MINIREVIEW

Cytoskeletal makeup of the synapse: Shaft versus spine

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Abstract
The ability of neurons to communicate and store information depends on the activity of synapses which can be located on small protrusions (dendritic spines) or directly on the dendritic shaft. The formation, plasticity, and stability of synapses are regulated by the neuronal cytoskeleton. Actin filaments together with microtubules, neurofilaments, septins, and scaffolding proteins orchestrate the structural organization of both shaft and spine synapses, enabling their efficacy in response to synaptic activation. Synapses critically depend on several factors, which are also mediated by the cytoskeleton, including transport and delivery of proteins from the soma, protein synthesis, as well as surface diffusion of membrane proteins. In this minireview, we focus on recent progress made in the field of cytoskeletal elements of the postsynapse and discuss the differences and similarities between synapses located in the spines versus dendritic shaft.

KEYWORDS
F-actin, microtubule, pyramidal neurons, septins, shaft synapse, spine cytoskeleton

1 | INTRODUCTION

The potential of the brain to acquire and consolidate information from the environment critically depends on the ability of neurons to communicate with each other by transmitting electro-chemical signals. A common class of neurons found in the cerebral cortex and subcortical structures, such as amygdala and hippocampus, comprises principal excitatory cells also known as pyramidal neurons. They are distinguished by their prominent basal and apical dendritic trees and single axon extending from the teardrop-like cell body as well as their use of glutamate as the major neurotransmitter. A different class of neurons involved in neuronal signaling is inhibitory cells releasing gamma-aminobutyric acid (GABA) as neurotransmitter and are frequently referred to as GABAergic interneurons. Their central function is to modulate the excitation coming from pyramidal cells by providing input-specific inhibition which is essential for forming functional neuronal networks (Contreras, Hines, & Hines, 2019). The interactions between neurons take place at synapses, highly specialized membrane compartments. The two major types of chemical synapses in the brain are excitatory glutamatergic synapses and inhibitory GABAergic synapses. Whereas the molecular machineries involved in presynaptic vesicle release have been studied in great detail, the organization of postsynaptic membranes and underlying scaffolds, which are important for synaptic receptor clustering, is less well understood (Wilhelm et al., 2014). In this minireview, we will put emphasis on the cytoskeletal organization of excitatory synapses located on spines and shafts of dendrites in principal neurons and provide future perspectives for extending synaptic cytoskeleton research to other types of neurons found in the brain.

2 | POSTSYNAPTIC ORGANIZATION OF EXCITATORY AND INHIBITORY SYNAPSES

Glutamatergic synapses are frequently located on small protrusions of dendrites called dendritic spines or can be found directly on the
dendritic shaft (Bourne & Harris, 2011; van Bommel, Konietzny, Kobler, Bär, & Mikhaylova, 2019). Although during early neuronal development the majority of glutamatergic synapses are located at dendritic shafts, following the maturation process they are gradually replaced by excitatory spine synapses. Nonetheless, some of them still remain on the shaft (Yuste & Bonhoeffer, 2004). In the adult rat hippocampus, approximately 10% of synapses are still located on the shaft and in cortical neurons, this number can reach up to 30% (Bourne & Harris, 2011; Reilly, Hanson, & Phillips, 2011). The morphology of spines and their distribution along dendritic branches plays a crucial role in synaptic plasticity, which is essential in learning and memory processes. Mature, mushroom-like spines have a bulbous head connected to the shaft by a thinner neck and represent the most common type of dendritic spines in mature pyramidal neurons (Hering & Sheng, 2001). These morphological traits have been shown to serve as structural compartmentalization factors by restricting molecular diffusion in and out of the dendritic spine. Interestingly, the most important parameter determining the degree of compartmentalization is the spine neck diameter. While long and thin spine necks profoundly limit the diffusion especially of larger proteins, the latter are able to move freely in and out of spines with larger neck diameters. It has been demonstrated that the neck of dendritic spines is a plastic structure which undergoes both shortening and widening upon induced spine potentiation. This leads to a significant drop in the electrical resistance of spine necks while the biochemical compartmentalization is largely preserved (Adrian et al., 2014; Tønnesen, Katona, Roza, & Nagerl, 2014). The stability of dendritic spines as well as rapid activity-dependent shape changes are mediated by the actin cytoskeleton followed by co-ordinated reorganization of the postsynaptic density (PSD) and will be discussed later in this review.

The PSD is one of the relevant key components in the organization of spines and shafts synapses. It consists of scaffolding proteins associated with cytoskeletal elements and is essential for anchoring of synaptic receptors, ion channels, and adhesion molecules. The most relevant PSD scaffolds of excitatory synapses are homer, GKAP, members of the membrane-associated guanylate kinases family (MAGUKs—such as PSD95 and SAP97), or SH3 domain and ankyrin repeat domain proteins (SHANKs). These proteins are organized in highly ordered nanodomains, which can anchor and cluster synaptic membrane proteins (Hurska, Henderson, Le Marchand, Jafari, & Dalva, 2018; MacGillavry, Song, Raghavachari, & Blanpied, 2013). Glutamate released from the excitatory presynaptic terminal can be bound by three distinct types of ionotropic glutamate receptors: 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid (AMPA), N-methyl-D-aspartate (NMDA), and kainate receptors located at the postsynaptic side. Additionally, L-type calcium channels and various types of adhesion molecules, such as neuroligin-1, NCAM1, and integrins are localized to dendritic spines and participate in postsynaptic signaling (Stefen, Chaichim, Power, & Fath, 2016). The surface expression, localization, and removal of glutamatergic receptors and adhesion molecules are regulated by interactions between postsynaptic scaffolds. For instance, glutamate receptor interacting protein (GRIK) regulates trafficking of AMPA receptors and is critical for synaptic development and several forms of synaptic plasticity (Anggono & Huganir, 2012; Geiger et al., 2014). Importantly, scaffolding proteins from the PSD such as SHANK and PSD95 are associated with the dendritic actin cytoskeleton through interaction with F-actin-binding proteins like cofilin and α-actinin. These interactions are crucial for spines to dynamically rearrange F-actin in order to maintain stability and flexibility (MacGillavry, Kerr, Kassner, Frost, & Blanpied, 2016; Matt et al., 2018). Accordingly, spine morphology, dynamics, and stability rely on interactions of the actin cytoskeleton with multiple actin regulating proteins, which are highly enriched in this compartment and can rapidly respond to synaptic activation (Bosch et al., 2014; Mikhaylova et al., 2018).

In contrast to excitatory synapses, the vast majority of inhibitory synapses are located at the dendritic shaft and the cell body, while only a few inhibitory synapses in the neocortex are spine synapses (Chiu et al., 2013). The main neurotransmitter receptors at inhibitory postsynapses are GABA_A and GABA_B. Gephyrin plays a determinant role in organizing the GABAergic PSD and is responsible for the localization and stabilization of inhibitory receptors at the synaptic membrane (Jacob, Moss, & Jurd, 2008). Additionally, gephyrin assembles various inhibitory synapse-specific proteins including neuroligin 2 and GABA_A receptor-associated protein into a hexagonal lattice, which contributes to intracellular signal transduction (Crosby et al., 2019; Sheng & Kim, 2011). Spatially, inhibitory synapses are in much closer proximity to the microtubules and intermediate filaments than excitatory spine synapses and therefore it is possible that a different type of cross-talk with other cytoskeletal elements will occur at these locations.

3 | SYNAPTIC ACTIN CYTOSKELETON

Actin is the main cytoskeletal element of dendritic spines. It is present in two forms: as monomers or filaments. Based on their turnover rate, actin filaments can be divided into dynamic and stable pools and based on the morphology described as linear or branched filaments. The controlled assembly and tread milling of F-actin are essential for plasticity and stability of synapses (MacGillavry et al., 2016; Mikhaylova et al., 2018; Mundhenk, Fusi, & Kreutz, 2019; Stefens et al., 2016). Importantly, a plethora of actin nucleation factors, severing, and capping proteins are involved in filament formation and regulation of filament stability (Hotulainen & Hoogenraad, 2010). A detailed overview of these factors can be found in the review by Konietzny, Bär, and Mikhaylova (2017). Moreover, in neurons F-actin can form higher-order structures such as bundles of linear filaments, a periodic F-actin lattice, axonal actin hotspots, and dendritic F-actin patches (Bär, Kobler, van Bommel, & Mikhaylova, 2016; D’Este, Kamin, Gottfert, El-Hady, & Hell, 2015; Ganguly et al., 2015; A. Konietzny et al., 2017; Korobova & Svitkina, 2010; Papandreou & Letererier, 2018; Sidenstein et al., 2016; van Bommel et al., 2019; Xu, Zhong, & Zhuang, 2013). The diversity of actin-based structures indicates the versatility of F-actin as a tool for creating subcellular compartments which is particularly evident when looking at the dendrites...
of principal neurons (A. Konietzny et al., 2017; Lanoue & Cooper, 2019; Papandreou & Leterrier, 2018). In this section, we will focus on the structure, organization, and function of F-actin at postsynaptic sites.

3.1 | Spinous actin

Dendritic spines are the most striking F-actin-based structures in neurons. Morphological changes of the spine head and neck are correlated with alterations in synaptic strength (Bosch et al., 2014; Racz & Weinberg, 2013). The ability of actin to rapidly polymerize and depolymerize provides a very efficient tool for coupling synaptic activity to structural adaptation of dendritic spines. Thus, the actin cytoskeleton, together with scaffolding molecules at the PSD, establishes spine architecture and enables tuning of synaptic efficacy. Besides, the precise nanoscale organization of the actin cytoskeleton within these compartments has a profound influence on synaptic function.

Earlier studies have described actin filaments of mixed polarity which are arranged roughly in longitudinal direction along the spine neck with predominant orientation of the barbed end pointing toward the PSD (Hotulainen & Hoogenraad, 2010). More recently, super-resolution microscopy techniques such as direct stochastic optical reconstruction microscopy (dSTORM) and stimulated emission depletion (STED) enabled high spatial resolution and multicolor imaging, uncovering the architecture of the actin cytoskeleton within different neuronal compartments. One of the most relevant and striking findings discovered by these techniques was the presence of a regularly spaced F-actin lattice located in axons and dendrites (Bär et al., 2016; D’Este et al., 2015; Sidenstein et al., 2016; Xu et al., 2013). These membrane-associated actin structures are not only present in the dendritic shaft but extend from the dendrite through the spine neck and fade away in the spine head, thus adding another structural element formed by F-actin in dendritic spines (Figure 1(a) / Bär et al., 2016). Interestingly, in contrast to longitudinal and branched actin filaments in the spines, periodic F-actin structures are very stable and resistant to depolymerization induced by treatment with Latrunculin A and B (Abouelezz, Micinski, Lipponen, & Hotulainen, 2019). Furthermore, F-actin forms a membrane-associated periodic lattice together with βII, βIII, and βIV spectrins, where actin filaments are spaced by spectrin

![Figure 1](wileyonlinelibrary.com)

**FIGURE 1**  Schematic illustrating the cytoskeletal architecture of spines and dendrites in principle neurons. Spine and shaft synapses differ in their cytoskeletal environment. (a) Different components of the neuronal cytoskeleton mediate cargo transport and/or provide mechanical support to the synapse. Actin filaments (yellow) are found as dense mesh within the spine head while periodic F-actin/spectrin lattice constricts the spine neck and dendrite. Septins (light green) serve as additional constriction and shaping factors at the base of dendritic spines. Microtubules (dark green) serve as “roads” for kinesin- and dynein-driven cargo transport. The fast-growing (+)-end of microtubules (+TIP) is decorated with end-binding proteins such as EB3. In response to synaptic activity, microtubules may enter dendritic spines. This is mediated by the actin-binding protein drebrin (brown). Furthermore, neurofilaments (NF; purple) are found within dendrites and participate in electrical and biochemical signal transduction. (b) Excitatory and inhibitory synapses: detailed overview [Color figure can be viewed at wileyonlinelibrary.com]
tetramers with a periodicity of approximately 190 nm (Bär et al., 2016; D’Este et al., 2016; Han, Zhou, Xia, & Zhuang, 2017). A very recent correlative dSTORM and electron microscopy study indicated that on an ultrastructural level, this periodically organized F-actin forms a braid-like structure containing two long, entwined actin filaments (Vassilopoulos, Gibaud, Jimenez, Caillol, & Leterrier, 2019). It has been suggested by Han et al., that spectrin isoforms partially exhibit a compartmentally distinct expression. While jll spectrin seems to be expressed uniformly within axons and dendrites, jlll spectrin is particularly enriched in dendrites (Han et al., 2017). Indeed, only a fraction of dendritic spines had a periodic lattice formed by F-actin/jlll spectrin (Bär et al., 2016). Although it has not been demonstrated directly by super-resolution imaging, it is likely that jlll spectrin-negative spine necks with a periodic F-actin pattern do contain jlll spectrin. The crucial role of this protein for shaping neuronal compartments has been further confirmed by showing that dendritic spines collapse to shaft synapses upon knockdown of jlll spectrin in primary neurons (Efimova et al., 2017). It is possible that the presence of the periodic actin/spectrin lattice in the spine neck provides elasticity and mechanical support to this fine structure. However, the question arises how the neck of dendritic spines can change rapidly in response to synaptic activity (Tønnesen et al., 2014)? In a very recent study from the Zhuang lab was shown that in axons the membrane-associated periodic skeleton serves as a structural platform to recruit certain G protein-coupled receptors, cell adhesion molecules, receptor tyrosine kinases (RTKs) and related signaling molecules. They describe a mechanism by which periodic F-actin/spectrin lattice-associated RTK transactivation induces downstream signaling of ERK. This subsequently leads to the disruption of this structure by calpain, thereby enabling remodeling of the actin/spectrin lattice (Zhou, Han, Xia, & Zhuang, 2019). It is very tempting to speculate that such mechanism might be relevant for regulating the structural plasticity of the spine neck.

Nonetheless, the most striking changes in response to synaptic activity occur at the spine head. Notably, Ca2+ influx through NMDARs is instructive for F-actin dynamics (Bosch et al., 2014; Okamoto, Narayanan, Lee, Murata, & Hayashi, 2007). Few years ago, Bosch and colleagues investigated the effect of long-term potentiation (LTP), induced at individual spines, on the concentration of different actin-binding proteins within spines. They found that following 2-photon glutamate uncaging, the F-actin severing protein cofilin-1 rapidly enters a stimulated spine, where cofilin-1 activity results in an increased number of F-actin barbed ends. Subsequently, F-actin branching (Arp2/3 complex) or capping (ABP1) proteins transiently increase within a few minutes, whereas actin-binding proteins involved in stabilizing the supra-structure of the actin cytoskeleton (cortactin, drebrin A, profilin 2A, or α-actinin 2) are transiently reduced (Bosch et al., 2014; Hering & Sheng, 2003). Such switch in equilibrium from actin stabilizers to actin modifiers generates a short time interval in which the spinous actin cytoskeleton undergoes major reorganization (Bosch et al., 2014). In order to avoid a total collapse of the structure, a stable synaptic pool of actin-binding proteins and actin filaments must exist. This elementary F-actin meshwork associated with the postsynapse is regulated by the Ca2+ sensor caldendrin that orchestrates nanodomain actin dynamics. At a mechanistic level, Ca2+ binding to caldendrin disrupts an intramolecular interaction and allows for binding of cortactin, which upon activation can interact with F-actin. In the proximity of the synaptic membrane, cortactin recruits the Arp2/3 complex via N-WASP and promotes formation of new actin branches (Helgeson & Nolen, 2013). At the spine base, caldendrin keeps cortactin in an active conformation and protects F-actin against cofilin-induced severing (Mikhaylova et al., 2018). In line with these findings, the stable pool of spinous actin is no longer preserved in caldendrin deficient mice. Additionally, the mice show an aberrant nanostructural organization of F-actin in dendritic spines, higher dynamics of spines, as well as reduction in the number of mature mushroom-like spines and failure to maintain LTP. Thus, the caldendrin-cortactin complex directly couples spinous Ca2+ concentrations to the stabilization of a minimal F-actin pool that is required for actin remodeling in the early phase of LTP (Mikhaylova et al., 2018).

Even though mushroom-like spines represent the vast majority of excitatory synapses in the adult brain, there is a morphological continuum of synaptic contacts. It will be an intriguing topic for future research to investigate if Ca2+ signaling via caldendrin will have similar implications for morphologically distinct synapses, especially if they are directly located at the dendritic shaft.

### 3.2 | Actin at the excitatory shaft synapse

In excitatory principal neurons a certain proportion of glutamatergic synapses remains located in the dendritic shaft in adulthood (Bourne & Harris, 2011; Reilly et al., 2011). Moreover, most GABAergic interneurons have smooth dendrites with no or only a few dendritic spines. Nonetheless, their dendritic shafts are densely covered with glutamatergic synapses (Figure 1(b) / He, Janssen, Vissavajhala, & Morrison, 1998).

F-actin is also an important element of shaft synapses. In a very recent work, the dendritic actin cytoskeleton of adult primary neurons has been mapped at the nanoscale level (van Bommel et al., 2019). STED nanoscopy revealed local enrichments of F-actin within the dendritic shaft or at the base of some dendritic spines. As the authors could show, these structures evolve during synaptogenesis and, like F-actin in dendritic spines, consist of a mixture of linear and branched filaments. Interestingly, actin patches can vary in size (from 0.1 to 2 μm). They invade deep into the dendritic shaft but are devoid of microtubules. During development, a delayed appearance of actin patches compared to microtubule bundles could give a possible explanation for such spatial segregation. The periodic F-actin lattice is interrupted near excitatory shaft synapses, thus the synapse-associated F-actin mesh seems not to overlap with the cortical periodic cytoskeleton. The interesting question arises whether such shaft synapses can transform into dendritic spines and if spines can retract to the shaft without stripping their synaptic contact. In this respect, co-ordinated remodeling of the actin cytoskeleton could provide a pushing force to form new spines when all other PSD components will be readily present at the synapse (Figure 2).
The presence of F-actin patches is very critical for regulating organelle transport, including lysosomal trafficking in dendrites, as lysosomes frequently stop and stall at these loci. It has been shown that F-actin patches form a physical barrier for kinesin-driven cargo and in addition, they can serve as organelle docking sites for cargo associated with the myosin V motor (van Bommel et al., 2019). Interestingly, in addition to synaptic delivery of recycling endosomes, myosin V is important for targeting of the spine apparatus, an endoplasmic reticulum-like specialization, into spine- and shaft synapses via association with synaptopodin. This indicates a pivotal role of F-actin in positioning of the spine apparatus (Konietzny et al., 2019). Future studies will show to what extent other membrane organelles or perhaps also dendritic mRNA can be affected by the presence of a dense F-actin mesh around shaft synapses, and if this is a general principle allowing for sorting and positioning of cargo in dendrites.

3.3 | Actin at the inhibitory shaft synapse

The diversity of F-actin-based structures implies specific functions of F-actin within different neuronal compartments. Nonetheless, the organization of the actin cytoskeleton at inhibitory synapses has not yet been investigated in detail. Among very few studies on this topic, the interaction between the actin-binding proteins profilin and Mena/VASP with gephyrin, which is thought to facilitate postsynaptic anchoring of glycine receptors (GlyRs) at inhibitory synapses, has been reviewed previously (Kneussel & Loebrich, 2007). Another study showed that differential expression of drebrin A influences both glutamatergic and GABAergic activities as well as synaptic density, which might be explained by the interaction with actin, microtubules or both (Ivanov, Esclapez, Pellegrino, Shirao, & Ferhat, 2009). Although the density of F-actin at inhibitory synapses may be low, it represents a critical factor in receptor localization at the PSD (Giesemann et al., 2003; van Bommel et al., 2019). The interesting question raises whether GABAergic synapses enriched in F-actin differ in their stability and plastic properties from inhibitory synapses without enrichment. This differential distribution and the functional consequences need to be addressed by future studies. It is still unknown whether acting rings, which are commonly found in axons, spines, and dendrites, are also present at inhibitory synapses and super-resolution imaging would be very helpful in exploring this in detail.

4 | MICROTUBULE CYTOSKELETON AND SYNAPSES

Microtubules are hollow polymers which are found in axons, dendrites and a subset of spines and are formed by the polymerization of α/β-tubulin heterodimers. GTP-bound tubulin heterodimers are mostly incorporated in the fast-growing plus-end. Microtubules can also grow from their minus-end, although this process is substantially slower compared to canonical plus-end growth. Furthermore, it has been shown that microtubules undergo dynamic growth and catastrophe, a process termed dynamic instability. Interestingly, the end-binding (EB) protein family modulates microtubule growth by selectively binding to distinct α-tubulin conformations at the (+)-end (Zhang, Alushin, Brown, & Nogales, 2015). On the other hand, microtubule (-)-ends are bound and stabilized by proteins of the calmodulin-regulated spectrin-associated protein (CAMSAP) family (Yau et al., 2014). From a regulatory perspective, the presence of various α- and β-tubulin isotypes as well as different post-translational modifications (PTMs) of tubulin such as phosphorylation, acetylation, detyrosination, or polyglutamylation generate a complex molecular pattern, which confers unique properties to individual microtubules (Eshun-Wilson et al., 2019; Gadadhar, Bodakuntla, Natarajan, & Janke, 2017). Therefore, the diversity of possible microtubule types led to the idea of the so-called tubulin code (Verhey & Gaertig, 2007). Moreover, individual

![FIGURE 2](https://example.com/figure2.png)
Microtubules PTMs might facilitate the recruitment of distinct microtubule-associated proteins (MAPs) for local regulation of microtubule function (Janke & Kneussel, 2010).

Microtubules mediate a plethora of different cellular functions including active transport of cargo by kinesin and dynein motors, chromosome segregation, or subcellular compartment specific regulation of neuronal activity. Additionally, microtubules regulate cellular polarity and morphology, which is crucial for the physiological function of neurons. However, mechanistic details of neuronal polarity establishment have been recently reviewed (Yoge & Shen, 2017). In mature neurons, microtubules are oriented in a characteristic manner. Axons exhibit microtubules with uniform polarity where the dynamic plus-ends point toward the periphery. Conversely, dendrites generally seem to have mixed microtubule polarities with more minus-end directed microtubules in the distal parts (Baas, Deitch, Black, & Banker, 1988; Baas & Lin, 2011; Kapitein & Hoogenraad, 2011; Tas et al., 2017).

Although microtubules are not the major spinous cytoskeletal element, they are able to enter dendritic spines in an NMDAR-dependent manner (Gu, Firestein, & Zheng, 2008; Hu, Vieselman, Nam, Merriam, & Dent, 2008; Jaworski et al., 2009; Mitsuyama et al., 2008). In this context, it has been shown that local actin polymerization at the head and neck of dendritic spines following NMDAR-dependent calcium spikes is sufficient to facilitate the entry of growing microtubules proximal to the active spine. Thereby drebrin, an actin-binding protein, establishes the crosslink between F-actin and growing microtubules by interacting with EB3, resulting in guidance of microtubule polymerization into the spine head (Merriam et al., 2013) (Figure 1a). On the other hand, it has been demonstrated recently, that spines seem to be the preferred loci for microtubule catastrophes in mature neurons (Schätzle et al., 2018). Nonetheless, a possible function of microtubules in spines may be the facilitation of AMPAR trafficking. AMPAR containing recycling endosomes have been shown to travel along both microtubules and filamentous actin (F-actin). Although spinous microtubule entry is not essential for synaptic delivery of AMPARs, it increases trafficking dynamics of Rab11-positive recycling endosomes (Esteves da Silva et al., 2015). This demonstrates the involvement of microtubules in regulated cargo delivery into spines.

In order to facilitate input-specific synaptic plasticity, precisely regulated cargo delivery is crucial. It is interesting to speculate which mechanisms might play a role to accomplish this task. Three distinct possible scenarios of microtubule guided cargo deposition near synaptic sites have been proposed (Dent, 2017). First, vesicles may be directly transported into the spine head by microtubule-bound kinesin motors, which detach from the microtubule within the spine thereby facilitating cargo release. Second, microtubule motors can deliver the cargo within dendrites, which is then released in proximity of a spine and diffuses into the spine head. Third, cargo, which is initially transported by kinesin along microtubule tracks within the dendrite, is handed off to myosin-motors at the neck of dendritic spines and is transported further into the spine head along actin filaments. The latter is of particular interest in the light of recent findings suggesting that F-actin patches are either located at the base of dendritic spines or around excitatory shaft synapses thereby playing a crucial role in positioning of organelles (van Bommel et al., 2019).

The microtubule environment of the shaft synapse is much less investigated compared to dendritic spines. Interestingly, it was demonstrated recently that F-actin patches surrounding excitatory shaft synapses are devoid of microtubules (van Bommel et al., 2019). It is possible that in analogy with F-actin at dendritic spines, these patches could participate in the modulation of local microtubule dynamics (see Figure 1a). In contrast, most inhibitory shaft synapses do not seem to be enriched in F-actin (van Bommel et al., 2019). Consistently, it has been shown that synaptic maintenance of gephyrin and GABAaRs does not depend on the actin or microtubule cytoskeleton (Allison, Chervin, Gelfand, & Craig, 2000). Nonetheless, it has been suggested that gephyrin and inhibitory glycine receptors (GlyRs) are co-transported along microtubule tracks by the dynein motor complex (Maas et al., 2006). This highlights a role of microtubules in the formation and dynamics of inhibitory shaft synapses. However, the precise organization of the microtubule cytoskeleton at inhibitory shaft synapses is an intriguing question for future studies.

5 | INTERMEDIATE FILAMENTs IN DENDRITES AND SYNAPSE DEVELOPMENT

Neurofilaments (NF) are members of the intermediate filaments family (IFs) composing the neuronal cytoskeleton. NFs regulate axonal growth, provide structural support essential for the electrical signal conduction velocity and participate in signaling (Yuan, Rao, Veeranna, & Nixon, 2017). But are they important for the synapse? The loss of function of NF proteins in the central nervous system profoundly disrupts synaptic plasticity and alters synapse morphology (Yuan et al., 2015). Yuan and colleagues found that synaptic NF proteins are more abundant in the postsynaptic area. However, only some synaptic NF subunits exist in polymerized form while pleura of monomeric IFs could contribute to synaptic signaling and have other noncytoskeletal functions. Along these lines, Karpova and colleagues demonstrated the presence of α-internexin in dendrites and PSD and showed that synaptic NMDA receptor activation induces phosphorylation of the protein messenger Jacob by ERK1/2. Jacob and ERK1/2 then associate with an α-internexin cleavage fragment and are protected from dephosphorylation during trafficking to the nucleus. Phosphorylated nuclear Jacob promotes cell survival and enhances synaptic plasticity, suggesting the relevant role of cytoskeletal intermediate filaments in synaptic signaling to the nucleus (Karpova et al., 2013). These findings strongly support the evidence that NF proteins play a determinant role for the proper neuronal morphology, and are integral components of synapses required to induce synaptic plasticity. However, it is still unclear whether IFs play a structural role at spine and shaft synapses or if their effects are mediated by engagement of monomeric proteins in different signaling pathways.
SEPTINS AND DEVELOPMENT OF DENDRITIC SPINES

Septins are a group of conserved guanine nucleotide binding proteins which recently began to be intensively studied as crucial components of the neuronal cytoskeleton (Ewers et al., 2014; Spiliotis, 2018; Yadav et al., 2017). Several septins have been found in the central nervous system of mammals including Sept4, 5, 6, 7, and 14. Interestingly, septins exist as hetero-oligomeric complexes forming higher-order structures such as filaments, meshes or rings. Commonly, they have been described as a mechanical support associated with the plasma membrane and are contributing to different cellular functions including cytokinesis, mitosis, exocytosis, and apoptosis. Structurally it can form arc-shaped or ring-like structures at the base of dendritic spines, which might contribute to establishment and stabilization of neuronal membrane curvature. However, in pyramidal neurons septins have been shown to promote axonal growth as well as dendrite extension (Ageta-Ishihara et al., 2013; Shinoda et al., 2010; Spiliotis, 2018). Sept7 is particularly interesting in the context of the synaptic cytoskeleton. Its expression is developmentally regulated in hippocampal neurons. Notably, in young neurons endogenous Sept7 is found in growth cones of dendrites extending from the cell body and at the base of filopodia-like dendritic protrusions (Tada et al., 2007). The expression of sept7 increases significantly after the early stages of development and the protein is not homogenously distributed along neurites, suggesting a role in dendritic morphogenesis. Upon downregulation of Sept7, dendritic branching is impaired and numerous changes of spine morphology have been demonstrated, indicating an important role of Sept7 in spine development (Ewers et al., 2014; Xie et al., 2007). Additionally, its depletion results in destabilization of dendritic protrusions, mislocalization of shaft-synapses and loss of compartmentalization of glutamatergic receptors. Apart from this, the direct phosphorylation of Sept7 by the serine/threonine protein kinase TAO2 (TAOK2) is required for spine maturation (Yadav et al., 2017). Taken together, Sept7 has been shown to be an important, developmentally regulated constituent of the neuronal cytoskeleton playing a crucial role in dendrite morphogenesis and synaptic stability (Yadav et al., 2017).

7 PERSPECTIVES

The direct relationship between synaptic morphology and function highlights the central role of processes regulating the synaptic cytoskeleton. Especially, progress in understanding the role of actin in formation, maintenance, and functioning of synapses has tremendously benefited from the development of super-resolution imaging. Kinetic studies allowing visualization of actin filament turnover and microtubule dynamics upon induction of synaptic activity brought another level of understanding of how a specific type of signal can trigger local changes in the cytoskeleton allowing fine-tuning of the inputs. It is still an open question why dendritic spines are only growing to a limited extend and what factors can control shape features such as the spine neck diameter or volume of the spine head. Can shaft synapses become spines and vice versa (Figure 2)? It is very likely that the synaptic cytoskeleton will play a key role in these processes. Another interesting question is activity-dependent reorganization of periodic actin and spectrin lattice at the spine neck. It is likely that calcium and MAPK signaling induced activation of calpain will lead to the rapid disassembly of spines or dendritic actin rings. However, such assumptions still require experimental proof.

Another important aspect is the cross-talk between different cytoskeletal components, such as microtubule entry in dendritic spines which depends on the presence of an F-actin mesh at the base of a target spine. This raises many interesting questions dealing with the function of microtubules in spines, the molecular mechanisms allowing co-ordinated action of the actin and microtubule cytoskeleton at the synapse in general, or how dynamic microtubules would interact with the F-actin mesh surrounding excitatory shaft synapses. A multitude of possible signaling pathways triggered by activation of glutamate, GABA or neurotrophine receptors could participate in fine-tuning this interaction. Similar questions could be applied to all other cytoskeletal components. The function of NFs is highly regulated by phosphorylation and dephosphorylation. It would be interesting to see in the future to what extend these processes occur at the synapse and if they have a structural role at the synapse.

Up to now, most of the studies were performed in spines principal neurons. It is very important to extend this type of research to the other neuronal cell types such as GABAergic interneurons, which are crucial for brain function.

Additionally, considering that the cytoskeleton is targeted in many neurological disorders, it would be extremely valuable to gain better understanding of its structure, function, and regulations at the synapse under physiological conditions.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

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