Synaptogenesis on Mature Hippocampal Dendrites Occurs via Filopodia and Immature Spines during Blocked Synaptic Transmission

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ABSTRACT

During development, dendritic spines emerge as stubby protrusions from single synapses on dendritic shafts or from retracting filopodia, many of which have more than one synapse. These structures are rarely encountered in the mature brain. Recently, confocal and two-photon microscopy have revealed a proliferation of new filopodia-like protrusions in mature hippocampal slices, especially when synaptic transmission was blocked. It was not known whether these protrusions have synapses nor whether they are accompanied by the other immature spine forms. Here, reconstruction from serial section electron microscopy (ssEM) was used to answer these questions. Acute hippocampal slices from mature male rats, ages 56 and 63 days, were maintained in vitro in control medium or in a nominally calcium-free medium with high magnesium, glutamate receptor antagonists, and sodium and calcium channel blockers. At the end of each 8-hour experiment, all slices were fixed, coded, and processed for ssEM. In agreement with light microscopy, there were more filopodia along dendrites in slices with blocked synaptic transmission. These filopodia were identified by their pointy tips and either the absence of synapses or presence of multiple synapses along them. There was also a proliferation of stubby spines. Filopodia along mature dendrites were typically shorter than developmental filopodia, with outgrowth likely being constrained by reduced extracellular space and compact neuropil, providing numerous candidate presynaptic partners in the vicinity of the mature dendrites. These findings suggest that synaptogenesis and spine formation are readily initiated under conditions of reduced activity in the mature brain. J. Comp. Neurol. 484:183–190, 2005.

Indexing terms: spinogenesis; structural synaptic plasticity; mature CA1 neurons; dendritic spines; serial section electron microscopy

Most excitatory synapses in the adult brain occur on dendritic spines (Gray, 1959; Sorra and Harris, 2000). Changes in dendritic spines alter synapse function and connectivity, thereby impacting information storage in the brain (Harris and Kater, 1994; Stepanyants et al., 2002). Despite more than a century of studying dendritic spines (Ramón y Cajal, 1891), the pivotal question of whether new spines form to support experience-dependent synaptic plasticity, especially in the mature brain, remains one of the most challenging questions in neuroscience and is crucial for our understanding of memory (Chang and Greenough, 1982; Desmond and Levy, 1990; Bailey and Kandel, 1993; Geinisman, 2000; Muller et al., 2002; Harris et al., 2003).

Recent findings show that new spines can indeed form on mature neurons. Spines increase on mature neurons with estrogen (Woolley et al., 1990; Gould et al., 1990), after exposure to enriched environments (Greenough and

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Bailey, 1988; Bailey and Kandel, 1993), with spatial learning (Moser et al., 1994), or with prolonged sensory stimulation (Knott et al., 2002). New spines form on neurons of mature mice in the barrel cortex in vivo over a period of weeks (Trachtenberg at al., 2002), but are more stable in primary visual cortex (Grutzendler et al., 2002). New spines emerge from the soma of dentate granule cells in mature hippocampal slices (Wenzel et al., 1994). More spines also occur along the dendrites of mature CA1 neurons in hippocampal slices during incubation in vitro (Kirov et al., 1999; Johnson and Ouimet, 2004). The new spines form rapidly, with more than half of them appearing during the first 30 minutes in vitro (Kirov et al., 2004a). Blocking synaptic transmission further elevates this spine number (Kirov and Harris, 1999; Kirov et al., 2004b). Nevertheless, little is known about the process of new spine formation and synaptogenesis in the mature brain.

Dendritic filopodia are the prominent structures during developmental synaptogenesis in the hippocampus (Dailey and Smith, 1996; Fiala et al., 1998; Boyer et al., 1998; Dunaevsky et al., 1999). During the first few postnatal days most of the synapses are located on dendritic shafts or filopodia, although there are also many filopodia without synapses (Fiala et al., 1998; Boyer et al., 1998). By postnatal day 15, short stubby spines predominate. These observations suggest that filopodia may be direct precursors of dendritic spines (Ziv and Smith, 1996; Marrs et al., 2001) and also serve to recruit nascent synapses to the dendritic shaft from which stubby spines emerge (Fiala et al., 1998). During the next week, thin and mushroom dendritic spines become prominent and are ultimately the dominant forms in adults (Harris et al., 1992; Fiala et al., 1998).

Confocal microscopy revealed the highest proliferation of filopodia-like protrusions when synaptic transmission was blocked on mature CA1 pyramidal cells (Kirov and Harris, 1999). Here, reconstruction from serial section electron microscopy (ssEM) revealed a selective elevation in dendritic protrusions with developmental features, suggesting a recapitulation of developmental synaptogenesis during periods of reduced synaptic transmission in the mature brain.

MATERIALS AND METHODS

Acute hippocampal slices

Hippocampal slices (400 μm) were prepared from mature male rats of the Long-Evans strain, ages 56 and 63 days according to standard protocols (Kirov and Harris, 1999). All procedures followed the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals* and all efforts were made to minimize animal suffering and to reduce the number of animals used. Animals were deeply anesthetized with metofane (methoxyflurane) and decapitated. The right hippocampus was mounted on an agar block in the slicing chamber of a vibrating-blade microtome (VT1000 S, Leica Instruments, Nussloch, Germany) containing partially frozen oxygenated saline with 234 mM sucrose, 5.3 mM KCl, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 8 mM $MgSO_4$, and 10 mM glucose, pH 7.4. During preparation the slices were transferred into 4°C oxygenated control and block mediums. The normal control medium contained 117 mM NaCl, 5.3 mM KCl, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, and 10 mM glucose, pH 7.4. Transverse slices were cut from the middle third of the hippocampus. The block medium had this same solution except that it had 0 mM Ca^{2+} and 8 mM Mg^{2+} and the sodium channel blocker tetrodotoxin (TTX, 1 μM), the ionotropic glutamate receptors antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM), D,L-2-amino-5-phosphonovaleric acid (APV, 50 μM), L-type calcium channels blocker nimodipine (5 μm), and the metabotropic glutamate receptor antagonist (S)-α-methyl-4-carboxyphenylglycine (MCPG, 500 μM).

Immediately after preparation, slices were transferred into the recording chamber (Stoelting, Wood Dale, IL) and incubated in the control or block medium for a total of 8 hours at an interface of humid atmosphere (95% O_2, 5% CO_2) at 32°C. Previously, we have demonstrated a significant increase in the protrusion number on mature dendrites during 8 hours of blocking synaptic transmission (Kirov and Harris, 1999). Here the same time point was chosen to obtain measurements using the more labor-intensive ssEM approach. After 8 hours the slices were immersed in mixed aldehydes (6% glutaraldehyde, 2% paraformaldehyde, 1 mM $CaCl_2$, and 2 mM $MgCl_2$, in 0.1 M cacodylate buffer at pH 7.4) and exposed for 8 seconds to microwave irradiation for rapid fixation.

To confirm slice viability field excitatory postsynaptic potentials (fEPSPs) were recorded in the control slices within an hour of fixation using the Axopatch 200 amplifier (Axon Instruments, Foster City, CA). Signals were filtered at 2 kHz, digitized at 10 kHz with Digidata 1200 D/A interface board (Axon Instruments), and analyzed with pClamp 8 software (Axon Instruments). The slope function (mV per ms) of the fEPSP was measured from the steepest 400 μs segment of the negative field potential over a series of stimulus intensities, and the half-maximal responses were used to monitor the stable fEPSPs. Slices maintained with activity antagonists were also tested to ensure that no synaptic response could be elicited, even at high stimulus intensities. Only experiments in which the control slices had a healthy sigmoidal input/output response function and a stable response at half maximal stimulation were used.

Blocking synaptic transmission with activity antagonists alone also causes an increase in the number of dendritic protrusions detected by confocal microscopy, although to a lesser extent than when extracellular calcium is controlled (Kirov and Harris, 1999). Due to the labor-intensive nature of analysis by ssEM, only the experimental treatment containing activity antagonists, high extracellular magnesium, and calcium-free medium was evaluated because it produced the greatest increase in spine density revealed by confocal microscopy (Kirov and Harris, 1999; Kirov et al., 2004b).

TTX was acquired from Calbiochem (La Jolla, CA), CNQX and nimodipine from Research Biochemicals (Natick, MA) and MCPG from Tocris Cookson (Bristol, UK). All other drugs and chemicals were from Sigma Chemical (St. Louis, MO). MCPG was solubilized at 100× final concentration in 1.1 eq. of NaOH. All other drugs were prepared at 1,000× concentration in stock solutions.

Electron microscopy (EM)

After fixation, the tissue was stored overnight at room temperature in the fixative solution. The next day, stan-

dard microwave-enhanced procedures were used to process the slices through osmium, uranyl acetate, dehydration, and embedding in resin (Fiala et al., 1998; Kirov et al., 1999). Briefly, the slice was manually trimmed under a dissecting microscope to a region containing only area CA1 and processed in potassium ferrocyanide-reduced osmium, osmium, and aqueous uranyl acetate, and dehydrated in a sequence of increasing acetone concentrations using microwave-enhanced processing and embedded in resins for 2 days at 60°C (Feinberg et al., 2001).

Ultrathin sections were cut from the middle of the slice ~200 μm from the air surface perpendicular to the apical dendritic field in *striatum radiatum* midway between area CA3 and the subiculum at a distance 150–200 μm from the CA1 pyramidal cell body layer. Sections were mounted on pioloform-coated (SPI Supplies, Westchester, PA) slot grids (Synaptek, Ted Pella, Redding, CA) and counterstained with saturated aqueous uranyl acetate, followed by Reynolds lead citrate, each for 5 minutes. The series of sections were photographed with a JEOL 1200EX or 2010KV electron microscope (JEOL, Peabody, MA) at 10,000× magnification. Calibration grids (Ernest Fullam, Latham, NY) were photographed with each series. Negatives were digitized at 1,000 dpi on a Sprintscan 45 large-format film scanner (Polaroid, Cambridge, MA). The serial sections images were aligned using ssEM Align software (the new software entitled Reconstruct is freely available at http://synapses.bu.edu/tools/index.htm). In total, four EM series from four slices from two animals (one slice per condition per each animal) were analyzed, ranging from 98–117 serial sections. Section thickness was obtained for each series by measuring the diameter of longitudinally sectioned mitochondria and dividing it by the number of sections mitochondria occupied (Kirov et al., 1999; Fiala and Harris, 2001). Section thickness ranged from 45–65 nm but was uniform within each series.

Dendrite analysis from serial section EM and statistics

Serial section analyses were performed using IGL Trace software (Reconstruct is the latest version). Serial sections were coded prior to analysis such that investigators were blind to experimental conditions. Only lateral apical dendritic segments having 6–27 cross-sectioned microtubules with a corresponding diameter of less than 1 μm were included in the analyses (Fiala et al., 2003). The primary apical dendrites were excluded from these analyses because they were too infrequent and spiny to be grouped with the thin lateral dendrites for statistical analysis (see also Kirov et al., 1999). The profiles of each reconstructed dendritic segment were systematically searched for dendritic protrusions. Dendritic spines, filopodia, synapses, and other protrusions were identified by standard criteria (Harris et al., 1992; Fiala et al., 1998; Kirov et al., 1999). Spines were classified as stubby, mushroom, thin, or branched and synapses were identified as macular or perforated by the shape of the postsynaptic density. Dendritic segment lengths were computed from the first section with a complete spine origin to the last section with a protrusion origin to compute densities per unit length of dendrite. Protrusion lengths were measured from the middle of their origins out to their tip. Twenty-six dendrites were analyzed in the control and 20 dendrites in the block condition. The dendrites for analysis were randomly assigned to each of the three investigators, such that each investigator analyzed the same number of dendrites in each series. Three-dimensional surface reconstructions created by IGL Trace were exported to 3D Studio Max (Discreet Logic, Montreal, Canada) for rendering of final figures.

Statistica (Statsoft, Tulsa, OK) was used to test differences in means between control and block conditions. Two-way analysis of variance (ANOVA) and covariance (ANCOVA) were used to evaluate what part of the variance between data arose from different experimental conditions and what part was due to differences between slices and animals. Dendrite segment length and number of microtubules were used as continuous predictor variables (covariates) to control for potential effects of inconsistency in length and diameter of dendrites included in the analysis. The significance criterion was set at $P < 0.05$. Data are presented as mean ± SEM.

RESULTS

Healthy dendrites were characterized by the presence of intact cytoplasm, uniformly spaced microtubules, dendritic spines, and distinct postsynaptic densities associated presynaptic axonal boutons containing evenly distributed vesicles. Most of the dendrites, axons, and synapses in mature hippocampal slices from both conditions were morphologically healthy (Fig. 1–4), consistent with their healthy electrophysiological responses. Occasionally, a dendrite or axon with dark compressed cytoplasm was encountered, as would be expected were it to have been cut at the surface of the slice.

In agreement with our previous studies from confocal and two-photon microscopy (Kirov and Harris, 1999; Kirov et al., 2004a,b), these ssEM analyses revealed a significant increase in the density of dendritic protrusions, including excitatory shaft synapses in slices incubated under conditions of blocked synaptic transmission (Fig. 1a, "All," $F_{1,40} = 4.21$, $P < 0.05$). Dendritic spines were grouped into thin (Fig. 1b,c), mushroom (Fig. 1d,e), or an "other" category with nonsynaptic and multisynaptic filopodia, stubby and branched spines, asymmetric shaft synapses, and incomplete spines. Thin and mushroom spines appeared similar in shape in the control (Fig. 1b,d) and block conditions (Fig. 1c,e). Thin spines predominated in both conditions but there was no change in either thin ($P = 0.93$) or mushroom ($P = 0.57$) spine densities during 8 hours of blocked synaptic transmission (Fig. 1a). In addition, there were no differences in the density of macular ($P = 0.34$) or perforated ($P = 0.67$) synapses between these conditions. Spine and protrusion lengths also did not differ between conditions (Fig. 1f; $P = 0.37$).

The density of protrusions and synapses in the other category was 68.1 ± 12.5% greater in the block condition than control slices (Fig. 1a, $F_{1,40} = 16.5$, $P < 0.0005$). A detailed analysis of the individual components of the "other" category revealed a remarkable similarity to those previously described during development (Fiala et al., 1998). Nonsynaptic filopodia were characterized by a cytoplasmic protrusion from the dendrite that did not have a synapse along its length (Fig. 2a). Upon reconstruction, these nonsynaptic filopodia were found to range in length from 0.11–1.48 μm (Fig. 2b). There was a marked increase in nonsynaptic filopodia, from only one observation (i.e., 0.2% of total) in the control condition to 23 observations at 3.2% in the block condition (Fig. 2c, $F_{1,40} = 18.12$, $P <$

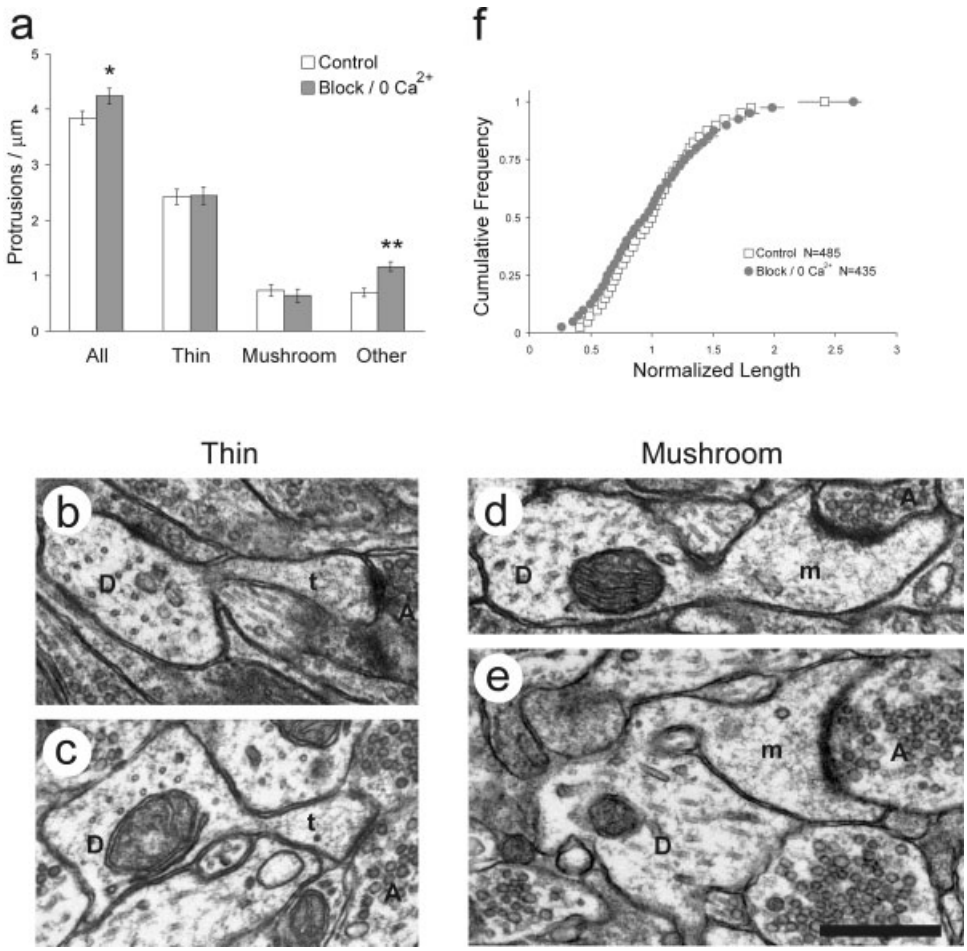

Fig. 1. Number and length of dendritic spines or "other" protrusions in mature hippocampal slices. **a:** Selective increase in the density of protrusions during blocked synaptic transmission. Twenty-six dendrites were analyzed in the control and 20 dendrites in the block conditions. The total number of protrusion origins and asymmetric shaft synapses was 527 in the control and 509 in the block condition. Asterisks indicate significant differences between block and control conditions (*$P < 0.05$; **$P < 0.0005$). Dendrites with a thin spine in **b**, control and in **c**, block conditions. Dendrites with a mushroom spine in **d**, control, and **e**, block conditions. (A axonal bouton; D dendrite; m mushroom spine; t thin spine). **f:** Cumulative frequency in the length of dendritic protrusions in the control and block conditions. Scale bar = 0.5 µm in e.

0.0005). Although the nonsynaptic filopodia along these mature dendrites were not as long as most filopodia during development (Fiala et al., 1998), their shorter length may reflect the compact nature of the mature neuropil, where dendritic protrusions need not elongate across vast expanses of extracellular space to encounter a presynaptic partner, as they do during development.

Multisynaptic filopodia have more than one presynaptic axonal bouton forming synapses along their lengths (Fig. 3a), which distinguishes them from spines that have only one synapse (Harris et al., 1992). 3D reconstructions revealed that the synapses on multisynaptic filopodia may be relatively small or nearly the same size as single synapses on mature thin or mushroom dendritic spines (Fig. 3b). Only two multisynaptic filopodia were observed in the control slices (i.e., 0.4% of total), whereas 22 (5.1%) were multisynaptic in the block condition (Fig. 3c; $F_{1,40} = 18.17$, $P < 0.0005$). Multiple synapses have also been observed early during development on both short and long filopodia (Fiala et al., 1998).

Stubby dendritic spines are about as long as they are wide and appeared similar in shape in the control and the block conditions (Fig. 4a,b). Only 12 stubby spines (2.5% of total) were encountered along dendrites from in the control sample while, 27 stubby spines (6.4%) were found in the block condition (Fig. 4c $F_{1,40} = 4.6$, $P < 0.05$).

Although there was a trend towards more of the rare asymmetric excitatory shaft synapses in the block condition (17 observations in control vs. 31 in block condition), it did not reach statistical significance ($P = 0.07$). Branched spines were also rare, with no change across conditions ($P = 1$). Finally, incomplete dendritic protrusions, i.e., protrusions that were not contained in their entirety among the serial sections, accounted for less than 8% overall and their frequency did not differ between conditions ($P = 0.7$).

DISCUSSION

Blocked synaptic transmission results in a pattern of synaptogenesis along mature hippocampal dendrites that recapitulates development with a proliferation of nonsynaptic filopodia, multisynaptic filopodia, and stubby spines. These observations raise many questions about what induces spine formation and synaptogenesis in the mature brain. Is their formation induced by a period of reduced activity, as we have seen here, and then preserved by subsequent activity that might accompany environmental enrichment or learning? How might the various stages of sleep and wakefulness regulate new spine formation?

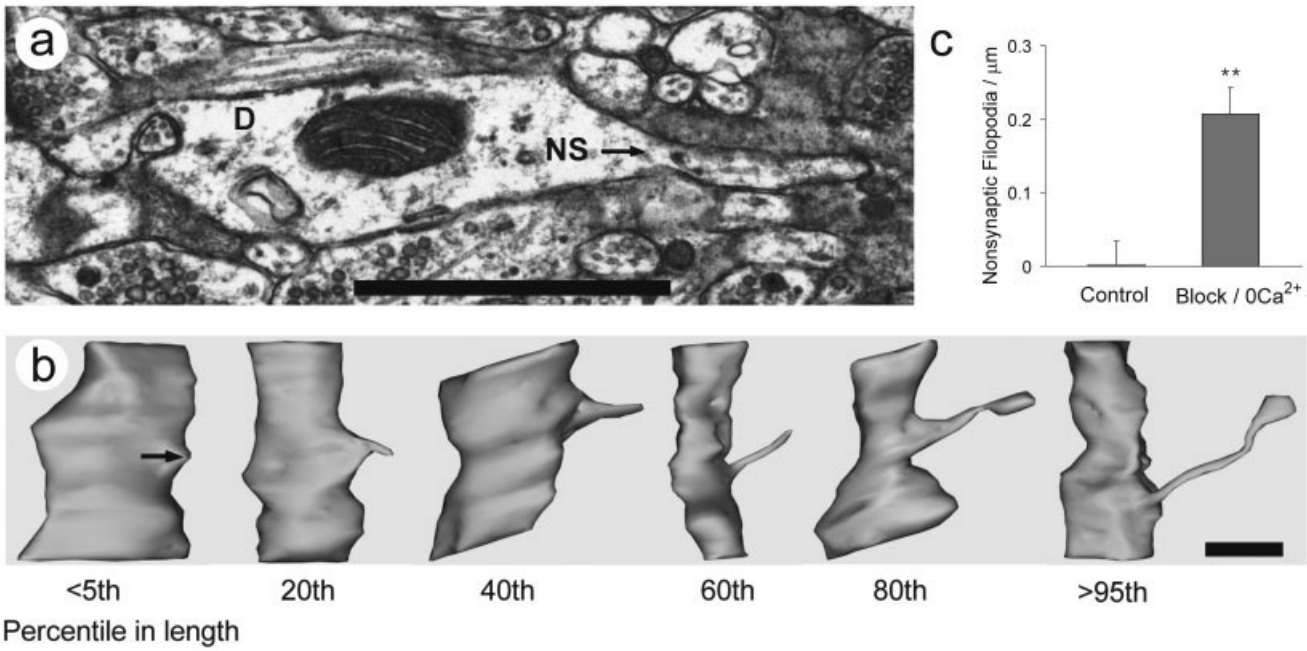

Fig. 2. Nonsynaptic filopodia in slices with blocked synaptic activity. **a:** A dendrite (D) with a nonsynaptic filopodium (NS). **b:** Representative 3D reconstructions of dendritic segments with nonsynaptic filopodia. Dendritic segments are displayed by percentile rank in the length of nonsynaptic filopodia in increments of twenty. Arrow points to the origin of the shortest filopodium on the first dendritic segment. **c:** There are more nonsynaptic filopodia in the block condition (**$P <$ 0.0005). Scale bars = 1 μm in a,b.

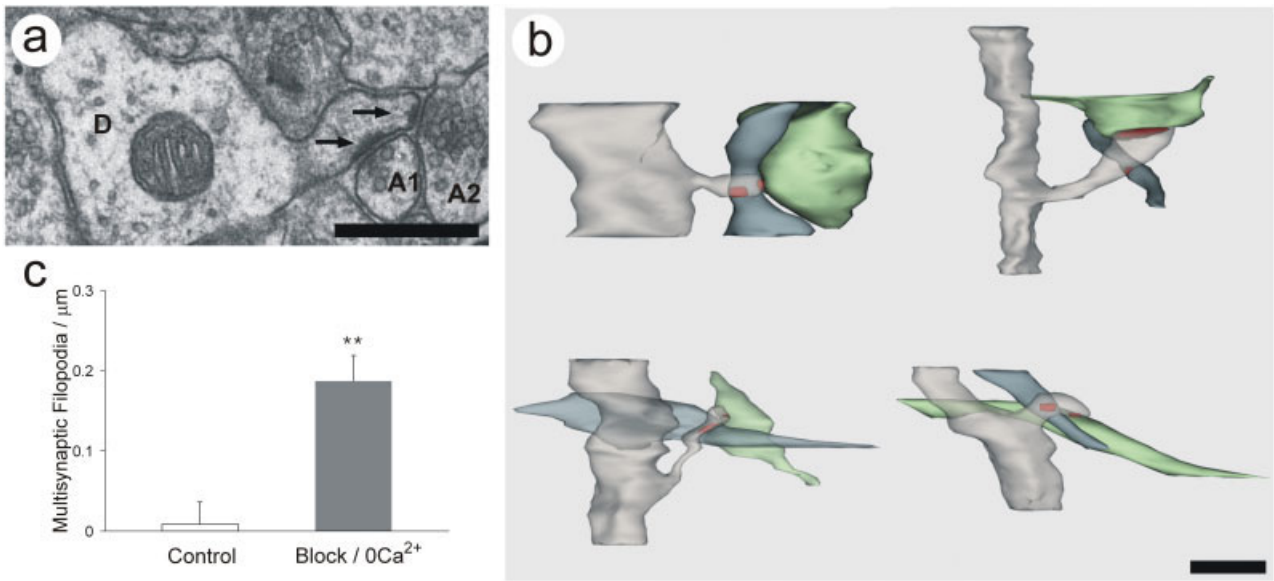

Fig. 3. Multisynaptic filopodia in slices with blocked synaptic transmission. **a:** Electron micrograph of a dendrite (D) with a multisynaptic filopodium. Arrows point to two synapses on the filopodium made with two different presynaptic axonal boutons (A1) and (A2). **b:** Representative 3D reconstructions of short dendritic segments illustrating multisynaptic filopodia and each of their postsynaptic densities (red) synapsing with different presynaptic axonal boutons (green and blue). **c:** The density of multisynaptic filopodia in the block condition is higher than in the control condition (**$P < 0.0005$). Scale bars = 1 μm in a,b.

Filopodial outgrowth, new spine formation, and synaptogenesis all depend on the exact level of change in intracellular calcium (Matus, 2000; Yuste et al., 2000; Segal, 2001). Release of calcium from intracellular stores during slice preparation might be just sufficient to induce filopodia and synaptogenesis on mature dendrites (Kirov et al., 2004a). Whether the filopodia and synapses are preserved or eliminated during development depends on the level of subsequent synaptic activation (Katz and Shatz, 1996). Prolonged silencing of cortical synapses during develop-

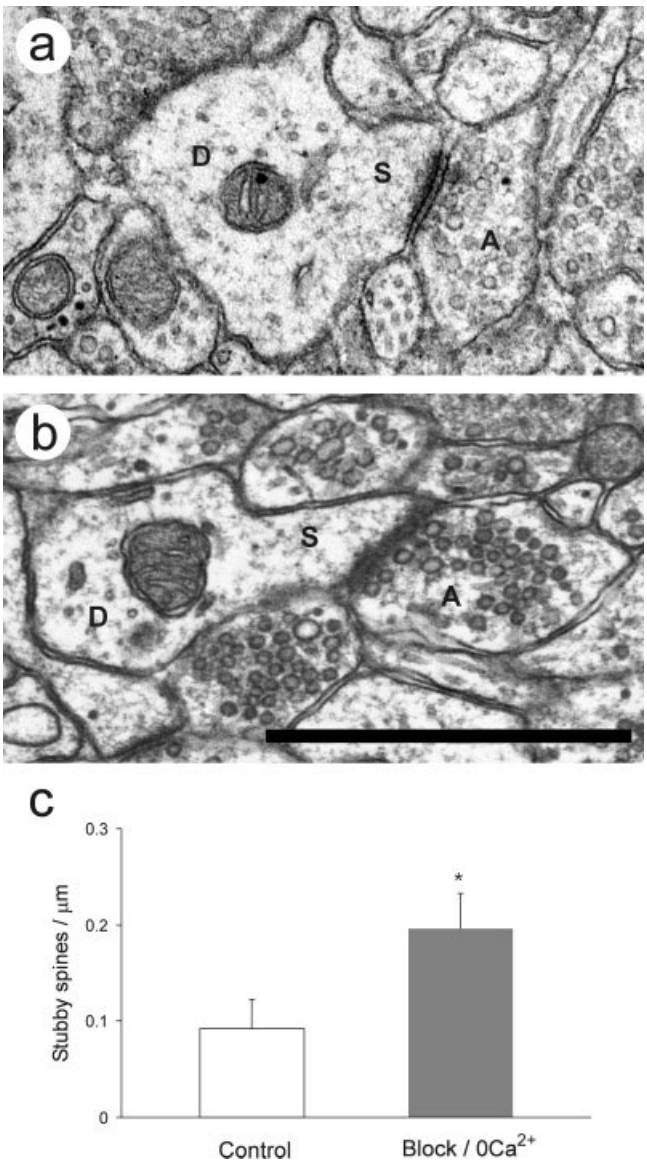

Fig. 4. The number of stubby dendritic spines is higher in slices with blocked synaptic transmission. **a:** Dendrite (D), stubby spine (S), and its presynaptic axonal bouton (A) in the control condition. **b:** Dendrite (D), stubby spine (S) and its presynaptic axonal bouton (A) in the block condition. **c:** Increased stubby spine density in the block condition (*$P < 0.05$). Scale bar = 1 µm.

ment in culture leads to receptor insertion and enlargement of synapses (Turrigiano and Nelson, 2000; Burrone and Murthy, 2003). Whether synapse enlargement also occurs on mature dendrites during acute synaptic silencing is not known, although synapses on the new immature forms of spines described here were not enlarged relative to synapses on the mature thin and mushroom spines. Coincident activation of synapses leads to their preservation and a concomitant elimination of neighboring synapses that were not appropriately activated during development (Katz and Shatz, 1996). Perhaps during slice recovery and incubation, the number of filopodia and other immature forms continues to increase when activity is blocked because they either do not mature or are not eliminated during preferential activation of neighboring synapses (Kirov and Harris, 1999; Turrigiano and Nelson, 2000; Kirov et al., 1999, 2004b).

Dendrites of mature CA1 pyramidal cells in slices maintained in normal artificial cerebrospinal fluid (ACSF) for 2–10 hours had more spines than hippocampus that is perfusion fixed in vivo (Kirov et al., 1999). ssEM revealed all of those spines to have normal synapses with presynaptic and postsynaptic elements. There was, however, a marked increase in the number of the immature stubby and the mature mushroom spines, but no significant change in mature thin spines (Kirov et al., 1999). Hence, the only immature spine type that remained with incubation in normal ACSF were the stubby spines. Nonsynaptic and multisynaptic filopodia were not elevated. These findings suggest that ongoing activity in the slice is sufficient to allow some of the new spines to achieve the more mature mushroom form within a relatively short time.

Live imaging shows dendritic filopodia on developing neurons to be transient structures, with a lifetime of less than 10 minutes (Dailey and Smith, 1996; Ziv and Smith, 1996; Parnass et al., 2000). Filopodia have a dynamic actin cytoskeleton (Wong et al., 2000; Portera-Cailliau et al., 2003) that responds rapidly to changes in neuronal activity and synaptic transmission (Portera-Cailliau et al., 2003; Konur and Yuste, 2004). Depending on the brain region, neuronal activity during development may increase or decrease the number of filopodia or alter their dynamic properties. For example, activation of NMDA receptors results in new filopodia and spines along developing CA1 hippocampal dendrites (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Fiala et al., 2002a). Blocking ionotropic glutamate receptors decreases dendritic filopodia, whereas blocking synaptic transmission with TTX or calcium-free medium increases filopodia on developing layer 5 pyramidal neurons in mouse neocortex (Portera-Cailliau et al., 2003). In contrast, blocking synaptic transmission on developing hippocampal CA1 neurons at postnatal days 6–7 had no effect on the number of filopodia, although they elongated (Kirov et al., 2004b).

Early during development dendritic filopodia form nascent synapses at contacts with axons or axonal filopodia (Saito et al., 1997; Fiala et al., 1998). Individual filopodia can have no, single, or even multiple synapses along their lengths or piled up at their bases (Fiala et al., 1998). Dendritic filopodia are thought to be involved in the process of locating and recognizing appropriate presynaptic partners. In some cases, filopodia may mature into dendritic spines with bulbous heads, while other filopodia may serve to guide nascent synapses to the dendritic shaft with subsequent spine outgrowth (Dailey and Smith, 1996; Ziv and Smith, 1996; Fiala et al., 1998; Marrs et al., 2002). Early during development the neuropil is rather loosely organized, with large areas of extracellular space; hence, the filopodia may need to elongate further across extracellular space to reach synaptic partners. In the mature hippocampus the neuropil is densely packed, with little extracellular space, thereby constraining outgrowth as well as providing axonal boutons in the immediate vicinity to make synapses if appropriate conditions are met.

Little is known about the dynamics of dendritic filopodia on mature neurons. Our ssEM analysis shows that the number of filopodia and stubby spines was increased dur-

ing 8 hours in vitro when synaptic transmission was blocked. These findings suggest that blocking synaptic transmission prolonged the process of synaptogenesis on mature hippocampal dendrites, preventing them from progressing to their more mature forms. An open question is how long can the immature forms remain synaptogenic before they are lost in the absence of synaptic activity? The observation that multiple synapses continue to form in the dentate gyrus of adult rats during several months after destruction of the entorhinal cortex (Steward and Vinsant, 1983) implies that under these conditions of reduced overall synaptic activity immature forms continue to appear for an extended period of time. Live-imaging with multiphoton microscopy shows that mature hippocampal dendrites also produce more elongated filopodia after exposure to cold for only 20 ± 13 minutes (Kirov et al., 2004a). Additional experiments are required to determine the exact dynamics and turnover rate of filopodia on mature hippocampal neurons and whether reinstatement of synaptic activity will induce spine maturation and/or competitive elimination of the immature forms.

Mature neurons of mentally retarded adults exhibit numerous dendritic filopodia (Fiala et al., 2002b), suggesting that the failure of filopodia to mature leads to aberrant synaptic networks. Further understanding of the exact mechanisms by which dendritic spines are induced and how they establish appropriate synaptic connections in the mature brain are crucial to the development of appropriate treatments. If, indeed, reduced synaptic activity is required in the intact mature brain, then knowing when to tap into this responsive synaptogenesis, for example, after a stroke, could also mean the difference between loss and recovery of function. Variation in brain activity during the sleep–wake cycle may also provide a mechanism for the induction and preservation of spine synapses or the elimination of unconsolidated synapses in the mature brain.

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