Plasticity of Synaptopodin and the Spine Apparatus Organelle in the Rat Fascia Dentata Following Entorhinal Cortex Lesion

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ABSTRACT
Synaptopodin is an actin-associated molecule essential for the formation of a spine apparatus in telencephalic spines. To study whether synaptopodin and the spine apparatus organelle are regulated under conditions of lesion-induced plasticity, synaptopodin and the spine apparatus were analyzed in granule cells of the rat fascia dentata following entorhinal denervation. Confocal microscopy was employed to quantify layer-specific changes in synaptopodin-immunoreactive puncta densities. Electron microscopy was used to quantify layer-specific changes in spine apparatus organelles. Within the denervated middle and outer molecular layers, the layers of deafferentation-induced spine loss, synaptogenesis, and spinogenesis, the density of synaptopodin puncta and the number of spine apparatuses decreased by 4 days postlesion and slowly recovered in parallel with spinogenesis by 180 days postlesion. Within the nondenervated inner molecular layer, the zone without deafferentation-induced spine loss, a rapid loss of synaptopodin puncta and spine apparatuses was also observed. In this layer, spine apparatus densities recovered by 14 days postlesion, in parallel with plastic remodeling at the synaptic level and the postlesional recovery of granule cell activity. These data demonstrate layer-specific changes in the distribution of synaptopodin and the spine apparatus organelle following partial denervation of granule cells: in the layer of spine loss, spine apparatus densities follow spine densities; in the layer of spine maintenance, however, spine apparatus densities appear to be regulated by other signals. J. Comp. Neurol. 499:471–484, 2006.

Spines are small protrusions of dendrites containing the postsynaptic elements of synapses (Gray, 1959). Although their precise role is not yet understood, recent studies have shown that the morphological parameters of spines, the density of receptors at their postsynaptic membrane, and the intracellular composition of spines determine how presynaptic neuronal activity is propagated to the postsynaptic neuron (Svoboda et al., 1996; Matsuzaki et al., 2001, 2004; Korkotian et al., 2004; for review see Hayashi and Majewska, 2005; Segal, 2005). A distinguishing and highly characteristic feature of a subpopulation of large mature spines is the presence of a cytoplasmic organelle, the spine apparatus (Gray, 1959; Spacek, 1985; Spacek and Harris, 1997). It 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 (Spacek, 1985; Spacek and Harris, 1997). Available data suggest that it could be involved in the sequestration and intracellular release of calcium (Burgoyne et al., 1983; Fikova et al., 1983; Korkotian and Segal, 1999) and, possibly, the synthesis of membrane-bound proteins (Pierce et al., 2000, 2001). Recently, we have reported that the spine apparatus is tightly associated with the actin-binding molecule synaptopodin (Mundel et al., 1997; Deller et al., 2000; Ansumana et al., 2005). Mice lacking synaptopodin do not form a spine apparatus and show deficits in long-term potentiation and spatial learning, suggesting that synaptopodin links the spine apparatus organelle to the spine cytoskeleton and implicating the spine apparatus organelle in synaptic plasticity (Deller et al., 2003).

At present, little is known about the cell biology of the spine apparatus. Because of its suggested role in synaptic plasticity, however, it is of interest to identify the signals that govern its formation, maintenance, and turnover in spines. In the present study, we addressed the specific question of whether afferent denervation affects the distribution of synaptopodin, an essential component of the spine apparatus organelle, in granule cells of the rat fascia dentata. These cells appear to be particularly well suited for this kind of analysis, because up to 37% of granule cell spines contain synaptopodin-positive spine apparatus organelles (Bas Orth et al., 2005). Entorhinal cortex lesions (ECL; Steward, 1991; Deller and Frotscher, 1997; Savaskan and Nitsch, 2001; Ladeby et al., 2005) were performed, causing a layer-specific denervation of the middle molecular layer (MML) and outer molecular layer (OML) of the fascia dentata, followed by a layer-specific loss of granule cell spines in the same zone (Parnavelas et al., 1974; Matthews et al., 1976a,b; Caceres and Steward, 1983). Immunolabeling for synaptopodin was employed to identify and quantify changes in synaptopodin-positive puncta via confocal microscopy (Deller et al., 2000; Bas Orth et al., 2005). Electron microscopic analysis was used to evaluate the density of the organelle (Spacek, 1985; Spacek and Harris, 1997; Deller et al., 2000). These data were compared with changes in synaptopodin and spine apparatuses in the nondegenerated inner molecular layer (IML) of the fascia dentata, a zone in which granule cells maintain their spines following denervation (Parnavelas et al., 1974; Caceres and Steward, 1983). Our data revealed layer-specific changes in the distribution of synaptopodin-positive puncta and the spine apparatus organelle following ECL, suggesting that their distribution is differentially regulated in denervated and nondener-

### MATERIALS AND METHODS

#### Animals and tissue preparation

Adult male Sprague-Dawley rats (250–350 g; Charles River, Sulzfeld, Germany) housed under standard labora-
tory conditions were used. For light microscopic immuno-
histochemistry, control rats (n = 4), and entorhinal cortex (EC)-lesioned rats surviving for 4 (n = 3), 7 (n = 3), 10 (n = 3), 14 (n = 5), 30 (n = 4), and 180 (n = 3) days postlesion were used. All animals were deeply anesthe-
tized with an overdose of Nembutal (300 mg/kg body weight) and transcardially perfused [fixative: 4% parafor-
maldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4]. All experiments were performed in accordance with the German law on the use of laboratory animals. Brains were removed and postfixed for 24 hours in 4% parafor-
maldehyde at 4°C. Serial frontal sections of the septal hippocampus were cut with a Vibratome (50 μm) and washed in PBS. Serial horizontal sections of the EC were cut for control of lesion quality.

For electron microscopy, control rats (n = 5) and EC-
lesioned rats surviving for 4 (n = 4), 10 (n = 4), 30 (n = 4), and 180 (n = 4) days postlesion were used. After anesthe-
sia (see above), animals were perfusion fixed (4% parafor-
maldehyde, 0.25% glutaraldehyde, in 0.1 M PBS, pH 7.4), and sections (50 μm) were cut on a Vibratome. For postembedding immunocytochemistry, lesioned rats sur-
vived for 14 (n = 2) or 30 (n = 2) days postlesion. Tissue preparation in these cases is described below.

For anterograde tracing of hippocampal afferents, unles-
ioned control rats were used. After tracer injection to
label commissural (n = 3) and entorhinal (n = 9) afferents, animals were perfusion fixed (4% parafomaldehyde; 0.1% glutaraldehyde, 0.15% picric acid, in 0.1 M PBS, pH 7.4), and sections (50 μm) were cut on a Vibratome.

#### Entorhinal cortex lesion

All surgical procedures were performed with animals
under deep sodium pentobarbitone (Nembutal, 50 mg/kg body weight) anesthesia. For adult animals, a standard electrocoagulator was used to make a unilateral cut in the frontal and sagittal plane between the entorhinal area and the hippocampus, which resulted in the complete destruction of the ipsilateral entorhinal afferents to the fascia dentata. The following coordinates were measured from the interaural line were used: Frontal cut with the knife angled backward against the frontal plane by 10°; AP 0, L 3–7, V down to the base of the skull; sagittal cut: AP +1 to +4, L 6.5, V down to the base of the skull.

Because lesion quality is essential, completeness of ECL was verified prior to analysis. On serial horizontal sec-
tions through the EC, the correct location and size of the lesion were verified. All animals with even the slightest injury to the temporal hippocampus were excluded. On serial septal sections, every fifth section was used for histochemical staining for acetylcholinesterase (AChE; see below). In the case of complete lesions, a dense AChE-
positive fiber band appears in the denervated outer two-
thirds of the molecular layer between 5 and 10 days postlesion (Nadler et al., 1977; Zimmer et al., 1986). Ani-
mals (5 days postlesion and later) without an appropriate AChE-positive fiber band were excluded. To control lesion quality in animals with shorter survival times postlesion, immunostaining with an antibody against calpain-cleaved spectrin was employed. This antibody labels degenerating axons and terminals and reliably demonstrates the zone of degeneration (Kiss et al., 1996).

#### Anterograde tracing

Anterograde tracing of entorhinal and commissural affer-
ters was performed as described elsewhere (Gerfen and Sawchenko, 1984; Deller et al., 1995, 1996), with minor
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AChE histochemistry

Sections were processed for AChE histochemistry by using a modified Karnovsky-Roots protocol (Mesulam et al., 1987; Deller et al., 1997). In short, 17.186 g maleic acid, 147 mg sodium citrate, 75 mg copper sulfate, and 16.4 mg potassium ferricyanide were added to 1,000 ml distilled water. The pH was adjusted (pH 8.0), and 106 mg acetylthiocholine iodide was added. Sections were mounted on slides, air dried, and rinsed in 0.1 M maleate buffer (MB; pH 8.0). Sections were incubated in the cholinesterase solution, rinsed in 0.1 M Tris buffer (pH 7.6), and intensified in a solution of 0.5% cobalt chloride in Tris buffer. Thereafter, sections were rinsed again and incubated in a DAB solution. After the sections were washed in Tris buffer, they were air dried, dehydrated, cleared, and coverslipped.

**Confocal laser scanning microscopy and quantitative image analysis**

Confocal microscopy was performed with a Zeiss LSM 510 laser scanning microscope, Zeiss ×63 oil immersion lens (NA 1.4), and ×2 scan zoom. Detector gain and amplifier offset were initially set to obtain pixel densities within a linear range. Images of 1-μm-thick optical sections of the layers of the fascia dentata (IML, MML, and OML) were recorded. All images were recorded with exactly the same settings.

The septal portion of the hippocampus was used for the quantitative analysis of synaptopodin puncta densities. Details of this method and its validation have been reported previously (Bas Orth et al., 2005). Control rats (n = 4) and EC-lesioned rats surviving for 4 (n = 3), 7 (n = 3), 10 (n = 3), 14 (n = 5), 30 (n = 4), and 180 (n = 3) days were used. On average, five septal sections (distance between each section approximately 100 μm) were analyzed per animal. Per section, three frames (IML, MML, and OML; frame size: 30 μm × 30 μm × 1 μm) were sampled. To obtain a representative average throughout the entire denervated zone, data from the MML and OML were pooled and compared with the nondenervated IML. Confocal images were analyzed with ImageJ software from the National Institutes of Health. First, a threshold was set manually (threshold level: 70), and all objects with intensity values lower than this threshold were set equal to background. Minimum size was set at 5 pixels. Finally, all objects defined by these criteria were counted automatically by the software. Values for threshold as well as minimum object size were kept constant for all measurements. The number of positive puncta and the standard error of the mean (SEM) were calculated and expressed as mean number of puncta per 1,000 μm² ± SEM.

On synaptopodin-labeled sections, the nondenervated IML and the denervated zone (MML and OML) could be readily distinguished. For each time point, the width of these zones was measured at the middle of the granule cell layer on 15 septal sections from three or four animals (×20 magnification images) by using the Zeiss LSM image browser. The average widths of the nondenervated IML, the denervated zone (MML + OML), and the total molecular layer (ML) were calculated for each time point and expressed as mean width ± SEM. The widths of these layers in control animals were set to 1.0, and the values for each time point were expressed relative to control values. For shrinkage correction of the synaptopodin-
density data, raw values were multiplied by this factor for each time point.

Postembedding immunohistochemistry for synaptopodin

For postembedding immunohistochemistry, an osmium-free method of Epon embedding was used that preserves both ultrastructure and antigenicity for postembedding immunohistochemistry (Phend et al., 1995). Details of this procedure have been published elsewhere (Deller et al., 2000). In short, rats were deeply anesthetized with pentobarbitone (300 mg/kg body weight) and transcardially perfused (2.5% glutaraldehyde, 1% paraformaldehyde, and 0.1% picric acid in 0.1 M PBS, pH 7.4). Brains were removed and postfixed in the same fixative, and sections (50 μm) were cut on a Vibratome. Selected sections were incubated in 1% tannic acid in 0.1 M MB (pH 6.0, 40 minutes), incubated in 1% uranyl acetate in MB, and incubated in 0.5% platinum chloride in MB. Thereafter, sections were dehydrated in graded ethanol solutions (the 70% ethanol also contained 1% p-phenylenediamine; Sigma, St. Louis, MO), incubated in 100% propyleneoxide (2 × 5 minutes), placed into resin (Durcupan, ACM), and embedded between liquid-release-coated slides and coverslips. Sections were reembedded for ultrathin sectioning, and serial thin sections were cut and mounted on nickel grids.

The immunogold staining procedure was carried out on droplets of Millipore-filtered solutions in humid Petri dishes. After a brief immersion in saturated sodiumethanolate, the sections were washed, transferred through a glycine sodium borohydride solution [0.05 M glycine, 0.1% sodium borohydride, 0.1% Triton X-100, in Trisbuffered saline (TBS); 10 minutes], washed in a blocking solution, and incubated in rabbit antisynaptopodin antiserum (Mundel et al., 1997; synaptopodin antisemur 26-1E 1:50, 2% human serum albumin, 0.1% Triton X-100 in TBS, 3 hours, room temperature). After intense washing, grids were incubated in the secondary antibody (goat anti-rabbit IgG-coated colloidal gold (10 nm; Amersham, Buckinghamshire, United Kingdom), 0.1% Triton X-100, in TBS) for 2 hours (1:50), washed, and air dried. Selected grids were contrasted with lead citrate and investigated with a Philips electron microscope.

Quantitative electron microscopic analysis

In unstained thin sections of the fascia dentata of control rats (n = 5 animals) and EC-lesioned rats (4, 10, 30, and 180 days; n = 4 animals for each time point; septal hippocampus), spine apparatus densities were quantified in the denervated zone (MML and OML) and the non-denervated IML. The layers were identified at low magnification with the outer border of the granule cell layer and the hippocampal fissure as reference. High-resolution photographs were randomly taken in the denervated and non-denervated layers and analyzed by an investigator blind to the lesioning time point. A spine apparatus was considered to be present if at least one dense plate and at least two tubules of smooth endoplasmic reticulum were detected in close apposition (cf. Deller et al., 2003). Per animal, 3,500 μm² were analyzed per layer. Data were expressed as spine apparatus densities (number of spine apparatuses per 10,000 μm²), relative changes of spine apparatuses, and relative changes corrected for shrinkage (see Fig. 6).

Statistical analysis

Statistical analysis of the light and electron microscopic data was performed as follows. Layer-specific control data were tested against layer-specific data obtained at 4 and 180 days postlesion (see Figs. 4, 6). Homogeneity of the variance was proved via Levene test. Because the variance of synaptopodin puncta densities and spine apparatus scores were not equal between case and control groups, the nonparametric Mann-Whitney U test was employed to determine the significance of difference. Tests were two-tailed, and significance was set at P ≤ 0.05. Computations were performed with the aid of SPSS for MS Windows, release 13 (SPSS Inc., Chicago, IL).

Digital illustrations

Confocal images were exported from the Zeiss LSM image browser and stored as TIFF files. Figures were prepared in Photoshop 6.0 (Adobe, San Jose, CA). Image brightness, contrast, and sharpness were adjusted.

RESULTS

Distribution of synaptopodin in the molecular layer of unlesioned control animals corresponds to the layer-specific termination of afferent fiber systems

In the rodent fascia dentata, synaptopodin is distributed in a layer-specific fashion (Deller et al., 2000, 2002; Roth et al., 2001). Punctate synaptopodin immunostaining is abundant throughout the molecular layer, weak in the granule cell layer, and only slightly stronger in the hilar region of the fascia dentata (Fig. 1a). In addition, a synaptopodin-poor line divides the IML and the MML (Fig. 1a,b). The anatomical localization of the synaptopodin-poor line suggests that it is situated right at the border between the IML and MML, i.e., the border formed by the commissural/associational and the entorhinal/entorhinal fiber systems. This could be confirmed via anterograde tracing of commissural and entorhinal fibers with PHAL. Commissural fibers terminated right below...
Figure 1
Middle and outer molecular layers of the fascia dentata are denervated by entorhinal cortex lesion

To study lesion-induced plasticity of synaptopodin-positive spines, the MML and OML were denervated by ECL. In all animals, lesion quality was tightly controlled (see Materials and Methods). Completely lesioned animals that survived for 5 days or longer after the lesion showed a dense plexus of AChE-positive fibers throughout the denervated zone (Fig. 2a,b). This fiber band is considered a reliable indicator for the extent of the lesion (Nadler et al., 1977; Zimmer et al., 1986). At shorter times postlesion, degenerating terminals and axons were directly visualized by using an antibody against calpain-cleaved spectrin (Kiss et al., 1996). This method revealed the layer-specific degeneration of entorhinal terminals in the MML and OML (Fig. 2c). It should be emphasized that the IML was essentially free of degeneration product, demonstrating that the hippocampus itself was not injured.

Distribution of synaptopodin is changed following entorhinal cortex lesion

Immunostaining for synaptopodin with DAB revealed a loss of staining intensity in the denervated MML and OML after survival times of 4 days or more (Fig. 1e–g). There was a concomitant relative increase in staining intensity in the IML (Fig. 1g). These differences in synaptopodin immunostaining in the different sublayers of the molecular layer normalized with long survival times (Fig. 1h), likely associated with postlesional reorganization processes. To characterize these qualitative changes in synaptopodin immunolabeling further, changes in synaptopodin-immunopositive puncta over time were quantified in the zone of denervation (MML and OML) and the nondenervated zone (IML).

Loss and reacquisition of synaptopodin puncta in the denervated zone

Quantification of synaptopodin-immunoreactive puncta in the denervated zone (MML and OML) was performed by confocal microscopy in combination with computer-based image analysis (Bas Orth et al., 2005). In the denervated zone, synaptopodin puncta density decreased to 13% of control values within the first days postlesion (Figs. 3a,b,d,e,g,h, 4a,c). Over the course of the first month postlesion, synaptopodin densities gradually recovered and reached 62% of control values by 30 days and 78% of control values by 180 days postlesion (Fig. 4b,c). If shrinkage is taken into account—additional synaptopodin puncta are formed.

Loss and reacquisition of synaptopodin puncta in the nondenervated inner molecular layer

The IML of the fascia dentata is not denervated by ECL (cf. Fig. 2c). Nevertheless, considerable changes in synaptopodin puncta densities were observed within this zone: synaptopodin densities decreased to 31% of control levels within the first days postlesion (Figs. 3a,b,j,k, 4a,c). Thereafter, synaptopodin densities rapidly recovered, reaching control levels by 14 days postlesion and 113% of control levels by 180 days postlesion. These changes in synaptopodin densities were accompanied by a significant expansion of the IML (Figs. 3a–c, 4b). By 180 days postlesion, the IML had expanded by 37 μm (158% of control width; Fig. 4b) and covered 46% of the total ML.

Similar to the situation in the denervated zone, puncta densities in the nondenervated IML were corrected for the expansion of the IML (Fig. 4c). Again, these changes in width were especially important during the late recovery phase. Our expansion-corrected data revealed that, by 180 days postlesion, synaptopodin densities reached 178% of control values. Thus, synaptopodin puncta are only transiently lost in the nondenervated IML. Control densities are rapidly regained, and—if the expansion of the IML is taken into account—additional synaptopodin puncta are formed.

Synaptopodin is associated with the spine apparatus postlesion

In control animals, synaptopodin is associated with the spine apparatus of granule cell spines (Fig. 5a,b). To verify that synaptopodin immunostaining also labels spine apparatuses postlesion, electron microscopy was performed of synaptopodin-immunostained sections of EC-lesioned animals. Similarly to the case in controls, synaptopodin labeled the spine apparatus organelle in the denervated outer two-thirds (Fig. 5c) as well as the nondenervated inner one-third (Fig. 5d) of the molecular layer. Thus, synaptopodin can be used as a marker for the spine apparatus organelle postlesion.

Loss and reacquisition of the spine apparatus organelle following entorhinal cortex lesion

To compare the light microscopic data with changes in the density of the spine apparatus organelle, spine apparatuses were quantified in the denervated layers and the nondenervated IML of the fascia dentata by using unstained electron micrographs (Fig. 6a). In the denervated zone, densities of spine apparatuses decreased to 52% of control values by 4 days postlesion (Fig. 6b,c) before returning to control values (85% by 10 days, 98% by 30 days, and 123% by 180 days postlesion). In the nondenervated zone, spine apparatus densities also decreased and dropped to 58% of control values by 4 days postlesion. Recovery appeared to be slightly faster (113% by 10 days, 105% by 30 days, and 119% by 180 days postlesion) than in the denervated zone (Fig. 6b,c). If shrinkage is taken into account (Fig. 6c), the density of spine apparatuses only partially recovers in the denervated zone (72% by 180 days) of prelesion density. Thus, synaptopodin puncta appear to be permanently lost in the denervated zone following denervation.
Fig. 2. Selective denervation of the outer molecular layer of the fascia dentata following entorhinal cortex lesion. a, b: Acetylcholinesterase (AChE) histochemistry of the rat fascia dentata. Compared with controls (a), animals 30 days postlesion (b) show an increased density of AChE-positive fibers (arrowheads) in the denervated middle molecular layer (MML) and outer molecular layer (OML). A dense AChE-positive fiber band throughout the MML and OML is typical for a complete ECL. GCL, granule cell layer; H, hilus; IML, inner molecular layer. c: Fascia dentata 4 days postlesion immunostained for calpain-cleaved spectrin. Degeneration products are observed throughout the denervated MML and OML (arrowheads). The non-denervated IML is essentially free of degenerating structures. Scale bars = 200 μm.
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DISCUSSION

In the present study, ECL was performed to analyze the effect of denervation on the distribution of synaptopodin and the spine apparatus organelle in granule cell spines. Our data can be summarized as follows: 1) In the denervated zone (MML and OML) of the dentate gyrus, where deafferentation-induced spine loss, synaptogenesis, and spinogenesis occurred, a rapid loss of synaptopodin and of spine apparatuses was observed. In this zone, synaptopodin-puncta and spine apparatus densities slowly recovered in parallel with spinogenesis. 2) In the nondenervated zone (IML), the layer without deafferentation-induced spine loss, a rapid loss of synaptopodin and of spine apparatuses also occurred. In this layer, the recovery of synaptopodin-puncta and spine apparatus densities was fast and correlated with plastic remodeling occurring at the synaptic level, as well as with postlesional recovery of spontaneous granule cell activity. Together, these data demonstrate layer-specific changes in the distribution of synaptopodin and the spine apparatus organelle following partial denervation of granule cells. We conclude that spine apparatus densities follow spine densities in layers of spine loss, whereas spine apparatus densities appear to be regulated by other signals in the layer of spine maintenance.

Methodological aspects

In the present study, layer-specific changes in the actin-associated molecule synaptopodin were analyzed following ECL. Because almost all synaptopodin puncta in the fascia dentata can be localized to granule cell spines (Bas Orth et al., 2005) and, within spines, synaptopodin is tightly associated with the spine apparatus organelle (Deller et al., 2000), synaptopodin puncta may be regarded as markers of spine density.
as light microscopic correlates of the spine apparatus organelle. To exclude the possibility, however, that the subcellular distribution of synaptopodin is changed following denervation, electron microscopy of synaptopodin-immunostained sections of EC-lesioned animals was performed. This approach demonstrated that synaptopodin immunostaining remains associated with the spine apparatus even after ECL. Thus, we conclude that the overwhelming majority of synaptopodin puncta represent spine apparatus organelles in control (Bas et al., 2005) as well as EC-lesioned animals (this study).

To compare our light microscopic data (synaptopodin-positive puncta) with changes of the spine apparatus organelle, electron microscopy was performed. This comparison revealed that layer-specific changes in synaptopodin puncta densities are qualitatively similar and follow the same time course as changes of the spine apparatus organelle (Figs. 4, 6). It also revealed that the loss of synaptopodin-positive puncta exceeds the loss of the organelle. A likely explanation for this phenomenon is that the light microscopic quantification does not take into account changes in the size of the synaptopodin puncta. If the size of a dot decreases, for example, because synaptopodin is only partially removed from a spine apparatus or because a spine apparatus shrinks, this dot will drop below the detection threshold level and will not be counted. In this context, it is important to keep in mind that it was not the aim of the present study to determine the precise number of synaptopodin puncta or spine apparatus organelles following ECL. Rather, our study was designed to investigate the effects of axonal denervation on synaptopodin, a molecule essential for the spine apparatus organelle, in spine-bearing neurons. Accordingly, our conclusions (see below) are based on the layer specificity and the time course of the changes that we observed.

In the denervated layers, changes in synaptopodin and spine apparatus organelles parallel spine loss and spineogenesis

The outer two-thirds of the molecular layer are heavily denervated following ECL. Plastic changes occurring within this zone have been thoroughly studied, and the loss and reacquisition of terminals, synapses, spines, and dendrites have been documented (Parnavelas et al., 1974; Matthews et al., 1976a,b; Caceres and Steward, 1983; Steward and Vinsant, 1983; Steward et al., 1988; Steward, 1991; Nitsch and Frotscher, 1993; Diekmann et al., 1996; Rappert et al., 2004). As far as changes in spine densities are concerned, Golgi studies indicate that up to 50% of spines are lost by 4 days postlesion (Parnavelas et al., 1974; Caceres and Steward, 1983; Steward, 1991). Concomitantly with the reinervation of the denervated zone, spine densities recover and return to control values (Caceres and Steward, 1983). One of the earliest studies (Matthews et al., 1976a) noted an apparent reduction of spine apparatus organelles following ECL.

In our study, which is the first to analyze systematically changes in synaptopodin and the spine apparatus organelle after ECL, changes in synaptopodin puncta densities and spine apparatus densities were observed in the denervated OML that followed a time course similar to the one reported for the entire spine population in this zone (Parnavelas et al., 1974; Caceres and Steward, 1983). This suggests that, in the OML, spine apparatuses are lost together with spines and, upon reinervation and spine reconstruction, are formed anew (Fig. 7a).

In the nondenervated inner molecular layer, changes in synaptopodin and spine apparatus organelles indicate a turnover of the spine apparatus

In contrast to the denervated zone, the IML of the fascia dentata is not directly denervated by ECL. Nevertheless, synaptic remodeling also occurs within this zone (Hoff et al., 1981; Marrone et al., 2004a,b), indicating that adjacent afferents and proximal dendrites of granule cells also react to ECL. However, these changes occur primarily at the level of the synapse, are largely over by 10–14 days postlesion, and may represent structural correlates of functional changes (Marrone et al., 2004a,b). These remodeling processes do not seem to affect the basic structural integrity of spines; postlesional changes in neither the density nor the shape of spines could be detected in the IML at the light microscopic level (Parnavelas et al., 1974; Caceres and Steward, 1983). Thus, granule cells maintain their spines in the nondenervated IML in spite of lesion-induced remodeling at the level of the synapse.

In our study, changes in synaptopodin puncta densities and spine apparatus densities were also observed in the nondenervated IML. These changes cannot be attributed to an accidental denervation of this layer because 1) only animals with an undamaged hippocampus were used for analysis, 2) immunostaining for cleaved spectrin demonstrated the absence of degenerating terminals in this layer, and 3) the time course of these changes corresponded to the time course reported for synaptic remodeling in the IML (Hoff et al., 1981; Marrone et al., 2004a,b) rather than to the time course reported for spine loss and spine reconstruction in the denervated zone (Parnavelas et al., 1974; Matthews et al., 1976a,b; Caceres and Steward, 1983). We conclude, that these changes in synaptopodin and spine apparatus densities are not caused by a lesion-induced loss of spines but, rather, the spine apparatus organelle is removed from intact spines and reconstructed within these spines in parallel with the synaptic remodeling of the IML (Fig. 7b). Conversely, our observations also imply that spines are structurally maintained following removal of a spine apparatus organelle, similar
to the situation in synaptopodin-deficient mice (Deller et al., 2003). Thus, the structural integrity of spines alone neither is sufficient for a spine apparatus organelle nor is the presence of a spine apparatus organelle apparently required for spine maintenance.

The turnover of spine apparatuses in the nondenervated IML following ECL raises the question of which signals regulate the loss and formation of spine apparatuses in these spines. An attractive hypothesis was proposed many years ago by Tarrant and Routtenberg (1979), who suggested that the presence of a spine apparatus organelle could be regulated by neuronal activity. In light of the recent data, which have demonstrated considerable dynamics of several intraneuronal organelles, such as the endoplasmic reticulum and elements of the Golgi apparatus (Horton and Ehlers, 2003; Toresson and Grant, 2005; Horton et al., 2005), this implies that the spine apparatus organelle underlies a physiological turnover depending on the state of activation of individual spines.

Although this hypothesis has not yet been verified, an activity-dependent regulation of synaptopodin has recently been demonstrated: After long-term potentiation (LTP), synaptopodin mRNA (Yamazaki et al., 2001) and protein (Fukazawa et al., 2003) are up-regulated by hippocampal neurons. Synaptopodin protein was sorted to the stimulated fiber layers together with F-actin (Fukazawa et al., 2003), indicating that synaptopodin accumulates in stimulated spines under conditions of synaptic strengthening.
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