How multiple-synapse boutons could preserve input specificity during an interneuronal spread of LTP

Kristen M. Harris

A model is proposed whereby the spread of long-term potentiation (LTP) between potentiated and neighboring neurons is initiated by a retrograde signal that is restricted to the synaptic clefts of the potentiated neurons. Next, a change, such as enhanced release of neurotransmitter, occurs in the presynaptic boutons that are associated with the potentiated synapses. This change affects all synapses that are located on the potentiated boutons, and leads to LTP at synapses on neighboring neurons that share multiple-synapse boutons with the initially potentiated neurons. In this model, restricting the retrograde signal to the potentiated synaptic clefts ensures the axonal-input specificity of LTP, and the induction of the secondary LTP requires the same cellular mechanisms as those of induction of the primary LTP.

Trends Neurosci. (1995) 18, 365–369

LONG-TERM POTENTIATION (LTP) is a form of synaptic plasticity that is accepted widely as a cellular model for stabilization of synapses during development, learning and memory. A characteristic of LTP is axonal-input specificity, such that only the activated synapses become potentiated while neighboring, naive synapses remain unchanged[1–4]. However, it has been shown recently that LTP can spread from potentiated neurons to neighboring neurons that were not otherwise activated to induce LTP (Refs 5–7). One model to explain this spread of LTP has been that the signal that is initiated at the potentiated postsynaptic neuron spreads intracellularly through the input axons for a limited distance to activate other cells that are associated with the same axons[5]. Another hypothesis has been that this interneuronal spread of LTP occurs by diffusion of a retrograde messenger beyond the activated synapses to neighboring synapses on other neurons[8–12]. Here, an alternative model is considered wherein the retrograde signal is restricted to the activated synaptic clefts, and the spread is initiated at multiple-synapse boutons (MSBs) of the activated inputs that synapse on both the potentiated and the neighboring neurons.

Physiological and structural evidence for the limited presynaptic spread of LTP

Two groups have presented evidence that LTP spreads to neighboring pyramidal cells in hippocampal area CA1 (Refs 5 and 6). In the first experiments, organotypic cultures of hippocampal slices were used in combination with intracellular, extracellular and optical recordings[5]. Two neighboring cells were impaled, and action potentials were elicited independently in each cell to establish that they were not synaptically or electronically coupled. Test stimuli were delivered extracellularly to axons that synapsed with both of the cells. Test stimuli alone did not produce potentiation in either cell; however, when one cell was depolarized during the test stimulation, both cells became potentiated, suggesting a spread of LTP to the neighboring cell. Optical recordings of the surrounding hippocampal cells, stained with a voltage-sensitive dye, suggested that the spread of LTP was limited to cells within 150 μm of the potentiated cell.

Later experiments[6] examined several conditions that were involved in this spread of LTP among CA1 pyramidal cells (Fig. 1). A similar paradigm was used in which two cells were impaled and one cell, referred to as the 'paired cell', was depolarized while test stimuli were delivered to the presynaptic axons of both cells. The responses of both cells were monitored for at least 1 h, and then biocytin was injected into both cells to define the extent of their dendritic arbors. If the two cell bodies were within 150 μm of one another, and had extensively intermingled dendrites, then the LTP spread from the paired cell to the close neighboring cell (Fig. 1A and B). Alternatively, if the two cells were farther apart and had no dendritic overlap, then LTP occurred in the paired cell only, and no change in response occurred in the distant neighbor (Fig. 1C and D). The primary potentiation averaged 184%, and ranged from no potentiation in three cells to 330% of baseline; while the secondary potentiation in the neighboring cells averaged 134%, and ranged from no potentiation in four cells to 253% of baseline (Fig. 1A; see also Fig. 1D of Ref. 6).

In both experiments, no monosynaptic connections or gap junctions occurred between the neighboring cells, hence it was concluded that the potentiation must spread via the input axons that formed synapses on the dendrites of both cells. Currently, there is wide agreement that the induction of LTP occurs postsynaptically[13–16]; therefore, if LTP is to spread to neighboring cells via the input axons, there must be a retrograde message from the potentiated cell to signal the presynaptic axons when LTP has occurred. Many candidates for retrograde signals exist, ranging from mechanochemical signaling via proteins that span the synaptic cleft[17,18] to diffusible molecules[9,10].

Kristen M. Harris is at the Dept of Neurology and Program in Neuroscience, Children's Hospital and Harvard Medical School, Enders 260, 300 Longwood Ave, Boston, MA 02115, USA.

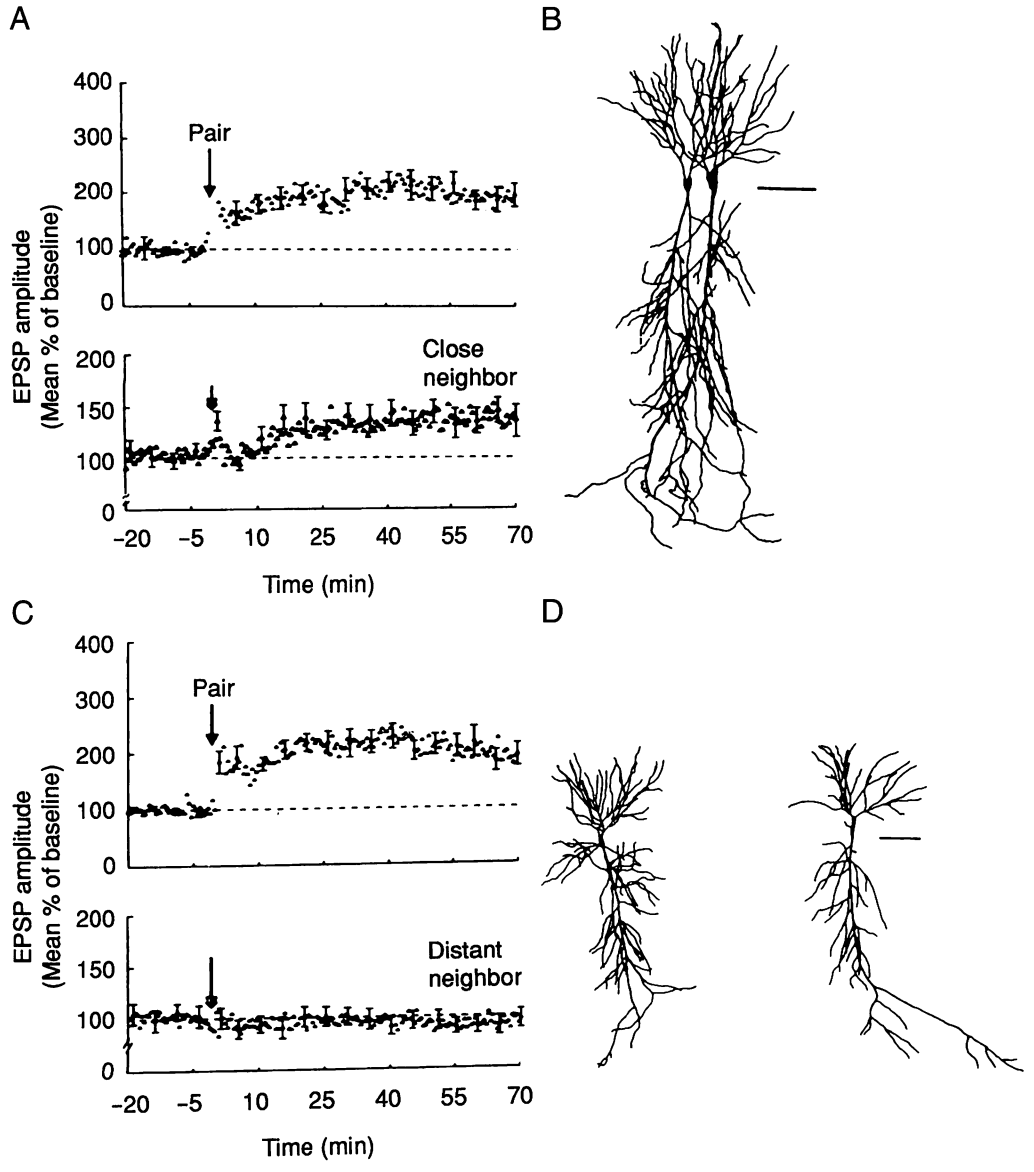

Fig. 1. ***Presynaptic spread of long-term potentiation (LTP) is limited to neighboring CA1 pyramidal cells with extensive dendritic overlap.*** **(A)** *Long-term potentiation was induced by pairing postsynaptic depolarization of one cell (Pair, arrow) with extracellular stimulation of axons that synapse on both cells. In the paired cell, the primary potentiation plateaued at an average of 200% baseline. After some delay, the close neighboring cell also became potentiated.* **(B)** *Camera lucida tracings of a pair of cells that contributed to the ensemble averages in* A. *Scale bar, 100 μm.* **(C and D)** *Distant cells having no intermingling of their dendritic arbors with the paired cell did not become potentiated. Scale bar, 100 μm.* Reproduced, with permission, from Ref. 6.

Candidate proteins that are thought to span the synaptic cleft include integrin-type molecules[19] and neural cell-adhesion molecules[20]; peptides that block a subclass of the integrins have been shown to disrupt the stabilization of LTP (Refs 21–23). Four diffusible molecules have been identified, including nitric oxide, carbon monoxide, arachidonic acid and platelet-activating factor[9,10]. While it is not clear what the exact action is for each of these molecules, nor when during the course of LTP they exert their main effects, evidence suggests that they might act individually, or in concert, to enhance conditions for either synaptic potentiation or depression[11,12,24].

The axonal-input specificity is restricted even among adjacent synapses in the neuropil, as demonstrated routinely in experiments of LTP where two stimulating electrodes, located 'on beam' across from one another, are used as the experimental and naive inputs, respectively[1–4]. Tetanus delivered to the experimental stimulating electrode produces LTP at that input only and not at the naive input. Since the axons between these two stimulating electrodes are highly intermingled, even synapses that are located right next to one another in the neuropil do not necessarily exhibit LTP from activation of a subset of the inputs. Hence, there must be a highly specific mechanism in place to ensure that either the retrograde signal does not spread beyond the cleft of the activated synapses, or that if it does spread beyond the cleft, it only causes potentiation at other synapses that are located specifically on the potentiated axonal inputs.

One possibility is that the retrograde signal works only at 'recently' activated synapses[25]. However, since neurons in the hippocampus can be active spontaneously, there could still be a nonspecific spread of LTP to axonal inputs that were not part of the potentiated pathway but that were active spontaneously just after the input pathway was activated. Here, an alternative model is proposed that ensures input specificity, and that is also consistent with current physiological data regarding the spread of LTP to neighboring neurons.

A model that involves MSBs in the spread of LTP

In hippocampal area CA1, most of the excitatory axons that course through the middle of the apical dendritic field arise from the pyramidal cells of the ipsilateral and contralateral area CA3. In Golgi impregnations, it was possible to show that a single branch of these axons can contact two to four different dendrites of a target CA1 cell[26] (Fig. 2A). These axons are known to be highly branched, and each branch runs approximately parallel to the others[27,28]; hence, the intermingled dendrites of neighboring CA1 cells are likely to be multiply innervated by each of the many axon collaterals. Electron microscopy revealed that a single presynaptic bouton in the stratum radiatum can make synapses with two or more dendritic spines that arise either from dendrites of the same cell or dendrites of neighboring cells[26,29]. These boutons are referred to as MSBs (Figs 2 and 3). Approximately 20–40% of the synapses in a typical plane of neuropil were found to occur on these MSBs (Ref. 26). Since the spines are too short to reach more than 1–2 μm from their parent dendrite, only pyramidal cells with highly intermingled dendritic arbors can share the same population of MSBs (Fig. 3).

The following estimates illustrate how the intermingled dendrites of close neighboring CA1 pyramidal

cells could share many of the MSBs that occur among them. In a typical plane through the middle of the s. radiatum, there are about 50–80 synapses per 100 μm^2 of neuropil[30]. Of these, 10–30 occur with MSBs, and the rest occur on boutons that have only one synapse[26]. Approximately 15–30 different dendritic segments give rise to the spines of these synapses, although the dendritic segments arise from fewer CA1 pyramidal cells, as determined by tracing several of the segments through serial sections to the same parent cell. Thus, in a single 100 μm^2 plane of neuropil, neighboring cells with intermingled dendrites could easily share one or more MSBs. The overlapping dendrites in the apical field of neighboring CA1 pyramidal cells occupy a column of neuropil with a width of about 150–300 μm, a height of about 300–600 μm, and a depth of about 100 μm. A single axon collateral passing through this column produces hundreds of synaptic varicosities that are separated from one another by a constriction that is 0.3–2 μm long and less than 0.5 μm in diameter (Fig. 3). Adding across the many axons that repeatedly intersect the intermingled dendrites while passing through this column of neuropil results in hundreds to thousands of MSBs that could be shared among neighboring cells with intermingled dendrites. Depending on the intensity of the extracellular stimulation, a large proportion of these MSBs can be activated during the induction of LTP.

With this possibility for a high degree of shared presynaptic connectivity, the following sequence of events is proposed (Fig. 4). Synapses of one cell are potentiated by pairing presynaptic activation with sufficient postsynaptic depolarization to induce LTP at the postsynaptic cell (step 1). Shortly after this postsynaptic induction of LTP, a retrograde signal(s) occurs at the potentiated synapses (step 2). The signal(s) needs only to be present in the synaptic cleft of the potentiated synapse for sufficient time to influence its own presynaptic bouton. Thus, any of the candidate retrograde messengers, even synaptic-cleft proteins that are anchored in the pre- or postsynaptic membrane, could signal the presynaptic axon when LTP had occurred.

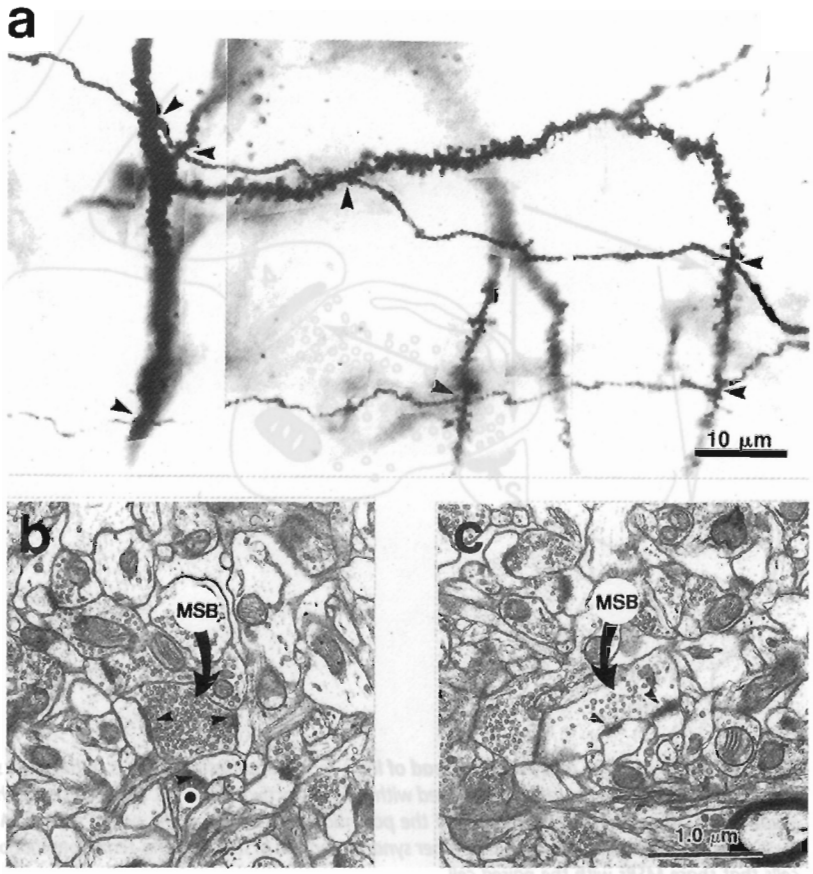

Fig. 2. *Appositions and multiple-synapse boutons (MSBs) in the stratum radiatum.* **(A)** *Photomontage of radiatum axons making three and four appositions, respectively (arrowheads), onto dendrites of the same CA1 pyramidal cell.* **(B and C)** *Multiple-synapse boutons in the middle of the stratum radiatum of area CA1 (curved arrows). Small arrows point to synapses on these MSBs from the presynaptic side.*

Subsequent events occurring in the presynaptic bouton (step 3) amplify the signal so that all synapses that occur on the MSB become potentiated. Some results suggest that either more neurotransmitter (glutamate) is released[31] or the probability of release increases with LTP, or both[14,32,33]. Several questions remain, however, regarding the interpretation of

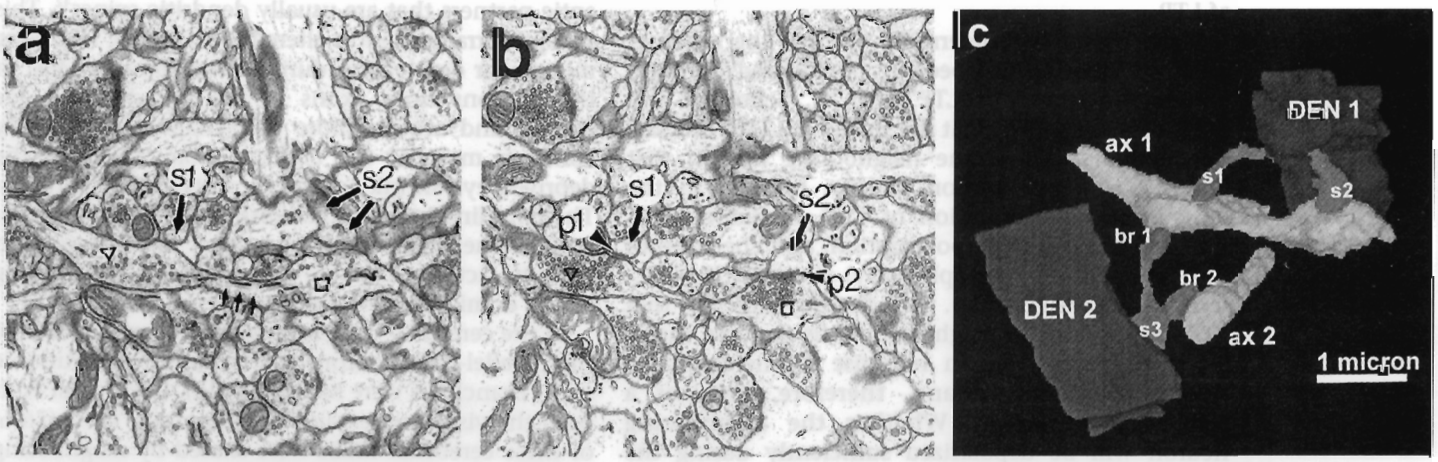

Fig. 3. *Adjacent axonal boutons synapsing with two dendritic spines from the same parent dendrite and with one dendritic spine from a different dendrite. The axon is sectioned longitudinally, and two adjacent axonal boutons are identified by open triangles and open squares.* **(A)** *In this section, the axonal boutons contain synaptic vesicles, and are connected by a constricted inter-varicosity region of the axon (three small arrows). The head of spine 1 (s1) and the neck of s2 are present.* **(B)** *The axonal boutons are filled with vesicles in this section, and appear as two separate profiles that synapse with the dendritic spine heads (s1 and s2). Synapses and postsynaptic densities (p1 and p2) are also indicated.* **(C)** *Three-dimensional reconstruction of these adjacent axonal boutons illustrating how spines of DEN1 synapse on adjacent boutons and a spine from DEN2 shares the presynaptic bouton of s1 from DEN1. The second head of the branched spine on DEN2 synapses with a different axon in the same field.*

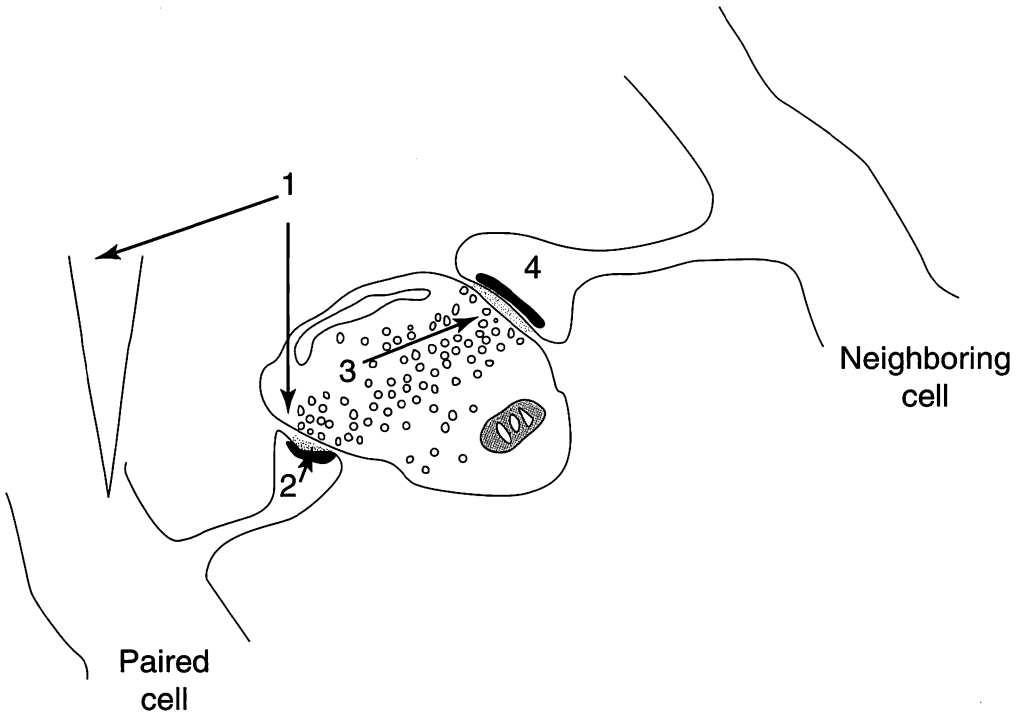

Fig. 4. ***Model of how the presynaptic spread of long-term potentiation (LTP) is initiated at shared multiple-synapse boutons (MSBs).*** *(1) Depolarization is paired with synaptic activation, and LTP is induced at the paired cell. (2) A retrograde signal occurs in the synaptic cleft of the potentiated synapses on the paired cell. (3) A presynaptic intracellular messenger initiates the spread of LTP to other synapses on the MSBs. (4) Long-term potentiation spreads to neighboring cells that share MSBs with the paired cell.*

these results[15,16,34]. A simple explanation for a presynaptic spread of LTP is that a retrograde signal triggers an elevation in the amount or probability of neurotransmitter release at synapses on the neighboring cells that share MSBs with the potentiated cell. This explanation is plausible because it is already known that local application of glutamate is sufficient, in the absence of any other stimulation, to induce LTP (Refs 35 and 36). For the model to work, however, other mechanisms could signal all of the synapses on the MSB that one of them had been potentiated, thereby initiating the presynaptic spread of LTP.

Many factors will determine whether the synapses on a neighboring cell become potentiated during a presynaptic spread of LTP (step 4). Schuman and Madison have shown that the spread of LTP is blocked by manipulations to the neighboring neuron that prevent its depolarization and an increase in the postsynaptic concentration of Ca^{2+}, including postsynaptic dialysis, hyperpolarization, and chelation of Ca^{2+} with *bis*-(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′,-tetraacetic acid[6] (BAPTA). This finding indicates that LTP only spreads to neighboring cells that can themselves be depolarized in response to the increased release of glutamate and, therefore, involves a Hebbian mechanism. Whether the neighboring neuron will be depolarized sufficiently during the increased release of glutamate will depend on the proportion of potentiated synapses that are shared by MSBs among the paired and neighboring cells. The finding that not all of the axonal boutons will be MSBs that share synapses among overlapping dendrites of neighboring cells is consistent with the physiological data that show that the secondary LTP in the neighboring cell is usually lesser in magnitude than the primary LTP.

The magnitude of LTP that occurs in the neighboring cell will also depend on whether the presynaptic messenger spreads beyond the initially potentiated MSBs to neighboring boutons along the stimulated axons (see Fig. 3, spines 1 and 2 of DEN1). If adjacent boutons are also affected, then the number of synapses that could become potentiated on the neighboring cells is expanded greatly. How far the presynaptic signal spreads will depend on the distance between synapses on the potentiated axon, and the diameter, length and composition of the axon between them. If the presynaptic messenger was Ca^{2+}, then a transient or sustained presynaptic increase in the concentration of Ca^{2+} might occur via influx through voltage-dependent channels[37] or via a presynaptic wave of Ca^{2+} triggered by an inositol 1,4,5-trisphosphate (IP_3)-mediated release from intraboutonal stores in the smooth endoplasmic reticulum (SER) and mitochondria[38–40]. The axonal SER and mitochondria might later sequester the Ca^{2+}, thereby restricting how far such a wave could spread.

Broader implications of MSBs in co-ordinating synaptic associations among neurons

Multiple-synapse boutons are found in most brain regions[41]. It has been suggested that the presynaptic spread of LTP might be important in co-ordinating the formation of appropriate functional units in visual cortex[7]. In the visual cortex of a normal adult cat, 60% of the axonal boutons are MSBs that have two postsynaptic partners that are usually dendritic spines[42]. This synaptic arrangement is altered dramatically following monocular deprivation during development. Most of the boutons from axons of the non-deprived eye enlarge and acquire more postsynaptic partners. By contrast, most of the boutons from axons of the deprived eye shrink and loose postsynaptic partners. These findings suggest that both the pre- and postsynaptic elements of MSBs are subject to alterations in synaptic activity that are important for establishing ocular dominance columns.

In the cerebellar cortex, only approximately 25% of the parallel-fiber boutons that synapse with Purkinje spiny branchlets were MSBs (Ref. 43). In contrast with both the visual cortex and hippocampus, most (76%) of these cerebellar MSBs were shared by neighboring dendritic spines that arose from the same Purkinje-cell dendrite. In hippocampal area CA3, nearly all of the mossy-fiber boutons made multiple synapses with different dendritic spines; however, all of the postsynaptic partners, reconstructed so far, are neighboring dendritic spines of the same CA3 pyramidal-cell dendrite[44]. This connectivity of cerebellar and CA3 MSBs

suggests that they would be unlikely to initiate a presynaptic spread of synaptic plasticity to neighboring cells. Whether activity-dependent changes in the structure or composition of MSBs occur with LTP in the hippocampus, or with long-term depression (LTD) in the cerebellum, deserves further consideration, as the types of changes that have been observed in visual cortex would also have important implications for the underlying mechanisms of LTP and LTD.

Several questions remain. What percentage of the synapses must be potentiated on the close neighboring cells to initiate a presynaptic spread of LTP? Does the extent of sharing of MSBs between neighboring cells establish whether, and to what degree, potentiation spreads to neighboring cells, as suggested by current data? Does the geometry and intracellular composition of the presynaptic axons facilitate or restrict the spread and stabilization of LTP? In addition, are there developmental differences in the occurrence of MSBs, and in the composition of axons that might facilitate or restrict the spread of LTP at crucial ages? Answers to these and related questions will provide a basis for understanding the role of synaptic structure in the formation, stabilization and loss of appropriate connections among neurons in both the developing and mature brain.

Acknowledgements

I thank Karin Sorra for her work on the original data that led to the hypothesis put forth here, and John Lisman and Frances Edwards for their valuable input on an earlier version of the manuscript. In addition, the anonymous reviewers of the manuscript helped further to shape the presentation, and I thank them for their input. This work is supported by NIH-NINDS #NS21184, and by the MR Center Grant #P30-HD18655 from NICHD.

Selected references

1 **Wigström, H. and Gustafsson, B.** (1985) *Acta Physiol. Scand.* 125, 159–172
2 **Wigström, H. and Gustafsson, B.** (1988) in *Long-term Potentiation: From Biophysics to Behavior* (Landfield, P.W. and Deadwyler, S.A., eds), pp. 73–108, Alan R. Liss
3 **Kelso, S.R., Ganong, A.H. and Brown, T.H.** (1986) *Proc. Natl Acad Sci. USA* 83, 5326–5331
4 **Muller, D., Hefft, S. and Figurov, A.** (1995) *Neuron* 14, 599–605
5 **Bonhoeffer, T., Staiger, V. and Aertsen, A.** (1989) *Proc. Natl Acad. Sci. USA* 86, 8113–8117
6 **Schuman, E.M. and Madison, D.V.** (1994) *Science* 263, 532–536
7 **Kossel, A., Bonhoeffer, T. and Bolz, J.** (1990) *NeuroReport* 1, 115–118
8 **Gally, J.A. *et al.*** (1990) *Proc. Natl Acad. Sci. USA* 87, 3547–3551
9 **Schuman, E.M. and Madison, D.V.** (1994) in *Molecular and Cellular Mechanisms of Neurotransmitter Release* (Stjarne, L. *et al.*, eds), pp. 507–520, Raven Press
10 **Hawkins, R.D., Zhuo, M. and Arancio, O.** (1994) *J. Neurobiol.* 25, 652–665
11 **Baudry, M.** (1994) in *Long-term Potentiation* (Baudry, M. and Davis, J.L., eds), pp. 45–51, MIT Press
12 **Murphy, K.P.S.J. *et al.*** (1994) *Neuropharmacology* 33, 1375–1385
13 **Bliss, T.V.P. and Collingridge, G.L.** (1993) *Nature* 361, 31–39
14 **Stevens, C.F. and Wang, Y.** (1994) *Nature* 371, 704–707
15 **Manabe, T. and Nicoll, R.A.** (1994) *Science* 265, 1888–1891
16 **Malinow, R.** (1994) *Science* 266, 1195–1196
17 **Lisman, J. and Harris, K.M.** (1993) *Trends Neurosci.* 16, 141–147
18 **Harris, K.M. and Kater, S.B.** (1994) *Annu. Rev. Neurosci.* 17, 341–371
19 **Bahr, B.A. and Lynch, G.** (1992) *Biochem. J.* 281, 137–142
20 **Persohn, E., Pollerberg, G.E. and Schachner, M.** (1989) *J. Comp. Neurol.* 288, 92–100
21 **Staubli, U., Vanderklish, P. and Lynch, G.** (1990) *Behav. Neural Biol.* 53, 1–5
22 **Xiao, P. *et al.*** (1991) *NeuroReport 2*, 461–464
23 **Luthi, A. *et al.*** (1995) *Nature* 372, 777–779
24 **Zhuo, M., Kandel, E.R. and Hawkins, R.D.** (1994) *NeuroReport* 5, 1033–1036
25 **Williams, J.H. *et al.*** (1989) *Nature* 341, 739–742
26 **Sorra, K.E. and Harris, K.M.** (1993) *J. Neurosci.* 13, 3736–3748
27 **Tamamaki, N. and Nojko, Y.** (1991) *J. Comp. Neurol.* 303, 435–442
28 **Tamamaki, N., Abe, K. and Nojyo, Y.** (1988) *Brain Res.* 452, 255–272
29 **Harris, K.M. and Stevens, J.K.** (1989) *J. Neurosci.* 9, 2982–2997
30 **Harris, K.M., Jensen, F.E. and Tsao, B.** (1992) *J. Neurosci.* 12, 2685–2705
31 **Bliss, T.V.P. *et al.*** (1986) *J. Physiol.* 377, 391–408
32 **Bekkers, J.M. and Stevens, C.F.** (1990) *Nature* 346, 724–729
33 **Malinow, R. and Tsien, R.W.** (1991) *Nature* 350, 282
34 **Jack, J.J.B. *et al.*** (1994) in *Molecular and Cellular Mechanisms of Neurotransmitter Release* (Stjarne, L. *et al.*, eds), pp. 275–299, Raven Press
35 **Cormier, R.J., Mauk, M.D. and Kelly, P.T.** (1993) *Neuron* 10, 907–919
36 **Malgaroli, A. and Tsien, R.W.** (1992) *Nature* 357, 134–139
37 **Llinás, R., Sugimori, M. and Silver, R.B.** (1992) *J. Physiol.* (Paris) 86, 135–138
38 **Amundson, J. and Clapham, D.** (1993) *Curr. Opin. Neurobiol.* 3, 375–382
39 **Berridge, M.J.** (1993) *Nature* 361, 315–325
40 **Berridge, M.J. and Dupont, G.** (1994) *Curr. Opin. Cell Biol.* 6, 267–274
41 **Peters, A., Palay, S.L. and Webster, H.DeF.** (1991) *The Fine Structure of the Nervous System: the Neurons and Supporting Cells*, W.B. Saunders
42 **Friedlander, M.J., Martin, K.A. and Wassenhove-McCarthy, D.** (1991) *J. Neurosci.* 11, 3268–3288
43 **Harris, K.M. and Stevens, J.K.** (1988) *J. Neurosci.* 8, 4455–4469
44 **Chicurel, M.E. and Harris, K.M.** (1992) *J. Comp. Neurol.* 325, 169–182