Synaptic plasticity and dynamic modulation of the postsynaptic membrane

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The biochemical composition of the postsynaptic membrane and the structure of dendritic spines may be rapidly modulated by synaptic activity. Here we review these findings, discuss their implications for long-term potentiation (LTP) and long-term depression (LTD) and propose a model of sequentially occurring expression mechanisms.

Dynamic changes in the structural characteristics of the postsynaptic membrane and, in particular, of dendritic spines have been a topic of great interest over the last decade (reviewed in refs. 1–7). Such changes are of particular interest because these tiny protrusions are the normal site of excitatory synaptic transmission in many regions of the brain, including the hippocampus. Furthermore, changes in efficacy at excitatory synapses are thought to underlie many forms of adaptive behavior, including learning and memory.

In the last few years, several studies using fluorescent imaging techniques as well as electron microscopy (EM) indicate that dendritic spines can change their morphology and ultrastructure more rapidly than previously envisioned. Furthermore, electrophysiological and immunohistochemical data suggest that glutamate receptors may be subject to rapid exo- and/or endocytosis, demonstrating that the biochemical composition of the postsynaptic membrane can be modified rapidly. Here we review these findings and put them into the context of synaptic plasticity, developing a scenario of successive events that may underlie the major forms of long-term potentiation (LTP) and long-term depression (LTD).

Filopodia are precursors of spines and synapses

During development, filopodial protrusions emerge from dendrites and change in size over the time course of minutes. Some of these may completely retract, whereas others may eventually stabilize to form spines[8] and functional synapses[9]. The growth of these filopodia can be modulated by synaptically released neurotransmitters such as glutamate. A recent study using GFP-transfected CA1 neurons in young organotypic hippocampal slice cultures showed that strong activation of *N*-methyl-D-aspartic acid (NMDA) receptors by repetitive stimulation of afferent fibers leads to filopodial growth within minutes[10]. These data suggest that filopodial protrusions are the precursors of dendritic spines during development[11], and that they can rapidly form and change shape in response to appropriate stimuli. However, although filopodia are undoubtedly important during development, their existence and roles in mature neurons are not established.

Spines undergo rapid shape changes

When dendritic spines of dissociated hippocampal cells[12] or organotypic slices in culture[13] are visualized using time-lapse fluorescent video microscopy, clear shape changes are observed. Spines seem to oscillate with a period of tens of seconds, but do not seem to change their cross-sections. Because these movements are reversibly blocked by cytochalasin D, the authors concluded that the shape changes are mediated by actin polymerization. Such 'dancing spines' have not been reported *in vivo* or in acute slice preparations, suggesting that, in a more natural environment where synapses often are tightly ensheathed by glial processes, movements may be more restricted. Nevertheless, these experiments demonstrate that constitutive actin polymerization occurs in dendritic spines and may have functional consequences. Indeed, loading CA1 pyramidal cells with agents that interfere with actin function (latrunculin B or phalloidin) decreases basal synaptic responses[14]. These data raise the possibility that in mature synapses, modification of actin filaments may be involved in remodeling of the postsynaptic membrane, which in turn may contribute to changes in synaptic efficacy.

Changes in dendritic spine shape and filopodial growth from dendrites do not seem to occur when NMDA receptors are blocked[10,15], perhaps because entry of Ca^{2+} through activated NMDA receptor channels is required to depolymerize postsynaptic actin[16]. The finding that the application of caffeine leads to growth of filopodia[17], an effect blocked by preincubation with ryanodine, indicates that release of Ca^{2+} from intracellular stores may be a particularly important source of Ca^{2+} for influencing production of dendritic filopodia and modifying dendritic spine

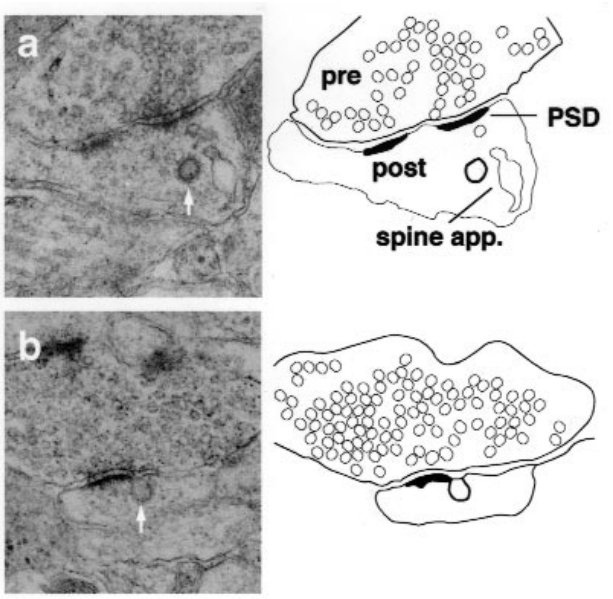

Fig. 1. Coated vesicles in dendritic spines. A fraction of spines contain coated vesicles (**a**, arrow), which seem to derive from the adjacent spine apparatus (spine app.). (**b**) Such a coated vesicle is in contact with the postsynaptic membrane immediately next to the postsynaptic density (PSD). These organelles constitute a possible ultrastructural correlate for the proposed constitutive recycling of AMPA receptors.

shape. It may be that NMDARs and intracellular stores can independently or synergistically, through calcium-induced calcium release[18], contribute to the calcium rise necessary to effect structural plasticity.

The role of NMDAR activation/Ca^{2+} signaling in influencing filopodia production and spine shape, however, is probably quite complex. Although the reports detailed above implicate NMDAR activation in the activity-induced growth of filopodia, exogenous application of NMDA causes spine retraction though a mechanism requiring the phosphatase calcineurin[16]. This bidirectional capacity of NMDARs to either promote or reduce spines is reminiscent of the bidirectionality of NMDAR-dependent LTP and LTD, and the calcineurin requirement for spine retraction further strengthens the resemblance. On a much longer time scale, spine density decreases in organotypic hippocampal slice cultures when, for several days, amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors are blocked or when vesicular release of glutamate is prevented by application of botulinum toxin[19]. The mechanisms underlying this slow, trophic effect (which does not seem to be present in mature neurons[20]) are unknown; however, because this effect is neither blocked by the sodium channel blocker tetrodotoxin (TTX) nor the NMDA receptor antagonist MK801, it seems unlikely that this process is engaged in activity-dependent plasticity of the sort induced by LTP or LTD. Taken together, these and other related results have led to a model proposing that changes in intracellular Ca^{2+} can have various effects on spines[2,21]: minimal synaptic activation is required for spine maintenance, moderate and brief increases in Ca^{2+} cause spine formation and growth, and very large and sustained increases cause spine retraction.

The detailed mechanisms by which NMDA receptor activation and the subsequent changes in intracellular Ca^{2+} influence the postsynaptic cytoskeleton and, thus, spine shape and filopodia formation remain unknown. A critical additional question is how these morphological changes of the postsynaptic membrane relate to long-lasting changes in synaptic efficacy elicited by synaptic activity, as during LTP. A possible answer is suggested by the dependence of LTP and LTD on the rapid redistribution of AMPA receptors into and out of the postsynaptic plasma membrane.

AMPA receptors recycle in the postsynaptic membrane

The idea that the number of glutamate receptors in the postsynaptic plasma membrane can be modified by synaptic activity and contribute to LTP was proposed over 15 years ago[22]. This idea, however, was largely ignored by the community of scientists actively investigating LTP over the ensuing decade. It resurfaced approximately five years ago with data consistent with the existence of synapses lacking functional AMPA receptors, the so-called 'silent synapses', and their conversion to functional synapses with LTP induction[23–25]. Subsequent reports indicated that *N*-ethylmaleimide-sensitive fusion protein (NSF), a protein involved in numerous membrane fusion events, interacts with the C terminus of the AMPA receptor subunit GluR2. The acute disruption of this NSF–GluR2 interaction results in a decrease in synaptic AMPAR responses[26–30], which further raises the possibility that AMPARs may be inserted into and removed from the postsynaptic membrane on a relatively rapid time scale. This hypothesis was tested by introducing into hippocampal CA1 pyramidal cells (the postsynaptic cell) compounds known to disrupt exocytosis or endocytosis[29] while monitoring excitatory postsynaptic currents. Blocking postsynaptic exocytosis led to an activity-independent decrease of AMPA receptor-mediated synaptic responses, presumably because endocytosis continued unabated[29]. Importantly, NMDA receptor-mediated responses were unaffected, indicating that these manipulations did not cause a nonspecific deterioration of synaptic functions. Conversely, blocking endocytosis increased AMPA receptor-mediated synaptic responses[29], presumably because AMPA receptors were constitutively inserted into the synaptic plasma membrane. These studies provide evidence for a mobile pool of AMPA receptors that constitutively cycle between a cytoplasmic and a surface-membrane compartment on a time scale of tens of minutes via exo- and endocytotic pathways.

Further support for this idea comes from immunocytochemical studies examining changes in the synaptic localization of AMPA and NMDA receptors following various experimental manipulations. Perhaps the most dramatic finding is that expression of the peptide that disrupts the NSF–GluR2 interaction in cultured hippocampal neurons causes a profound loss of surface AMPA receptor clusters, but has no effect on NMDA receptor clusters[26,29]. Loss of surface AMPA receptors, but not NMDA receptors, also occurs rapidly in response to bath application of glutamate[31]. This seems to be due to dynamin-dependent endocytosis of surface AMPA receptors[32]. Evidence for activity-dependent surface delivery of AMPA receptors in organotypic cultures of the hippocampus was provided using antibodies against GFP fused at the N terminus of the GluR1 AMPA receptor subunit[33]. Moreover, tetanus toxin-sensitive, Ca^{2+}-dependent postsynaptic exocytosis of trans-Golgi network-derived organelles was observed using the styryl dye FM1-43 as a probe for dendritic membrane dynamics[34].

AMPA receptor redistribution during LTP and LTD

Does activity-dependent redistribution of AMPA receptors into and out of the postsynaptic plasma membrane play a role

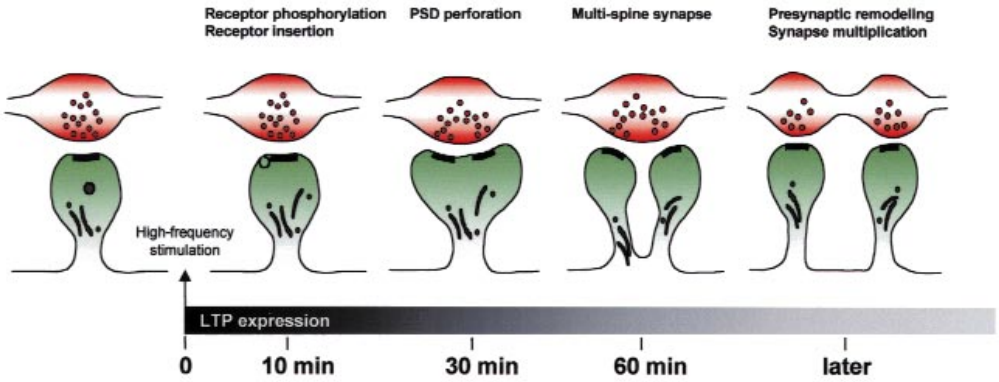

Fig. 2. Proposed sequence of mechanisms involved in expression of LTP. Within ten minutes of LTP induction, activation of Ca^{2+}-dependent signal-transduction pathways results in phosphorylation of AMPA receptors and an increase in their single-channel conductance. In addition, the size of the spine apparatus increases, and AMPA receptors are delivered to the postsynaptic membrane by exocytosis of coated vesicles. This membrane insertion also leads to an increase in the size of the postsynaptic density (PSD) and, eventually, to the production of perforated synapses within the first 30 minutes. At one hour, through an unknown mechanism, some synapses use the expanded membrane area to generate multi-spine synapses (where two or more spines contact the same presynaptic bouton). Concomitant retrograde communication, possibly through cell-adhesion molecules, would trigger appropriate presynaptic structural changes, eventually increasing the total number of synapses.

in NMDA receptor-dependent LTP and LTD, respectively? As mentioned above, electrophysiological evidence that LTP expression is associated with the insertion of AMPA receptors was provided by the demonstration of functionally silent synapses (synapses yielding only NMDA receptor-mediated responses). When subjected to an LTP-induction protocol, these silent synapses became functional and expressed AMPA receptor-mediated responses[23,24]. Consistent with a role of exocytosis in this process, introduction into CA1 pyramidal cells of substances such as botulinum toxin strongly impaired or prevented LTP[35]. To directly visualize the potential movement of AMPA receptors during LTP, cells in organotypic hippocampal slice cultures were transfected with a green fluorescent protein-tagged AMPA receptor subunit (GFP-GluR1)[33]. Under baseline conditions, the GFP-GluR1 was found mainly in dendritic shafts; within 15 minutes of a tetanic stimulation that induced robust LTP, fluorescence developed in dendritic spines that previously had not shown fluorescence. Furthermore, fluorescent intensity increased in spines that did show some fluorescence before the tetanus. These changes in the distribution of GFP-GluR1 due to tetanic stimulation were prevented by the NMDA receptor antagonist D-2-amino-5-phosphonopentanoic acid (D-APV), suggesting that NMDA receptor activation can lead to the delivery of AMPA receptors into dendritic spines. Recent work confirms that these receptors are actually inserted into the membrane at a synaptic location[36].

There also is evidence that LTD involves increased endocytosis of AMPA receptors. In cultured hippocampal neurons, immunocytochemical analysis demonstrated that NMDA receptor-dependent LTD was accompanied by a decrease in the number of synaptic surface AMPA receptors, with no significant effect on the distribution of NMDA receptors[37]. Consistent with this finding, loading CA1 pyramidal cells with inhibitors of endocytosis prevented the generation of LTD[29], as did the peptide that interferes with the NSF–GluR2 interaction[29,38]. Furthermore, following the saturation of NMDA receptor-dependent LTD, this peptide no longer caused a decrease in the AMPA receptor-mediated EPSC[38]. More recently, two additional reports confirmed the importance of clathrin-dependent endocytosis of AMPA receptors in the hippocampus[39] and in mediating LTD at excitatory synapses on cerebellar Purkinje cells[40].

Remodeling of postsynaptic densities with LTP

The AMPA receptors that respond to synaptically released glutamate are found in the postsynaptic density (PSD) of dendritic spines, an electron-dense thickening closely associated with the inside of the postsynaptic plasma membrane, across from the presynaptic active zone. Given that the PSD either contains or is closely associated with receptors, scaffolding molecules, signal transduction machinery and cytoskeletal elements such as actin (reviewed in refs. 6, 41–43), it is difficult to envision how AMPA receptors can be inserted into or removed from the PSD without some sort of significant structural modifications. A possible relationship between changes in spine shape and the redistribution of AMPA receptors is supported by EM analyses.

EM pictures of spines, especially when reconstructed from serial sections, reveal that spines have a complex ultrastructure and come in a variety of shapes[1]. For several decades, the effect of activity on spine shape and synapse ultrastructure has been a topic of great interest. One specific morphological modification repeatedly associated with increased activity seems to involve reorganization of the PSD. Specifically, it was suggested that LTP is associated with an increase in the fraction of synapses containing discontinuities within their postsynaptic densities (termed 'perforated synapses')[44–46]. This was recently confirmed using a calcium-precipitation protocol that identified recently activated synapses, at which Ca^{2+} accumulated in storage organelles[47,48]. In these studies, LTP was associated with a rapid and transient increase in the proportion of perforated synapses, which showed larger total PSD surface areas when compared with non-perforated synapses.

What might cause the growth of PSDs and their eventual perforation? Several lines of evidence suggest that these processes may be initiated by increasing the number of AMPA receptors in the PSD. First, recent immunogold labeling studies confirm that larger PSDs almost always contain AMPA receptors[49,50] and that perforated synapses express more AMPA receptors than synapses with simple PSDs[51]. Second, an ultrastructural characteristic of perforated synapses is that they contain a higher proportion of smooth endoplasmic reticulum and are more likely to show a spine apparatus[52]. These are organelles thought to be involved in membrane synthesis and storage of calcium that can be released in response to the appropriate stimulus, which are probably required for the transport of AMPA receptors to the

surface of the spine. Indeed, sparse immunogold labeling of AMPA receptors is observed in the spine apparatus[49]. Third, a fraction of spines clearly contain coated vesicles[52], some of which contact the postsynaptic membrane (**Fig. 1**). This may represent an ultrastructural correlate for the proposed constitutive recycling of AMPA receptors[29] and regulated Ca^{2+}-evoked dendritic exocytosis of trans-Golgi network-derived organelles revealed using FM1-43 (ref. 34). However, complete characterization of the protein cargo of coated vesicles and the other intracellular organelles localized to dendritic spines will require further work.

LTP and formation of new spine synapses

The occurrence of perforated synapses and the insertion of new receptors at the postsynaptic membrane could also be interpreted as reflecting early events in a process leading to the splitting or budding of existing spines[4,53]. Consistent with this idea, increases in spine density are reported following LTP induction[54–56], as are increases in the frequency of multiple-spine synapses, in which two adjacent spines arising from the same dendrite contact a single presynaptic bouton[47]. The lack of alteration in overall synapse density two hours after LTP[57], as estimated from serial EM sections, is not surprising, as only a fraction of activated synapses undergo spine duplication[47]. However, an alternative hypothesis also consistent with these observations is that LTP induces *de novo* spine formation at the dendritic shaft[58]. Indeed, direct support for this model was provided by two-photon confocal microscopy of labeled CA1 neurons, which revealed the formation of new spines about one hour after application of an LTP-inducing protocol at sites where no spine was visible previously[59]. However, the observation that spine duplication occurs after LTP induction does not rule out concomitant PSD splitting; in any case, because these changes occur with a substantial delay after LTP induction, the *de novo* formation of synapses can only contribute to a later stage of LTP and thus may represent a way of consolidating changes in synaptic efficacy that are initially produced, at least in part, by receptor insertion.

Morphological remodeling and signal transduction of LTP

Taken together, the data discussed so far lead to the conclusion that morphological remodeling of the postsynaptic membrane and functional changes in synaptic strength are related. Induction of LTP, through a rise in postsynaptic calcium concentration and activation of various signaling cascades, would both enhance actin-dependent dynamics in the spines and promote endo-/exocytotic mechanisms leading to modifications of receptor properties and insertion of new receptors in the postsynaptic membrane. These actions would be visualized as increased spine motility, growth of thin filopodial protrusions and formation of synapses with perforated PSDs. At a later stage, these mechanisms could eventually result in duplication of spine synapses or the formation of new ones, which could contribute to the increase in synaptic efficacy by increasing the number of release sites between individual synaptically coupled cell pairs.

A first step of LTP expression might involve calcium-dependent activation of second messenger cascades and, in particular, the activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII; reviewed in ref. 60). Calcium entering through NMDA receptors activates CaMKII rapidly and leads to autophosphorylation of Thr286 (refs. 61–63). This causes CaMKII to translocate to the PSD[64] in a constitutively active state, resulting in the phosphorylation of the GluR1 AMPA receptor subunit[61,65]. Phosphorylation of AMPA receptors, in particular GluR1, enhances AMPA receptor-mediated currents[61] due to an increase in single-channel conductance[61,66–68]. Furthermore, a similar increase in AMPA receptor single-channel conductance was observed soon after the induction of LTP[69]. In this study, however, changes in single-channel conductance were observed in approximately half of the cases. The remaining cells either showed no changes at all or changed only the proportion of synaptic failures. These observations are consistent with the rapid insertion of AMPA receptors of unaltered average single-channel conductance at both functional and silent synapses, which do or do not contain AMPA receptors in the PSD, respectively. Direct evidence that, in addition to changing channel properties, CaMKII also drives AMPA receptors into synapses is corroborated by the observation that cotransfection with activated CaMKII and GFP-GluR1 increases not only the amplitude but also the rectification of synaptic responses, a property of the newly inserted AMPARs containing only the subunit GluR1. Furthermore, delivery of a mutated GluR1 subunit lacking a PDZ interaction site (derived from the proteins PSD-95, Dlg and ZO1, which contain the domain) was abolished, whereas a mutation of the CaMKII phosphorylation site allowed normal insertion. Taken together, these experiments indicate a delivery mechanism requiring the association between GluR1 and a PDZ-domain protein in response to CaMKII (ref. 36).

This insertion of AMPA receptors would clearly increase synaptic efficacy but, in addition, as discussed above, may also cause changes in the structure of the PSD leading to an increase in its size and, in most cases, to its perforation[46]. The characteristics of perforated synapses are consistent with this scenario. That CaMKII is also an essential trigger for this structural change is supported by the finding that inhibition of CaMKII prevents not only the measured increase in synaptic strength during LTP but also the increase in the occurrence of perforated synapses[47].

How might these initial changes in AMPA receptor content and PSD structure relate to the new spine formation thought to occur during later stages of LTP? In the two studies that visualized dendritic structure using two-photon laser-scanning microscopy, the activity-dependent growth of new filopodia or spines accompanying LTP seemed to occur *de novo* from the dendritic shaft or existing spines[10,59]. In both studies, the authors observed highly dynamic dendrites that changed their morphology within 10–30 minutes after the application of a high-frequency, LTP-inducing stimulation protocol. Although one study using very young organotypic cultures (2–7 days *in vitro* from postnatal day 7 rats) observed mostly transient filopodial growth and spine formation in only 27% of cases[10], the other study using 2–4-week-old cultures reported almost exclusively the stable formation of new spines[59]. Despite these developmental differences, both groups used preparations in which a significant proportion of synapses were found directly on dendritic shafts, but not on well formed spines. Thus it is conceivable that the sites at which new outgrowths occurred were actually sites containing clustered receptors in preformed PSDs. Recent EM studies of LTP in organotypic slice cultures reporting increases in both perforated synapses and multiple-spine synapses from the same parent dendrite[47] suggest a splitting or budding process through which a perforated synapse could be transformed into two distinct, independent spines.

The detailed mechanisms underlying new spine formation in response to activity remain unclear. Furthermore, it is not firmly established that the newly formed spines observed in imaging studies are actually functional. The only evidence of their function comes from an experiment in which a high-frequency stimulation protocol was applied an hour after LTP induction, revealing

Ca^{2+} precipitates in both components of multiple spine synapses in subsequent EM analysis[47]. Assuming that the newly formed spines are indeed functional, another important question arises: what is the computational implication of having multiple spines connecting to the same presynaptic bouton? What could be the advantage of having two distinct spines, which, on all accounts, would be activated synchronously by glutamate release from the same terminal? Is this a stable endpoint of synaptic plasticity, or are presynaptic release sites remodeled as well?

After the first 30–50 minutes, LTP seems to be accompanied by an increase in the number of synapses; one direct test of this idea would be to record glial transporter currents. These are sensitive to changes in the number of active synapses, but remain unchanged during the first 30 minutes after the triggering of LTP[70,71]. Another very valuable but technically challenging approach would involve the development of techniques that permit the imaging of spines together with their cognate presynaptic boutons. Assuming presynaptic structural changes occur during LTP, which seems probable, an important area of investigation will be the identification of the retrograde messengers required to signal from postsynaptic to presynaptic elements. Prime candidates for such a role are cell-adhesion molecules such as cadherins and neural cell-adhesion molecules in conjunction with proteases modifying the extracellular matrix, all previously implicated in LTP[72], as well as β-neurexins[73] and neuroligins[74] which also provide a structural link across the synaptic cleft.

Another important topic about which even less is known concerns structural changes during LTD. By analogy with LTP, a very simple model would predict that, following removal or endocytosis of AMPA receptors, the PSD would shrink, eventually leading to the loss of the dendritic spine and its corresponding presynaptic bouton. A very similar sequence of events occurs at the neuromuscular junction during development[75]. It will therefore be interesting to apply some of the approaches discussed thus far to LTD and to determine whether spine production and loss occurs much more frequently in response to changes in patterns of synaptic activation than previously envisioned.

A model of sequentially occurring expression mechanisms

We present a model, inspired by previous ideas[4] and supported by much current evidence, that suggests a succession of different expression mechanisms during the first hour of LTP (**Fig. 2**). These lead to structural modification of the postsynaptic membrane and the production of new dendritic spines. Initially, activation of Ca^{2+}-dependent signal-transduction pathways, most notably CaMKII, results in phosphorylation of GluR1-containing AMPA receptors and an increase in their single-channel conductance. In addition, AMPA receptors are translocated into the spine and PSD via a mechanism analogous to exocytosis. These events occur at active synapses as well as at silent synapses lacking surface AMPA receptors. The associated trafficking events lead to an increase in the size of the PSD and, eventually, to the production of perforated synapses. Using the expanded membrane area, some perforated spines, through an unknown mechanism, would split to generate a second spine still contacting the same presynaptic bouton. Concomitant retrograde communication, possibly through cell-adhesion molecules, would trigger appropriate presynaptic structural changes. This entire sequential cascade of events would not necessarily occur at all synapses; depending on the state of the synapse and the pattern of activity to which it is exposed, an individual synapse might express only a subset of these initial changes. According to this model, LTD would involve the converse of these events—loss or endocytosis of AMPA receptors from the PSD, followed by a decrease in PSD size and, eventually, complete loss of the dendritic spine. We emphasize that the large amount of experimental evidence accumulated over the last year or two puts this model on a firm footing.

Important areas of future research include further study of the interplay between pre- and postsynaptic structural changes occurring at synapses expressing LTP and LTD and the roles of the cytoskeleton and cell-adhesion molecules in mediating these changes. In addition, there is probably an important role of protein synthesis that will need to be addressed. How do these changes in synaptic structure and function mediate learning, memory and other behavioral changes? We believe that finding the answer to this question will be facilitated by the development of hypotheses such as that presented here.

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