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Invited Review

Morphological changes associated with long-term potentiation

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Summary. Long-term potentiation (LTP) is a long-lasting form of synaptic plasticity induced by brief repetitive afferent stimulation that is thought to be associated with learning and memory. It is most commonly studied in the hippocampus where it may last for several weeks, and involves the synthesis of new proteins that might play a structural role. In this review we summarize the evidence in favor of modifications of neuronal architecture during LTP. We focus our attention on changes occurring at the level of single synapses, including components of postsynaptic dendrites (dendritic spines, the postsynaptic density, and synaptic curvature), of presynaptic terminals, and the formation of new synapses. We conclude that although many morphological changes at various sites have been observed during LTP, there is no definitive proof in favor of structural changes associated with LTP. However, morphological modifications remain a valid candidate for mechanisms of learning and memory.

Key words: Synaptic plasticity, Synapse formation, Structural changes, Dendritic spines, Postsynaptic density, Presynaptic terminal

Introduction

Plastic changes in the strength of synaptic connections are believed to be fundamental to information processing in the nervous system. Long-term potentiation (LTP), an increase in the efficacy of synaptic transmission following brief repetitive presynaptic stimulation, has become the dominant model of activity-dependent synaptic plasticity in the brain and a leading candidate to play a role in the formation and storage of memory.

LTP was first described in the hippocampus (Lømo, 1966; Bliss and Lømo, 1973) and it has since been found in other regions of the brain. It has been most extensively studied at the Schaffer collaterals, axons of CA3 cells that form synaptic contacts on the apical dendrites of CA1 pyramidal neurons. It is induced at these synapses by a local postsynaptic increase in the levels of Ca^{2+}, triggered by the activation of glutamate receptors of the N-methyl-D-aspartate (NMDA) subtype (Lynch et al., 1983; Bliss and Collingridge, 1993). This transient elevation of calcium is converted into an enduring increase in the efficacy of synaptic transmission by the activation of a series of enzymes, particularly protein kinases. The phosphorylation reactions catalyzed by these molecules affect critical synaptic proteins, most of which remain unidentified, that are the presumptive effectors of the plastic change (for a review see Bailey et al., 1996).

There has been a great deal of controversy about the site of expression of LTP. Some studies support the idea that LTP involves changes in the properties of the postsynaptic spine such as modifications in the number or in the affinity of the receptors for glutamate (Isaac et al., 1996; Malenka and Nicoll, 1997). Others contend that the presynaptic terminal is the site where the long-lasting changes take place. Specifically, the increase in Ca^{2+} in the postsynaptic terminal is thought to produce LTP by triggering the synthesis of nitric oxide (Böhme et al., 1991; O'Dell et al., 1991; Schuman and Madison, 1991; Arancio et al., 1996), a soluble gas that is proposed to diffuse back to the presynaptic terminal and activate guanylyl-cyclase (Haley et al., 1992; Zhuo et al., 1994; Arancio et al., 1995) and cGMP-dependent protein kinases (Zhuo et al., 1994; Arancio et al., 1997), and after a series of hitherto unidentified presynaptic modifications, cause an activity-dependent increase in transmitter release (Hawkins, 1996).

These mechanistic differences notwithstanding, both models of LTP expression share the same molecular logic. They explain the change in synaptic efficacy by reducing it to the occurrence of covalent modifications of preexisting synaptic proteins. However, LTP is not a unitary process. Its prolonged duration has made it possible to fractionate it into at least two separate stages: an early phase that is solely dependent on those covalent

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reactions, and a late phase that requires transcription and translation (for a review see Huang et al., 1996).

What role do newly-synthesized proteins play in the late phase of LTP? Several hypotheses have been advanced to answer this question. For instance, it has been proposed that they could decrease the turnover rate of the enzymes involved in covalent reactions that occur during the early phase, a possibility that has obtained support in other models of plasticity (Hegde et al., 1997). Alternatively, new proteins could play a structural role. They could constitute the building blocks necessary for the creation of new synaptic contacts. Such morphological changes might explain some of the contradictory electrophysiological evidence about whether the expression of LTP is pre- or postsynaptic (Edwards, 1995).

The purpose of this review is to summarize the evidence that supports the idea that activity can be accompanied by modifications of neuronal architecture with a special emphasis on changes observed at the level of single synaptic contacts. We will focus primarily on three complementary processes: the morphological modifications in the components of postsynaptic sites (dendritic spines, the postsynaptic density, and synaptic curvature), of presynaptic terminals, and the formation of new synapses (Tsukahara, 1981).

Postsynaptic changes

(i) Dendritic spines

Axons may form synaptic contacts directly on the dendritic shaft or on thorny protrusions of the dendrite, called spines. Dendritic spine morphology varies greatly. They can be long or short, stumpy or thin, and with or without a head shaped like a mushroom (Gray, 1959; Kaiserman-Abramof, 1969; Peters and Kaiserman-Abramof, 1969, 1970; Purpura, 1974; Chang and Greenough, 1984; Spacek, 1987). A possible spine function that might be related with plastic changes is to influence the amplitude of the excitatory postsynaptic potential (EPSP) through variations of the spine neck resistance. Any increase in the neck resistance, due to a decrease of its diameter, its partial occlusion, or due to an increase in its length, could cause a decrease in synaptic efficacy (Pongrácz, 1985; Lisman, 1989). However, according to more recent studies, spine neck resistance would be too low to modulate synaptic currents effectively (Yuste and Denk, 1995). Alternatively, spines could be necessary for compartmentalizing biochemical changes occurring inside them (Gamble and Koch, 1987; Wickens, 1988; Koch and Zador, 1993; Svoboda et al., 1996). For instance, a localized elevation of Ca^{2+} ions has been shown in dendritic spines of CA1 cells in brain slices (Yuste and Denk, 1995). The function of this compartmentalization would be to restrict plastic changes to spines that have detected release of neurotransmitter from the presynaptic terminal (Rall, 1974; Lisman, 1989). Thus, spines could detect the coincidence of the activity in the presynaptic neuron with biochemical changes occurring in the postsynaptic cell.

Several studies on spine shape modifications during LTP have been performed during the last two decades. It has been demonstrated that high-frequency stimulation of the perforant path is able to cause an increase in the mean area of the distal spines (Van Harreveld and Fifková, 1975), in the width of the spine head and the spine neck, and a decrease in the length of the spine neck (Fifková and Anderson, 1981). These changes started two minutes after the end of the stimulation and lasted at least 60 minutes (Van Harreveld and Fifková, 1975). Other studies on the same synapses (Desmond and Levy, 1983, 1986, 1988) showed an increase in the number of large, concave spine synapses with large postsynaptic densities. This increase was accompanied by a decrease in the number of less complex, convex spines, starting a few minutes after the potentiating stimulus and lasting for at least 60 minutes. These results suggest an interconversion from non-concave to concave spines during LTP (Desmond and Levy, 1986).

However, when LTP was evoked between the Schaffer-collateral commissural pathway and CA1 pyramidal neurons in hippocampal rat slices, there was no change in the mean width of dendritic spine stalks and mean area of dendritic spines (Lee et al., 1980). The only changes present were an increase in the number of shaft synapses, and a reduction in the coefficient of variation of: (1) the area of dendritic spines, (2) the length of postsynaptic densities on spines, and (3) the width of spine stalks (Lee et al., 1980). A different study on the same preparation showed an increase in the number of short and stumpy spines (Chang and Greenough, 1984). When LTP was chemically induced with a superfusate containing elevated Ca^{2+}, reduced Mg^{2+}, and tetraethylammonium in order to affect all potentiable synapses in the slice, there was an increase in the number of small spines, and a change in the angle between the spine and dendritic shaft in CA1 pyramidal neurons (Hosokawa et al., 1995). These morphological differences between CA1 and dentate gyrus might be explained by the different protocols used to induce LTP in the two areas (Chang and Greenough, 1984).

How do spine morphology modifications occur? It has been suggested that the entrance of Ca^{2+} during LTP induction triggers a series of reactions involving modifications of cytoskeletal proteins. Actin (Crick, 1982), myosin (Morales and Fifková, 1989), brain spectrin, microtubule-associated protein (Aoki and Siekevitz, 1985, 1988; Lynch and Baudry, 1987), and calpain (Lynch and Baudry, 1987) have all been implicated in the change in spine shape and total spine area occurring during LTP. Orientation of actin filaments within spines might influence spine conformation (Markham and Fifková, 1986). For instance, in short and stumpy spines, actin filaments are oriented perpendicular to the long axis of the dendritic shaft, while in more complex shaped spines, actin filaments have a very high

density, and are tightly knit (Markham and Fifková, 1986). Calpain, a calcium activated protease located in the postsynaptic density, would be able to degrade cytoskeletal proteins, producing changes in spine conformation (Aoki and Siekevitz, 1985, 1988; Lynch and Baudry, 1987). Levels of an extracellular protease, tissue plasminogen activator, also increase after LTP induction (Qian et al., 1993). Moreover, amounts of two membrane-spanning glycoproteins associated with the maintenance of intercellular interactions, neural-cell adhesion molecule and amyloid precursor protein increase in push-pull superfusates of the dentate gyrus after LTP induction (Fazeli et al., 1994).

LTP is thought to be associated with behavioral changes related with memory formation and storage. Changes in spine shape and density have been observed in several behavioral studies. For instance, forager honeybees show spines with larger profile areas and shorter stems than newly-emerged and nurse honeybees (Coss et al., 1980). Furthermore, passive avoidance training in one day-old chicks causes an increase in spine density and in spine head diameter and a decrease in spine stem length (Lowndes and Stewart, 1994).

(ii) Postsynaptic density

Another synaptic element that might change after synaptic plasticity is the postsynaptic density, an electron-dense area found in the postsynaptic region. It is formed by a planar array of spherical subunits of (18 nm in diameter (Kennedy, 1997) and contains many proteins, including actin (Cohen et al., 1977), cAMP-dependent protein kinase (Ueda et al., 1979), Ca^{2+} calmodulin-dependent protein kinase (Grab et al., 1981a,b), and a calmodulin-activated cAMP-dependent Ca^{2+} pump (Papazian et al., 1984). It is very likely that the postsynaptic density influences the shape of the terminal by controlling the size and orientation of filaments linking it to the surrounding cytoplasm (Siekevitz, 1985).

Evidence relating changes in the postsynaptic density to LTP has been obtained in studies on the hippocampal dentate gyrus (Desmond and Levy, 1983, 1986, 1988), where there was an increase in the number of spines with large postsynaptic densities following LTP-inducing stimuli. Furthermore, chronic electrical stimulation of the deafferented cerebral cortex has been shown to cause an increase in the size of postsynaptic densities (Rutledge, 1978).

(iii) Synaptic curvature

Another parameter that might vary during LTP is the curvature of the membrane above the postsynaptic density. If the membrane curvature is convex with regard to the presynaptic terminal, it is referred to as positive curvature; if it is concave with regard to the presynaptic terminal, it is considered as a negative curvature (Jones and Devon, 1978). Desmond and Levy (1983, 1988) found that the number and area occupied by negatively curved synapses increased during LTP induced by high frequency stimulation of the perforant pathway to the hippocampal dentate gyrus.

Presynaptic changes

LTP is thought to be associated not only with postsynaptic changes, but also with an increase in probability of transmitter release from the presynaptic terminal, and is accompanied by presynaptic morphological changes. Repetitive hippocampal stimulation causes a decrease in total vesicle density in addition to an increase in spine volume (Fifková and van Harreveld, 1977). Synaptic vesicles within the presynaptic terminal are redistributed during LTP induced through stimulation of the Schaffer collateral-commissural pathway of the hippocampus (Applegate et al., 1987). The number of synaptic vesicles attached to the active zone membrane is significantly increased together with the percentage of vesicles adjacent to the active zone (Applegate et al, 1987). Similar results have been obtained in the forebrain of the chick after passive avoidance training (Rusakov et al., 1995), suggesting a role for presynaptic terminals in behavioral changes. Two regions in the chick forebrain, the intermediate and medial hyperstriatum ventrale and the lobus parolfactorius, are important loci for memory storage. Stochastic analysis revealed a re-distribution of synaptic vesicles between two spatial pools relative to synaptic apposition zones resulting in a larger number of synaptic vesicles closer to synaptic apposition zones (Rusakov et al., 1995).

Presynaptic modifications have also been observed after electrophysiological stimulation of the reticular formation in rabbit sensorimotor cortex. Short term stimulation caused a 16% increase in the number of synaptic vesicles per terminal, while long-term stimulation induced an 11% decrease (Artyukhina and Ryabinina, 1980).

Synapse number

LTP may also involve the formation of new synapses. This idea is supported by electrophysiological results of a recent study by Bolshakov et al. (1997), who found that the synapses between individual CA3 and CA1 neurons normally release only a single quantum of transmitter, and the early phase of LTP is associated with an increase in the probability of release. However, the late phase of LTP at those synapses is associated with an increase in the number of quanta released, presumably due to an increase in the number of sites of synaptic transmission. Formation of new synapses might explain some of the seemingly contradictory electrophysiological data on whether the expression of LTP is pre- or post-synaptic, because it would have to involve coordinated changes on both sides of the synapses (see Fig. 1) (Edwards, 1995).

New synapse formation might involve intermediate

stages such as perforated synapses. According to a model proposed by Carlin and Siekevitz (1983), presynaptic stimulation causes a spinule to appear at a perforation in the postsynaptic density. The next step would be the invagination of the spinula into the presynaptic terminal, followed by the formation of two release sites. Although this has not yet been unequivocally demonstrated, this model is supported by experiments where application of KCl on slices of cerebral cortex (Van Harreveld and Trubatch, 1975), or epileptic seizures (Nitsch and Rinne, 1981) cause an increase in the number of spinules. A related mechanism that might give rise to a change in synapse number is spine branching. Two recent studies have provided evidence for spine branching associated with either an increase or decrease in spine density during LTP in the dentate gyrus (Trommald, 1990; Trommald et al., 1996; Rusakov et al., 1997).

(i) Perforated synapses

Discontinuities of the postsynaptic densities, also known as perforated synapses, have often been associated with LTP and learning. They were first observed by Peters and Kaiserman-Abramof (1969) in pyramidal neurons in layers II and III of the rat parietal cortex. Postsynaptic densities had the form of a disc in the smallest dendritic spines. The disc was perforated to form a ring in the synapses of larger spines, and a number of perforations were present in the largest spines. The distance between pre- and postsynaptic membrane in the perforations resembles that of the adjacent active zone (Sirevaag and Greenough, 1985), but perforations are not associated with cleft material,

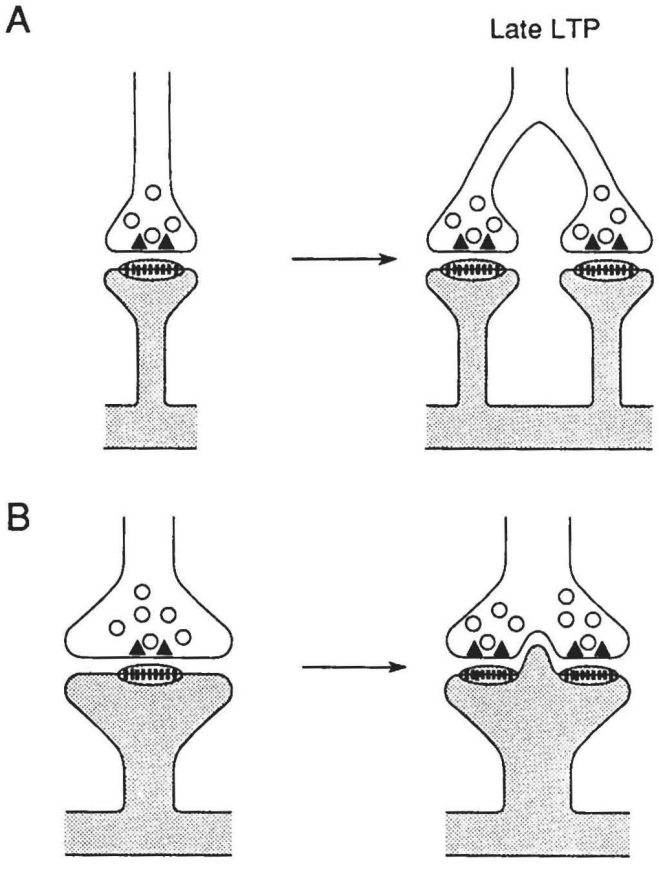

Fig. 1. LTP may involve the formation of new synapses. The diagram presents two models of synapse formation. In **A** a new branch sprouts from an existing presynaptic terminal; in **B** a spinule in the postsynaptic membrane protrudes into the presynaptic terminal to form a new synapse.

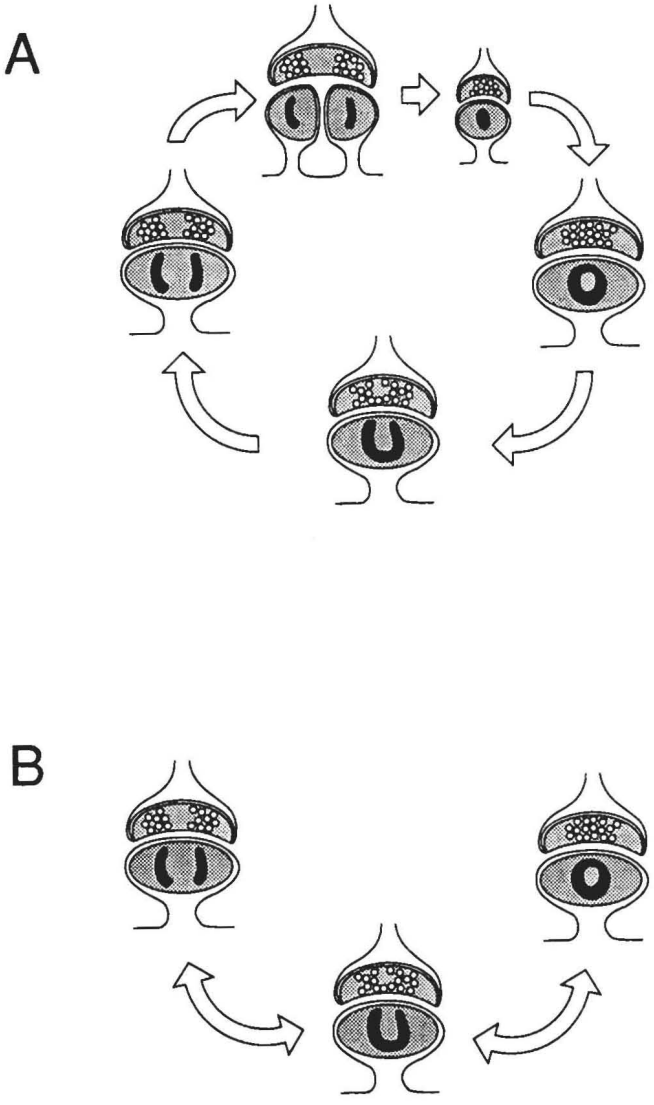

Fig. 2. Two models of formation of new synapses during LTP (Redrawn from Geinisman et al., 1991). In **A** a non-perforated synapse enlarges, fenestrates at the level of the postsynaptic density, and finally splits into two smaller non-perforated synapses. In **B** preesisting perforated synapses split into two non-perforated synapses. Presynaptic terminals are shown in cross section, whereas postsynaptic spines are shown in an en face view.

dense projections or synaptic vesicles in the presynaptic terminal (Cohen et al., 1977; Greenough et al., 1978; Vrensen and Nunes Cardozo, 1981; Chen and Hillman, 1982). The function of perforated synapses is not yet clear. They might be caused by splitting postsynaptic densities that are going to form new synapses, in which case they could be related to synaptic plasticity (Greenough et al., 1978). Alternatively, Jones (1993) has suggested that perforated and nonperforated synapses constitute separate populations formed early in the development, each representing complementary forms of synaptic plasticity. According to another hypothesis, they might be caused by postsynaptic densities that increase in size before breaking down into several fragments, which may or may not give rise to a new simple synapse (Hoff and Cotman, 1982).

High-frequency stimulation of the Schaffer-collateral commissural pathway in the hippocampus causes an increase in the number of perforated synapses (see Fig. 2) (Geinisman et al., 1991; Buchs and Muller, 1996). In the latter study there is an elegant demonstration that this change occurred at synapses where potentiation had occurred, as identified by Ca^{2+} staining. At those synapses the apposition zones between pre- and postsynaptic structures were also larger, postsynaptic densities were longer, and spine profiles were enlarged (Buchs and Muller, 1996).

Several behavioral studies have also supported a relationship between perforated synapses and learning and memory. Aged rats that exhibit a deficit in spatial memory showed a reduction in the number of perforated synapses in the dentate gyrus of the hippocampus formation in comparison with either young adults or aged rats with good memory (Geinisman et al., 1986). This finding suggests that perforated synapses in the hippocampus might be related to spatial memory. Furthermore, rats raised in a complex environment showed 25% more perforated synapses in cortical layers I, III and IV, than rats raised under impoverished conditions (Greenough et al., 1978).

(ii) Formation of new synapses

Formation of new types of synapses has been observed anatomically after high frequency stimulation of the medial perforant pathway in young rats. Perforated axospinous synapses with a segmented postsynaptic density were increased (see Fig. 2), and this increase was confined to the area where LTP had occurred (Geinisman et al., 1991; Hawrylak et al., 1993). Kindling, another phenomenon that has been related to plastic changes, also caused an increase in synapse number, which involved only perforated axospinous synapses with a segmented post-synaptic density (Geinisman et al., 1990, 1992; Morrell et al., 1991).

New synapse formation has also been observed in behavior experiments. One of the most important alterations observed during passive avoidance learning in the chick is an increase in synapse number in the lobus parolfactorius coupled with alterations in dendritic spine number (Stewart et al., 1984, 1987; Patel et al., 1988a,b; Lowndes and Stewart, 1994). These morphological changes were detected after 24 hrs, whereas the associated biochemical changes disappeared after 3 hrs. Similar to the late phase of LTP in hippocampus, this increase in synaptic density is blocked by the protein synthesis inhibitor, anisomycin (Sojka et al., 1995). Further evidence for an increase in synapse number comes from studies on brain areas involved on vocal control in birds. Some bird species modify their songs according to their age or to their hormonal levels. This behavior is mediated by brain nuclei whose dimensions increase in males which sing compared to females which do not sing, and also in males with complex song repertoires (Brenowitz and Arnold, 1986; Juraska et al., 1980), and during the singing season (Gurney and Konishi, 1980; Nottebohm, 1981).

Conclusion

A large variety of studies has been performed over the last two decades on morphological changes accompanying various forms of synaptic plasticity such as LTP which are thought to be associated with learning and memory. Structural modifications have been hard to investigate because of the inability to examine and follow dynamic events in the CNS with ultrastructural methods. Information on dynamic changes has been acquired through inferences drawn from morphometric analysis. However, synapses are continuously re-modelled, probably as part of the normal functioning of neurons in the brain. Therefore, it is difficult to have a reliable image of the structural components of the synapses as they change. Morphological studies also usually examine all synapses on a cell, whereas changes are generally induced at only a very small proportion of synapses. Thus, an additional difficulty to overcome in morphological studies is the identification of potentiated synapses. Moreover, it is not yet clear if the morphological modifications associated with plasticity are the cause of the potentiation, or its consequence. Despite all these difficulties, structural changes remain a valid candidate for mechanisms of learning and memory.

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