RAPID COMMUNICATION

Polyribosomes are Increased in Spines of CA1 Dendrites 2 h After the Induction of LTP in Mature Rat Hippocampal Slices

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ABSTRACT: Enduring long-term potentiation (LTP) requires immediate protein synthesis, hence we assessed whether more polyribosomes are present in dendritic spines of mature hippocampal dendrites after the induction of LTP. Reconstructions from serial section transmission electron microscopy (sSTEM) revealed more dendritic polyribosomes 2 h posttetanus, relative to low-frequency stimulation (LFS). Polyribosomes were present in spines of all shapes with larger postsynaptic densities after 2 h, suggesting a coordinated local protein synthesis among many synapses to replenish proteins utilized during an earlier phase of LTP. © **2006 Wiley-Liss, Inc.**

KEY WORDS: protein synthesis; LTP; three-dimensional reconstructions; adult; ultrastructure

LTP is an enduring increase in synaptic efficacy that models learning and memory by persisting for many hours in vitro (Kelleher et al., 2004) and a year in vivo (Abraham et al., 2002). When protein synthesis is inhibited during induction, the synapses remain potentiated for about an hour; longer-lasting LTP requires new protein synthesis at induction (Otani et al., 1989; Frey and Morris, 1997). These findings suggest that some plasticity-related proteins are available early but then need replenishment to sustain LTP (Routtenberg and Rekart, 2005).

Immature CA1 dendrites recruit local protein synthesis to dendritic spines with enlarged synapses after the induction of LTP (Ostroff et al., 2002). Whether local protein synthesis is similarly upregulated in mature hippocampal spines remains an open and somewhat controversial question. Physiological studies show that protein synthesis is required for enduring LTP in the mature hippocampus (Kelleher et al., 2004; Sajikumar et al., 2005; Vickers et al., 2005); however, initial observations from single EM sections suggested that fewer polyribosomes occur in granule cell dendrites after LTP induction in vivo (Desmond and Levy, 1990). Differences in age, brain region, and analytical methods may account for these discrepancies. Here, reconstructions from sSTEM were analyzed to determine whether polyribosomes are present in spines on mature dendrites 2 h after the induction of LTP in hippocampal slices (Sorra and Harris, 1998) that were prepared identically to previous studies (Ostroff et al., 2002).

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Hippocampal slices were obtained from 60- to 70-day-old male Long-Evans rats in accordance with NIH guidelines (Sorra and Harris, 1998). Slices were equilibrated at 31°C at the interface of a static bath of ACSF (in mM: 116.4 NaCl, 5.4 KCl, 3.2 $CaCl_2$, 1.6 $MgSO_4$, 26.2 $NaHCO_3$, 1.0 NaH_2PO_4, and 10 D-glucose) and humidified 95% O_2–5% CO_2. Two concentric bipolar stimulating electrodes were positioned in CA1 *stratum radiatum* 300–400 μm on either side of an extracellular recording electrode. Following a stable 30 min baseline, three low-frequency stimuli (LFS) were delivered to one electrode (2 trains, 5 Hz for 20 s, 5 min interval), and then three pairs of tetanic stimulation were delivered at 10 min intervals (2 trains of 100 Hz for 1 s, 20 s interval) to induce LTP at the other electrode. Input-specific responses were monitored for 2 h with no change at the LFS input and robust LTP (∼160%) at the tetanized input (Sorra and Harris, 1998). Slices were rapidly fixed in mixed aldehydes under microwave irradiation and processed for EM (Jensen and Harris, 1989). All processing, photography, reconstruction, and analyses were done blind as to experimental condition. Series of 152–214 sections were photographed at a depth ∼100–180 μm from the air surface beneath the LFS or LTP stimulating electrodes; digitized, aligned, and traced using Reconstruct (http://synapses.bu.edu) (Fiala, 2005). Pixel size was computed based on a calibration grid and section thickness was calculated as 43–58 nm by the cylindrical diameters method (Fiala and Harris, 2001). The reconstructed dendritic segments were similar for the LFS ($n = 15$, diameter $= 0.54 \pm 0.03$ μm, length $= 7.8 \pm 0.6$ μm) and LTP conditions ($n = 14$, diameter $= 0.56 \pm 0.03$ μm, length $= 8.3 \pm 0.21$ μm). Overall there were 776 dendritic spines, 959 synapses (including branched heads, multisynaptic spines, and shaft synapses), and 146 polyribosomes. One-way ANOVAs were performed on dendritic segment or individual spines (Statistica, StatSoft, Tulsa, OK).

Polyribosomes constituted three or more ribosomes, with opaque 15–30 nm centers surrounded by lighter edges and forming a spiral, staggered line or irregular cluster (Fig. 1) (Steward and Schuman, 2001; Ostroff et al., 2002). Polyribosomes occurred in the base (Figs. 1a,b), neck, and head of spines (Figs. 1c,d) and in dendritic shafts (Figs. 1e,f). Three-dimensional

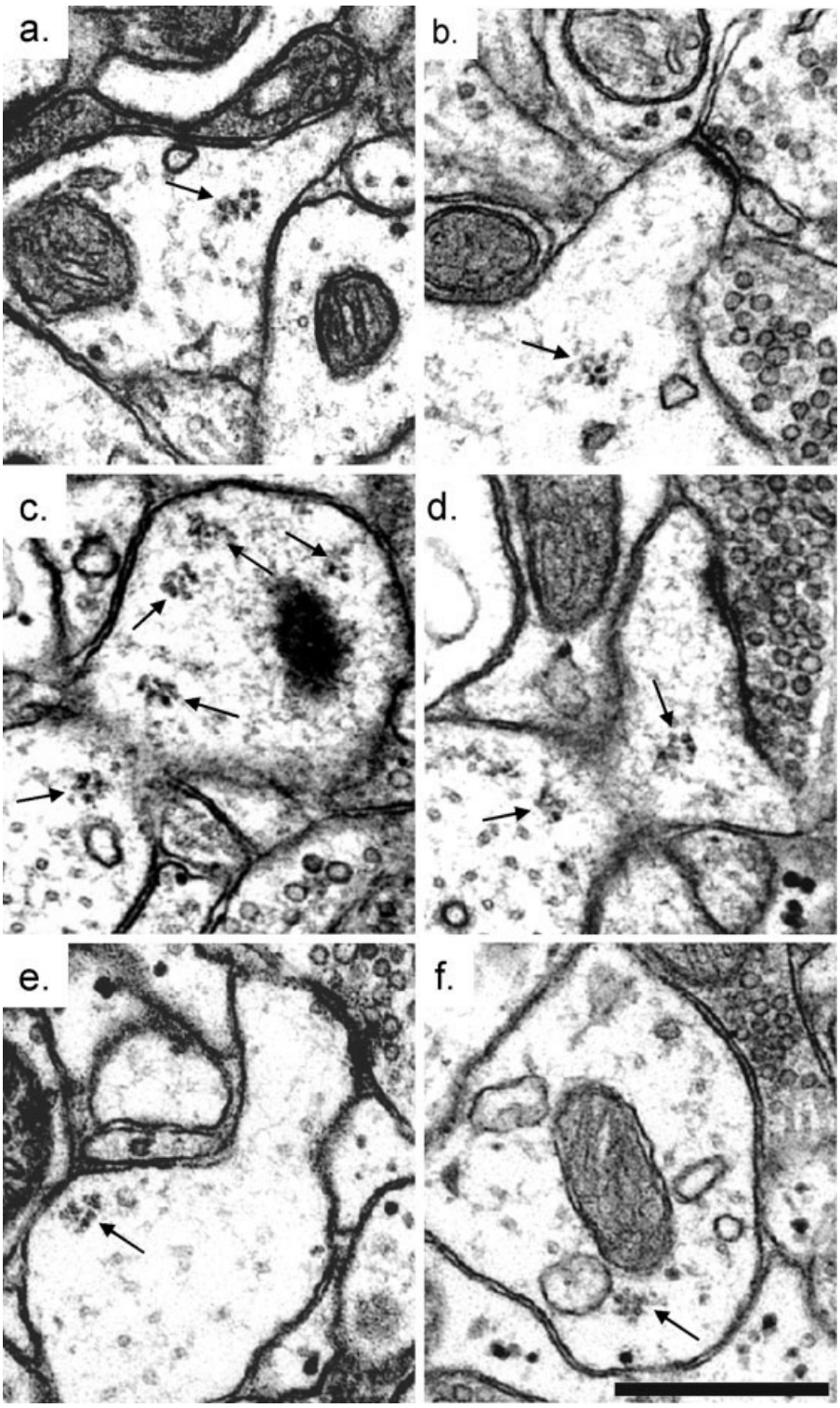

FIGURE 1. Location of polyribosomes (arrows) in mature CA1 dendrites: (a,b) at spine bases, (c,d) throughout a spine (illustrated in nonsequential sections), and (e,f) in dendritic shafts. Scale = 0.5 μm.

reconstructions illustrate the range in polyribosome densities (Fig. 2a), which were higher 2 h post-tetanus (0.92 ± 0.17 per μm) than after control LFS (0.34 ± 0.04 per μm, Fig. 2b, $P < 0.01$). More polyribosomes were observed in all spine types after LTP induction (Fig. 2c). Our original study from shorter series reported no significant differences in spine densities or postsynaptic density (PSD) areas across the LTP and LFS conditions (Sorra and Harris, 1998). Here longer reconstructions

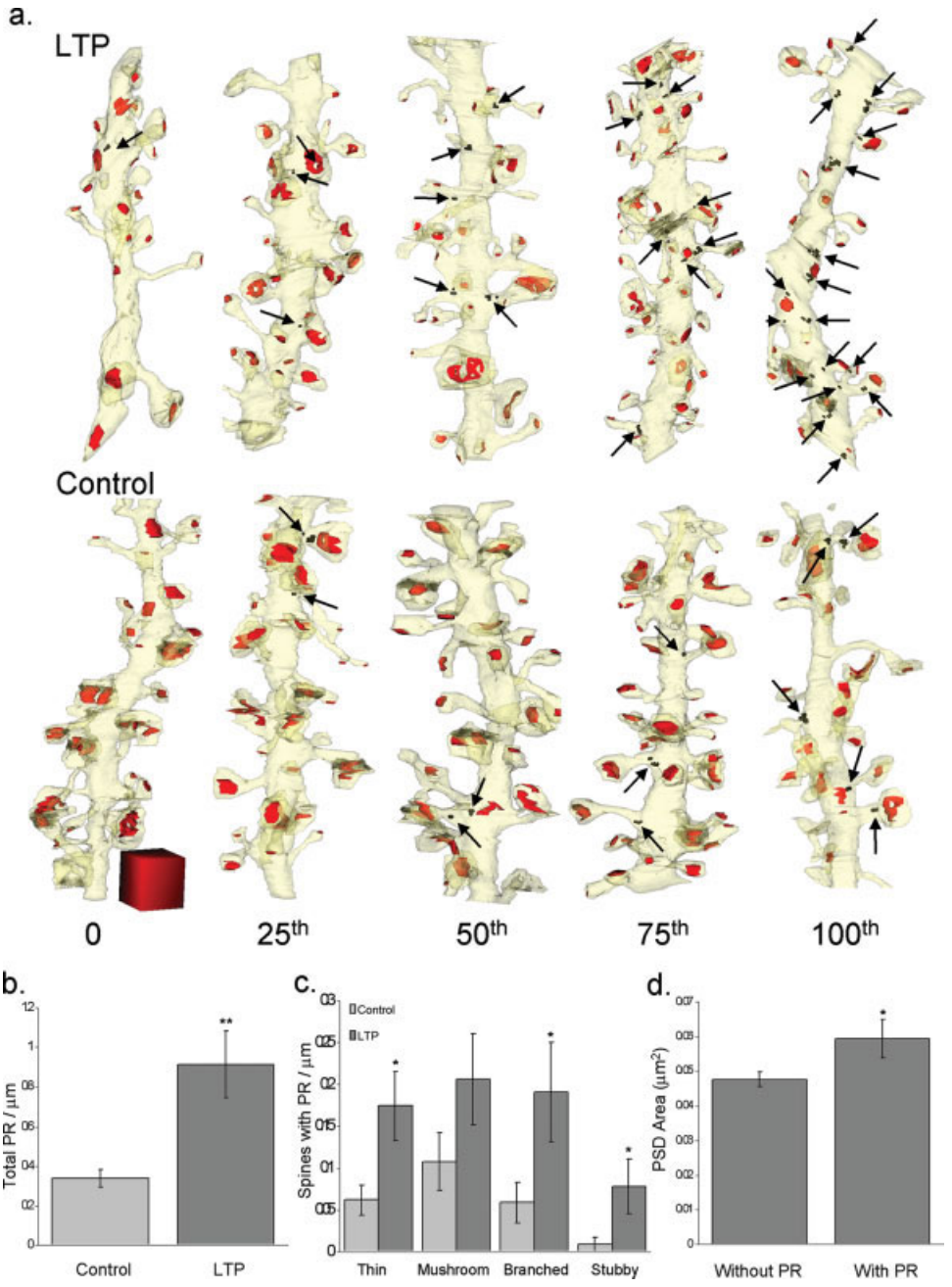

FIGURE 2. Dendrites had more polyribosomes 2 h after the induction of LTP. (a) Dendrites illustrated by percentile rank in frequency of polyribosomes (arrows). Scale = 1.0 µm³. (b) Density of polyribosomes was higher in LTP than control LFS dendrites ($P < 0.01$). (c) Polyribosomes were elevated in several spine types: thin (*$P < 0.02$), mushroom ($P = 0.13$), branched (*$P < 0.05$), and stubby (*$P < 0.05$). (d) PSDs were larger on spines with polyribosomes ($n = 71$) than spines without polyribosomes ($n = 367$) at 2 h after induction of LTP (*$P < 0.05$); but not LFS ($P = 0.2$; data not shown). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]**

revealed a somewhat higher spine density in the LFS (3.73 ± 0.18 per µm) than in the LTP condition (3.18 ± 0.18 per µm, $P = 0.03$), and there were more large PSDs in the LFS (median: 0.041 µm^2, range: 0.003–0.57 µm^2, $n = 521$ synapses) than the LTP condition (median: 0.034 µm^2, range: 0.004–0.22 µm^2, $n = 438$ synapses; $P < 0.05$). Spines containing polyribosomes had significantly larger PSDs than spines without polyribosomes at 2 h post-tetanus (Fig. 2d), but not after LFS.

These findings show that mature CA1 dendrites can recruit polyribosomes into dendritic spines in the vicinity of potentiated synapses, allowing for local protein synthesis to remain elevated at least 2 h after the induction of LTP. Polyribosomes were present in spines of diverse sizes and shapes, consistent with the hypothesis that local changes in protein synthesis may be coordinated among multiple synapses (Steward and Schuman, 2001; Kelleher et al., 2004). Polyribosomes appear to be

recruited independently of spine density and existing PSD size, although PSD area was higher following the induction of LTP only on spines that contained polyribosomes, like developing CA1 dendrites (Ostroff et al., 2002). Preparation of mature hippocampal slices with ice-cold ACSF results in robust spinogenesis relative to perfusion fixed hippocampus (Kirov et al., 1999), possibly confounding the ability to detect plasticity-related changes in spine density (Bourne et al., 2007). Since the control sites in these hippocampal slices also had a higher spine density than in vivo, the lower spine density observed 2 h after LTP induction may have resulted from elimination of nonpotentiated synapses. The increase in polyribosomes associated with spines having enlarged PSDs after 2 h of LTP supports the hypothesis that the potentiated synapses recruited the cellular resources necessary to maintain enhanced synaptic efficacy. Further study is needed to determine which proteins are altered in spines with polyribosomes and whether protein composition changes as synapse size changes after the induction of LTP.

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