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A role for synaptopodin and the spine apparatus in hippocampal synaptic plasticity ☆

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Summary

Spines are considered sites of synaptic plasticity in the brain and are capable of remodeling their shape and size. A molecule that has been implicated in spine plasticity is the actin-associated protein synaptopodin. This article will review a series of studies aimed at elucidating the role of synaptopodin in the rodent brain. First, the developmental expression of synaptopodin mRNA and protein were studied; secondly, the subcellular localization of synaptopodin in hippocampal principal neurons was analyzed using confocal microscopy as well as electron microscopy and immunogold labelling; and, finally, the functional role of synaptopodin was investigated using a synaptopodin-deficient mouse. The results of these studies are: (1) synaptopodin expression by hippocampal principal neurons develops during the first postnatal weeks and increases in parallel with the maturation of spines in the hippocampus. (2) Synaptopodin is sorted to the spine compartment, where it is tightly associated with the spine apparatus, an enigmatic organelle believed to be involved in calcium storage or local protein synthesis. (3) Synaptopodin-deficient mice generated by gene targeting are viable but lack the spine apparatus organelle. These mice show deficits in synaptic plasticity as well as impaired learning and memory. Taken together, these data implicate synaptopodin

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and the spine apparatus in the regulation of synaptic plasticity in the hippocampus. Future studies will be aimed at finding the molecular link between synaptopodin, the spine apparatus organelle, and synaptic plasticity.

Spines are sites of neuronal plasticity in brain

Spines are small appendages of dendrites that contain the postsynaptic elements of asymmetric synapses (Gray, 1959). In recent years, it has been well documented that spines are structurally highly dynamic and that their morphological parameters, i.e. their length and head size, influence the propagation of neuronal activity from the pre- to the postsynaptic neuron (Svoboda et al., 1996; Matsuzaki et al., 2001, 2004; Korkotian et al., 2004; Hayashi and Majewska, 2005; Segal, 2005). Since the morphological dimensions of spines are, in turn, regulated by afferent synaptic activity (Fifkova and van Harreveld, 1977; Korkotian and Segal, 1999a; Matsuzaki et al., 2004), presumably via changes in intracellular calcium (Yuste et al., 2000; Fischer et al., 2000; Korkotian et al., 2004; Brunig et al., 2004; Segal, 2005; Oertner and Matus, 2005), changes in spine geometry may be the means by which the postsynaptic neuron is able to adapt to long-lasting changes in presynaptic activity. This interdependency of neuronal activity and spine morphology has led to the prevailing notion that spines are major sites of functional and structural plasticity in the brain.

Yet what is the biological relevance of spine plasticity? Because the number, shape, and size of spines depend on various factors such as neuronal activity, hormonal and environmental stimuli (Valverde, 1967; Frotscher et al., 1977; Woolley and McEwen, 1993; Murphy and Segal, 1996; McKinney et al., 1999; Harris, 1999; Muller et al., 2000; Buchs and Muller, 2002; Kretz et al., 2004), it has been proposed that spines could be involved in learning and memory processes. Some authors even suggested on the basis of novel imaging, electrophysiological, and molecular data, that spines could represent the morphological correlates of memory traces in brain (Matsuzaki et al., 2001, 2004). Others, however, have pointed out that the data on the role of spines in learning and memory are ambiguous and have suggested that spines may primarily serve to biochemically isolate excitatory synapses from the dendrite, thereby protecting neurons from excitatory damage (Segal, 2005). In any case, understanding structural spine plasticity at the molecular and cellular level promises fundamental insights into the physiological regulation of neuronal communication. In the present report, we will, therefore, summarize our findings on synaptopodin, an actin-associated molecule, that could be involved in the regulation of structural spine plasticity in the brain of adult vertebrates.

The structural plasticity of spines depends on actin and Ca^{2+}

In recent years, cell biological studies have provided new insights into the molecular machinery regulating spine plasticity. As far as the rearrangement of the cytoskeleton is concerned, actin certainly plays the central role (Adam and Matus, 1996; Matus et al., 2000; Dillon and Goda, 2005). Actin is abundant in the head and neck of spines (Fifkova and Delay, 1982; Matus et al., 1982; Cohen et al., 1985; Morales and Fifkova, 1989), and imaging studies have provided direct evidence that spine motility depends on actin polymerization (Fischer et al., 1998; Dunaevsky et al., 1999; Fischer et al., 2000). In contrast, even though myosin is also present in dendritic spines (Drenckhahn and Kaiser, 1983), myosin is not required for spine motility (Fischer et al., 1998).

Not surprisingly, actin polymerization in spines is a tightly regulated process and several regulatory molecules have been identified that regulate actin polymerization in spines and, as a consequence, structural spine plasticity (Adam and Matus, 1996; Matus et al., 2000; Dillon and Goda, 2005). It is likely to be modified by microfilament-associated proteins, such as α-actinin (Wyszynski et al., 1997; Wyszynski et al., 1998), drebrin (Hayashi et al., 1996), fodrin (Carlin et al., 1983), and gelsolin (Furukawa et al., 1997), which are only a selection of the many candidate regulatory molecules (Dillon and Goda, 2005). Importantly, the biochemical properties of actin and of many of its regulatory molecules depend on the levels of intracellular calcium, linking structural spine plasticity tightly to changes in synaptic activity at the axo-spinous synapse that can regulate the concentration of this ion in the spine compartment (Volfovsky et al., 1999; Korkotian and Segal, 1999b; Yuste et al., 2000; Fischer et al., 2000; Korkotian et al., 2004;

Brunig et al., 2004; Holcman et al., 2005; Oertner and Matus, 2005).

Synaptopodin is an actin-associated molecule found in kidney podocytes and neurons

Synaptopodin is an actin-associated molecule that could potentially be involved in the actin-based remodeling of spine morphology. It was characterized by Mundel and co-workers (1997) who described it in the kidney and in the brain. In the kidney, synaptopodin is found in the cytoskeleton of podocyte foot processes where it regulates stress fiber formation and may be involved in the regulation of foot process effacement (Mundel et al., 1997; Asanuma et al., 2005, 2006). In the adult mouse and rat brain, synaptopodin mRNA is expressed by neurons in the olfactory bulb, cerebral cortex, striatum, and hippocampus. In all of these regions, synaptopodin is sorted to a subpopulation of dendritic spines (Mundel et al., 1997; Deller et al., 2000). After the synaptopodin gene was cloned, other members of the synaptopodin family of proteins were identified in muscle cells: myopodin, which is expressed in skeletal and heart muscle (Weins et al., 2001), and fesselin, which is expressed in smooth muscle and appears to be involved in the regulation of actin polymerization (Leinweber et al., 1999).

In a recent study, the mouse synaptopodin gene was analyzed in depth and it was shown that it contains three exons (Asanuma et al., 2005). In wild-type mouse, two proteins were found that are alternative splice products of the synaptopodin gene: a 110 kD isoform found in the kidney and a 100 kD form expressed in brain. Interestingly, the two synaptopodin isoforms contain high amounts of proline, which is almost evenly distributed throughout the molecules. This prevents the formation of globular domain structures and, thus, the synaptopodin proteins appear to have an almost rod-like structure. Synaptopodin tightly associates with actin, as could be shown using the actin-depolymerizing drug cytochalasin B, which disrupted the normal distribution of actin and synaptopodin in vitro (Mundel et al., 1997), and recently in two other studies using the expression of synaptopodin in vitro (Asanuma et al., 2005) and a co-sedimentation assay (Kremerskothen et al., 2005). The tight association of synaptopodin with actin, the molecule centrally involved in spine plasticity, makes it certainly attractive to unravel the role of synaptopodin in the spine compartment.

To understand the functional role of synaptopodin in spines, it is important to know more about its potential interaction partners. At present, our knowledge in this respect is very limited and we do not yet understand how synaptopodin fits into the prevailing models of actin-based spine dynamics (Dillon and Goda, 2005). So far, it has been shown that brain synaptopodin interacts with α-actinin-2 (Asanuma et al., 2005; Kremerskothen et al., 2005), and possibly also with MAGI, a member of the MAGUK scaffolding proteins frequently found at postsynaptic sites (Kremerskothen et al., 2005). In vitro, the interaction of synaptopodin with α-actinin-2 results in the elongation of actin filaments and the formation of parallel actin fiber bundles (Asanuma et al., 2005), suggesting that synaptopodin regulates the formation of the parallel actin fibers typically found in the spine neck (Fifkova and Delay, 1982; Matus et al., 1982; Cohen et al., 1985; Morales and Fifkova, 1989). The kidney-specific synaptopodin isoform also regulates stress fiber formation in podocytes by competitive blocking of Smurf1-mediated ubiquitination of RhoA, thereby preventing the targeting of RhoA for proteasomal degradation (Asanuma et al., 2006). Since recent data also implicate RhoA in spine plasticity (Kennedy et al., 2005; Carlisle and Kennedy, 2005), it is conceivable that synaptopodin may also influence GTPases in this compartment.

The localization of synaptopodin in two motile compartments; i.e. the foot processes of kidney podocytes and dendritic spines of neurons, and the similarities between the two cell types at the morphological, cellular, and molecular level (Kobayashi, 2002) suggested that synaptopodin could be involved in the regulation of the actin-based structural plasticity of dendritic spines. Since many of the functional studies on spine plasticity have been conducted on hippocampal neurons, we performed a detailed analysis of (1) the developmental expression of synaptopodin in the hippocampus (Czarnecki et al., 2005), (2) the cellular and subcellular distribution of synaptopodin and its mRNA in the adult hippocampal formation (Deller et al., 2000, 2002, 2006; Bas Orth et al., 2005), and (3) studied the effect of a targeted deletion of synaptopodin on the morphology and function of hippocampal neurons (Deller et al., 2003).

Synaptopodin expression during development correlates with the maturation of neurons and spines

In the earliest publication on synaptopodin (Mundel et al., 1997), it was noted that the expression of synaptopodin in the brain occurs

rather late during brain development. Synaptopodin was first detected by Western blot analysis at day 15 and maximal levels were obtained in the adult animal. Recently, the developmental expression of synaptopodin mRNA and protein was studied in depth in the postnatal rat hippocampus (Czarnecki et al., 2005). In this brain region, synaptopodin mRNA is already present at birth in CA3 pyramidal neurons (Fig. 1A). At postnatal day (P) 6, it is also found in CA1 pyramidal neurons and granule cells of the suprapyramidal blade of the dentate gyrus, although synaptopodin mRNA expression remains strongest in CA3 (Fig. 1B). At P9, virtually all principal neurons expressed synaptopodin in a pattern similar to the adult situation (Fig. 1C). In the adult (Fig. 1D, E), synaptopodin mRNA was strongly expressed by hippocampal principal neurons, spinebearing mossy cells, and spine-bearing interneurons in stratum lucidum (Deller et al., 2000, 2002; Czarnecki et al., 2005; Bas Orth et al., 2005). A small subpopulation of non-spiny interneurons also showed synaptopodin expression in the adult (Czarnecki et al., 2005). To study whether the expression levels of synaptopodin differ between the hippocampal subfields, the principal cell layers of the hippocampus were harvested using laser microdissection and analyzed by quantitative RT PCR. This demonstrated that the expression levels of synaptopodin mRNA are comparable between the different hippocampal regions in the adult animal (Bas Orth et al., 2005).

Immunostaining for synaptopodin protein was also performed and the developmental time course of synaptopodin in the hippocampus was analyzed. In contrast to the mRNA signal, which was exclusively observed in the soma of neurons, the immunocytochemical signal was found in the dendritic layers of the hippocampus. At birth, no immunoreactivity was visible, while at P5 a weak staining was observed in stratum oriens. At P9, immunolabeling was still strongest in stratum oriens followed by the molecular layer of the dentate gyrus. The adult pattern (see below) with strong labeling of all dendritic layers was reached by P12. Interestingly, no immunolabeling for synaptopodin was observed in identified interneurons, confirming that synaptopodin protein is mainly present in spinebearing principal cells.

Comparison of the development of synaptopodin expression in the hippocampus with the major steps of hippocampal principal cell differentiation revealed that synaptopodin expression follows the well-known sequence of hippocampal principal neuron development (Bayer, 1980a, b). In addition, the developmental time course coincides with the presence of mature spines in vivo (Harris et al., 1989, 1992) as well as in vitro (Ziv and Smith, 1996; Dailey and Smith, 1996). Thus, synaptopodin appears to be expressed during late stages of neuronal development and spine differentiation and persists in the adult hippocampus.

Synaptopodin is distributed in a lamina-specific pattern in the adult hippocampus

To study the distribution of synaptopodin protein in the adult hippocampus, hippocampal sections were stained for synaptopodin and analyzed using light- and electron microscopy (Deller et al., 2000, 2002, 2003; Bas Orth et al., 2005). In rat as well as mouse hippocampus, immunolabeling was strongest in dendritic areas of the hippocampus, where high densities of heavily immunoreactive synaptopodin-positive puncta were observed at high magnifications. Interestingly, these immunoreactive puncta were inhomogenously distributed and showed a characteristic region- and lamina-specific pattern (Deller et al., 2000, 2002; Bas Orth et al., 2005). To verify this observation, confocal microscopy was used to quantify synaptopodin-positive puncta densities in the different layers of the hippocampus (Bas Orth et al., 2005). This sensitive approach confirmed our qualitative initial observations and demonstrated that synaptopodin is sorted into the different fiber layers of the hippocampus in a layer-specific fashion.

What could be the significance of such a layer-specific distribution? The fiber layers of the hippocampus are formed by the major glutamatergic afferents to the hippocampus that terminate in a highly laminated manner on the dendrites of the hippocampal principal neurons. The observation that the distribution of the postsynaptic molecule synaptopodin respects the laminar organization of the hippocampus, strongly suggests that synaptopodin is sorted within hippocampal dendrites in response to afferent synaptic activity. This interpretation – based on our anatomical data – is in line with other studies which showed lamina-specific alterations in the distribution of synaptopodin in the hippocampus following kainic acid injection (Roth et al., 2001) or the induction of hippocampal long-term potentiation in vivo (Yamazaki et al., 2001; Fukazawa et al., 2003). Interestingly, upregulation of synaptopodin in stimulated fiber layers coincided with the upregulation of other cytoskeletal molecules, notably actin, in the same layers, suggesting that actin and synaptopodin play an important role in spine plasticity under conditions of synaptic strengthening (Fukazawa et al., 2003).

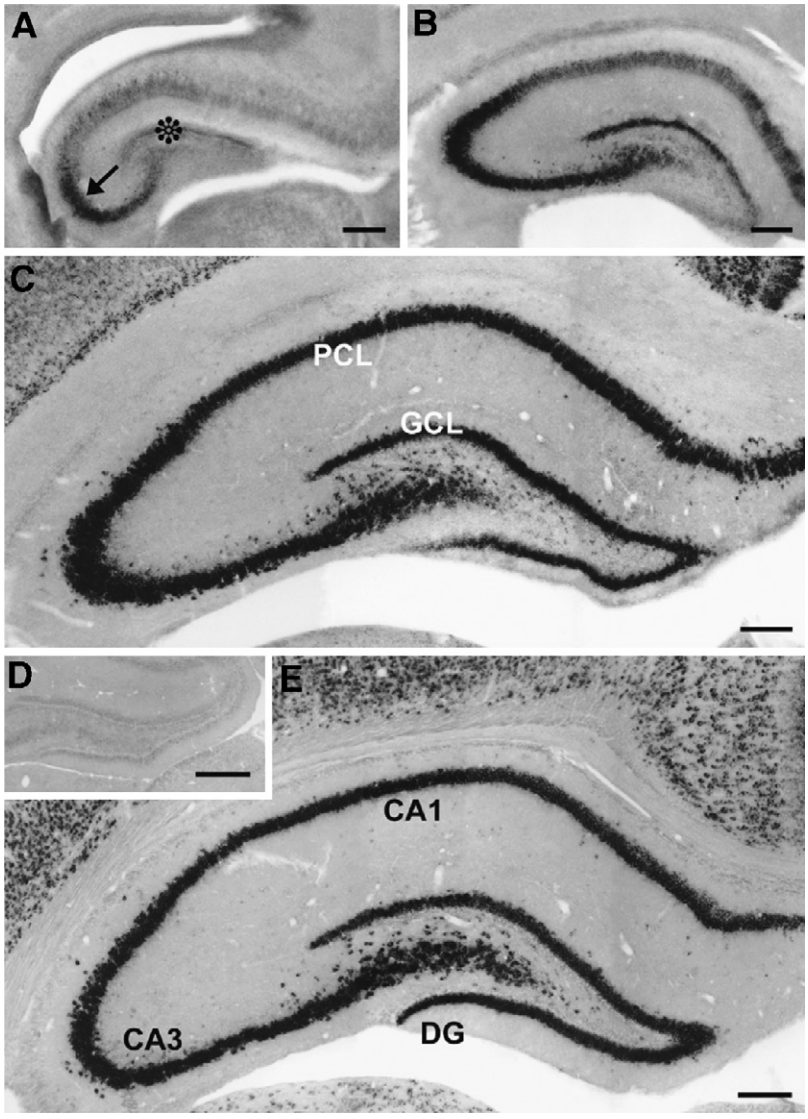

Figure 1. Expression pattern of synaptopodin mRNA during postnatal development of the rat hippocampus (A) At P1, strongly labeled cells can be observed in the pyramidal cell layer of CA3 (arrow), whereas the later developing CA1 region shows weaker staining. In the dentate gyrus, synaptopodin mRNA expression is restricted to the suprapyramidal blade of the granule cell layer (asterisk). (B) At P6, the overall synaptopodin mRNA expression has increased in the pyramidal cell layer; there is still a stronger signal in CA3 compared to CA1. In situ hybridization labeling is evident in the suprapyramidal blade of the dentate gyrus. Single labeled cells are seen outside the principal cell layers in the hilus, stratum oriens, and stratum lucidum, indicating synaptopodin expression in interneurons. (C) At P19, synaptopodin mRNA expression closely resembles the pattern seen in adult rats. PCL, pyramidal cell layer; GCL, granule cell layer. (D) Sense control. (E) In the adult rat, all principal cell layers show a strong signal intensity for synaptopodin mRNA. Outside the principal cell layers, single labeled cells can be seen in the hilus, stratum oriens, stratum lucidum, and stratum radiatum. Labeling is always restricted to the cytoplasm of neurons and is not seen in dendrites or axons. CA1, CA3, hippocampal regions CA1 and CA3; DG, dentate gyrus. Scale bars = 200 μm, except in d = 400 μm. Reprinted from Czarnecki et al. (2005).

Synaptopodin is localized to the spine apparatus and essential for spine formation in neurons

Next, we wanted to know the subcellular localization of synaptopodin in neurons. Accordingly, electron microscopy of synaptopodin-immunostained hippocampal sections was performed to study the localization of synaptopodin in the hippocampus (Deller et al., 2000). Standard electron microscopy with diaminobenzidine as a chromogen, pre-embedding immunogold labeling, and

postembedding-immunogold labeling were employed (Deller et al., 2000, 2002, 2003). All three electron microscopic techniques revealed that synaptopodin is primarily located in dendritic spines and only rarely found in dendritic shafts of hippocampal neurons. Within spines, synaptopodin was most abundant in the spine neck, where it was regularly associated with the spine apparatus organelle (Fig. 2A). Neither spines lacking a spine apparatus nor axon terminals forming contacts with spines were immunoreactive for synaptopodin (Deller et al., 2000).

Next, we addressed the question, how many spines of a single neuron contain synaptopodin. For such a quantitative analysis, classical methods such as electron microscopic reconstructions or the Golgi technique are of limited use, since these methods cannot be used to analyze a large number of cells. Therefore, we employed recently generated transgenic mice, which express fluorescent proteins in single neurons of the brain (Feng et al., 2000). Brain sections of these mice can be stained using immunofluorescence techniques, which makes it possible to study the localization of antigens within single EGFP-labeled cells using confocal microscopy. One of these mouse mutant strains, the so-called Thy1-GFP mouse (M-line), is of considerable interest, because it expresses enhanced green fluorescent protein (EGFP) in single principal neurons of the hippocampal formation. These neurons are intensely fluorescent and their dendrites are stained in a Golgi-like manner. Using synaptopodin-immunostained hippocampal sections of these mice for three-dimensional confocal reconstruction (Fig. 3A–D), we analyzed hippocampal principal cells and determined the percentage of synaptopodin-positive puncta in dendrites and spines, as well as the percentage of spines containing a synaptopodin-positive spine apparatus organelle (Bas Orth et al., 2005). As suggested in our electron microscopic preparations, synaptopodin puncta were primarily found in dendritic spines ($>95\%$) and only rarely in dendritic shafts. Interestingly, synaptopodin-positive puncta were unevenly distributed within the dendritic arbor of an identified neuron: The analysis of dendritic segments revealed a lamina-specific sorting of synaptopodin in hippocampal neurons (Fig. 1E). Densities of synaptopodinpositive spines ranged between 37% in the outer molecular layer of the dentate gyrus and 14% in stratum oriens of CA1. This confirmed and extended our previous data on the region and lamina-specific distribution of synaptopodin in the mouse hippocampus and demonstrated that a lamina-specific distribution of synaptopodin is also found within dendritic segments of identified hippocampal principal neurons. In addition, the three-dimensional reconstructions of synaptopodin puncta within spines underscored the usefulness of synaptopodin as a bona fide indicator of the spine apparatus organelle. We concluded, that the spine apparatus organelle is distributed in a region- and layer-specific fashion in the rodent hippocampus (Bas Orth et al., 2005).

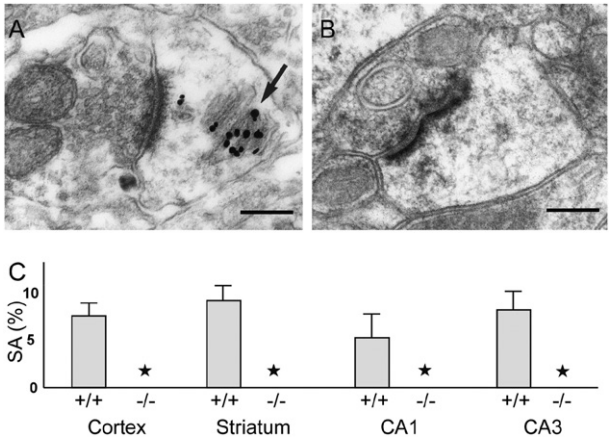

Figure 2. Synaptopodin is essential for the formation of a spine apparatus (A) Spine apparatus (arrow) in a wild-type hippocampal neuron immunolabeled for synaptopodin protein (1.4 nm gold grains, silver-intensified). Scale bar: 0.2 μm. (B) Absence of spine apparatus (and of synaptopodin protein) in a hippocampal neuron from a synaptopodin-deficient mouse. Scale bar: 0.2 μm. (C) Average percentage (plus SD) of spines with a spine apparatus (SA) in wild-type (+/+) animals ($n = 5$) and synaptopodin-deficient (−/−) mice ($n = 5$). Number of spines analyzed (+/+ versus −/−): Cortex (2396/1889); striatum (2218/1985); hippocampal area CA1 (2488/2218); hippocampal area CA3 (1742/1635). In synaptopodin- deficient mice (asterisks) the spine apparatus is absent in all regions analyzed. Reprinted from Deller et al. (2003). Copyright (2003) National Academy of Sciences, USA.

The question remained, however, whether synaptopodin is also required for the formation of a spine apparatus organelle. This question was addressed using mice homozygous for a targeted deletion of the synaptopodin gene (Deller et al., 2003). Synaptopodin-deficient mice were viable, did not show a pathological kidney phenotype under physiological conditions (Asanuma et al., 2005), and had grossly normal brains (Deller et al., 2003). No pathology was observed at the light microscopic level. An extensive electron microscopic analysis performed in the striatum, cortex, and hippocampus, however, revealed that telencephalic neurons of these mice completely lack spine apparatuses (Fig. 2B, C). In these animals, spine

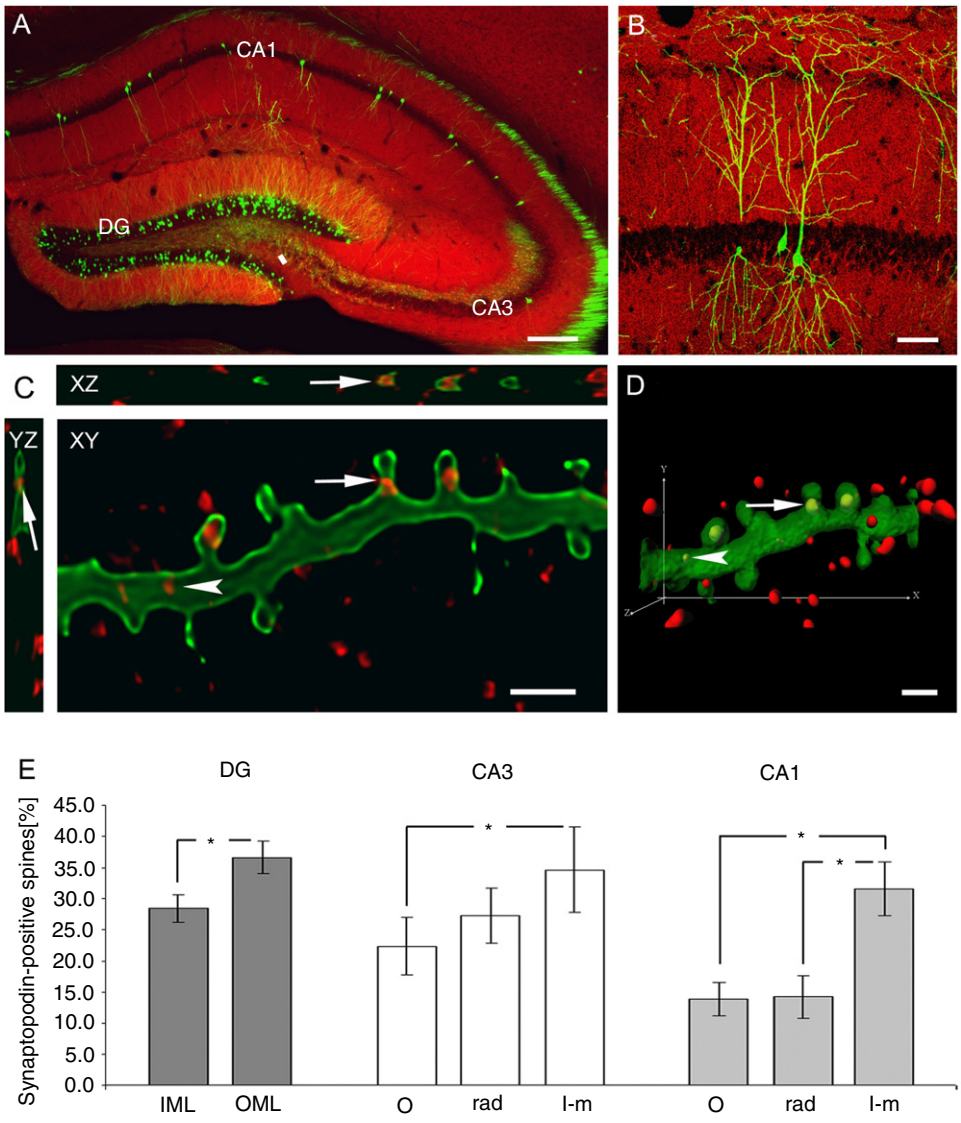

Figure 3. Laminar distribution of synaptopodin-positive puncta in dendritic segments of identified hippocampal principal cells (A) Frontal section of the hippocampus (Thy1-GFP transgenic mouse) stained for synaptopodin (red). Several EGFP-labeled neurons (green) are visible in the dentate gyrus (DG), in subfield CA3, and in subfield CA1. (B) Higher magnification of EGFP-labeled neurons in area CA1. The entire dendritic arbor of EGFP-labeled neurons is stained in a Golgi-like fashion. (C) Three-dimensional analysis of identified dendritic segments was performed using confocal imaging. The intracellular location of synaptopodin-puncta could be resolved by analyzing single sections from a confocal image stack in the *XY*-, *XZ*- and *YZ*-planes. In this pyramidal cell dendrite (CA1, *stratum oriens*), several synaptopodin-positive puncta are located in spines. The arrows indicate a synaptopodin-positive structure located within a spine neck. Occasionally, synaptopodin-positive puncta were found in dendrites (arrowhead). (D) Three-dimensional reconstruction of the dendritic segment shown in C. Synaptopodin-positive structures located within the EGFP-labeled neuron are coded in yellow. (E) Percentage of synaptopodin-positive spines (± 2 SEM) in identified dendritic segments (IML, inner molecular layer; l-m, stratum lacunosum-moleculare; o, stratum oriens; OML, outer molecular layer; rad, *stratum radiatum*). Asterisks indicate significant differences between the layers. DG, dentate gyrus; area CA3, and area CA1. Scale bars: $A = 200\,\mu m$; $B = 50\,\mu m$; C, $D = 1\,\mu m$. Reprinted from Bas Orth et al. (2005).

morphology and density appeared to be normal, suggesting that synaptopodin may act within spines to form a cytoskeletal scaffold for the spine apparatus organelle. Although this mechanism is yet hypothetical and requires further analysis, we could conclude that synaptopodin is essential for the formation of a spine apparatus (Deller et al., 2003).

The biological role of the spine apparatus is poorly understood

What is the role of the spine apparatus in neurons? At present, very little is known about the function of this enigmatic organelle that has been described many years ago (Gray, 1959). Its morphology has been analyzed in considerable detail and its distribution in CA1 pyramidal neurons has been studied using three-dimensional reconstructions of ultrathin sections (Spacek, 1985; Spacek and Harris, 1997). These investigations revealed that the spine apparatus is composed of tightly packed stacks of smooth endoplasmic reticulum laminated with densely stained material. Extensions of the dense material tether the spine apparatus to the postsynaptic density and some of the cisternae of smooth endoplasmic reticulum extend back to the smooth endoplasmic reticulum of the dendrite. Interestingly, and in line with our observations, these earlier studies (Spacek and Harris, 1997) showed that the spine apparatus is present only in a subpopulation of spines, predominantly the large and mushroom-shaped variant, that are functionally the most active (Harris and Stevens, 1989; Matsuzaki et al., 2001; Noguchi et al., 2005).

The biological significance of this organelle is, however, controversially discussed. On the one hand, available data suggest that the spine apparatus is involved in the intracellular release or the intracellular sequestration of calcium (Fifkova et al., 1983; Burgoyne et al., 1983; Fifkova, 1985). Since spines may act as biochemical compartments to protect the dendrite from damaging increases in intracellular calcium, the spine apparatus could play a crucial role in this context (Segal, 1995, 2005). If this hypothesis is true and the spine apparatus acts as a calcium store or a calcium sink, then calcium transients of individual spines should depend on the presence of a spine apparatus organelle. This hypothesis can be tested using synaptopodin-deficient mice that lack a spine apparatus organelle. Studies are ongoing that address this issue by using two-photon microscopy and calcium-sensitive dyes.

On the other hand, the spine apparatus has also been suggested to play a role in the synthesis of membrane bound proteins (Pierce et al., 2001, 2000). It can be labeled with markers for the secretory pathway, such as the translocon-subunit sec61α or the Golgi markers α-mannosidase II and giantin, which suggests that it could be involved in local protein synthesis or the posttranslational modification of proteins. In this context, it is interesting that synaptopodin and the spine apparatus organelle may be linked to the postsynaptic density, for example the NMDA receptor via α-actinin-2 (Wyszynski et al., 1997, 1998; Racca et al., 2000; Asanuma et al., 2005). Thus, it is attractive to speculate that the spine apparatus could play a role in postsynaptic receptor trafficking or receptor modification. Regardless of its precise role, however, the available data strongly implicate the spine apparatus in spine plasticity since both, changes in calcium transients (Denk et al., 1996; Svoboda and Mainen, 1999; Matus et al., 2000; Segal, 2005), as well as changes in local protein synthesis (Steward and Schuman, 2001; Pierce et al., 2001) have been implicated in plastic processes at axo-spinous synapses.

Synaptopodin and the spine apparatus organelle may play a role in learning and memory

The morphological observations reviewed in the previous paragraphs lead to the question about the functional role of synaptopodin and the spine apparatus in spines. Because of the sorting of synaptopodin to spines, the pivotal role of actin in spine plasticity, and the proposed role of the spine apparatus in calcium storage and local protein synthesis (see previous paragraph), we hypothesized that synaptopodin and the spine apparatus could be involved in the regulation of synaptic plasticity at the axo-spinous synapse. To test this hypothesis, we used synaptopodin-deficient mice and studied changes in synaptic plasticity at the Schaffer collateral-CA1 synapse in acute hippocampal slice preparations (Deller et al., 2003). After verifying that basal synaptic transmission was normal at this synapse, we analyzed long-term potentiation using theta-burst as well as tetanus stimulation protocols. Under both conditions, the increase in the slope of the field excitatory postsynaptic potential (fEPSP) was impaired in the synaptopodin-deficient mouse compared to the wild-type control. Although synaptopodin-deficient mice could be potentiated, impairment of the slope-LTP became significant after approximately 1 hour. Thus, both the early (E-LTP) as well as the late phase of LTP (L-LTP) were altered in synaptopodin-deficient mice.

The observed changes in LTP prompted us to perform a variety of behavioral tests with the synaptopodin-deficient animals. Most relevant to the electrophysiological data, significant differences between synaptopodin mutants and controls were observed in the radial arm maze (Deller et al., 2003). Synaptopodin-deficient mice were impaired

in their spatial learning ability after the third day of training. Thus, we observed a strong correlation between changes in hippocampal synaptic plasticity and a hippocampus-dependent spatial learning task.

Conclusions and outlook

The findings of the studies that were reviewed in this paper can be summarized as follows: (1) Synaptopodin is an actin-associated molecule that is expressed by neurons and kidney podocytes. During development, it is expressed fairly late in the hippocampus, in a sequence that correlates with the regional differentiation of the hippocampal formation and the appearance of mature spines. (2) In the adult brain, synaptopodin is preferentially sorted to spines. In spines, synaptopodin is tightly associated with the spine apparatus and can be used as a bona fide marker for this organelle. The density of synaptopodin-positive spines varies between the layers of the hippocampus, suggesting that the distribution of synaptopodin and the spine apparatus organelle in single neurons depends on presynaptic signals, very likely on afferent activity. (3) To study the functional role of synaptopodin in neurons, a synaptopodin-deficient mouse was generated. These mice do not form a spine apparatus organelle, demonstrating that synaptopodin is required for the formation of a spine apparatus. Importantly, synaptopodin-deficient mice showed impaired synaptic plasticity at the CA3–CA1 synapse in the hippocampus and an impairment in a hippocampus-dependent learning task. We conclude from these findings, that synaptopodin and the spine apparatus play a role in synaptic plasticity in the CA1 region of the hippocampus. Further studies are underway to unravel the underlying mechanisms and to dissect whether the alterations in synaptic plasticity and learning and memory observed in synaptopodin-deficient mice are caused by the absence of the spine apparatus organelle, a yet unidentified regulatory role of synaptopodin in the regulation of the spine actin cytoskeleton, or both.

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