Biochimica et Biophysica Acta 1838 (2014) 2391–2398

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Structural dynamics of dendritic spines: Molecular composition, geometry and functional regulation

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ARTICLE INFO

Article history:
Received 5 February 2014
Received in revised form 18 May 2014
Accepted 2 June 2014
Available online 8 June 2014

Keywords:
Filopodia
Dendritic spine
BAR domain protein
Postsynaptic membrane
Membrane receptor
Synaptic pathology

ABSTRACT

The development of dendritic spines with specific geometry and membrane composition is critical for proper synaptic function. Specific spine membrane architecture, sub-spine microdomains and spine head and neck geometry allow for well-coordinated and compartmentalized signaling, disruption of which could lead to various neurological diseases. Research from neuronal cell culture, brain slices and direct *in vivo* imaging indicates that dendritic spine development is a dynamic process which includes transition from small dendritic filopodia through a series of structural refinements to elaborate spines of various morphologies. Despite intensive research, the precise coordination of this morphological transition, the changes in molecular composition, and the relation of spines of various morphologies to function remain a central enigma in the development of functional neuronal circuits. Here, we review research so far and aim to provide insight into the key events that drive structural change during transition from immature filopodia to fully functional spines and the relevance of spine geometry to function.

1. Introduction

Dendritic spines are elaborate structural units with a specific architecture that allows for rapid and compartmentalized neuronal signal transmission [1]. The cytoplasm of dendritic spines is enriched with filamentous actin (F-actin) and regulation of its dynamics is critical for morphological changes, maturation, and stability of spines [2]. Rho GTPases regulate actin dynamics *via* multiple effectors, including NWASP, WAVE, and the LIM kinase–cofilin pathway, and are major regulators of dendritic filopodia and spine development [3]. The activity of Rho GTPases is regulated by guanine-exchange factors (GEFs) and GTPase activating proteins (GAPs), which are known to be important in the fine-tuning of spine morphology [3]. Indeed, genetic mutations of these regulators are reported to be present in the genome of patients with various forms of mental retardation [4]. Simple growth and volume expansion of immature spines may be explained by actin polymerization and bundling, whereas the unique negative curvature of the spine neck may require structural determinants other than F-actin. Studies using cultured fibroblasts show a clear role for the BAR (Bin–Amphiphysin–Rvs167) superfamily of proteins in the formation and remodeling of specific membrane curvatures [5–7]. BAR family members may also play a role in neuronal morphogenesis and spine maturation [8,9]. However, apart from membrane-associated molecular cues from the presynaptic compartment, a possible role for membrane modulators in actin and Rho GTPases mediated filopodia initiation and spine membrane construction is not clear.

Classification of spines in fixed preparations, according to their shape and size, is a popular strategy to evaluate maturation and pathological changes of neurons. Spines are often classified into three morphological categories; thin, stubby, and mushroom types. Others prefer to add two additional categories; branched and cup-shaped spines [10–14]. An important question is whether the categorization of spines according to their morphology represents a rigid classification of distinct entities or tentative labeling of transient spine states. Live-cell imaging studies clearly show that spines are very dynamic and undergo reversible transformation between thin and mushroom morphologies in a time scale of minutes to hours [15,16]. These observations support the view that categorization only reflects the transient characteristics of spine shape. On the other hand, long-term two-photon imaging of individual spines presented evidence for a persistent morphology of large mushroom spines over months *in vivo* [17]. This suggests the presence of a distinct spine subtype with a specific morphology *in vivo*, with little transformation between morphological categories. Possibly, both views are correct in specific contexts and morphological categorization of spines is useful only with a clear knowledge about their dynamics and developmental history.

Morphological categorization of spines in mature neurons may shed light on the relationship between spine morphology and synaptic efficacy. One obvious example of a relationship between structure and function is a positive correlation between spine size and synaptic strength [1,18], as the volume of a spine head may directly correlate with the amount of postsynaptic density (PSD) [19]. Another example

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http://dx.doi.org/10.1016/j.bbamem.2014.06.002
0005-2736/

is the geometry of the spine neck, which may be critical in the spread of multiple signaling molecules. The spine neck restricts the spread of Ca^{2+} signaling into the neighboring dendritic shaft [1,18]. A study showed a 15-fold difference in Ca^{2+} inside the spine head in spines with a neck diameter of 0.55 μm compared to those with 0.1 μm [20]. This result clearly indicates the importance of spine neck shape in the retention of calcium ions. In addition to small signaling molecules, activated signaling proteins and their complex can escape through spine necks to parental dendritic shafts. An example is the spread of the Rho family of GTPases into the surrounding dendritic shaft and adjacent spines [21,22]. The relationship between spine neck shape and confinement of activated protein molecules, such as autophosphorylated CaMKII and the GTP-form of Rho family proteins, is an important topic. If spines with thin and long necks can retain activated signaling molecules for a prolonged time period, the same amount of activation in signaling molecules may have a differential impact on spines dependent on their neck shape. Because proteins are larger than calcium and the reduction in diffusion through a narrow space is larger for bulky molecules, thin spine necks may be more effective in the retention of proteins compared with that of calcium ions. In support of this, spines with longer necks retain photoactivatable (PA) GFP slightly longer than those with shorter necks [23]. Another parameter that can influence the diffusion of molecules through spine necks is the ongoing activity of synapses and neurons. Previous studies indicated an increase in the retention of calcium ions and protein-based fluorescent probes in spines that were activated by tetanic stimulation or glutamate uncaging respectively [20,23]. This increase in molecular retention in spines may be associated with an increase of filamentous actin within spine necks. These examples of an intimate relationship between form and function of spine synapses lead to the hypothesis that spines of different morphologies may have distinct functional properties and their morphological and functional diversity may be established *via* multiple regulatory mechanisms operating during development. So, how does this complex spine shape form during development and how do the spines of various morphologies relate to function?

In this review, by analyzing the literature and the various approaches used to study spine development and function, we aim to provide insight into the molecular basis of what structural and compositional changes take place during transition from filopodia to spines of various morphologies and how construction of certain spine geometry may relate to synaptic function.

2. What structural refinements take place from filopodia to spines of different geometry?

2.1. Structural coupling of membrane dynamics and the molecular mechanism of filopodia initiation

Highly dynamic dendritic filopodia are postulated to be precursors of dendritic spines [24–27]. Dendritic filopodia are short-lived structures and only a small fraction of filopodia will be converted to mature spines. It is also argued that spines can be generated by a gradual expansion of stubby protrusions formed at the sites of axonal contacts. The complex relationship between axon–dendrite contacts, filopodial protrusions, and spine synapse formation can be classified into three models based on observations of different subsets of synapses present in different brain regions [28]. Based on observations of synapse development in the cerebellum, Sotelo proposed that Purkinje cell spines form independent of axonal counterparts [29]. Miller and Peters proposed that axonal contact triggers protrusions from dendrites and subsequent maturation into spines in the pyramidal neurons of the visual cortex [30]. Alternatively, randomly generated filopodia from dendrites may search the surrounding environment, and form synapses when they encounter appropriate target axons (the filopodia model) [31]. The Miller–Peters model and the filopodia model provide different views on the initiation of dendritic protrusions in the cortical pyramidal neurons. Recently, Kwon and Sabatini showed that local two-photon laser uncaging of glutamate can induce *de novo* and rapid formation of functional spines within seconds [32]. This observation suggests that spine formation triggered by presynaptically released glutamate can bypass the filopodial stage, thereby supporting the Miller–Peters model.

What are the core structural changes that occur in the dendritic membrane and the cytoskeleton for the initiation and development of spines? A study using electron microscopy of hippocampal dissociated neurons showed the origin of dendritic filopodia as cross-linked actin rich patches often resembling lamellipodial actin networks [33]. However, possible changes in the composition or structure of the dendritic shaft membrane in these regions have not been investigated. One attractive hypothesis is that the accumulation of actin patches at sites along dendrites leads to the recruitment of molecules required for the initiation and elongation of dendritic filopodia. These molecules can include Rho GTPase family members and their upstream and downstream factors as well as membrane remodeling proteins of the BAR superfamily.

The role of BAR family members and their interaction with actin or actin remodeling proteins as well as Rho GTPases have been extensively studied in a wide variety of non-neuronal cells [5–7]. BAR proteins may play a critical role in actin nucleation in multiple ways; as effectors or regulators of Rho GTPases, independently of Rho GTPases, or by direct binding and facilitating the function of actin nucleating proteins such as WASP/WAVE [5,34]. Therefore, it is possible that BAR family proteins may functionally interact with Rho GTPase family members and related actin nucleating and cross-linking proteins at the site of actin patches, and bend the membrane to facilitate filopodia/lamellipodia protrusion. Indeed, two BAR family members IRSP53 and srGAP2 are known to functionally interact with different Rho GTPases as well as actin remodeling proteins [35–37]. Interestingly, one member of the BAR family, IRSP53 is shown to induce dynamic membrane protrusions independently of actin in mammalian cells [38–40]. Similarly, a study by Guerrier and colleagues showed that another BAR family member, srGAP2 is sufficient to induce filopodia-like membrane protrusions even when actin polymerization is inhibited [8]. Although these studies showed a clear role for IRSP53 and srGAP2 in filopodia formation, whether these proteins could function upstream of actin patch formation during neuronal filopodial/lamellipodial formation remains to be investigated. Altogether, studies so far suggest a common role for the BAR family proteins in filopodia initiation in both fibroblasts and neurons by interacting with and facilitating the function of various actin remodeling proteins and bending the dendritic shaft membrane.

Filopodia formation in fibroblasts and neurons may require different modes of actin polymerization and organization. Filopodia in non-neuronal cells are composed of parallel actin bundles that grow in one direction from their tip [41], while neuronal filopodia are made up of cross-linked actin bundles that are able to grow at both ends *via* the use of a different Rho GTPase, Rif and its effector mDia2 [42]. A plausible hypothesis based on these observations is that BAR family proteins initiate membrane protrusions and subsequently recruit specific Rho GTPases and actin modulating proteins leading to neuron-specific filopodia formation. However, one remaining interesting question is; what factors determine the timing of localization and function of these regulators of filopodia and subsequent dendritic spine development? A good candidate can be the MAGUK protein family, including PSD95 [43,44] which is shown to localize to the dendritic plasma membrane during synaptogenesis [45]. A study showed that IRSP53 binds to PSD95 and another PSD protein, PSD93, and that its knock-down leads to a decrease in spine density, length and width in a Cdc42 and Rac dependent manner [46]. Therefore, it will be interesting to determine if localization of a member of the MAGUK protein family during filopodia initiation could lead to the recruitment of BAR domain proteins and the subsequent initiation of filopodia and synaptogenesis. Altogether, these studies indicate that proteins of the BAR family are attractive candidates as modulators of membrane dynamics from the

initial steps of filopodia initiation to the construction of functional spines (Fig. 1). Understanding if and how membrane remodeling is coupled to cytoskeletal dynamics and various molecular pathways to initiate filopodia formation, and how structural membrane compartmentalization occurs from initiating filopodia to spines of various morphologies would provide valuable insight into the process of synaptogenesis and the functional relevance of spine architecture.

3. From filopodia to spines: microsecond snapshots or highly dynamic structural units?

In the introduction, we briefly discussed the two contrasting hypotheses on the diversity of spine morphology. One argues that spine morphology helps rigid classification of distinct entities of spine subtypes. The other postulates that spine shape changes continuously and shape-based classification does not reflect the presence of distinct subtypes. Ideally, this controversy can be solved by taking time-lapse images of spines *in vivo* and estimating the rate of transitions between different spine subtypes. However, time-lapse imaging experiments *in vivo* are technically demanding and high-frequency imaging of single spines for a prolonged time period *in vivo* has not yet been achieved. Therefore, accumulating data on spine morphological changes are limited to low frequency, long-term imaging *in vivo* (several imaging frames over weeks or months) or high frequency, short-term imaging *in vitro* (imaging every few minutes for several days). For example, live imaging of hippocampal dissociated culture neurons imaged for 2 min showed changes in shape without significant changes in size [15]. Another study imaging cortical, cerebellar, and hippocampal slice culture neurons for a period of 90 min showed that spine dynamics is higher in young neurons compared with older neurons [16]. The spectrum of morphological features in these time-lapse imaging studies are in good agreement with previous electron microscopic studies, including the cat neocortex [10], pyramidal neurons of the rat cerebral cortex [47] and rat hippocampal slices, which described various spine morphologies from thin filopodia-like spines to large-headed mushroom spines [13]. Frequent changes in spine shape and reversible transition between different morphological categories (for example, thin spines can expand to the mushroom type, but they can reversibly shrink to their original thin morphology) observed in culture preparations favor the idea that no real distinction exists between spines with different shapes. However, direct *in vivo* imaging of the mouse barrel cortex followed by serial section electron microscopy showed that spine growth and establishment of complete synaptic contact would take up to four days without significant changes in spine morphology [48]. The slow spine dynamics they observed in this study was consistent with previous *in vivo* studies [17,49,50].

A critical question is whether spine behavior *in vivo* is fundamentally different from *in vitro* preparations. It has been argued that rapid structural remodeling of spines observed in culture preparations is difficult to detect *in vivo*, using transcranial two-photon imaging of the mouse neocortex. But this may be mainly due to a lower resolution of two-photon imaging, which was convincingly demonstrated by the recent imaging of rapid spine structural remodeling *in vivo* with stimulated-emission-depletion (STED) scanning microscopy [51]. Even if rapid morphing of spine shape exists both *in vitro* and *in vivo*, transition between different morphological categories may be greatly suppressed *in vivo*. This view is supported by previous imaging studies showing persistent morphology of large mushroom spines over months *in vivo* [17]. Persistence of large spines in the mature neocortex also illustrates their distinct dynamic property. Altogether, time-lapse imaging studies of spines both *in vitro* and *in vivo* revealed two important aspects of spine morphological dynamics. First, rapid morphing of spines, possibly based on actin remodeling is a common property of spines both *in vitro* and *in vivo*. This dynamic property may reflect activity-dependent response of individual spines. Suppression of spine morphing by the application of glutamate receptor agonists and volatile anesthetics supports this view [52]. Second, transition of spines between different morphological categories is common in culture preparations, but may be highly suppressed in the mature neocortex *in vivo*. An intriguing hypothesis is that suppression in spine morphological transition is regulated by sensory experiences. A study by Yang and colleagues compared spine turnover of mice in a standard housing environment to

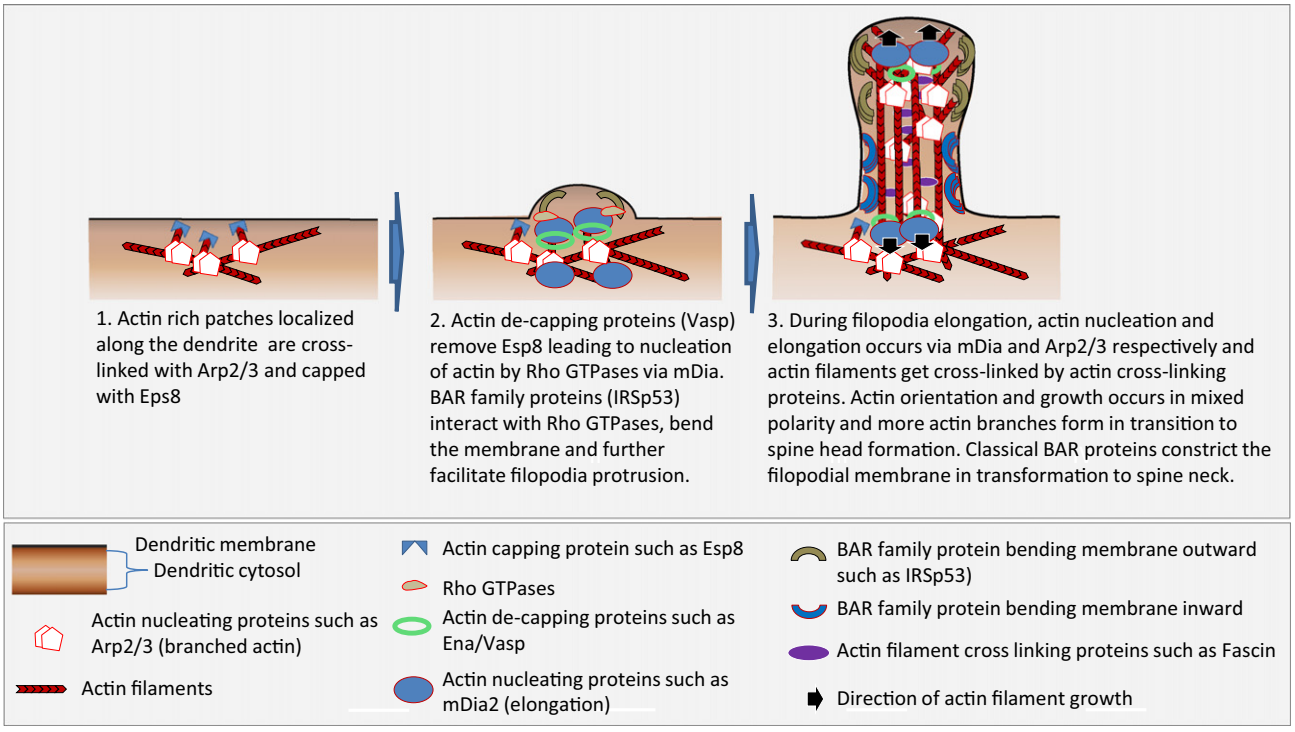


Fig. 1. A model for neuronal dendritic filopodia formation.

those in an enriched environment over a period of weeks to months [49]. Their study showed that an enriched environment increased the stability of newly formed spines, of which are mainly categorized as large spines. Enhanced stability of newly formed spines may indicate sensory experience-dependent or motor activity-dependent enhancement of spine morphological transition from thin to mushroom spines. An important question in the future is how morphological transition of spines is suppressed *in vivo*. The enduring storage of our memory is based on the maintenance of synaptic connectivity and efficacy, which should be based on the structural stability of spines.

4. How do the differences in spine architecture relate to function?

4.1. Are spines of different developmental ages and brain regions structurally and functionally different?

Serial electron microscopy reconstruction [53–56] and three-dimensional analyses of Golgi impregnated cells combined with electron microscopy [57] showed that spine geometry differ depending on brain region. A similar approach by Harris and Tsao showed that the number of dendritic spines in the hippocampal CA1 region of rats can double from postnatal day 15 to adult ages [58]. They also reported that each spine type showed a different extent of developmental increase. Thin spines, branched spines and perforated PSD-containing mushroom spines increased 4-fold. On the other hand, there was no change in mushroom spines with muscular PSDs and stubby spines decreased to about 50%. Thus composition of spine types may differ in different brain regions and may change as development proceeds. Whether this is directly related to the function of the various brain regions remains unknown and will require further functional and behavioral studies of each region during various developmental ages.

Research so far indicates that even on the same dendritic segment, incoming axons from different brain areas may generate postsynaptic spines with different morphology and function. A study by Humeau and colleagues showed a tendency for cortical inputs to synapse on thin spines and thalamic inputs to synapse on mushroom spines in the lateral nucleus of the amygdala [59]. As shown and discussed by the authors, the differences in spines may allow cortico- and thalamo-amygdala synapses to express different types of voltage gated Ca^{2+} channels (VGCCs). The spines innervated by thalamic input are large and express R-type VGCCs allowing greater Ca^{2+} transients and the induction of LTP or LTD when pulses of excitatory postsynaptic potentials (EPSPs) are paired with backpropagating action potentials (APs) or when pulses of APs are paired with EPSPs respectively. However, these same stimulations did not lead to LTP or LTD in cortico-amygdala inputs. Therefore, their data showed that not only spine size but also the content of specific ion channels may make spines functionally different (discussed in more detail below). On the other hand, the same inputs can synapse on both thin and mushroom spines in the hippocampal CA3 region [54]. Another study showed that both LTP and LTD can be induced in CA3 neurons by repeatedly pairing presynaptic potentials with postsynaptic pulses, and LTD can be induced by the reverse [60] similar to the thalamo-amygdala synapses discussed above. Since both studies on hippocampal CA3 neurons did not investigate a potential difference in receptor content in thin and large spines, whether the thin spines are functionally different or are simply immature spines in transition to developing into large spines remains unknown. In addition, a study using Ca^{2+} signaling showed that smaller spines were better able to restrict Ca^{2+} signaling within the spine in CA1 hippocampal slices compared to larger spines [61]. Therefore, it would be tempting to hypothesize that, similar to the thin spines of the lateral amygdala, the thin spines of the CA1 region may be functionally different from the mushroom spines, and those of the hippocampal CA3 region may be either functionally different or simply less mature synapses with the potential to grow into mushroom spines. Further functional characterization of these spines could shed light onto their possible functional relevance and differences in various brain regions.

4.2. How does spine head and neck geometry and membrane composition relate to functional specificity and strength?

The size and geometry of the spine head and neck are believed to be main determinants in synaptic signal compartmentalization, strength and specificity [1,18]. A study by Noguchi and colleagues using two-photon glutamate uncaging of single dendritic spines in acute slices of CA1 neurons showed that NMDA receptor-mediated Ca^{2+} signaling compartmentalization depends strongly on the radius and length of the spine neck [61]. In this study, they observed that even the smallest spines possessed NMDA receptors and were able to elicit calcium signaling. However, their results indicated that smaller spines were able to restrict signaling while Ca^{2+} signaling in larger spines was more readily able to spread into the surrounding dendritic shaft. The kinetics and amount of Ca^{2+} entry into a spine can differ greatly among different receptors; NMDA receptors allow a much slower kinetics for Ca^{2+} transients compared to Ca^{2+}-permeable AMPA receptors and VGCCs [62–64]. Therefore, it is not surprising that Ca^{2+} entering *via* NMDA receptors has more time to diffuse while that entering *via* AMPA receptors is readily utilized within the spine. However, AMPA receptors are also able to depolarize the synaptic membrane leading to NMDA-mediated Ca^{2+} entry which could diffuse into the surrounding dendrite. As discussed above, VGCCs can also act as a route of entry of Ca^{2+} into the cell [59,61]. The kinetics, amount and compartmentalization of Ca^{2+} are further regulated by the large heterogeneity of these receptors on different spines even on the same dendrite [65–67].

In addition to membrane receptors, there are a large variety of proteins that act as buffer molecules and bind and regulate Ca^{2+} inside the spine. These can activate, inactivate or spatially restrict Ca^{2+} signaling within microdomains of a dendritic spine and hence specify its signaling to give specific outcomes as well as minimizing its diffusion into the proximal dendrite [68]. It is believed that only about 1 to 5% of Ca^{2+} that enters the cell remains unbound [68]. The kinetics and function of free intracellular Ca^{2+} depends greatly on the affinity of available buffers. High affinity buffers sequester free Ca^{2+} more effectively but are more prone to saturation [64,68–75]. Calmodulin (CaM) is a good example of a Ca^{2+} buffer and responds to a wide range of intracellular Ca^{2+} concentrations. Different intracellular Ca^{2+} concentrations lead to multiple outcomes of CaM-dependent signaling [64]. A study by Faas and colleagues showed that CaM can directly bind Ca^{2+} upon entry with much higher affinity than other Ca^{2+} binding proteins and thus may play a crucial role in determining free intracellular Ca^{2+} levels and its downstream signaling outcomes [72]. The authors hypothesized that CaM binds to the higher Ca^{2+} levels while other buffers may bind the lower levels of free Ca^{2+}. Their data also indicated that the kinetics and function of Ca^{2+} bound to CaM may be subject to further regulation by other buffers. An interesting property of Ca^{2+}-bound CaM is that it can lead to the two opposite outcomes of LTP and LTD [76]. This differential function of CaM is due to the existence of its N-terminal and C-terminal lobes that have different kinetics of Ca^{2+} binding and activate different downstream molecules [77–80]. Recent studies suggest that the N-terminal and C-terminal lobes may bind Ca^{2+} independently and that this may greatly depend on their location within the spine, such that N-terminal binding may be triggered by uniform localization of CaM close to Ca^{2+} channels while C-terminal binding may be triggered mainly by localization at the PSD [70,71]. These findings provide an interesting example of how Ca^{2+} within specific microdomains inside the spine may be regulated. Therefore, a combination of the ratio and type of receptors expressed on the membrane as well as the distribution of various Ca^{2+} regulating and buffering proteins determines the strength and specificity of signaling and its compartmentalization within the spine head.

In addition to biochemical properties associated with the spine head, spine neck morphology can also be highly dynamic and may influence compartmentalization of Ca^{2+} [81] and other signaling molecules. A study showed that two-photon glutamate uncaging leads to the spread of RhoA activity out of the spine into the surrounding dendrite, while Cdc42 activity largely remained within the spine head [22]. Similar experimental approaches revealed that HRas activity spread from the spine while CaMKII activity was restricted within the spine [21]. One possibility is that the different molecules may be differentially bound and restricted by anchoring molecules within the spine. It will be interesting to investigate the relationship between spine neck morphology and the confinement of signaling molecules, such as RhoA and HRas taking into account possible restriction by binding partners. These experiments will clarify whether spines of different morphologies are functionally distinct in transmission of local synaptic signaling to nearby dendritic segments and spines (Fig. 2).

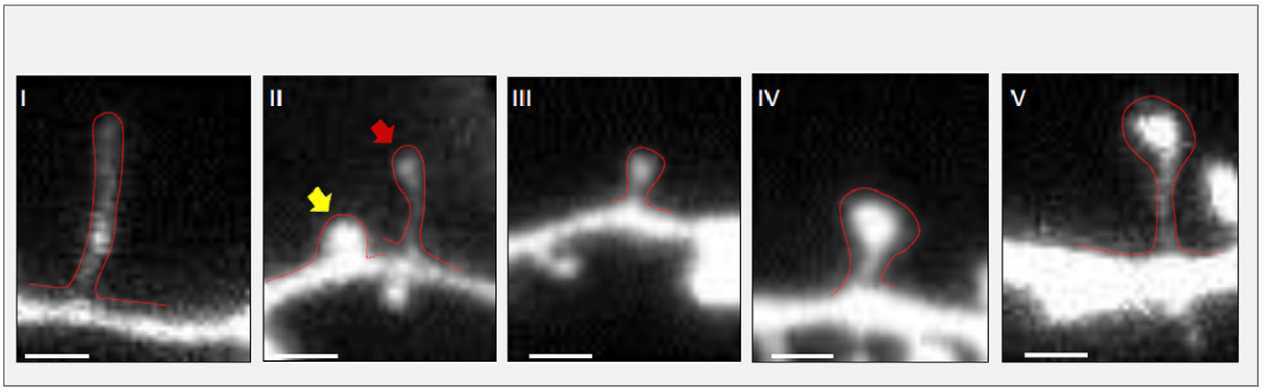
5. PSD and the cytoskeleton in regulating spine membrane composition and geometry

As mentioned above, the synaptic membrane expresses a variety of receptors heterogeneously in different spines. One interesting question is then, how do different spines express different proportions and/or types of receptors? Receptors at excitatory synapses are clustered at the PSD which also contains scaffolding proteins, actin, dynamic microtubules and signaling molecules [82–85]. The PSD is located just below the plasma membrane in dendritic spines and its size directly correlates with the volume of the spine head [54]. PSD components and the cytoskeleton have been known to play a major role in dendritic spine construction, activity-dependent membrane protein localization and in particular the synaptic localization of AMPA and NMDA receptors [86–88]. PSD scaffolding components, PSD95, Homer, Shank and GKAP link NMDA receptors and metabotropic glutamate receptors at the PSD and maintain them in the spine [89,90]. PSD95 has also been shown to be required for AMPA receptor localization at the postsynaptic membrane *via* TARPs (Transmembrane AMPA receptor regulatory proteins) [91,92]. In addition to PSD scaffolding molecules, there is increasing evidence for a role for spine actin in regulating the localization of AMPA receptors and some PSD proteins [2] while dynamic microtubule invasion into spines is shown to lead to spine enlargement [85]. Finally, the size and area of PSD varies greatly among different spines possibly due to both activity and intrinsic factors [54,93]. This may therefore lead to a difference in the number and activity of different receptors at the postsynaptic membrane making dendritic spines functionally diverse.

6. Dendritic spine abnormalities in pathology of neuronal diseases

6.1. Aberrant dendritic spine number in post-mortem human brains with neuronal diseases

Abnormalities in dendritic spine number were first reported using the Golgi method in post-mortem brains of human patients with various forms of mental retardation [94,95]. The relationship between neurological and psychiatric disorders and spine density is complex and interpretation requires careful considerations of multiple factors, including disease history of patients, previous medications, direct cause of death, and the preservation of the brain tissue [96–98]. The neuropathological changes associated with neurodegenerative disorders including Alzheimer's disease have been described extensively; however, the data on spine density have only started to accumulate recently. Currently, available data suggest a possible decline of spine density in Alzheimer's disease [99,100]. It has been proposed that impairment in synaptic function may precede accumulation of beta-amyloid (Aβ) deposits and neurofibrillary tangles, which are the hallmarks of the disease pathology [100,101]. Consistent with this synapse-pathology hypothesis, recent studies of post-mortem brains detected spine loss in patients in the early phase of Alzheimer's disease [102–104]. The study of spine density and shape in post-mortem brains of patients with psychiatric diseases have been initiated recently and more data may be required to reach a consensus about spine pathology. In the case of autism spectrum disorders (ASDs), a recent report suggests an increase in spine density using histological examination of ASD patients with ages ranging from 10 to 45 [105]. Fragile X syndrome (FXS), a genetic disorder with a mutation of fragile X mental retardation 1 (FMR1) gene, is known to be associated with an increase in spine density [106]. This spine phenotype is also present in a mouse model of FXS, in which FMRP gene is deleted [107]. Because FXS patients



Images of typical dendritic spines of a hippocampal CA1 region slice culture taken at various stages of development using a confocal fluorecence microscope:
(I) A filopodia can develop into various types of spines (II-V). (II) A thin spine (red arrow) with a small head may transduce week signal. A stubby spine (yellow arrow) with a large head and no neck may elicit strong signal diffusing through the surrounding dendrite. (III) A relatively small mushroom spine which may elicit a small amount of signal. (IV) A Large mushroom spine with a short neck that elicits strong signal which may diffuse through the surrounding dendrite. (V) A Large mushroom spine with a long and thin neck allowing for strong compartmentalized signal within the spine head. The white bar in each panel represents 1 μm.

Fig. 2. Dendritic spines of various geometry.

often show autism features and about 5% of ASD cases are associated with FXS mutations, the spine phenotype seen in FXS may also be linked to autistic behaviors [108–111]. In addition, mutations in genes encoding for various Shank proteins are reported in autistic patients [112–114]. It is reasonable to hypothesize that clinical manifestations of psychiatric disorders can be explained by circuit-level dysfunctions. Spine density changes seen in patients of psychiatric disorders may be either primary changes associated with disease etiology or the outcome of circuit dysfunctions caused by other factors, such as imbalance of excitatory and inhibitory circuits and dysfunctions of monoamine systems. A study using post-mortem brains of humans with a history of anxiety and depression showed a decrease in dendritic spine density. In order to advance our understanding in the etiology of psychiatric disorders and the contribution of spine pathology, complex interactions between genetic background and environmental factors influencing the proper function of neural circuits should be clarified in the future.

6.2. Dendritic spine pathology in animal models of neuronal diseases

Genomic studies of neurological and psychiatric disorders have revealed candidate gene mutations and copy number variations in the human genome. Based on the genomic information, a number of model mice for neuronal diseases have been generated, with special interests in the identification of any impairments in synaptic functions and associated spine pathology [99,115]. We do not intend to make a comprehensive list of spine phenotypes seen in these mouse models in this review; however, we will introduce examples illustrating the important contribution of spine morphology and function in pathophysiology. Transgenic mice of both amyloid precursor protein and presenilin-1 show extensive accumulation of Aβ deposits and decreased spine density in the area of Aβ deposits [116]. Detailed inspection of spine morphology in the hippocampus of the transgenic mice revealed clear alterations in spine morphology outside of the Aβ deposits. Namely, in the stratum oriens of the hippocampal CA1, spine necks were significantly shorter without changes in spine head size. On the other hand, the *stratum radiatum* spines showed no change in spine necks but had much smaller heads compared to wild-type mice. Although it is not clear if and how overproduction of Aβ may cause changes in spine geometry, these findings suggest that changes in spine number may depend on direct contact with amyloid plaques, while changes in spine shape may be triggered by soluble oligomers of Aβ in an area and layer-dependent manner.

For the studies of psychiatric disorders, many useful animal models have been established. A mouse model of Angelman syndrome showed no detectable differences in the organization, branch number and morphology of Purkinje cells [117], but there exists a clear spine phenotype in this disease model. Cerebellar Purkinje cells, and hippocampal and cortical neurons show a reduced spine density, thinner spine morphology, and a high degree of variability in spine neck and head size [118]. Furthermore, as described previously, both human patients of FXS and the FMRP knock-out mouse model show a significant increase in the density of thin and long dendritic spines [106,107]. Given the importance of spine head and neck size in synaptic signaling [1,18], it would be intriguing to investigate if the changed synapses are functional.

In researches of drug abuse, animal models are also indispensable. Mouse studies provided a strong evidence for a role for PSD proteins such as Esp8, believed to be part of the NMDAR complex, in alcohol addiction, and Homer and PSD95 in psychostimulant drug addiction [119–122]. Studies using psychostimulant drug treatment of rats have also yielded interesting results on dendritic spine development and experience dependent synaptic plasticity. Jedynak and colleagues showed that repeated treatment of rats with methamphetamine leads to a significant increase in mushroom and thin spines on dorsolateral striatum medium spiny neurons while they observed a decrease in mushroom spines in dorsomedial striatum [123]. A similar study showed that repeated amphetamine or cocaine intake greatly reduces experience-dependent increase in dendritic spines in rats in medium spiny neurons of the nucleus accumbens and pyramidal cells in the parietal cortex [124]. Collectively, the above studies show the important role of dendritic spines to a wide variety of mental and neurodegenerative diseases.

Understanding the possible functional significance of aberrant synapses may shed light onto the mechanism of neurological diseases and prove useful in identifying more effective drug targets.

7. Conclusions

Decades of research using animal models, cell culture and post-mortem human brains have led to accumulating evidence for possible functional differences in dendritic spines of various morphologies in synaptic plasticity and pathology. Evidence suggests that membrane remodeling proteins of the BAR family may facilitate protrusion of dendritic filopodia from actin rich patches that can then develop into spines with different head and neck geometry which are thought to be important factors in the compartmentalization of synaptic signals. In addition to spine morphology, various factors such as buffer molecules that restrict the activity, localization and diffusion of Ca^{2+} signaling as well as the content, ratio and subunit composition of membrane receptors at the postsynaptic membrane are essential in determining the strength and specificity of signaling in a brain area and layer-dependent manner. Studies on human disease brains have provided useful clues in the involvement of various molecules that function in dendritic spines in the pathology of brain diseases; however, the difficulty and shortcomings of preserving post-mortem human brains and complex patient medical history need to be carefully considered. The use of various animal models has provided useful insight into the neuronal pathology of diseases such as Alzheimer's, schizophrenia and many forms of mental retardation. Future studies carefully examining the interaction of membrane molecules and spine geometry are required in order to determine if spines of different geometry have altered circuit level functions in disease and may facilitate our understanding of synaptic pathology and drug discovery based on these new findings.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research (22650070 to S.O.) and Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. This work has also been supported by Grant-in-Aid for Scientific Research on Innovative Areas, 'Glial assembly: a new regulatory machinery of brain function and disorders'. A part of this study is the result of 'Development of biomarker candidates for social behavior' carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan (S.O.).

References

[1] V.A. Alvarez, B.L. Sabatini, Anatomical and physiological plasticity of dendritic spines, Annu. Rev. Neurosci. 30 (2007) 79–97.
[2] P. Hotulainen, C.C. Hoogenraad, Actin in dendritic spines: connecting dynamics to function, J. Cell Biol. 189 (2010) 619–629.
[3] E.E. Govek, S.E. Newey, L. Van Aelst, The role of the Rho GTPases in neuronal development, Genes Dev. 19 (2005) 1–49.
[4] S.E. Newey, V. Velamoor, E.E. Govek, L. Van Aelst, Rho GTPases, dendritic structure, and mental retardation, J. Neurobiol. 64 (2005) 58–74.
[5] A. Frost, V.M. Unger, P. De Camilli, The BAR domain superfamily: membrane-molding macromolecules, Cell 137 (2009) 191–196.
[6] S. Suetsugu, K. Toyooka, Y. Senju, Subcellular membrane curvature mediated by the BAR domain superfamily proteins, Semin. Cell Dev. Biol. 21 (2010) 340–349.
[7] M. Masuda, N. Mochizuki, Structural characteristics of BAR domain superfamily to sculpt the membrane, Semin. Cell Dev. Biol. 21 (2010) 391–398.
[8] S. Guerrier, J. Coutinho-Budd, T. Sassa, A. Gresset, N.V. Jordan, K. Chen, W.L. Jin, A. Frost, F. Polleux, The F-BAR domain of srGAP2 induces membrane protrusions required for neuronal migration and morphogenesis, Cell 138 (2009) 990–1004.

[9] C. Charrier, K. Joshi, J. Coutinho-Budd, J.E. Kim, N. Lambert, J. de Marchena, W.L. Jin, P. Vanderhaeghen, A. Ghosh, T. Sassa, F. Polleux, Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation, Cell 149 (2012) 923–935.

[10] E.G. Jones, T.P. Powell, Morphological variations in the dendritic spines of the neocortex, J. Cell Sci. 5 (1969) 509–529.

[11] F.L. Chang, W.T. Greenough, Transient and enduring morphological correlates of synaptic activity and efficacy change in the rat hippocampal slice, Brain Res. 309 (1984) 35–46.

[12] K.M. Harris, S.B. Kater, Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function, Annu. Rev. Neurosci. 17 (1994) 341–371.

[13] H. Hering, M. Sheng, Dendritic spines: structure, dynamics and regulation, Nat. Rev. Neurosci. 2 (2001) 880–888.

[14] J.N. Bourne, K.M. Harris, Balancing structure and function at hippocampal dendritic spines, Annu. Rev. Neurosci. 31 (2008) 47–67.

[15] M. Fischer, S. Kaech, D. Knutti, A. Matus, Rapid actin-based plasticity in dendritic spines, Neuron 20 (1998) 847–854.

[16] A. Dunaevsky, A. Tashiro, A. Majewska, C. Mason, R. Yuste, Developmental regulation of spine motility in the mammalian central nervous system, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 13438–13443.

[17] A.J. Holtmaat, J.T. Trachtenberg, L. Wilbrecht, G.M. Shepherd, X. Zhang, G.W. Knott, K. Svoboda, Transient and persistent dendritic spines in the neocortex in vivo, Neuron 45 (2005) 279–291.

[18] Y. Hayashi, A.K. Majewska, Dendritic spine geometry: functional implication and regulation, Neuron 46 (2005) 529–532.

[19] J.I. Arellano, R. Benavides-Piccione, J. Defelipe, R. Yuste, Ultrastructure of dendritic spines: correlation between synaptic and spine morphologies, Front. Neurosci. 1 (2007) 131–143.

[20] J.I. Gold, M.F. Bear, A model of dendritic spine Ca^{2+} concentration exploring possible bases for a sliding synaptic modification threshold, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 3941–3945.

[21] C.D. Harvey, R. Yasuda, H. Zhong, K. Svoboda, The spread of Ras activity triggered by activation of a single dendritic spine, Science 321 (2008) 136–140.

[22] H. Murakoshi, H. Wang, R. Yasuda, Local, persistent activation of Rho GTPases during plasticity of single dendritic spines, Nature 472 (2011) 100–104.

[23] B.L. Bloodgood, B.L. Sabatini, Neuronal activity regulates diffusion across the neck of dendritic spines, Science 310 (2005) 866–869.

[24] J.C. Fiala, M. Feinberg, V. Popov, K.M. Harris, Synaptogenesis via dendritic filopodia in developing hippocampal area CA1, J. Neurosci. 18 (1998) 8900–8911.

[25] N.E. Ziv, S.J. Smith, Evidence for a role of dendritic filopodia in synaptogenesis and spine formation, Neuron 17 (1996) 91–102.

[26] S. Okabe, A. Miwa, H. Okado, Spine formation and correlated assembly of presynaptic and postsynaptic molecules, J. Neurosci. 21 (2001) 6105–6114.

[27] C. Portera-Cailliau, D.T. Pan, R. Yuste, Activity-regulated dynamic behavior of early dendritic protrusions: evidence for different types of dendritic filopodia, J. Neurosci. 23 (2003) 7129–7142.

[28] R. Yuste, Dendritic Spines, The MIT Press, Cambridge, Massachusetts, 2009.

[29] C. Sotelo, Cerebellar synaptogenesis: what we can learn from mutant mice, J. Exp. Biol. 153 (1990) 225–249.

[30] M. Miller, A. Peters, Maturation of rat visual cortex. II. A combined Golgi–electron microscope study of pyramidal neurons, J. Comp. Neurol. 203 (1981) 555–573.

[31] J.E. Vaughn, Fine structure of synaptogenesis in the vertebrate central nervous system, Synapse 3 (1989) 255–285.

[32] H.B. Kwon, B.L. Sabatini, Glutamate induces de novo growth of functional spines in developing cortex, Nature 474 (2011) 100–104.

[33] F. Korobova, T. Svitkina, Molecular architecture of synaptic actin cytoskeleton in hippocampal neurons reveals a mechanism of dendritic spine morphogenesis, Mol. Biol. Cell 21 (2010) 165–176.

[34] S.B. Padrick, H.C. Cheng, A.M. Ismail, S.C. Panchal, L.K. Doolittle, S. Kim, B.M. Skehan, J. Umetani, C.A. Brautigam, J.M. Leong, M.K. Rosen, Hierarchical regulation of WASP/WAVE proteins, Mol. Cell 32 (2008) 426–438.

[35] G. Scita, S. Confalonieri, P. Lappalainen, S. Suetsugu, IRSp53: crossing the road of membrane and actin dynamics in the formation of membrane protrusions, Trends Cell Biol. 18 (2008) 52–60.

[36] S. Ahmed, W.I. Goh, W. Bu, I-BAR domains, IRSp53 and filopodium formation, Semin. Cell Dev. Biol. 21 (2010) 350–356.

[37] F.M. Mason, E.G. Heimsath, H.N. Higgs, S.H. Soderling, Bi-modal regulation of a formin by srGAP2, J. Biol. Chem. 286 (2011) 6577–6586.

[38] K.B. Lim, W. Bu, W.I. Goh, E. Koh, S.H. Ong, T. Pawson, T. Sudhaharan, S. Ahmed, The Cdc42 effector IRSp53 generates filopodia by coupling membrane protrusion with actin dynamics, J. Biol. Chem. 283 (2008) 20454–20472.

[39] P.K. Mattila, A. Pykalainen, J. Saarikangas, V.O. Paavilainen, H. Vihinen, E. Jokitalo, P. Lappalainen, Missing-in-metastasis and IRSp53 deform PI(4,5)P2-rich membranes by an inverse BAR domain-like mechanism, J. Cell Biol. 176 (2007) 953–964.

[40] J. Saarikangas, H. Zhao, A. Pykalainen, P. Laurinmaki, P.K. Mattila, P.K. Kinnunen, S.J. Butcher, P. Lappalainen, Molecular mechanisms of membrane deformation by I-BAR domain proteins, Curr. Biol. 19 (2009) 95–107.

[41] C. Le Clainche, M.F. Carlier, Regulation of actin assembly associated with protrusion and adhesion in cell migration, Physiol. Rev. 88 (2008) 489–513.

[42] P. Hotulainen, O. Llano, S. Smirnov, K. Tanhuanpaa, J. Faix, C. Rivera, P. Lappalainen, Defining mechanisms of actin polymerization and depolymerization during dendritic spine morphogenesis, J. Cell Biol. 185 (2009) 323–339.

[43] K.O. Cho, C.A. Hunt, M.B. Kennedy, The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs-large tumor suppressor protein, Neuron 9 (1992) 929–942.

[44] U. Kistner, B.M. Wenzel, R.W. Veh, C. Cases-Langhoff, A.M. Garner, U. Appeltauer, B. Voss, E.D. Gundelfinger, C.C. Garner, SAP90, a rat presynaptic protein related to the product of the *Drosophila* tumor suppressor gene dlg-A, J. Biol. Chem. 268 (1993) 4580–4583.

[45] S. Okabe, H.D. Kim, A. Miwa, T. Kuriu, H. Okado, Continual remodeling of postsynaptic density and its regulation by synaptic activity, Nat. Neurosci. 2 (1999) 804–811.

[46] J. Choi, J. Ko, B. Racz, A. Burette, J.R. Lee, S. Kim, M. Na, H.W. Lee, K. Kim, R.J. Weinberg, E. Kim, Regulation of dendritic spine morphogenesis by insulin receptor substrate 53, a downstream effector of Rac1 and Cdc42 small GTPases, J. Neurosci. 25 (2005) 869–879.

[47] A. Peters, I.R. Kaiserman-Abramof, The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines, Am. J. Anat. 127 (1970) 321–355.

[48] G.W. Knott, A. Holtmaat, L. Wilbrecht, E. Welker, K. Svoboda, Spine growth precedes synapse formation in the adult neocortex in vivo, Nat. Neurosci. 9 (2006) 1117–1124.

[49] B. Lendvai, E.A. Stern, B. Chen, K. Svoboda, Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo, Nature 404 (2000) 876–881.

[50] J.T. Trachtenberg, B.E. Chen, G.W. Knott, G. Feng, J.R. Sanes, E. Welker, K. Svoboda, Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex, Nature 420 (2002) 788–794.

[51] S. Berning, K.I. Willig, H. Steffens, P. Dibaj, S.W. Hell, Nanoscopy in a living mouse brain, Science 335 (2012) 551.

[52] M. Fischer, S. Kaech, U. Wagner, H. Brinkhaus, A. Matus, Glutamate receptors regulate actin-based plasticity in dendritic spines, Nat. Neurosci. 3 (2000) 887–894.

[53] K.M. Harris, J.K. Stevens, Dendritic spines of rat cerebellar Purkinje cells: serial electron microscopy with reference to their biophysical characteristics, J. Neurosci. 8 (1988) 4455–4469.

[54] K.M. Harris, J.K. Stevens, Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics, J. Neurosci. 9 (1989) 2982–2997.

[55] J. Spacek, M. Hartmann, Three-dimensional analysis of dendritic spines. I. Quantitative observations related to dendritic spine and synaptic morphology in cerebral and cerebellar cortices, Anat. Embryol. (Berl.) 167 (1983) 289–310.

[56] C.J. Wilson, P.M. Groves, S.T. Kitai, J.C. Linder, Three-dimensional structure of dendritic spines in the rat neostriatum, J. Neurosci. 3 (1983) 383–388.

[57] K. Hama, T. Arii, T. Kosaka, Three-dimensional morphometrical study of dendritic spines of the granule cell in the rat dentate gyrus with HVEM stereo images, J. Electron Microsc. Tech. 12 (1989) 80–87.

[58] K.M. Harris, F.E. Jensen, B. Tsao, Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation, J. Neurosci. 12 (1992) 2685–2705.

[59] Y. Humeau, C. Herry, N. Kemp, H. Shaban, E. Fourcaudot, S. Bissiere, A. Luthi, Dendritic spine heterogeneity determines afferent-specific Hebbian plasticity in the amygdala, Neuron 45 (2005) 119–131.

[60] D. Debanne, B.H. Gahwiler, S.M. Thompson, Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures, J. Physiol. 507 (Pt 1) (1998) 237–247.

[61] J. Noguchi, M. Matsuzaki, G.C. Ellis-Davies, H. Kasai, Spine–neck geometry determines NMDA receptor-dependent Ca^{2+} signaling in dendrites, Neuron 46 (2005) 609–622.

[62] R. Schneggenburger, Z. Zhou, A. Konnerth, E. Neher, Fractional contribution of calcium to the cation current through glutamate receptor channels, Neuron 11 (1993) 133–143.

[63] D.E. Jane, D. Lodge, G.L. Collingridge, Kainate receptors: pharmacology, function and therapeutic potential, Neuropharmacology 56 (2009) 90–113.

[64] V. Raghuram, Y. Sharma, M.R. Kreutz, Ca(2+) sensor proteins in dendritic spines: a race for Ca(2+), Front. Mol. Neurosci. 5 (2012).

[65] Y. He, W.G. Janssen, J.H. Morrison, Synaptic coexistence of AMPA and NMDA receptors in the rat hippocampus: a postembedding immunogold study, J. Neurosci. Res. 54 (1998) 444–449.

[66] H. Monyer, N. Burnashev, D.J. Laurie, B. Sakmann, P.H. Seeburg, Developmental and regional expression in the rat brain and functional properties of four NMDA receptors, Neuron 12 (1994) 529–540.

[67] A. Sobczyk, K. Svoboda, Activity-dependent plasticity of the NMDA-receptor fractional Ca^{2+} current, Neuron 53 (2007) 17–24.

[68] M.J. Higley, B.L. Sabatini, Calcium signaling in dendritic spines, Cold Spring Harb. Perspect. Biol. 4 (2012) a005686.

[69] M.J. Higley, B.L. Sabatini, Calcium signaling in dendrites and spines: practical and functional considerations, Neuron 59 (2008) 902–913.

[70] D.X. Keller, K.M. Franks, T.M. Bartol Jr., T.J. Sejnowski, Calmodulin activation by calcium transients in the postsynaptic density of dendritic spines, PLoS One 3 (2008) 0002045.

[71] Y. Kubota, M.N. Waxham, Lobe specific Ca^{2+}-calmodulin nano-domain in neuronal spines: a single molecule level analysis, PLoS Comput. Biol. 6 (2010) 1000987.

[72] G.C. Faas, S. Raghavachari, J.E. Lisman, I. Mody, Calmodulin as a direct detector of Ca^{2+} signals, Nat. Neurosci. 14 (2011) 301–304.

[73] S.H. Lee, C. Rosenmund, B. Schwaller, E. Neher, Differences in Ca^{2+} buffering properties between excitatory and inhibitory hippocampal neurons from the rat, J. Physiol. 525 (Pt 2) (2000) 405–418.

[74] M. Maravall, Z.F. Mainen, B.L. Sabatini, K. Svoboda, Estimating intracellular calcium concentrations and buffering without wavelength ratioing, Biophys. J. 78 (2000) 2655–2667.

[75] B.L. Sabatini, T.G. Oertner, K. Svoboda, The life cycle of Ca(2+) ions in dendritic spines, Neuron 33 (2002) 439–452.
[76] Z. Xia, D.R. Storm, The role of calmodulin as a signal integrator for synaptic plasticity, Nat. Rev. Neurosci. 6 (2005) 267–276.
[77] C.B. Klee, T.C. Vanaman, Calmodulin, Adv. Protein Chem. 35 (1982) 213–321.
[78] S. Linse, A. Helmersson, S. Forsen, Calcium binding to calmodulin and its globular domains, J. Biol. Chem. 266 (1991) 8050–8054.
[79] S.P. Robertson, J.D. Johnson, J.D. Potter, The time-course of Ca^{2+} exchange with calmodulin, troponin, parvalbumin, and myosin in response to transient increases in Ca^{2+}, Biophys. J. 34 (1981) 559–569.
[80] J.D. Johnson, C. Snyder, M. Walsh, M. Flynn, Effects of myosin light chain kinase and peptides on Ca^{2+} exchange with the N- and C-terminal Ca^{2+} binding sites of calmodulin, J. Biol. Chem. 271 (1996) 761–767.
[81] A. Majewska, A. Tashiro, R. Yuste, Regulation of spine calcium dynamics by rapid spine motility, J. Neurosci. 20 (2000) 8262–8268.
[82] C.W. Cotman, G. Banker, L. Churchill, D. Taylor, Isolation of postsynaptic densities from rat brain, J. Cell Biol. 63 (1974) 441–455.
[83] N. Hirokawa, The arrangement of actin filaments in the postsynaptic cytoplasm of the cerebellar cortex revealed by quick-freeze deep-etch electron microscopy, Neurosci. Res. 6 (1989) 269–275.
[84] R.S. Cohen, F. Blomberg, K. Berzins, P. Siekevitz, The structure of postsynaptic densities isolated from dog cerebral cortex. I. Overall morphology and protein composition, J. Cell Biol. 74 (1977) 181–203.
[85] J. Jaworski, L.C. Kapitein, S.M. Gouveia, B.R. Dortland, P.S. Wulf, I. Grigoriev, P. Camera, S.A. Spangler, P. Di Stefano, J. Demmers, H. Krugers, P. Defilippi, A. Akhmanova, C.C. Hoogenraad, Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity, Neuron 61 (2009) 85–100.
[86] R.H. Scannevin, R.L. Huganir, Postsynaptic organization and regulation of excitatory synapses, Nat. Rev. Neurosci. 1 (2000) 133–141.
[87] W. Xu, PSD-95-like membrane associated guanylate kinases (PSD-MAGUKs) and synaptic plasticity, Curr. Opin. Neurobiol. 21 (2011) 306–312.
[88] S. Okabe, Molecular anatomy of the postsynaptic density, Mol. Cell. Neurosci. 34 (2007) 503–518.
[89] H.C. Kornau, L.T. Schenker, M.B. Kennedy, P.H. Seeburg, Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95, Science 269 (1995) 1737–1740.
[90] J.C. Tu, B. Xiao, S. Naisbitt, J.P. Yuan, R.S. Petralia, P. Brakeman, A. Doan, V.K. Aakalu, A.A. Lanahan, M. Sheng, P.F. Worley, Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins, Neuron 23 (1999) 583–592.
[91] G.A. Yudowski, O. Olsen, H. Adesnik, K.W. Marek, D.S. Bredt, Acute inactivation of PSD-95 destabilizes AMPA receptors at hippocampal synapses, PLoS One 8 (2013) e53965.
[92] A.E. El-Husseini, E. Schnell, D.M. Chetkovich, R.A. Nicoll, D.S. Bredt, PSD-95 involvement in maturation of excitatory synapses, Science 290 (2000) 1364–1368.
[93] K.E. Sorra, K.M. Harris, Overview on the structure, composition, function, development, and plasticity of hippocampal dendritic spines, Hippocampus 10 (2000) 501–511.
[94] M. Marin-Padilla, Pyramidal cell abnormalities in the motor cortex of a child with Down's syndrome. A Golgi study, J. Comp. Neurol. 167 (1976) 63–81.
[95] P.R. Huttenlocher, Dendritic and synaptic pathology in mental retardation, Pediatr. Neurol. 7 (1991) 79–85.
[96] P.J. Harrison, The neuropathological effects of antipsychotic drugs, Schizophr. Res. 40 (1999) 87–99.
[97] M.R. Hynd, J.M. Lewohl, H.L. Scott, P.R. Dodd, Biochemical and molecular studies using human autopsy brain tissue, J. Neurochem. 85 (2003) 543–562.
[98] I. Ferrer, G. Santpere, T. Arzberger, J. Bell, R. Blanco, S. Boluda, H. Budka, M. Carmona, G. Giaccone, B. Krebs, L. Limido, P. Parchi, B. Puig, R. Strammiello, T. Strobel, H. Kretzschmar, Brain protein preservation largely depends on the postmortem storage temperature: implications for study of proteins in human neurologic diseases and management of brain banks: a BrainNet Europe Study, J. Neuropathol. Exp. Neurol. 66 (2007) 35–46.
[99] P. Penzes, M.E. Cahill, K.A. Jones, J.E. VanLeeuwen, K.M. Woolfrey, Dendritic spine pathology in neuropsychiatric disorders, Nat. Neurosci. 14 (2011) 285–293.
[100] D.J. Selkoe, Alzheimer's disease is a synaptic failure, Science 298 (2002) 789–791.
[101] T. Arendt, Synaptic degeneration in Alzheimer's disease, Acta Neuropathol. 118 (2009) 167–179.
[102] E. Masliah, M. Mallory, M. Alford, R. DeTeresa, L.A. Hansen, D.W. McKeel Jr., J.C. Morris, Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease, Neurology 56 (2001) 127–129.
[103] S.W. Scheff, D.A. Price, F.A. Schmitt, E.J. Mufson, Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment, Neurobiol. Aging 27 (2006) 1372–1384.
[104] S.W. Scheff, D.A. Price, F.A. Schmitt, S.T. DeKosky, E.J. Mufson, Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment, Neurology 68 (2007) 1501–1508.
[105] J.J. Hutsler, T. Love, H. Zhang, Histological and magnetic resonance imaging assessment of cortical layering and thickness in autism spectrum disorders, Biol. Psychiatry 61 (2007) 449–457.
[106] S.A. Irwin, R. Galvez, W.T. Greenough, Dendritic spine structural anomalies in fragile-X mental retardation syndrome, Cereb. Cortex 10 (2000) 1038–1044.
[107] T.A. Comery, J.B. Harris, P.J. Willems, B.A. Oostra, S.A. Irwin, I.J. Weiler, W.T. Greenough, Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 5401–5404.
[108] R. Muhle, S.V. Trentacoste, I. Rapin, The genetics of autism, Pediatrics 113 (2004) e472–e486.
[109] G.B. Schaefer, N.J. Mendelsohn, Genetics evaluation for the etiologic diagnosis of autism spectrum disorders, Genet. Med. 10 (2008) 4–12.
[110] N.J. Mendelsohn, G.B. Schaefer, Genetic evaluation of autism, Semin. Pediatr. Neurol. 15 (2008) 27–31.
[111] D.B. Budimirovic, W.E. Kaufmann, What can we learn about autism from studying fragile X syndrome? Dev. Neurosci. 33 (2011) 379–394.
[112] R. Moessner, C.R. Marshall, J.S. Sutcliffe, J. Skaug, D. Pinto, J. Vincent, L. Zwaigenbaum, B. Fernandez, W. Roberts, P. Szatmari, S.W. Scherer, Contribution of SHANK3 mutations to autism spectrum disorder, Am. J. Hum. Genet. 81 (2007) 1289–1297.
[113] S. Berkel, C.R. Marshall, B. Weiss, J. Howe, R. Roeth, U. Moog, V. Endris, W. Roberts, P. Szatmari, D. Pinto, M. Bonin, A. Riess, H. Engels, R. Sprengel, S.W. Scherer, G.A. Rappold, Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation, Nat. Genet. 42 (2010) 489–491.
[114] A.M. Grabrucker, M.J. Schmeisser, M. Schoen, T.M. Boeckers, Postsynaptic ProSAP/Shank scaffolds in the cross-hair of synaptopathies, Trends Cell Biol. 21 (2011) 594–603.
[115] M.R. Kreutz, C. Sala, Postsynaptic molecular mechanisms. Preface, Adv. Exp. Med. Biol. 970 (2012) v–vi.
[116] P. Merino-Serrais, S. Knafo, L. Alonso-Nanclares, I. Fernaud-Espinosa, J. DeFelipe, Layer-specific alterations to CA1 dendritic spines in a mouse model of Alzheimer's disease, Hippocampus 21 (2011) 1037–1044.
[117] Y.H. Jiang, D. Armstrong, U. Albrecht, C.M. Atkins, J.L. Noebels, G. Eichele, J.D. Sweatt, A.L. Beaudet, Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation, Neuron 21 (1998) 799–811.
[118] S.V. Dindot, B.A. Antalffy, M.B. Bhattacharjee, A.L. Beaudet, The Angelman syndrome ubiquitin ligase localizes to the synapse and nucleus, and maternal deficiency results in abnormal dendritic spine morphology, Hum. Mol. Genet. 17 (2008) 111–118.
[119] A. Soetanto, R.S. Wilson, K. Talbot, A. Un, J.A. Schneider, M. Sobiesk, J. Kelly, S. Leurgans, D.A. Bennett, S.E. Arnold, Association of anxiety and depression with microtubule-associated protein 2- and synaptopodin-immunolabeled dendrite and spine densities in hippocampal CA3 of older humans, Arch. Gen. Psychiatry 67 (2010) 448–457.
[120] N. Offenhauser, D. Castelletti, L. Mapelli, B.E. Soppo, M.C. Regondi, P. Rossi, E. D'Angelo, C. Frassoni, A. Amadeo, A. Tocchetti, B. Pozzi, A. Disanza, D. Guarnieri, C. Betsholtz, G. Scita, U. Heberlein, P.P. Di Fiore, Increased ethanol resistance and consumption in Eps8 knockout mice correlates with altered actin dynamics, Cell 127 (2006) 213–226.
[121] K.K. Szumlinski, M.H. Dehoff, S.H. Kang, K.A. Frys, K.D. Lominac, M. Klugmann, J. Rohrer, W. Griffin III, S. Toda, N.P. Champtiaux, T. Berry, J.C. Tu, S.E. Shealy, M.J. During, L.D. Middaugh, P.F. Worley, P.W. Kalivas, Homer proteins regulate sensitivity to cocaine, Neuron 43 (2004) 401–413.
[122] W.D. Yao, R.R. Gainetdinov, M.I. Arbuckle, T.D. Sotnikova, M. Cyr, J.M. Beaulieu, G.E. Torres, S.G. Grant, M.G. Caron, Identification of PSD-95 as a regulator of dopamine-mediated synaptic and behavioral plasticity, Neuron 41 (2004) 625–638.
[123] J.P. Jedynak, J.M. Uslaner, J.A. Esteban, T.E. Robinson, Methamphetamine-induced structural plasticity in the dorsal striatum, Eur. J. Neurosci. 25 (2007) 847–853.
[124] B. Kolb, G. Gorny, Y. Li, A.N. Samaha, T.E. Robinson, Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10523–10528.