity by regulating actin dynamics, spine size, and the organization of the postsynaptic density.

## 6.5 MECHANISMS OF SPINE FORMATION

There appear to be at least two different processes through which new excitatory spine synapses can be formed. One includes as an initial step the growth of a very motile protrusion, the filopodium, which extends from the dendrite up to several microns and then makes contact with potential partners before eventually retracting into the form of a dendritic spine. Filopodia are mainly seen during early phases of synaptic development, when the distance to potential partners is greater (Ziv and Smith, 1996). Due to their high motility, they are ideally suited to explore the space around the dendrites searching for appropriate binding partners.

How exactly they contribute to synapse formation remains poorly understood. Filopodia have been shown to make repeated, transient contacts with axons, which in some cases can be stabilized, over a range of minutes, through the generation of calcium transients (Lohmann and Bonhoeffer, 2008). This implies a capacity

of filopodia to sense their environment. It does not seem that this signaling is mediated through the neurotransmitter glutamate, since glutamate receptor antagonists do not affect contact formation by filopodia. However, specific recognition mechanisms likely play a role, since filopodia are able to discriminate between partners and never make stabilized contacts with inhibitory axons (Lohmann and Bonhoeffer, 2008). Once a contact has been made, filopodia may eventually be transformed into spines (De Roo et al., 2008a; Maletic-Savatic et al., 1999; Marrs et al., 2001; Trachtenberg et al., 2002; Zuo et al., 2005). The success rate of this process, however, seems to be variable. Imaging studies in young mice or in hippocampal slice cultures suggest that only 10–20% of filopodia may actually be transformed into spines and that most of these spines still disappear within subsequent days (De Roo et al., 2008a; Zuo et al., 2005). Filopodia might however play a more important role during early phases of development, when the probability to reach a partner is lower and the motility of the filopodium is an advantage. Recent work in fact showed that decreasing filopodia motility by interfering with EphrinB signaling reduced the rate of synaptogenesis. This effect was notably prominent in early, but not late, development (Kayser et al., 2008). This is consistent with the notion that the contribution of filopodia to synapse formation may be restricted to early development.

Several different molecules have been identified as regulating the motility and behavior of filopodia. These can be classified in two categories: “accelerators” and “brakes”. The accelerators include calcium/calmodulin-independent protein kinase II (CaMKII) (Jourdain et al., 2003), syndecan-2 (Ethell and Yamaguchi, 1999; Lin et al., 2007), and paralemmin-1 (Arstikaitis et al., 2008), which enhance filopodia formation and further accelerate spine maturation. In striking contrast, the brakes are molecules that not only induce but also maintain dendritic filopodia, thus slowing spine maturation and sometimes even causing spine-to-filopodia reversion (Furutani et al., 2007; Kumar et al., 2005; Matsuno et al., 2006; Pak and Sheng, 2003; Vazquez et al., 2004). A particularly good example of a brake molecule is telencephalin (Yoshihara et al., 2009).

In more mature tissue, time-lapse imaging has shown that new protrusions may also directly appear as spines (Engert and Bonhoeffer, 1999; Trachtenberg et al., 2002). This process, which occurs within minutes, probably accounts for about half of all protrusions formed in young hippocampal tissue (De Roo et al., 2008a; Engert and Bonhoeffer, 1999; Jourdain et al., 2003). Typically, these new spines have long necks and small heads, which sometimes makes them difficult to distinguish from filopodia, except that they are less motile. In young neurons, new spines and filopodia are produced at a high rate and seemingly in a random fashion (Lendvai et al., 2000).

Also, a significant proportion are essentially transient and tend to disappear within hours (Cruz-Martin et al., 2010; De Roo et al., 2008a; Holtmaat et al., 2005). The reasons for this are still unknown, but could be linked to a failure to find a proper partner, to establish a contact, or to sense or relay activity. There might also be specific conditions in very young tissue that do not favor the induction of plasticity and thus reduce the probability that these new spines will become stabilized (Ehrlich et al., 2007).

What are the different steps that lead to formation of a synaptic contact at nascent synapses? 3D EM reconstruction of newly formed spines *in vitro* and *in vivo* or following LTP-inducing protocols in slices has revealed that they do not seem initially to express a PSD (De Roo et al., 2008a; Knott et al., 2006; Nagerl et al., 2007). Consistent with this, EM analyses in the cortex or hippocampus have shown the existence of a small population of spine-like protrusions devoid of PSD or even without presynaptic partners, suggesting that spine growth could precede synapse formation (Arellano et al., 2007; De Roo et al., 2008a; Knott et al., 2006). The speed of this process appears to be quite variable. Based on morphological criteria (presence of a postsynaptic density on 3D EM reconstruction or expression of PSD-95-EGFP), the formation of mature synapses appears to require between 5 and 24 h. However, functional analyses of spine sensitivity to glutamate uncaging suggest that a new protrusion could respond to released transmitter much faster, within tens of minutes (Zito et al., 2009). This suggests that receptors may be present or sense transmitter before a morphologically mature PSD is apparent. One principal takeaway, however, is that formation of new, functional synapses can be a fast process, taking just minutes to hours.

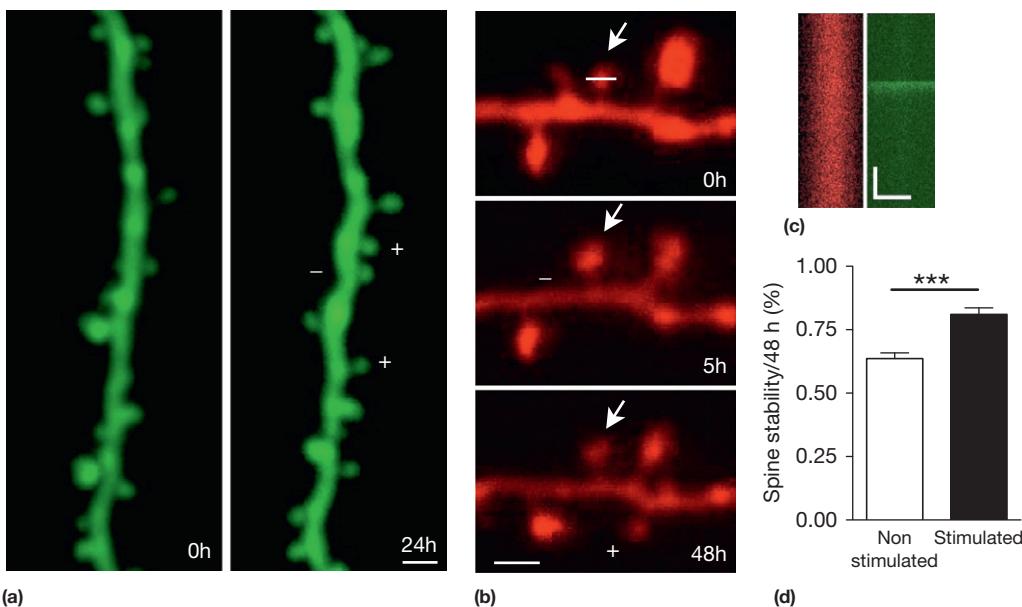
Another interesting set of questions pertains to the respective roles of the pre- and postsynaptic partners in regulating the formation of the new synapse. The observation that new protrusions initially grow without expressing a PSD indicates that the growth of the protrusion is probably initiated postsynaptically. Similarly, recent evidence indicating that the control of the number of PSDs and contacts made on a spine is regulated by the postsynaptic release of nitric oxide (NO) further suggests a primary role for the postsynaptic side in the control of the formation of a contact with a given partner (Nikonenko et al., 2008). However, there is also evidence indicating a role for active axon terminals in the promotion of spine growth. Notably, presynaptic activity and glutamate have been shown to promote spine growth (Maletic-Savatic et al., 1999; Richards et al., 2005). In addition, new protrusions seem to prefer to grow towards axonal boutons with active synapses (Knott et al., 2006; Toni et al., 2007); conversely, activated presynaptic boutons may grow filopodia-like protrusions that

establish synaptic contacts (Nikonenko et al., 2003). These observations therefore suggest that glutamate may also act as a trophic factor that could guide new protrusions to active boutons. Additionally, a great variety of adhesion and signaling molecules expressed either on pre- or postsynaptic structures have been shown to promote synapse formation even between non-neuronal partners (Dalva et al., 2007; Han and Kim, 2008). The process of synapse formation is therefore very likely to depend upon complex signaling mechanisms linked to numerous secreted or membrane-bound molecules present on both pre- and postsynaptic structures.

## 6.6 SPINE DYNAMICS AND DEVELOPMENT OF SYNAPTIC NETWORKS

*In vivo* analyses of spine behavior over periods ranging from minutes to days and months has revealed that spines are very dynamic structures that exhibit various forms of motility and morphological remodeling. A first type of motility initially reported in cultures, but also described *in vivo*, relates to fast (seconds to minutes) twitching of spine heads that has been linked to a continuous rearrangement of the actin cytoskeleton (Fischer et al., 1998; Majewska et al., 2006). Although this form of motility has been associated with activity and experience-dependent plasticity (Oray et al., 2004), its significance in terms of synapse function and properties remains unclear. One possibility is that this spine twitching relates to the mechanisms of protein turnover, which is particularly high in dendritic spines. Proteins such as PSD-95, Ras, or Shank3 have been shown to redistribute between synaptic and dendritic pools within a few minutes to a few hours (Gray et al., 2006; Tsuriel et al., 2006). These trafficking mechanisms might possibly require a continuous rearrangement of the actin cytoskeleton.

On a different timescale, spines have also been shown to undergo a continuous turnover through a process of formation and elimination (Figure 6.3(a)). The rate of spine growth and disappearance is particularly high during development and critical periods when the main organization of the cortical synaptic network is established (Cruz-Martin et al., 2010; Lendvai et al., 2000; Zuo et al., 2005). Spine turnover then decreases with brain maturation but remains effective at a low level even in adult mice (Grutzendler et al., 2002; Trachtenberg et al., 2002). Quantification of spine turnover in various brain regions has been difficult and shows great variability. In mice, turnover rate can affect as many as 5–20% of total spines per hour during the first weeks after birth, decreasing to about 5–10% per week at 1 month of age and 1–2% per month in adult



**FIGURE 6.3** Confocal images illustrating spine dynamics in hippocampal slice cultures. (a) Dendritic segment of a CA1 pyramidal neuron expressing eGFP and imaged at 24 h interval. Plus and minus signs indicate newly formed and lost spines. Scale bar: 1  $\mu$ m. (b) Enlargement and stabilization of a stimulated spine in a cell expressing mRFP and loaded with the calcium indicator Fluo4-AM. Line scan analysis of the spine observed at time 0 h showed an increased calcium transient upon electrical stimulation of a group of CA3 cells. Theta burst activation of this spine resulted in an enlargement of the spine head 5 h later and promoted the persistence of the spine over the next 48 h. Scale bar: 1  $\mu$ m. (c) Line scan analyses through the spine illustrated in (b) and showing the red (mRFP) and green (Fluo4-AM) signals. Scale bars: 0.5  $\mu$ m; 0.5 s. (d) Stimulated spines show a significantly increased stability over the next 48 h than non-stimulated spines (n=37 and 44 spines analyzed).

tissue (Cruz-Martin et al., 2010; Holtmaat and Svoboda, 2009); however, these values vary quite substantially depending on the cortical region or cell type analyzed, the criteria used for analysis, and the experimental approach. For example, the possible effects of anesthesia must be considered (De Roo et al., 2009). Despite this variability, the important message delivered by these studies is that remodeling of synaptic connections is very substantial during development and remains a significant phenomenon throughout life. Further interesting information provided by these experiments concerns the lifetime of a synapse. Analyses of spine stability over weeks or months have shown that not all spines have the same lifespan. While many new spines appear to be very transient, others may persist for prolonged periods of time, up to years under *in vivo* conditions (Grutzendler et al., 2002; Holtmaat et al., 2005). Consistent with the changes in spine dynamics, the fraction of persistent spines also increases as a function of development, with more than 95% of spines surviving months in adult tissue.

An important issue regarding the mechanisms of spine dynamics pertains to their contribution to the function and development of brain networks. Repetitive imaging approaches have shown that stimulation protocols that are used to induce LTP are associated with the growth of new protrusions (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Nagerl et al., 2007;

Toni et al., 1999). This growth process can, however, also be associated with an increased elimination of spines and thus involves a more subtle control of spine dynamics (De Roo et al., 2008b; Nagerl et al., 2004). Sensory stimulation and motor learning have both been reported to stimulate an increase in spine turnover, leading to modifications of network organization (Holtmaat et al., 2006; Wilbrecht et al., 2010; Xu et al., 2009; Yang et al., 2009). Similarly, in the visual cortex, monocular deprivation and restoration of binocular vision are associated with marked changes in spine dynamics and spine density (Hofer et al., 2009). All of these results suggest a strong link between functional plasticity and specific synaptic rearrangements.

The question, however, is to understand the specificity of these synaptic rearrangements and how they may affect brain networks at a functional level. An important hint is provided by studies of spine dynamics in relation to induction of synaptic plasticity. When LTP is induced at a group of synapses, several structural rearrangements can be observed (Figure 6.3(b–c)). First, stimulated synapses enlarge (De Roo et al., 2008b; Ehrlich et al., 2007; Harvey and Svoboda, 2007; Kopec et al., 2007; Matsuzaki et al., 2004), a process closely correlated with the accumulation of AMPAR at the synapse (Zito et al., 2009) and the reorganization of the actin cytoskeleton (Honkura et al., 2008). Spine size and synaptic efficacy are thus tightly linked.

A second important characteristic of potentiated synapses is that they are switched to a stable state (De Roo et al., 2008b). This effect is very specific for potentiated synapses since unstimulated synapses tend to undergo elimination (Figure 6.3(d)). How this activity-dependent structural remodeling of spines confers stability on them is still unclear. The close correlation existing between spine head size, PSD size, receptor number, and stability suggests that the phenomenon could be non-specifically related to the amount of receptors and proteins accumulated at the synapse: larger spines with larger PSDs express more adhesion and cross-linking molecules and are more stable. An important aspect to consider in this interpretation is that the size of spine heads shows continuous fluctuations over time (De Roo et al., 2008b; Holtmaat et al., 2005; Yasumatsu et al., 2008). Small spines can enlarge and retract over periods of days. This would suggest that stability, together with PSD size and strength, could continuously fluctuate according to the activity history of the synapse. Another interpretation, however, might be that there exist specific molecular events able to reinforce spine stability. For example, it has been suggested by ultrastructural analyses that stabilization could be provided through the acquisition by the potentiated spines of the machinery for mRNA translation. This would allow local regulation of protein synthesis and trafficking and thus confer independence and stability to the spine (Bramham, 2008; Ostroff et al., 2002; Steward and Falk, 1985). Additionally, this could be associated with the expression at the synapse of specific proteins able to stabilize the cytoskeleton or anchor pre- and postsynaptic structures. Recent evidence suggests that N-cadherin could play such a role (Mendez et al., 2010a). N-cadherin is expressed on both sides of the synaptic cleft and is involved in the formation of homophilic binding domains, which might account for the periodic protein complexes observed across the cleft (Zuber et al., 2005). Expression of dominant-negative mutant N-cadherin or knockdown of N-cadherin expression leads to the formation of unstable synapses. Conversely, expression of N-cadherin is associated with a synapse-specific increase in stability. Additionally, N-cadherin expression is regulated by synaptic activity, and it is selectively expressed in potentiated synapses upon induction of LTP, leading to a switch in stability of synapses. A main function of LTP during development might therefore be to promote stabilization through a change in the composition of the PSD and, importantly, an increase in the expression of the adhesion molecule N-cadherin.

The third important structural change induced by induction of LTP is an increase in spine dynamics. This process also shows specificity. Following induction of plasticity, growth predominantly takes place at the

vicinity of stimulated synapses, promoting in this way the formation of clusters of activated synapses (De Roo et al., 2008b). This might have important consequences, particularly for spines on remote dendrites, where spatiotemporal clustering of synaptic activity may play an important role for information processing (Nevian et al., 2007). Additionally, spine elimination also occurs in a very selective manner, since the process concerns mainly unstimulated spines. Thus, the changes in spine dynamics triggered by patterns of activity will affect differentially synaptic connections by favoring clustered activity with new partners, while eliminating inactive synapses. Together, these studies indicate that during development, plasticity mechanisms not only change synaptic strength, but also act as a major process organizing the wiring of synaptic networks. By switching stimulated synapses to a persistent state and additionally promoting the replacement of unstimulated synapses by new ones, LTP operates as a selection mechanism that maintains connections mediating coherent, synchronized activity, while, at the same time, favoring adaptation of the network. This fine-tuning process could be particularly important during critical periods, ensuring the specificity of network development, and may serve as an efficient learning and memory system. In keeping with this interpretation, *in vivo* work analyzing spine dynamics associated with a learning task in motor cortex found that memory of the task was associated with an increase in spine turnover and a selective stabilization of newly formed spines over the course of months (Xu et al., 2009; Yang et al., 2009).

In addition to activity patterns inducing synaptic plasticity, spine growth or elimination appears to be also regulated by a number of other homeostatic processes. One factor that seems to be very important for the regulation of spine dynamics during critical periods of development is the balance between excitation and inhibition (Hensch et al., 1998; Morishita and Hensch, 2008). In the visual cortex, alteration of this balance markedly affects the onset, formation, and plasticity of ocular dominance columns. This process, which is directly related to mechanisms of spine formation and pruning (Hofer et al., 2009), is regulated by GABAergic inhibition. Recent work has identified an important functional, bidirectional recruitment of fast-spiking interneurons during experience-dependent plasticity (Yazaki-Sugiyama et al., 2009). Also, in a manner consistent with this, the application of anesthetics to developing cortical tissue promotes a rapid enhancement of spine and synapse formation leading to a marked increase in spine density on pyramidal neurons (De Roo et al., 2009). Interestingly, this control by the excitation/inhibition balance could still persist even in the adult visual cortex. Recent experiments show that the removal of a molecular brake responsible for the age-dependent loss of visual plasticity demonstrates

the persistence of a control by the excitation/inhibition balance and suggests that the mechanisms which reduce adult structural plasticity could work by adjusting this balance (Morishita et al., 2010).

In addition, growth factors and hormones are also very likely to participate in the regulation of spine dynamics. For example, there is strong evidence suggesting an important role for estrogens in the regulation of spine formation and density (McEwen, 2010). The effect is rapid and affects spine growth but not spine elimination (Mendez et al., 2010b); it may thus represent a homeostatic mechanism for adapting spine density and the complexity of synaptic networks through changes in spine dynamics.

## 6.7 SPINE ALTERATIONS AND BRAIN DISEASE

Alterations of dendritic spine morphology or function represent key features of various developmental psychiatric disorders and neurodegenerative diseases. In the last two decades, progress concerning the identification of genetic alterations associated with mental retardation and autism spectrum disorders has revealed that, in many instances, these defects concern genes coding for synaptic proteins or proteins involved in the regulation of synaptic properties (Bourgeron, 2009; Laumonnier et al., 2007; Ropers, 2006). Analyses of the underlying mechanisms using gain and loss of function approaches have revealed various kinds of alterations, especially defects affecting spine morphology, spine density, or synaptic plasticity. One of the best examples of intellectual disability is probably the fragile X syndrome linked to the silencing of the fmr1 gene (Bagni and Greenough, 2005). The protein coded by the fmr1 gene (FMRP) is associated with polyribosomes in dendrites and spines, and can be found in dendritic RNA granules that travel on microtubules in dendrites. FMRP is thus believed to play a role in the regulation of local translation at individual synapses, acting both as translational suppressor and/or activator. The main defects reported in Fmr1 knockout mice include an increased spine density with more long, thin, tortuous spines reminiscent of immature filopodia-like spines, as well as defects in synaptic plasticity characterized by impaired cortical LTP and enhanced metabotropic glutamate receptor (mGluR)-mediated long-term depression (LTD). This has led to the hypothesis that FMRP could function as a negative feedback mechanism to suppress mGluR-stimulated translation implicated in LTD and the endocytosis of AMPARs (Huber et al., 2002). As such, knockdown of FMRP is believed to result in an increased mGluR-dependent removal of surface AMPARs, and evidence suggests that this defect could be corrected by antagonists of metabotropic receptors (Dolen et al., 2007).

Several other mutations of synaptic proteins identified as associated with cognitive disabilities, autism spectrum disorders, or even schizophrenia also result in dendritic spine abnormalities. This is the case for oligophrenin, PAK3, ARGHEF6, SynGAP, Shank, and EPAC2, but defects have also been reported in a mouse model of the 22q11 syndrome, a condition at high risk for schizophrenia, or following interference with disrupted-in-schizophrenia 1 (DISC1) (Boda et al., 2004; Govek et al., 2004; Node-Langlois et al., 2006; Sala et al., 2001; Vazquez et al., 2004; Woolfrey et al., 2009). In many of these cases, the synaptic defects include an increase in the fraction of long, thin and immature spines, and this is also often associated with alteration of glutamate receptor recycling. These results have therefore led to the hypothesis that cognitive disability could primarily result from alterations of dendritic spine plasticity, either because of defects in receptor recycling mechanisms that affect synaptic strength or because of alterations in spine formation, maturation, or dynamics that could interfere with the development and specificity of synaptic networks (Boda et al., 2010).

In addition to these developmental psychiatric disorders, spine pathology has also been observed in association with degenerative diseases such as Alzheimer's disease (Selkoe, 2002; Spires et al., 2005), Parkinson's disease (Day et al., 2006), or prion disease (Fuhrmann et al., 2007). In mouse models of Alzheimer's disease, the vicinity of amyloid plaques is characterized by highly dysmorphic neurites and spine loss, a phenotype that could be caused by the inhibition by amyloid oligomers of LTP mechanisms and the promotion of LTD (Wei et al., 2010). These results thus also suggest that changes in spine dynamics may provide an important contribution to the alterations and dysfunctions of neuronal networks in degenerative and memory disorders.

## 6.8 CONCLUSION

Dendritic spines are remarkable structures that exhibit a high degree of structural and functional specialization. They are tightly controlled by activity and signaling mechanisms that determine their properties of plasticity and their long-term maintenance in brain networks. The complexity of these regulations and their impact on the formation and function of brain circuits clearly highlight the key role played by dendritic spines in the processing of information by neuronal networks.

## References

- Aoto, J., Ting, P., Maghsoodi, B., Xu, N., Henkemeyer, M., Chen, L., 2007. Postsynaptic ephrinB3 promotes shaft glutamatergic synapse formation. *Journal of Neuroscience* 27, 7508–7519.

- Arellano, J.I., Espinosa, A., Fairen, A., Yuste, R., DeFelipe, J., 2007. Non-synaptic dendritic spines in neocortex. *Neuroscience* 145, 464–469.
- Arstikaitis, P., Gauthier-Campbell, C., Carolina Gutierrez Herrera, R., et al., 2008. Paralemmin-1, a modulator of filopodia induction is required for spine maturation. *Molecular Biology of the Cell* 19, 2026–2038.
- Bagni, C., Greenough, W.T., 2005. From mRNP trafficking to spine dysmorphogenesis: The roots of fragile X syndrome. *Nature Reviews Neuroscience* 6, 376–387.
- Bats, C., Groc, L., Choquet, D., 2007. The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. *Neuron* 53, 719–734.
- Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature* 361, 31–39.
- Boda, B., Alberi, S., Nikonenko, I., et al., 2004. The mental retardation protein PAK3 contributes to synapse formation and plasticity in hippocampus. *Journal of Neuroscience* 24, 10816–10825.
- Boda, B., Dubos, A., Muller, D., 2010. Signaling mechanisms regulating synapse formation and function in mental retardation. *Current Opinion in Neurobiology* 20, 519–527.
- Borgdorff, A.J., Choquet, D., 2002. Regulation of AMPA receptor lateral movements. *Nature* 417, 649–653.
- Bourgeron, T., 2009. A synaptic trek to autism. *Current Opinion in Neurobiology* 19, 231–234.
- Bourne, J., Harris, K.M., 2007. Do thin spines learn to be mushroom spines that remember? *Current Opinion in Neurobiology* 17, 1–6.
- Bourne, J.N., Sorra, K.E., Hurlburt, J., Harris, K.M., 2007. Polyribosomes are increased in spines of CA1 dendrites 2 h after the induction of LTP in mature rat hippocampal slices. *Hippocampus* 17, 1–4.
- Bramham, C.R., 2008. Local protein synthesis, actin dynamics, and LTP consolidation. *Current Opinion in Neurobiology* 18, 524–531.
- Chang, F.L., Greenough, W.T., 1984. Transient and enduring morphological correlates of synaptic activity and efficacy change in the rat hippocampal slice. *Brain Research* 309, 35–46.
- Cooke, S.F., Bliss, T.V., 2006. Plasticity in the human central nervous system. *Brain* 129, 1659–1673.
- Cooney, J.R., Hurlburt, J.L., Selig, D.K., Harris, K.M., Fiala, J.C., 2002. Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. *Journal of Neuroscience* 22, 2215–2224.
- Cruz-Martin, A., Crespo, M., Portera-Cailliau, C., 2010. Delayed stabilization of dendritic spines in fragile X mice. *Journal of Neuroscience* 30, 7793–7803.
- Dalva, M.B., McClelland, A.C., Kayser, M.S., 2007. Cell adhesion molecules: Signalling functions at the synapse. *Nature Reviews Neuroscience* 8, 206–220.
- Day, M., Wang, Z., Ding, J., et al., 2006. Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nature Neuroscience* 9, 251–259.
- De Roo, M., Klauser, P., Mendez, P., Poglia, L., Muller, D., 2008a. Activity-dependent PSD formation and stabilization of newly formed spines in hippocampal slice cultures. *Cerebral Cortex* 18, 151–161.
- De Roo, M., Klauser, P., Muller, D., 2008b. LTP promotes a selective long-term stabilization and clustering of dendritic spines. *PLoS Biology* 6, e219.
- De Roo, M., Klauser, P., Briner, A., et al., 2009. Anesthetics rapidly promote synaptogenesis during a critical period of brain development. *PLoS One* 4, e7043.
- Dolen, G., Osterweil, E., Rao, B.S., et al., 2007. Correction of fragile X syndrome in mice. *Neuron* 56, 955–962.
- Dudek, S.M., Bear, M.F., 1993. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *Journal of Neuroscience* 13, 2910–2918.
- Ehrlich, I., Klein, M., Rumpel, S., Malinow, R., 2007. PSD-95 is required for activity-driven synapse stabilization. *Proceedings of the National Academy of Sciences of the United States of America* 104, 4176–4181.
- Emes, R.D., Pocklington, A.J., Anderson, C.N., et al., 2008. Evolutionary expansion and anatomical specialization of synapse proteome complexity. *Nature Neuroscience* 11, 799–806.
- Engert, F., Bonhoeffer, T., 1999. Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* 399, 66–70.
- Ethell, I.M., Yamaguchi, Y., 1999. Cell surface heparan sulfate proteoglycan syndecan-2 induces the maturation of dendritic spines in rat hippocampal neurons. *The Journal of Cell Biology* 144, 575–586.
- Fischer, M., Kaech, S., Knutti, D., Matus, A., 1998. Rapid actin-based plasticity in dendritic spines. *Neuron* 20, 847–854.
- Frischknecht, R., Heine, M., Perrais, D., Seidenbecher, C.I., Choquet, D., Gundelfinger, E.D., 2009. Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. *Nature Neuroscience* 12, 897–904.
- Fuhrmann, M., Mitteregger, G., Kretzschmar, H., Herms, J., 2007. Dendritic pathology in prion disease starts at the synaptic spine. *Journal of Neuroscience* 27, 6224–6233.
- Furutani, Y., Matsuno, H., Kawasaki, M., Sasaki, T., Mori, K., Yoshihara, Y., 2007. Interaction between telencephalin and ERM family proteins mediates dendritic filopodia formation. *Journal of Neuroscience* 27, 8866–8876.
- Ganeshina, O., Berry, R.W., Petralia, R.S., Nicholson, D.A., Geinisman, Y., 2004. Differences in the expression of AMPA and NMDA receptors between axospinous perforated and nonperforated synapses are related to the configuration and size of postsynaptic densities. *The Journal of Comparative Neurology* 468, 86–95.
- Geinisman, Y., de Toledo-Morrell, L., Morrell, F., Heller, R.E., Rossi, M., Parshall, R.F., 1993. Structural synaptic correlate of long-term potentiation: Formation of axospinous synapses with multiple, completely partitioned transmission zones. *Hippocampus* 3, 435–445.
- Genoud, C., Quairiaux, C., Steiner, P., Hirling, H., Welker, E., Knott, G.W., 2006. Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. *PLoS Biology* 4, e343.
- Govek, E.E., Newey, S.E., Akerman, C.J., Cross, J.R., Van der Veken, L., Van Aelst, L., 2004. The X-linked mental retardation protein oligophrenin-1 is required for dendritic spine morphogenesis. *Nature Neuroscience* 7, 364–372.
- Gray, N.W., Weimer, R.M., Bureau, I., Svoboda, K., 2006. Rapid redistribution of synaptic PSD-95 in the neocortex in vivo. *PLoS Biology* 4, e370.
- Grutzendler, J., Kasthuri, N., Gan, W.B., 2002. Long-term dendritic spine stability in the adult cortex. *Nature* 420, 812–816.
- Gundelfinger, E.D., Frischknecht, R., Choquet, D., Heine, M., 2010. Converting juvenile into adult plasticity: A role for the brain's extracellular matrix. *European Journal of Neuroscience* 31, 2156–2165.
- Han, K., Kim, E., 2008. Synaptic adhesion molecules and PSD-95. *Progress in Neurobiology* 84, 263–283.
- Harris, K.M., Stevens, J.K., 1989. Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: Serial electron microscopy with reference to their biophysical characteristics. *Journal of Neuroscience* 9, 2982–2997.
- Harvey, C.D., Svoboda, K., 2007. Locally dynamic synaptic learning rules in pyramidal neuron dendrites. *Nature* 450, 1195–1200.
- Haydon, P.G., 2001. GLIA: Listening and talking to the synapse. *Nature Reviews Neuroscience* 2, 185–193.
- Hensch, T.K., Fagiolini, M., Mataga, N., Stryker, M.P., Baekkeskov, S., Kash, S.F., 1998. Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science* 282, 1504–1508.
- Hofer, S.B., Mrsic-Flogel, T.D., Bonhoeffer, T., Hubener, M., 2009. Experience leaves a lasting structural trace in cortical circuits. *Nature* 457, 313–317.

- Holtmaat, A., Svoboda, K., 2009. Experience-dependent structural synaptic plasticity in the mammalian brain. *Nature Reviews Neuroscience* 10, 647–658.
- Holtmaat, A.J., Trachtenberg, J.T., Wilbrecht, L., et al., 2005. Transient and persistent dendritic spines in the neocortex *in vivo*. *Neuron* 45, 279–291.
- Holtmaat, A., Wilbrecht, L., Knott, G.W., Welker, E., Svoboda, K., 2006. Experience-dependent and cell-type-specific spine growth in the neocortex. *Nature* 441, 979–983.
- Honkura, N., Matsuzaki, M., Noguchi, J., Ellis-Davies, G.C., Kasai, H., 2008. The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. *Neuron* 57, 719–729.
- Hotulainen, P., Hoogenraad, C.C., 2010. Actin in dendritic spines: Connecting dynamics to function. *The Journal of Cell Biology* 189, 619–629.
- Hu, X., Ballo, L., Pietila, L., et al., 2011. BDNF-induced increase of PSD-95 in dendritic spines requires dynamic microtubule invasions. *Journal of Neuroscience* 31, 15597–15603.
- Huber, K.M., Gallagher, S.M., Warren, S.T., Bear, M.F., 2002. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proceedings of the National Academy of Sciences of the United States of America* 99, 7746–7750.
- Jaworski, J., Kapitein, L.C., Gouveia, S.M., et al., 2009. Dynamic microtubules regulate dendritic space morphology and synaptic plasticity.
- Jourdain, P., Fukunaga, K., Muller, D., 2003. Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation. *Journal of Neuroscience* 23, 10645–10649.
- Kayser, M.S., Nolt, M.J., Dalva, M.B., 2008. EphB receptors couple dendritic filopodia motility to synapse formation. *Neuron* 59, 56–69.
- Kelleher Jr., R., Govindarajan, A., Jung, H.Y., Kang, H., Tonegawa, S., 2004. Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell* 116, 467–479.
- Kielland, A., Bochorishvili, G., Corson, J., et al., 2009. Activity patterns govern synapse-specific AMPA receptor trafficking between deliverable and synaptic pools. *Neuron* 62, 84–101.
- Knott, G.W., Quairiaux, C., Genoud, C., Welker, E., 2002. Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. *Neuron* 34, 265–273.
- Knott, G.W., Holtmaat, A., Wilbrecht, L., Welker, E., Svoboda, K., 2006. Spine growth precedes synapse formation in the adult neocortex *in vivo*. *Nature Neuroscience* 9, 1117–1124.
- Kopec, C.D., Real, E., Kessels, H.W., Malinow, R., 2007. GluR1 links structural and functional plasticity at excitatory synapses. *Journal of Neuroscience* 27, 13706–13718.
- Kumar, V., Zhang, M.X., Swank, M.W., Kunz, J., Wu, G.Y., 2005. Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. *Journal of Neuroscience* 25, 11288–11299.
- Laumonnier, F., Cuthbert, P.C., Grant, S.G., 2007. The role of neuronal complexes in human x-linked brain diseases. *American Journal of Human Genetics* 80, 205–220.
- Lendvai, B., Stern, E.A., Chen, B., Svoboda, K., 2000. Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex *in vivo*. *Nature* 404, 876–881.
- Lin, Y.L., Lei, Y.T., Hong, C.J., Hsueh, Y.P., 2007. Syndecan-2 induces filopodia and dendritic spine formation via the neurofibromin-PKA-Ena/VASP pathway. *The Journal of Cell Biology* 177, 829–841.
- Lisman, J., Schulman, H., Cline, H., 2002. The molecular basis of CaMKII function in synaptic and behavioural memory. *Nature Reviews Neuroscience* 3, 175–190.
- Lohmann, C., Bonhoeffer, T., 2008. A role for local calcium signaling in rapid synaptic partner selection by dendritic filopodia. *Neuron* 59, 253–260.
- Lohmann, C., Finski, A., Bonhoeffer, T., 2005. Local calcium transients regulate the spontaneous motility of dendritic filopodia. *Nature Neuroscience* 8, 305–312.
- Lushnikova, I., Skibo, G., Muller, D., Nikonenko, I., 2009. Synaptic potentiation induces increased glial coverage of excitatory synapses in CA1 hippocampus. *Hippocampus* 19, 753–762.
- Majewska, A.K., Newton, J.R., Sur, M., 2006. Remodeling of synaptic structure in sensory cortical areas *in vivo*. *Journal of Neuroscience* 26, 3021–3029.
- Makino, H., Malinow, R., 2009. AMPA receptor incorporation into synapses during LTP: The role of lateral movement and exocytosis. *Neuron* 64, 381–390.
- Malenka, R.C., Nicoll, R.A., 1999. Long-term potentiation—a decade of progress? *Science* 285, 1870–1874.
- Maletic-Savatic, M., Malinow, R., Svoboda, K., 1999. Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* 283, 1923–1927.
- Malinow, R., Malenka, R.C., 2002. AMPA receptor trafficking and synaptic plasticity. *Annual Review of Neuroscience* 25, 103–126.
- Marrs, G.S., Green, S.H., Dailey, M.E., 2001. Rapid formation and remodeling of postsynaptic densities in developing dendrites. *Nature Neuroscience* 4, 1006–1013.
- Matsuno, H., Okabe, S., Mishina, M., Yanagida, T., Mori, K., Yoshihara, Y., 2006. Telencephalin slows spine maturation. *Journal of Neuroscience* 26, 1776–1786.
- Matsuzaki, M., Honkura, N., Ellis-Davies, G.C., Kasai, H., 2004. Structural basis of long-term potentiation in single dendritic spines. *Nature* 429, 761–766.
- McEwen, B.S., 2010. Stress, sex, and neural adaptation to a changing environment: Mechanisms of neuronal remodeling. *Annals of the New York Academy of Sciences* 1204 (Suppl), E38–E59.
- Mendez, P., De Roo, M., Poglia, L., Klausner, P., Muller, D., 2010. N-cadherin mediates plasticity-induced long-term spine stabilization. *The Journal of Cell Biology* 189, 589–600.
- Mendez, P., Garcia-Segura, L.M., Muller, D., 2010. Estradiol promotes spine growth and synapse formation without affecting pre-established networks. *Hippocampus* 28, 8.
- Mitsuyama, F., Niimi, G., Kato, K., 2008. Redistribution of microtubules in dendrites of hippocampal CA1 neurons after tetanic stimulation during long-term potentiation. *Ital J Anat Embryol* 113, 17–27.
- Morishita, H., Hensch, T.K., 2008. Critical period revisited: Impact on vision. *Current Opinion in Neurobiology* 18, 101–107.
- Morishita, H., Miwa, J.M., Heintz, N., Hensch, T.K., 2010. Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. *Science* 330, 1238–1240.
- Nagerl, U.V., Eberhorn, N., Cambridge, S.B., Bonhoeffer, T., 2004. Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron* 44, 759–767.
- Nagerl, U.V., Kostinger, G., Anderson, J.C., Martin, K.A., Bonhoeffer, T., 2007. Protracted synaptogenesis after activity-dependent spinogenesis in hippocampal neurons. *Journal of Neuroscience* 27, 8149–8156.
- Nevian, T., Larkum, M.E., Polsky, A., Schiller, J., 2007. Properties of basal dendrites of layer 5 pyramidal neurons: A direct patch-clamp recording study. *Nature Neuroscience* 10, 206–214.
- Nicoll, R.A., Malenka, R.C., 1995. Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* 377, 115–118.
- Nicoll, R.A., Tomita, S., Bredt, D.S., 2006. Auxiliary subunits assist AMPA-type glutamate receptors. *Science* 311, 1253–1256.
- Nikonenko, I., Jourdain, P., Muller, D., 2003. Presynaptic remodeling contributes to activity-dependent synaptogenesis. *Journal of Neuroscience* 23, 8498–8505.
- Nikonenko, I., Boda, B., Steen, S., Knott, G., Welker, E., Muller, D., 2008. PSD-95 promotes synaptogenesis and multiinnervated spine

- formation through nitric oxide signaling. *The Journal of Cell Biology* 183, 1115–1127.
- Node-Langlois, R., Muller, D., Boda, B., 2006. Sequential implication of the mental retardation proteins ARHGEF6 and PAK3 in spine morphogenesis. *Journal of Cell Science* 119, 4986–4993.
- Opazo, P., Labrecque, S., Tigaret, C.M., et al., 2010. CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. *Neuron* 67, 239–252.
- Oray, S., Majewska, A., Sur, M., 2004. Dendritic spine dynamics are regulated by monocular deprivation and extracellular matrix degradation. *Neuron* 44, 1021–1030.
- Ostroff, L.E., Fiala, J.C., Allwardt, B., Harris, K.M., 2002. Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* 35, 535–545.
- Pak, D.T., Sheng, M., 2003. Targeted protein degradation and synapse remodeling by an inducible protein kinase. *Science* 302, 1368–1373.
- Park, M., Salgado, J.M., Ostroff, L., et al., 2006. Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. *Neuron* 52, 817–830.
- Penzes, P., Jones, K.A., 2008. Dendritic spine dynamics—a key role for kalirin-7. *Trends in Neurosciences* 31, 419–427.
- Poglia, L., Muller, D., Nikonenko, I., 2010. Ultrastructural modifications of spine and synapse morphology by SAP97. *Hippocampus* 21, 990–998.
- Racz, B., Blanpied, T.A., Ehlers, M.D., Weinberg, R.J., 2004. Lateral organization of endocytic machinery in dendritic spines. *Nature Neuroscience* 7, 917–918.
- Richards, D.A., Mateos, J.M., Hugel, S., et al., 2005. Glutamate induces the rapid formation of spine head protrusions in hippocampal slice cultures. *Proceedings of the National Academy of Sciences of the United States of America* 102, 6166–6171.
- Ropers, H.H., 2006. X-linked mental retardation: Many genes for a complex disorder. *Current Opinion in Genetics and Development* 16, 260–269.
- Sala, C., Piech, V., Wilson, N.R., Passafaro, M., Liu, G., Sheng, M., 2001. Regulation of dendritic spine morphology and synaptic function by Shank and Homer. *Neuron* 31, 115–130.
- Selkoe, D.J., 2002. Alzheimer's disease is a synaptic failure. *Science* 298, 789–791.
- Spacek, J., Harris, K.M., 1997. Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *Journal of Neuroscience* 17, 190–203.
- Spires, T.L., Meyer-Luehmann, M., Stern, E.A., et al., 2005. Dendritic spine abnormalities in amyloid precursor protein transgenic mice demonstrated by gene transfer and intravital multiphoton microscopy. *Journal of Neuroscience* 25, 7278–7287.
- Steward, O., Falk, P.M., 1985. Polyribosomes under developing spine synapses: Growth specializations of dendrites at sites of synaptogenesis. *Journal of Neuroscience Research* 13, 75–88.
- Tolias, K.F., Bikoff, J.B., Kane, C.G., Tolias, C.S., Hu, L., Greenberg, M.E., 2007. The Rac1 guanine nucleotide exchange factor Tiam1 mediates EphB receptor-dependent dendritic spine development. *Proceedings of the National Academy of Sciences of the United States of America* 104, 7265–7270.
- Toni, N., Buchs, P.A., Nikonenko, I., Bron, C.R., Muller, D., 1999. LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. *Nature* 402, 421–425.
- Toni, N., Buchs, P.A., Nikonenko, I., Povilaitite, P., Parisi, L., Muller, D., 2001. Remodeling of synaptic membranes after induction of long-term potentiation. *Journal of Neuroscience* 21, 6245–6251.
- Toni, N., Teng, E.M., Bushong, E.A., et al., 2007. Synapse formation on neurons born in the adult hippocampus. *Nature Neuroscience* 10, 727–734.
- Toresson, H., Grant, S.G., 2005. Dynamic distribution of endoplasmic reticulum in hippocampal neuron dendritic spines. *European Journal of Neuroscience* 22, 1793–1798.
- Trachtenberg, J.T., Chen, B.E., Knott, G.W., et al., 2002. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* 420, 788–794.
- Triller, A., Choquet, D., 2008. New concepts in synaptic biology derived from single-molecule imaging. *Neuron* 59, 359–374.
- Tsurui, S., Geva, R., Zamorano, P., et al., 2006. Local sharing as a predominant determinant of synaptic matrix molecular dynamics. *PLoS Biology* 4, e271.
- Vazquez, L.E., Chen, H.J., Sokolova, I., Knuesel, I., Kennedy, M.B., 2004. SynGAP regulates spine formation. *Journal of Neuroscience* 24, 8862–8872.
- Volterra, A., Meldolesi, J., 2005. Astrocytes, from brain glue to communication elements: The revolution continues. *Nature Reviews Neuroscience* 6, 626–640.
- Wang, X.B., Bozdagi, O., Nikitczuk, J.S., Zhai, Z.W., Zhou, Q., Huntley, G.W., 2008. Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. *Proceedings of the National Academy of Sciences of the United States of America* 105, 19520–19525.
- Wei, W., Nguyen, L.N., Kessels, H.W., Hagiwara, H., Sisodia, S., Malinow, R., 2010. Amyloid beta from axons and dendrites reduces local spine number and plasticity. *Nature Neuroscience* 13, 190–196.
- Wilbrecht, L., Holtmaat, A., Wright, N., Fox, K., Svoboda, K., 2010. Structural plasticity underlies experience-dependent functional plasticity of cortical circuits. *Journal of Neuroscience* 30, 4927–4932.
- Witcher, M.R., Kirov, S.A., Harris, K.M., 2007. Plasticity of perisynaptic astroglia during synaptogenesis in the mature rat hippocampus. *Glia* 55, 13–23.
- Woolfrey, K.M., Srivastava, D.P., Photowala, H., et al., 2009. Epac2 induces synapse remodeling and depression and its disease-associated forms alter spines. *Nature Neuroscience* 12, 1275–1284.
- Xu, T., Yu, X., Perlik, A.J., et al., 2009. Rapid formation and selective stabilization of synapses for enduring motor memories. *Nature* 462, 915–919.
- Yang, G., Pan, F., Gan, W.B., 2009. Stably maintained dendritic spines are associated with lifelong memories. *Nature* 462, 920–924.
- Yasumatsu, N., Matsuzaki, M., Miyazaki, T., Noguchi, J., Kasai, H., 2008. Principles of Long-Term Dynamics of Dendritic Spines. *Journal of Neuroscience* 28, 13592–13608.
- Yazaki-Sugiyama, Y., Kang, S., Cateau, H., Fukai, T., Hensch, T.K., 2009. Bidirectional plasticity in fast-spiking GABA circuits by visual experience. *Nature* 462, 218–221.
- Yoshihara, Y., De Roo, M., Muller, D., 2009. Dendritic spine formation and stabilization. *Current Opinion in Neurobiology* 19, 146–153.
- Zito, K., Scheuss, V., Knott, G., Hill, T., Svoboda, K., 2009. Rapid functional maturation of nascent dendritic spines. *Neuron* 61, 247–258.
- Ziv, N.E., Smith, S.J., 1996. Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. *Neuron* 17, 91–102.
- Zuber, B., Nikonenko, I., Klauser, P., Muller, D., Dubochet, J., 2005. The mammalian central nervous synaptic cleft contains a high density of periodically organized complexes. *Proceedings of the National Academy of Sciences of the United States of America* 102, 19192–19197.
- Zuo, Y., Lin, A., Chang, P., Gan, W.B., 2005. Development of long-term dendritic spine stability in diverse regions of cerebral cortex. *Neuron* 46, 181–189.