

Structural changes at dendritic spine synapses during long-term potentiation

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Two key hypotheses about the structural basis of long-term potentiation (LTP) are evaluated in light of new findings from immature rat hippocampal slices. First, it is shown why dendritic spines do not split during LTP. Instead a small number of spine-like dendritic protrusions may emerge to enhance connectivity with potentiated axons. These 'same dendrite multiple synapse boutons' provide less than a 3% increase in connectivity and do not account for all of LTP or memory, as they do not accumulate during maturation. Second, polyribosomes in dendritic spines served to identify which of the existing synapses enlarged to sustain more than a 30% increase in synaptic strength. Thus, both enhanced connectivity and enlarged synapses result during LTP, with synapse enlargement being the greater effect.

Keywords: synapse; serial electron microscopy; three-dimensional reconstruction; dendritic spine; plasticity; development

1. NO SPINE SPLITTING

Dendritic spines are tiny protrusions that stud the surface of neurons and form the postsynaptic component of most of the excitatory synaptic connections in the brain (Harris & Kater 1994). It has long been suggested that increasing the size and/or number of dendritic spines would enhance the strength of connections between neurons. This process is thought to underlie cellular mechanisms of learning and memory such as LTP in the hippocampus and elsewhere. Splitting existing synapses has been an attractive model for increasing synaptic coupling between neurons (Lusher *et al.* 2000), because input specificity would be preserved if the daughter spines retain synaptic connections with the parent axon.

Despite the simplicity and elegance of this model, little has been done to test its accuracy. A three-dimensional analysis of interconnectivity in hippocampal neuropil was needed to determine whether spine splitting is a viable mechanism. Dendrites, axons and synapses were reconstructed from hippocampal CA1 neurons with a special emphasis on detecting the various steps that would be required for spines and synapses to split (figure 1*a*). The first step in the proposed sequence is perforation of the synapse. Second, the dendritic spine begins to divide, thereby transiently forming a branched spine with two heads synapsing on the same presynaptic axon. Finally, the spine completes its division, resulting in two or more spines from the same dendrite synapsing with the same presynaptic axon, the so-called sdMSB.

Perforated synapses occur on mushroom-shaped dendritic spines that synapse with a single presynaptic bouton

in support of step one. They represent *ca.* 10–15% of mature synapses in hippocampal area CA1 (Harris & Stevens 1989). To test the second step in the spine-splitting hypothesis more than 100 branched dendritic spines have been reconstructed on mature hippocampal CA1 neurons (Sorra *et al.* 1998) and subsequently on immature PN15 and PN21 neurons (unpublished reconstructions). In no case did two or more heads of branched spines synapse with the same presynaptic axon. The different heads of a single branched spine had simple, perforated or segmented synapses. These findings provide morphological evidence that spine branches are not simple daughter spines arising from the splitting of an existing synapse. If sdMSBs arise during synaptic plasticity they do so by a mechanism that leaves no trace of splitting spines associated with an existing presynaptic bouton.

Under control conditions in perfusion fixed brain sdMSBs are rarely observed (Sorra & Harris 1993). Following LTP, we and other researchers have identified a small (less than 3% of all synapses) number of spines from a single dendrite that formed synapses on sdMSBs (figure 1*b*; Toni *et al.* 1999; Fiala *et al.* 2002). However, reconstructions revealed that long, mature axons and dendrites always passed between the neighbouring spines, apparently precluding their formation via splitting (figure 1*b,c*).

These results were obtained in acute hippocampal slices from postnatal day 15 rats (Fiala *et al.* 2002) or organotypic slices from immature rats (Toni *et al.* 1999). In these immature preparations, synaptogenesis is ongoing, so it could be argued that axons and dendrites passing between the spines grew there after the spines split. To test this hypothesis we measured the gap between the neighbouring spines and compared it with the dimensions of axonal growth cones found in the same slices (figure 2*a*). Seven sdMSBs were detected in the LTP condition in slices, and in addition, 10 sdMSBs were reconstructed

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One contribution of 30 to a Theme Issue 'Long-term potentiation: enhancing neuroscience for 30 years'.

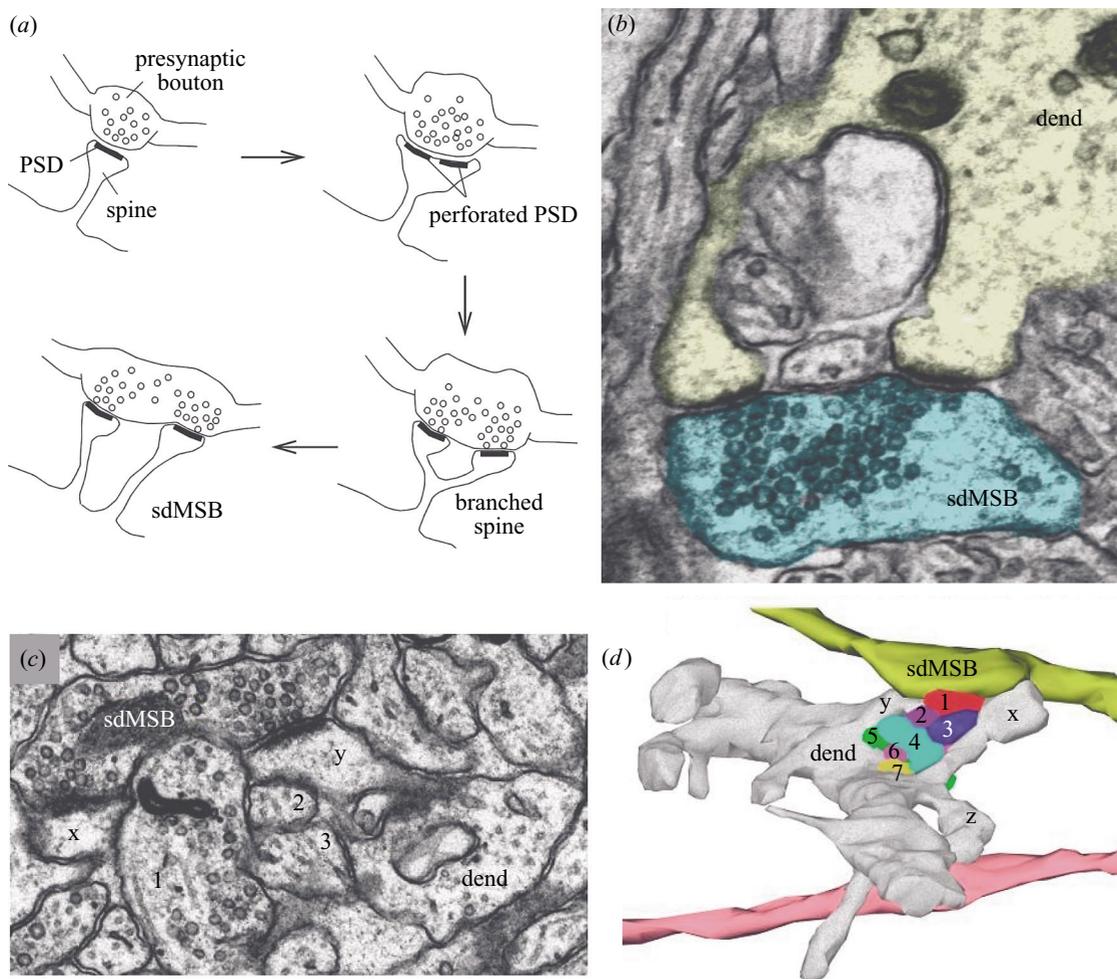


Figure 1. Spine splitting and LTP. (a) Model of spine splitting to enhance connectivity between hippocampal neurons. (b) Fortuitous longitudinal section through two spines arising from the same dendrite (yellow, dend) to synapse on a multiple synapse bouton (green, sdMSB). (c,d) Example section and reconstruction of sdMSB, dendritic spines and parent dendrite that synapse with the sdMSB, and axons (numbered) that pass between the spines that synapse on the sdMSB. One of the spines on the sdMSB is itself branched (x, z) but the two heads synapse on different axons, so that it does not represent synapse splitting.

from postnatal day 21 hippocampus, *in vivo*. The gap between the spines averaged $0.6 \mu\text{m}$ at both ages, and the average number of mature axons traversing the gap was 3.1 at PN15 and 3.7 at PN21. A growth cone was reconstructed from one of the PN15 slices demonstrating the typically large dimensions with a diameter greater than the width of the gap (figure 2b). Other gaps had spiny dendrites passing through them. These observations suggest that it is unlikely that axons and dendrites grew through the gap after the spines had split, via the mechanisms outlined in figure 1a.

2. SPINE OUTGROWTH

How then do sdMSBs form if not by spine splitting? An alternative mechanism is via spine outgrowth (figure 2c). During LTP, spine-like dendritic protrusions without synapses were discovered that could weave through the neuropil to encounter presynaptic axons already synapsing with their neighbouring spines (figure 2d). This mechanism would not require spine splitting, yet input specificity could be preserved if the potentiated presynaptic axons were more attractive to the emerging spines.

The formation of sdMSBs does not seem to account for the magnitude of LTP. Even during LTP less than 3% of synapses are of this type. LTP can involve a 100% increase in synaptic efficacy suggesting that some additional mechanism might be involved. Furthermore, if the formation of sdMSBs were a major mechanism to enhance connectivity between neurons and store memories, one would expect sdMSBs to accumulate with maturation. Instead, less than 2% of mature synapses occur on dendritic spines arising from the same dendrite and sharing the same presynaptic axon (Sorra & Harris 1993).

3. PROTEIN SYNTHESIS-MEDIATED SYNAPSE ENLARGEMENT

Enlargement of existing synapses is another favoured model for enhancing synaptic efficacy during LTP (Yuste & Bonhoeffer 2001). This hypothesis has also eluded an unequivocal answer because it has been impossible to distinguish potentiated synapses from neighbouring synapses that were not potentiated (Sorra & Harris 1998). Even approaches labelling sites of calcium accumulation (Toni *et al.* 1999) have been inadequate because the cal-

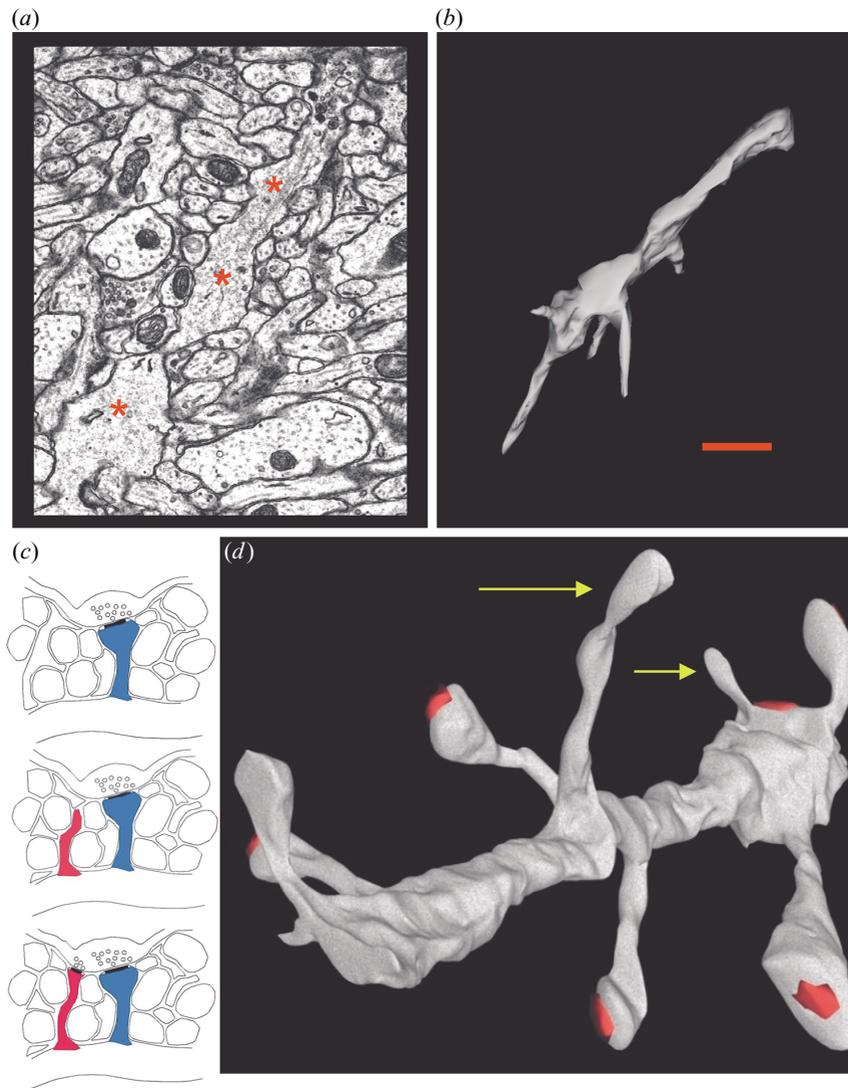


Figure 2. (Caption overleaf.)

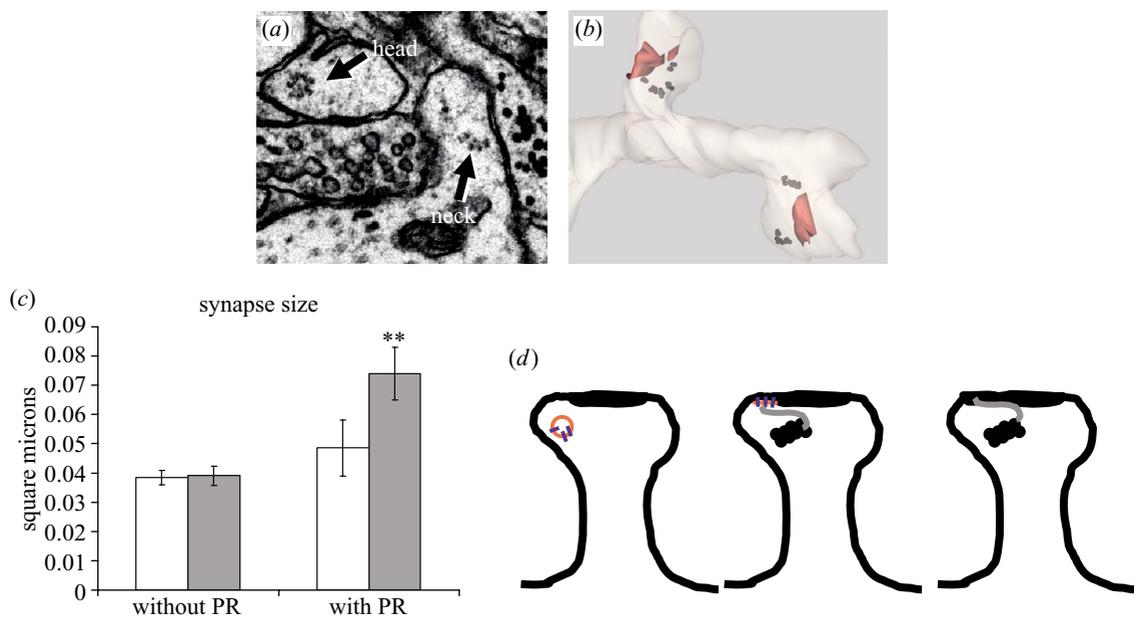


Figure 3. (Caption overleaf.)

Figure 2. Spine outgrowth to form sdMSBs. (a) Axonal growth cone (red asterisks). (b) Reconstruction of the axonal growth cone illustrated in (a). Scale bar, 0.5 μm . (c) Model of spine outgrowth to form sdMSBs. (d) Reconstruction of dendritic segment with two non-synaptic dendritic protrusions (yellow arrows).

Figure 3. Protein-synthesis-dependent synapse enlargement during LTP. (a) Polyribosomes in a dendritic spine head and a different spine neck. (b) Three-dimensional reconstruction of dendritic spines containing polyribosomes (grey spheres) and having large synapses (red). (c) Spines without polyribosomes had synapses of the same size under both LTP (grey bars) and control (open bars) conditions. Spines with polyribosomes had larger synapses under the LTP condition only (** $p < 0.02$). (d) Model illustrating how glutamatergic receptors (blue) located in postsynaptic vesicles (red) are inserted into the plasma membrane soon after induction of LTP. The new protein synthesis then adds postsynaptic density proteins to stabilize these receptors in the membrane.

cium precipitate is only detected above background in SER, hence only the 10–15% of spines that contain SER could be labelled though a larger percentage may have undergone LTP.

Results from many studies indicate that enduring LTP requires new protein synthesis (Nguyen *et al.* 1994; Frey & Morris 1997), and recent studies suggest that translation will occur near the specific synapses that undergo LTP (Steward & Worley 2001). Polyribosomes are distinctive ultrastructural features that are required for new protein synthesis. In fact, they are clear indicators of exactly where translation is occurring at the time of fixation (Steward & Schuman 2001). It is thus reasonable to assume that the presence of polyribosomes in particular dendritic spines would be an accurate marker of which spines had recently undergone protein-synthesis-dependent LTP.

Hippocampal dendrites were examined in three-dimensional reconstructions to determine the precise location of every polyribosome (figure 3*a,b*; Ostroff *et al.* 2002). Only $12 \pm 4\%$ of dendritic spines contained polyribosomes under control conditions whereas $39 \pm 4\%$ of spines contained them during LTP. A commensurate loss of polyribosomes from dendritic shafts accompanied this increase in spines with polyribosomes during LTP. Postsynaptic densities on dendritic spines that contained polyribosomes were larger during LTP (figure 3*c*), suggesting that local changes in protein synthesis serve to stabilize stimulation-induced growth of the synapse (figure 3*d*). This coincidence in polyribosomes and synapse enlargement suggests they mark the specific spines that are expressing LTP.

4. SUMMARY

Together these findings support the hypothesis that LTP uses two structural mechanisms to strengthen synap-

tic connections. The primary mechanism is a protein-synthesis-dependent enlargement of existing synapses. A few non-synaptic dendritic protrusions may also be captured to form additional synapses with potentiated boutons.

This work was supported by NIH grants NS21184, NS33574 and MH/DA57351 funded jointly by NIMH and NIDA.

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GLOSSARY

- LTP: long-term potentiation
 sdMSB: same dendrite multiple synapse bouton
 SER: smooth endoplasmic reticulum