11

SYNAPTIC PLASTICITY

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The most fascinating and important property of the mammalian brain is its remarkable plasticity, which can be thought of as the ability of experience to modify neural circuitry and thereby to modify future thought, behavior, and feeling. Thinking simplistically, neural activity can modify the behavior of neural circuits by one of three mechanisms: (a) by modifying the strength or efficacy of synaptic transmission at preexisting synapses, (b) by eliciting the growth of new synaptic connections or the pruning away of existing ones, or (c) by modulating the excitability properties of individual neurons. *Synaptic plasticity* refers to the first of these mechanisms, and for almost 100 years, activity-dependent changes in the efficacy of synaptic communication have been proposed to play an important role in the remarkable capacity of the brain to translate transient experiences into seemingly infinite numbers of memories that can last for decades. Because of its fundamental importance, there has been an enormous amount of work describing the many forms of synaptic plasticity and their underlying mechanisms.

Synaptic transmission can either be enhanced or depressed by activity, and these alterations span temporal domains ranging from milliseconds to enduring modifications that may persist for days or weeks and perhaps even longer. Transient forms of synaptic plasticity have been associated with short-term adaptations to sensory inputs, transient changes in behavioral states, and short-lasting forms of memory. More lasting changes are thought to play important roles in the construction of neural circuits during development and with long-term forms of memory in the mature nervous system. Given these diverse functions, it is not surprising that many forms and mechanisms of synaptic plasticity have been described. In this chapter, I provide a brief overview of some of the forms of synaptic plasticity found at excitatory synapses in the mammalian brain, focusing on long-term potentiation (LTP) and long-term depression (LTD).

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SHORT-TERM SYNAPTIC PLASTICITY

Virtually every synapse that has been examined in organisms ranging from simple invertebrates to mammals exhibits numerous different forms of short-term synaptic plasticity that last on the order of milliseconds to a few minutes (for detailed reviews, see 1 and 2). In general, these result from a short-lasting modulation of transmitter release that can occur by one of two general types of mechanisms. One involves a change in the amplitude of the transient rise in intracellular calcium concentration that occurs when an action potential invades a presynaptic terminal. This occurs because of some modification in the calcium influx before transmitter release or because the basal level of calcium in the presynaptic terminal has been elevated because of prior activity at the terminal. A second mechanism occurs downstream of calcium elevation in the presynaptic terminal and involves some modulation of the biochemical processes involved in synaptic vesicle exocytosis.

Paired-Pulse Facilitation and Depression

When two presynaptic stimuli are delivered within a short interval, the synaptic response to the second stimulus can be either enhanced or depressed relative to the first stimulus. Paired-pulse depression is commonly observed at all synapses at short (less than 20 milliseconds) interstimulus intervals. It may result from inactivation of voltage-dependent sodium or calcium channels or from a transient depletion of the synaptic vesicles that are ''docked'' adjacent to the presynaptic plasma membrane, waiting to be released. Many synapses at longer interstimulus intervals (20 to 500 milliseconds) exhibit paired-pulse facilitation that is thought to result from the influx of calcium that occurs in response to the first action potential. One simple idea is that the ''residual'' calcium left over from the first action potential combines with the calcium influx during the second action potential, and because the relationship between calcium concentration in the terminal and release is highly nonlinear, this small increase in resting calcium may cause substantial facilitation. However, with a single action potential, the increase in resting calcium concentration is very small, and

thus additional mechanisms are likely involved. Currently, there is much interest in the possibility that transient modulation, by activation of protein kinases, of some of the presynaptic phosphoproteins that are known to be involved in the control of transmitter release may play an important role in very short-term synaptic plasticity. For example, knockout mice lacking one or more of the synapsins (3, 4), or lacking the small guanosine triphosphate–binding protein rab3A (5,6), exhibit abnormal short-term synaptic plasticity.

Whether a specific synapse displays paired-pulse facilitation or depression depends on the initial state of the synapse and its recent history of activation. Because these forms of plasticity largely result from changes in the probability of transmitter release, synapses that begin with a very high probability of release tend to show depression, whereas those with a low probability of release exhibit facilitation. Consistent with this idea, activation of presynaptic receptors that cause a decrease in transmitter release almost always causes an increase in the magnitude of paired-pulse facilitation (or even a conversion of paired-pulse depression to paired-pulse facilitation).

Facilitation and Depression Following Trains of Stimuli

Longer-lasting forms of plasticity are observed following repetitive or tetanic stimulation of synapses with prolonged (approximately 200-millisecond to 5-second) trains of stimuli applied at high frequencies (10 to 200 Hz). *Augmentation* and *posttetanic potentiation* refer to enhancements of transmitter release that can last anywhere from seconds (augmentation) to several minutes (posttetanic potentiation). They are thought to result in large part to the buildup of calcium concentration in the presynaptic terminal during the trains of stimuli. This residual calcium may both combine with the calcium influx elicited by subsequent single action potentials and lead to biochemical modifications of proteins in the presynaptic terminal. At some synapses, repetitive activation leads to depression that can last for several seconds or even minutes. As in paired-pulse depression, this generally occurs at synapses that exhibit a high probability of release and is thought to result, at least in part, from a transient depletion of the synaptic vesicles that are poised to be released by an action potential.

In large part because of these short-term forms of synaptic plasticity, the strength of communication between pairs of neurons can be modified even during short bursts of presynaptic activity (e.g., five to ten action potentials at 20 to 50 Hz) (7). The functional relevance of such short-term synaptic dynamics has received much less attention than long-lasting forms of synaptic plasticity and is just beginning to be explored (8). One potential role of these short-term forms of synaptic plasticity is to transform incoming information in the temporal domain into a spatially distributed code (9,10). Furthermore, given that presynaptic proteins that may be involved in short-term plasticity may be abnormal in neuropsychiatric disorders (11), it is not unreasonable to speculate that abnormal short-term synaptic dynamics in specific neural circuits may contribute to the pathophysiology of any number of mental illnesses.

LONG-TERM SYNAPTIC PLASTICITY

During the last decade, there was enormous interest in elucidating the mechanisms responsible for activity-dependent long-lasting modifications in synaptic strength. The great interest in this topic is largely based on the simple idea that external and internal events are represented in the brain as complex spatiotemporal patterns of neuronal activity, the properties of which result from the pattern of synaptic weights at the connections made between the neurons that are contributing to this activity. The corollary to this hypothesis is that new information is stored (i.e., memories are generated) when activity in a circuit causes a long-lasting change in the pattern of synaptic weights. This simple idea was put forth by Ramon y Cajal almost 100 years ago, but experimental support for such a process was lacking until the early 1970s, when it was demonstrated that repetitive activation of excitatory synapses in the hippocampus caused an increase in synaptic strength that could last for hours or even days (12,13). This long-lasting synaptic enhancement, LTP, has been the object of intense investigation because it is widely believed that LTP provides an important key to understanding the molecular mechanisms by which memories are formed (14,15) and, more generally, by which experience modifies behavior. Furthermore, the activity- and experience-dependent refinement of neural circuitry that occurs during development shares features with learning, and thus a role for LTP in this process has been proposed (16–18).

Long-Term Potentiation

No form of synaptic plasticity has generated more interest and has been more extensively studied than LTP in the CA1 region of the hippocampus. The excitement surrounding this phenomenon derives mainly from four sources. First, there is compelling evidence from studies in rodents and higher primates, including humans, that the hippocampus is a critical component of a neural system involved in various forms of long-term memory (19). Second, several properties of LTP make it an attractive cellular mechanisms for information storage (20,21). Like memories, LTP can be generated rapidly and is prolonged and strengthened with repetition. It is also input specific in that it is elicited at the synapses activated by afferent activity and not at adjacent synapses on the same postsynaptic cell. This feature dramatically increases the storage capacity of individual neurons

TABLE 11.1. AREAS OF BRAIN IN WHICH LTP HAS BEEN DEMONSTRATED

Hippocampus	Amygdala
Dentate gyrus	Cerebellum
CA1	Thalamus
CA3	Striatum
Cerebral cortex	Nucleus acumbens
Visual	Ventral tegmental area
Somatosensory	
Motor	
Prefrontal	

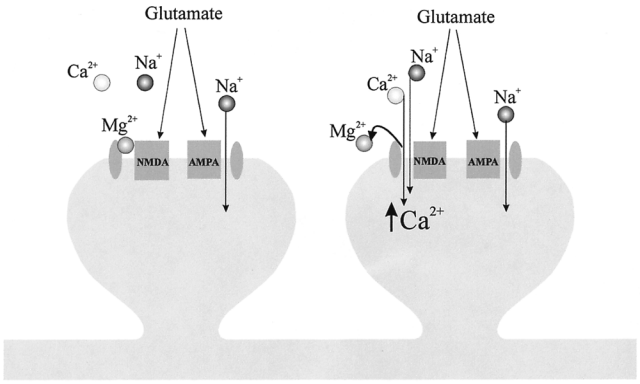

FIGURE 11.1. Model for the induction of long-term potentiation (LTP). During normal synaptic transmission (*left*), synaptically released glutamate acts on both NMDA and AMPA receptors. Na^+ flows through the AMPA receptor channel but not through the NMDA receptor channel because of the Mg^{2+} block of this channel. Depolarization of the postsynaptic cell (*right*) relieves the Mg^{2+} block of the NMDA receptor channel and allows Na^+ and Ca^{2+} to flow into the cell. The resultant rise in Ca^{2+} in the dendritic spine is a necessary trigger for the subsequent events leading to LTP.

that, because synapses can be modified independently, can participate in the encoding of many different bits of information. Third, LTP is readily generated in *in vitro* preparations of the hippocampus, thus making it accessible to rigorous experimental analysis. Indeed, much of what we know about the detailed mechanisms of LTP derives from studies of LTP at excitatory synapses on CA1 pyramidal cells in hippocampal slices. Fourth, LTP has been observed at virtually every excitatory synapse in the mammalian brain that has been studied. Table 11.1 gives a list of the brain regions in which LTP has been demonstrated, and it can be seen that regions thought to be particularly important for various forms of learning and memory are prominent. Although LTP is not a unitary phenomenon, most synapses appear to express a form of LTP that is identical or highly analogous to the LTP found at excitatory synapses on CA1 pyramidal cells. Thus, this form of LTP is the focus of the remainder of this section.

Triggering of LTP: A Critical Role for NMDA Receptors and Calcium

It is well established that the triggering of LTP requires synaptic activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors, a subtype of ionotropic glutamate receptor (see Chapter 6) and postsynaptic depolarization, which is accomplished experimentally by repetitive tetanic stimulation of synapses or by directly depolarizing the cell while continuing low-frequency synaptic activation (a so-called "pairing protocol"). How do these requirements explain the properties of LTP? During basal low-frequency synaptic transmission, synaptically released glutamate binds to two different subtypes of ionotropic glutamate receptor, termed AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA receptors, which are often, but not always (see later), co-localized on individual dendritic spines. The AMPA receptor has a channel that is permeable to monovalent cations (Na^+ and K^+), and activation of AMPA receptors provides most of the inward current that generates the excitatory synaptic response when the cell is close to its resting membrane potential (Fig. 11.1). In contrast, as described in Chapter 6, the NMDA receptor exhibits a strong voltage dependence because of the block of its channel at negative membrane potentials by extracellular magnesium. As a result, NMDA receptors contribute little to the postsynaptic response during basal synaptic activity. However, when the cell is depolarized, magnesium dissociates from its binding site within the NMDA receptor channel and allows calcium as well as sodium to enter the dendritic spine (Fig. 11.1). The resultant rise in intracellular calcium is a necessary and perhaps sufficient trigger for LTP. This local source of calcium within the dendritic spine accounts for the input specificity of LTP.

The evidence in support of this model for the initial triggering of LTP is compelling. Specific NMDA receptor antagonists have minimal effects on basal synaptic transmission but block the generation of LTP (22,23). Preventing the rise in calcium by loading cells with calcium chelators blocks LTP (24,25), whereas directly raising intracellular calcium in the postsynaptic cell mimics LTP (25,26). Furthermore, imaging studies have demonstrated that NMDA receptor activation causes a large increase in calcium level within dendritic spines (see 23 for references). The exact properties of the calcium signal that is required to trigger LTP are unknown, but a transient signal lasting only 1 to 3 seconds appears to be sufficient (27). Whether additional sources of calcium, such as release from intracellular stores, are required for the generation of LTP is unclear. It is also uncertain whether additional factors provide by synaptic activity are required. Various neurotransmitters found in the hippocampus such as acetylcholine and norepinephrine can modulate the ability to trigger LTP, and such modula-

tion may be of great importance for the functional in vivo roles of LTP. However, there is no compelling evidence to suggest that any neurotransmitter other than glutamate is required to trigger LTP.

Signal Transduction Mechanisms in LTP

A bewildering array of signal transduction molecules has been suggested to play a role in translating the calcium signal that is required to trigger LTP into a long-lasting increase in synaptic strength (28). However, for only a few of these has compelling evidence of a mandatory role in LTP been presented. A major limitation of much of the work on this topic is that investigators have not adequately distinguished molecules that are key components of the signal transduction machinery absolutely required for LTP from biochemical processes that modulate the ability to generate LTP or play a permissive role. For example, any manipulation that modifies the activity of NMDA receptors may affect LTP. Therefore, several requirements must be met for a signaling molecule to be considered a key component of the biochemical machinery that triggers LTP. First, it must be activated or produced by stimuli that trigger LTP but not by stimuli that fail to do so. Second, inhibition of the pathway in which the molecule participates should block the generation of LTP. Third, activation of the pathway should lead to LTP.

Strong evidence indicates that calcium/calmodulin–dependent protein kinase II (CaMKII) fulfills these requirements and is a key component of the molecular machinery for LTP. Inhibiting its activity pharmacologically by directly loading postsynaptic cells with CaMKII inhibitors or genetic knockout of a critical CaMKII subunit blocks the ability to generate LTP (29–31). Conversely, acutely increasing the postsynaptic concentration of active CaMKII increases synaptic strength and occludes LTP (32,33). Furthermore, CaMKII undergoes autophosphorylation after the triggering of LTP (34,35). That this autophosphorylation is required for LTP was demonstrated by the finding that genetic replacement of endogenous CaMKII with a form lacking the autophosphorylation site prevented LTP (36).

Several other protein kinases have also been suggested to play roles in the triggering of LTP, but the experimental evidence supporting their role is considerably weaker than for CaMKII. Activation of the cyclic adenosine monophosphate–dependent protein kinase (PKA), perhaps by activation of a calmodulin-dependent adenylyl cyclase, has been suggested to boost the activity of CaMKII indirectly by decreasing competing protein phosphatase activity (37,38). This presumably happens by phosphorylation of inhibitor-1, an endogenous protein phosphatase inhibitor (see section on LTD later). Protein kinase C may play a role analogous to CaMKII, whereas the tyrosine kinases Fyn and Src may indirectly modulate LTP by affecting NMDA receptor function (see 23 for references). The mitogen-activated protein kinase (MAPK) has also been suggested to be important for LTP, albeit in unknown ways.

Expression Mechanisms and LTP

In the 1990s, tremendous confusion and controversy surrounded the seemingly simple issue of whether LTP is caused primarily by presynaptic or postsynaptic modifications. The great challenge to answering this question largely stemmed from the great technical difficulties inherent in examining the changes the occur at individual synapses that are embedded in a complex network in which each cell receives 10,000 or more synapses. Most neurobiologists studying this question agree that the simplest postsynaptic change that could cause LTP would be a change in AMPA receptor function or number, whereas the simplest presynaptic change would be an increase in the probability of neurotransmitter release.

Most studies examining this issue have used electrophysiologic assays, and most of these are inconsistent with the hypothesis that the release of glutamate increases significantly during LTP (23,39). For example, changes in transmitter release probability invariably influence various forms of short-term synaptic plasticity such as paired-pulse facilitation, yet these phenomena are not affected by LTP. To measure glutamate release more directly, two approaches were used. One took advantage of the finding that glial cells tightly ensheath synapses and respond to synaptically released glutamate by activation of electrogenic transporters that generate a current directly proportional to the amount of glutamate released (40,41). The other took advantage of use-dependent antagonists of the NMDA receptor or of a mutant AMPA receptor that lacks the GluR2 subunit. These antagonists decrease synaptic currents at a rate that is directly proportional to the probability of transmitter release (42,43). LTP had no discernible effect on these measures, even though they were affected in the predicted fashion by manipulations known to increase transmitter release.

In addition to these negative findings, certain electrophysiologic and biochemical measures were found to increase during LTP. An increase in the amplitude of miniature electrophysiologic synaptic currents (mEPSCs), which represent the postsynaptic response to the spontaneous release of a single quantum of neurotransmitter, normally indicates an increase in the number or function of postsynaptic neurotransmitter receptors. Such an increase occurs during LTP (44), as well as after manipulations that load dendritic spines with calcium (45,46). A more direct way of monitoring changes in AMPA receptors is to measure the postsynaptic response to direct application of agonist,

and such responses have also been reported to increase, albeit gradually (47).

That LTP is caused by a modification of AMPA receptors is supported by the finding that LTP causes an increase in the phosphorylation of the AMPA receptor subunit GluR1 at the site that is known to be phosphorylated by CaMKII (as well as PKC) (35,48,49). Using expression systems, this phosphorylation has been shown to increase the single-channel conductance of AMPA receptors (50). Because an increase in single-channel conductance of AMPA receptors has been reported to occur during LTP (51), one mechanism that seems likely to contribute to LTP is CaMKII-dependent phosphorylation of GluR1. Consistent with this idea, genetic knockout of GluR1 has been found to prevent the generation of LTP (52).

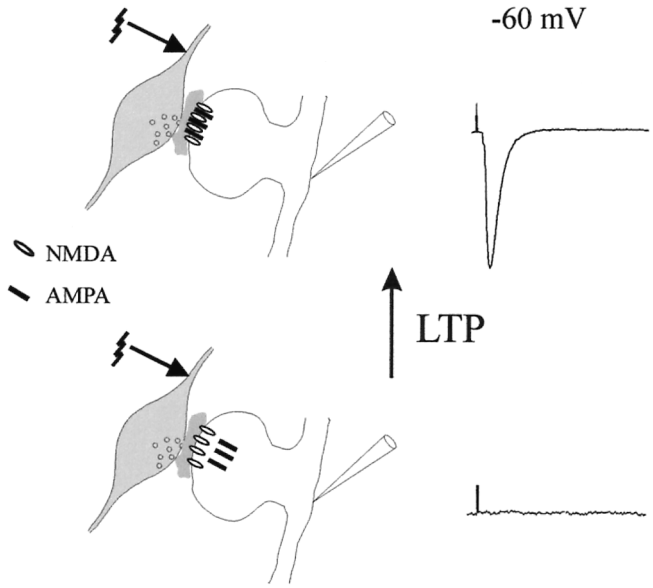

FIGURE 11.2. Diagram of the silent synapse hypothesis. A synapse is functionally silent when it expresses NMDA receptors but not AMPA receptors in its plasma membrane (bottom). The induction of LTP causes the insertion of AMPA receptors (top) from a putative cytosolic pool. To the right of each diagram are the synaptic currents (i.e., EPSCS) that would be recorded from the corresponding synapse.

Silent Synapses and Quantal Synaptic Transmission

Although the evidence presented thus far makes a strong case for postsynaptic changes contributing to LTP, there remained one reproducible experimental result that was difficult to reconcile with this idea. This result derived from experiments that took advantage of the finding that the action potential-dependent release of quanta of neurotransmitter at individual synapses is probabilistic, and therefore release occurs only 10% to 40% of the time. Therefore, if a single synapse or a very small number of synapses is activated once every few seconds, on some of the trials no postsynaptic response is recorded, that is, a so-called failure occurs. An extensively replicated finding is that LTP causes a decrease in the proportion of failures that occur (see 53 for review). Because these failures were assumed to result from failures of neurotransmitter release, it was concluded that LTP involves an increase in the probability of transmitter release.

How can this result be reconciled with all the evidence suggesting that LTP is caused by modulation of AMPA receptors and is not accompanied by an increase in glutamate release? One straightforward idea to explain this apparent discrepancy is the *silent synapse hypothesis* (54), which predicts that some synapses express only NMDA receptors, whereas others express both AMPA and NMDA receptors (Fig. 11.2). Synapses with only NMDA receptors would be functionally silent at hyperpolarized membrane potentials, and thus, when transmitter is released, they would not yield a response. However, LTP at such silent synapses could occur by the rapid expression of AMPA receptors, and such a mechanism would account for the apparent change in failure rate.

There is now strong evidence to support this model of LTP. First, it is possible to record EPSCs that are mediated solely by NMDA receptors, and applying an LTP induction protocol at such synapses causes the rapid appearance of AMPA receptor-mediated EPSCs (55,56). Second, immunocytochemical analysis demonstrates that AMPA receptors are not found at a significant percentage of hippocampal synapses, whereas all synapses appear to contain NMDA receptors (see 23 for references). Third, LTP has been shown to cause the delivery of green fluorescent protein (GFP)-tagged AMPA receptors to dendritic spines and the insertion of recombinant AMPA receptors into the synaptic plasma membrane (57,58). Fourth, AMPA and NMDA receptors interact with different proteins at the synapse (59), a finding suggesting that they are regulated independently. Fifth, interference with membrane fusion and presumably exocytosis in the postsynaptic cells impairs LTP (60) and AMPA receptors can interact with proteins involved in membrane fusion (61). These findings are consistent with the idea that membrane fusion may be an important mechanism for the delivery of AMPA receptors to the synaptic plasma membrane.

Virtually all the data presented thus far are consistent with the simple model that LTP, at least initially, is caused by the phosphorylation of AMPA receptors and the delivery or clustering of AMPA receptors within the synaptic plasma membrane (23). These events will presumably occur both at synapses that already contain functional AMPA receptors and ones that are functionally silent. As discussed later, LTD appears to involve the converse process, that is, the removal or endocytosis of AMPA receptors. At the end of this chap-

ter, I discuss how these changes in the number of AMPA receptors at individual synapses may lead to more permanent structural changes, which, in turn, may mediate long-lasting forms of experience-dependent plasticity.

Long-Term Depression

Like LTP, LTD has been demonstrated in a large number of different brain regions and comes in a variety of different forms (62–64). This section focuses on the NMDA receptor-dependent form of LTD found at excitatory synapses on CA1 pyramidal cells and that appears to result, in large part, from a reversal of the processes that mediate LTP.

Triggering of LTD: A Critical Role for NMDA Receptors and Calcium

LTD is normally generated by prolonged (3- to 15-minute) low-frequency (1- to 5-Hz) afferent stimulation or by a pairing protocol during which the cell is held at approximately −50 mV. It shares many features with LTP including input specificity, and it can completely reverse LTP, a process often termed *depotentiation.* Surprisingly, the triggering of LTD requires NMDA receptor activation and an increase in postsynaptic calcium concentration (65,66). This can occur because at resting membrane potentials, the voltage-dependent block of the NMDA receptor channel by magnesium is not 100% effective, and thus, each stimulus will cause a very small amount of calcium entry. Current evidence suggests that the specific properties of the intracellular calcium signal dictate whether LTP or LTD is generated by a specific pattern of synaptic activity, with LTD requiring a modest rise in calcium (67) and LTP requiring a large rise beyond some critical threshold value (68). The temporal characteristics of this calcium signal may also be important.

Signal Transduction Mechanisms in LTD: A Role for Protein Phosphatases

If calcium is the critical triggering signal for LTD, it must be capable of activating biochemical processes that reverse LTP. Because LTP results, at least in part, from activation of protein kinases, a reasonable hypothesis is that LTD is caused by preferential activation of protein phosphatases, several of which are known to be found at excitatory synapses (69). This idea was first proposed in a theoretic article (70) that presented a specific model that accounted for the bidirectional control of synaptic strength by calcium (Fig. 11.3). It proposed that a balance between CaMKII activity and protein phosphatase 1 (PP1) influenced synaptic strength by controlling the phosphorylation state of unidentified synaptic phosphoproteins. Small rises in calcium favored activation of PP1, whereas large rises were required to increase CaMKII activity. Because PP1 is not directly influenced by calcium, a well-established calcium-dependent phosphatase cascade was invoked to translate the calcium signal into increased PP1 activity (69). This cascade (Fig. 11.3) begins with activation of the calcium/calmodulin–dependent phosphatase calcineurin (also known as protein phosphatase 2B or PP2B). PP2B then dephosphorylates inhibitor 1 (I1), a phosphoprotein that, in its phosphorylated state, is a potent inhibitor of PP1. Thus, activa-

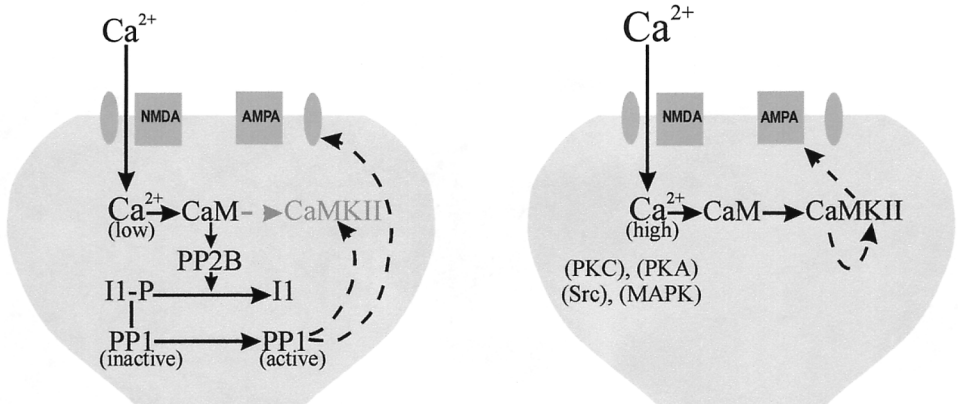

FIGURE 11.3. Model for the signaling cascades responsible for LTD and LTP. LTD is generated when a low rise in Ca^{2+} binds to calmodulin (cam) and activates calcineurin (PP2B). Calcineurin then dephosphorylates inhibitor 1 (I1), which therefore no longer inhibits protein phosphatase 1 (PP1). Active PP1 may act on any number of substrates including camkii or AMPA receptors (*left*). LTP is generated when a high rise in Ca^{2+} activates camkii. Other protein kinases that may also be involved in triggering LTP are protein kinase C (PKC), cAMP-dependent protein kinase (PKA), the tyrosine kinase src (Src), and MAP kinase (MAPK)(*right*).

tion of PP2B causes an increase in PP1 activity through a mechanism of disinhibition. An attractive feature of this model is that the affinity of PP2B for calcium/calmodulin is significantly greater than that of CaMKII. Therefore, PP2B will be preferentially activated by small increases in synaptic calcium levels. Furthermore, a large rise in calcium will preferentially increase protein kinase activity not only by directly activating CaMKII but also by leading to the activation of PKA, which phosphorylates I1 and thereby further inhibits PP1.

Several experimental results are consistent with this model, which currently remains the leading hypothesis for the triggering of LTD. Pharmacologic inhibitors of PP1 or PP2B, when loaded directly into CA1 pyramidal cells, prevent the generation of LTD (71,72). Furthermore, loading cells with the phosphorylated form of I1 blocked LTD. However, although the results of such inhibitor studies are consistent with an important role for protein phosphatases in triggering LTD, other interpretations are possible, and more experimental work testing this hypothesis needs to be performed. Most notably, if PP1 plays a role in LTD analogous to that played by CaMKII in LTP, it should be possible to increase PP1 activity directly in postsynaptic cells and to mimic LTD.

Expression Mechanisms of LTD: A Role for Endocytosis of AMPA Receptors

The silent synapse hypothesis discussed earlier suggested that LTP involves the insertion of AMPA receptors into the synaptic plasma membrane. Consistent with the idea that LTD is a reversal of LTP, there is now considerable evidence that LTD involves removal (i.e., endocytosis) of synaptic AMPA receptors. The first direct evidence that the synaptic localization of AMPA receptors could be rapidly modified was the demonstration that, in cultured hippocampal neurons, short application of glutamate receptor agonists caused rapid a loss of synaptic AMPA receptors with no significant effect on the synaptic localization of NMDA receptors (73). This agonist-induced loss of synaptic AMPA receptors was subsequently shown to result from dynamin-dependent endocytosis (74). Perhaps more important, synaptically triggered LTD in the cultured neurons was accompanied by a decrease in the number of synaptic surface AMPA receptors with no discernible effect on the distribution of NMDA receptors (75). Consistent with these findings, loading CA1 pyramidal cells with inhibitors of endocytosis prevented the generation of LTD (76). These inhibitors also caused a gradual increase in the size of the synaptic responses, whereas inhibitors of exocytosis caused a gradual decrease (76). These results suggest that there is a pool of AMPA receptors that cycle into and out of the synaptic plasma membrane fairly rapidly and that LTP and LTD may involve a modification of the kinetics of these processes.

Structural Changes and Long-Term Synaptic Plasticity

How are the changes in synaptic strength that occur following the triggering of LTP or LTD maintained for periods lasting weeks or perhaps even years? Although the answer to this question is unknown, recent evidence suggests that the mechanisms described previously may be the initial steps in a more profound anatomic restructuring of synapses, including perhaps the growth of new synapses and the pruning away of preexisting ones. Dendritic spines, the postsynaptic sites that presynaptic boutons contact, have a complex ultrastructure and come in a large variety of shapes (77). With technical advances in microscopy and the use of recombinant fluorescent proteins such as GFP, it has become possible to image individual spines in living neurons. Such experiments have shown that spines are not static but can undergo rapid shape changes (78,79) that are influenced by activity (80). Furthermore, strong synaptic activation of the type that triggers LTP causes an NMDA receptor-dependent growth of spines as well as filopodia, which may be the precursors of spines (81,82). Prolonged application of NMDA to cultured neurons also can cause the loss of spines (83), a finding indicating that, like synaptic strength, the growth and loss of dendritic spines may be under the control of NMDA receptors.

Changes in synapse structure in response to activity also have been extensively explored using more standard electron microscopic techniques. One specific morphologic modification repeatedly associated with increased neuronal activity involves a reorganization of the postsynaptic density (PSD), the electron-dense thickening that contains synaptic glutamate receptors. Specifically, it has been suggested that LTP is associated with an increase in the fraction of synapses that contain discontinuities in their PSDs, termed *perforated synapses* (see 84 for references). This idea is strongly supported by studies in which the synapses activated by strong tetanic stimulation were identified in electron microscopic sections and were found to have larger total PSD surface areas and a larger proportion of perforated synapses (85, 86). Several lines of evidence suggest that this growth of PSDs and their eventual perforation may be initiated by increasing the number of AMPA receptors in the PSD (see 84 for references).

The insertion of new AMPA receptors in the PSD and the generation of perforated synapses may also be early events in the generation of new synapses by a process of splitting or duplication of existing spines (87,88). Consistent with this hypothesis, LTP may be associated with an increase in spine density (84), as well as the frequency of multiple-spine synapses in which two adjacent spines arising from the same dendrite contact a single presynaptic bouton

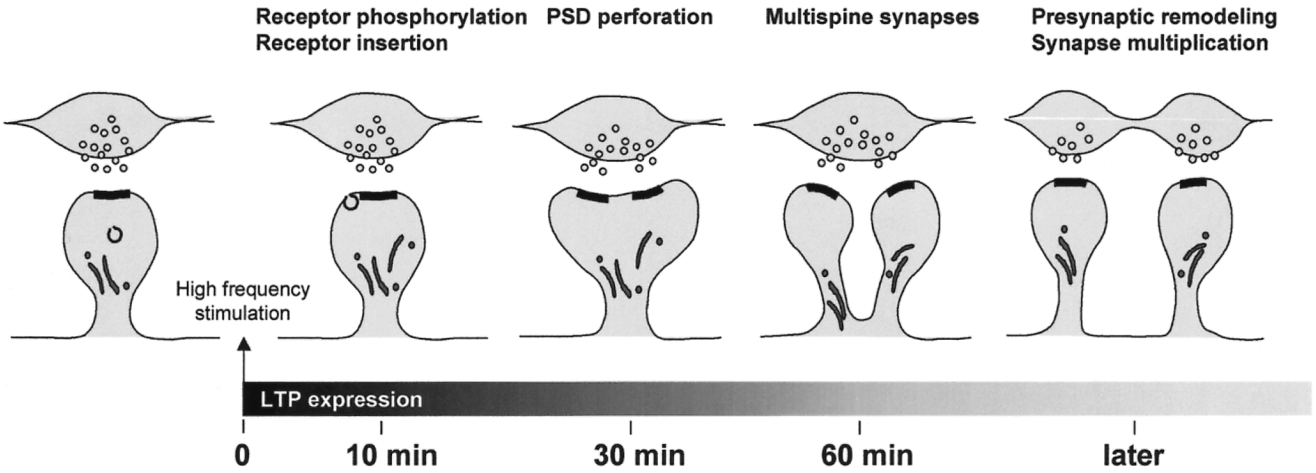

FIGURE 11.4. Model for sequence of events leading to structural changes following triggering of LTP. Within 10 minutes of LTP induction, AMPA receptors are phosphorylated and inserted into the postsynaptic membrane. This process leads to an increase in the size of the postsynaptic density (PSD) and the production of perforated synapses within 30 minutes. By 1 hour, some perforated synapses split and form multispine synapses. Eventually, retrograde communication, perhaps involving cell-adhesion molecules, leads to presynaptic structural changes and the production of new synapses.

(86). These types of observations have led to a model (Fig. 11.4) (84) that proposes a sequence of events by which the insertion of AMPA receptors leads to a growth and perforation of the PSD and eventually to multiple-spine synapses. Subsequently, retrograde communication perhaps involving cell adhesion molecules would cause appropriate presynaptic structural chances such that a completely new, independent synapse is formed. An attractive corollary to this hypothesis is that LTD involves a shrinkage of the PSD and eventually leads to a complete loss of the dendritic spine and its corresponding presynaptic bouton. However, minimal work on the structural changes associated with LTD has been performed.

An attractive feature of incorporating structural changes into the mechanisms of long-term synaptic plasticity is that it provides a straightforward means by which the activity generated by experience can cause very long-lasting modifications of neural circuitry. Structural changes also may explain the well-known requirement of long-lasting forms of synaptic plasticity for new protein synthesis and gene transcription (see 89 to 91 for reviews).

CONCLUSIONS

This is a brief review of some of the most common forms of synaptic plasticity found at excitatory synapses throughout the mammalian brain. Although relatively little is known about the functional roles of these phenomena, such changes in synaptic function and structure remain the leading candidates for some of the fundamental mechanisms by which experiences of any type cause the reorganization of neural circuitry and thereby modify thoughts, feelings, and behavior. One hopes that it is also apparent why understanding the mechanisms of synaptic plasticity has important implications for many branches of clinical neuroscience. For example, the development of many pathologic behaviors, such as drug addiction, likely depends on the maladaptive use of neural mechanisms that normally are used for adaptive learning and memory (92). Similarly, the recovery of function following brain injury or the successful pharmacologic and behavioral treatment of mental illness also certainly must result from the reorganization of neural circuitry that is in part achieved by synaptic plasticity mechanisms. Thus further elucidation of the mechanisms of phenomena such as LTP and LTD will continue to have implications for all those interested in the neural basis of cognition and behavior.

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