REVIEW: NEUROSCIENCE

Long-Term Potentiation—A Decade of Progress?

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Long-term potentiation of synaptic transmission in the hippocampus is the leading experimental model for the synaptic changes that may underlie learning and memory. This review presents a current understanding of the molecular mechanisms of this long-lasting increase in synaptic strength and describes a simple model that unifies much of the data that previously were viewed as contradictory.

Throughout the 20th century, since the classic descriptions of nervous system structure and function by Ramon y Cajal and Sherrington, the remarkable capacity of the brain to translate transient experiences into seemingly infinite numbers of memories that can last for decades has been attributed to long-lasting, activity-dependent changes in the efficacy of synaptic communication. Experimental support for such a process was lacking, however, until the early 1970s when it was shown that repetitive activation of excitatory synapses in the hippocampus, a brain region long known to be essential for learning and memory, caused an increase in synaptic strength that could last for hours or even days (*1*). Over the past 15 to 20 years, this long-lasting synaptic enhancement, known as long-term potentiation (LTP), has been the object of intense investigation because it is widely, although not universally, believed that LTP provides an important key to understanding the cellular and molecular mechanisms by which memories are formed and stored (*2*). 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 (*3*). Indeed, the enormous interest in LTP is illustrated by the fact that a simple MEDLINE search with the keywords "long-term potentiation" retrieves more than 3000 papers published this decade—almost one per day.

Despite the enormous interest in LTP, it has proven difficult to elucidate the detailed cellular and molecular changes that underlie LTP. In fact, for over a decade, there has been a vigorous, highly visible debate about whether the changes that occur soon after the generation of LTP happen on the pre- or postsynaptic side of the synapse. Although it seems a simple question, the answer constrains the types of molecular changes that underlie LTP and therefore may be responsible for key aspects of memory storage. Recent experimental evidence suggests that a resolution is close. In this review, we briefly summarize the current understanding of the mechanisms underlying LTP and present a simple hypothesis that unifies much of the data, which have previously been viewed as contradictory (*4*).

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Basic Properties of LTP

Because the vast majority of experimental work aimed at understanding the mechanisms of LTP has been performed on excitatory synapses in the hippocampus, specifically on the synapses between the Schaffer collateral and commissural axons and the apical dendrites of CA1 pyramidal cells, this review focuses on findings at these synapses. Although other forms of LTP display different properties (*2*), the LTP at CA1 synapses, which release the neurotransmitter glutamate, appears to be identical (or very similar) to the LTP observed at glutamatergic excitatory synapses throughout the mammalian brain, including the cerebral cortex (*5*). Indeed, the fact that LTP can be most reliably generated in brain regions involved in learning and memory is often used as evidence for its functional relevance. Whether LTP is triggered during learning and is causally related to memory formation is an actively debated topic that is beyond the scope of this review (*6*). In our view, LTP is a fundamental property of the majority of excitatory synapses in the mammalian brain and, as such, is likely to subserve many functions, including underlying some forms of learning and memory.

In the CA1 region of the hippocampus, LTP is input-specific, which means that when generated at one set of synapses by repetitive activation, the increase in synaptic strength does not normally occur in other synapses on the same cell. This property is advantageous because it greatly increases the storage capacity of individual neurons. LTP is also associative, that is, strong activation of one set of synapses can facilitate LTP at an independent set of adjacent active synapses on the same cell if both sets of synapses are activated within a finite temporal window. Associativity has often been viewed as a cellular analog of associative or classical conditioning.

Although LTP is triggered rapidly (within seconds), it can last for hours in in vitro preparations and days in vivo. As do long-lasting forms of memory, the late phases of LTP appear to require gene transcription and new protein synthesis (*2*). If LTP contributes to memory formation by triggering long-lasting, perhaps permanent, changes in neural circuitry, understanding the molecular mechanisms by which this occurs clearly is important, although technically difficult. Our discussion will be limited to the events that occur during the first ~60 minutes of LTP.

Triggering Mechanisms

It is well accepted that the triggering of LTP requires synaptic activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors, a subtype of glutamate receptor. This activation requires depolarization of the postsynaptic cell, which is usually accomplished experimentally by repetitive tetanic stimulation of synapses or by directly depolarizing the cell while continuing low-frequency synaptic activation (a "pairing protocol") (*2*). How do these requirements account for the properties of LTP? During low-frequency synaptic transmission, the neurotransmitter glutamate binds to two different subtypes of receptor that are often, but not always (see below), colocalized on individual dendritic spines, the small (~1 μm^3) outgrowths from the dendritic shaft that are the postsynaptic site of synaptic contacts (Fig. 1). The first is the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptor, which has a channel permeable to monovalent cations (Na^+ and K^+) and which provides the majority of inward current for generating synaptic responses when the cell is close to its resting membrane potential. The second is the NMDA receptor, which exhibits a profound voltage dependence because of the blocking of its channel by extracellular Mg^{2+}, such that it contributes little to the basal postsynaptic response during low-frequency synaptic transmission. However, when the postsynaptic cell is depolarized during the induction of LTP, Mg^{2+} dissociates from its binding site within the NMDA receptor channel, allowing Ca^{2+} as well as Na^+ to enter the dendritic spine. The consequent rise of intracellular Ca^{2+} is the critical trigger for LTP. This local source of

Ca^{2+} within the dendritic spine accounts for the input specificity of LTP. Associativity occurs because strong activation of one set of synapses depolarizes adjacent regions of the dendritic tree.

The evidence in support of this model is compelling and almost universally agreed upon. Specific NMDA receptor antagonists have minimal effects on basal synaptic transmission but completely block the generation of LTP (*2, 7*). Similarly, preventing the rise in postsynaptic Ca^{2+} with Ca^{2+} chelators blocks LTP, whereas directly raising the amount of postsynaptic Ca^{2+} by photolysis of caged Ca^{2+} can mimic LTP (*8*). Furthermore, imaging studies have demonstrated directly increases in Ca^{2+} within dendritic spines due to NMDA receptor activation (*9*). Currently, it is thought that a short-lasting (1- to 3-s) threshold level of Ca^{2+} must be reached to trigger LTP. Whether the influx of Ca^{2+} alone is adequate or an amplification due to Ca^{2+} release from intracellular stores is also required for triggering LTP remains unclear (*10*). Another important unresolved issue is whether an increase in Ca^{2+} alone is sufficient to trigger LTP or whether additional factors, presumably provided by synaptic activity, are required (*2*). One candidate for such additional input is a family of G protein–coupled receptors known as metabotropic glutamate receptors. These receptors are found at most excitatory synapses, but their activation does not appear to be absolutely required for the generation of LTP in CA1 pyramidal cells. They may, however, modulate the triggering of LTP (*11*).

Increases in postsynaptic Ca^{2+} that are NMDA receptor–dependent and that do not reach the threshold for LTP can generate either a short-term potentiation (STP) that decays to baseline over the course of 5 to 20 minutes or long-term depression (LTD), a long-lasting decrease in synaptic strength that may be due to a reversal of the mechanisms underlying LTP (*12*). Thus, any manipulation that influences the magnitude or dynamics of Ca^{2+} increases within dendritic spines may profoundly influence the form of the resulting synaptic plasticity. Although NMDA receptors are the primary source of Ca^{2+} entry into spines, activation of dendritic voltage-dependent Ca^{2+} channels also substantially raises Ca^{2+} levels and can generate LTP, STP, or LTD. Perhaps because of the distinct subcellular localization of Ca^{2+} channels, however, the LTP due to activation of Ca^{2+} channels may use mechanisms distinct from NMDA receptor–dependent LTP (*13*) and will not be considered further here.

Signal Transduction Mechanisms

What biochemical pathways are activated by Ca^{2+} and are required for translating the Ca^{2+} signal into an increase in synaptic strength? A review of the literature generates an enormous, even bewildering, list of candidate signal transduction molecules. However, this research has not 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 (*14*). There are only a few molecules for which the evidence of a key, mandatory role in LTP is compelling.

Overwhelming evidence implicates α-calcium-calmodulin–dependent proteinkinase II (CaMKII) as a key component of the molecular machinery of LTP (*2, 15*). CaMKII is found in high concentrations in the postsynaptic density, a submembrane component of the dendritic spine that also contains the glutamate receptors that mediate synaptic transmission (*16*). Postsynaptic injection of inhibitors of CaMKII or genetic deletion of a critical CaMKII subunit blocks the ability to generate LTP (*17*). That CaMKII plays a direct, causal role in LTP is strongly supported by the finding that synaptic transmission is enhanced and LTP is occluded by increasing the concentrations of constitutively active CaMKII in CA1 cells (*18*).

An important property of CaMKII is that when autophosphorylated on Thr^{286}, its activity is no longer dependent on Ca^{2+}-calmodulin (CaM) (*15, 19*). This allows its activity to continue long after the Ca^{2+} signal has returned to baseline. Biochemical studies have demonstrated that this autophosphorylation does in fact occur after the triggering of LTP (*20, 21*). That CaMKII autophosphorylation is required for LTP was convincingly demonstrated by an elegant use of molecular genetic techniques in which replacement of endogenous CaMKII with a form of CaMKII containing a Thr^{286} point mutation blocked LTP (*22*). A final important piece of evidence implicating CaMKII in LTP is that it can directly phosphorylate the AMPA receptor subunit, GluR1, in situ, and this has been shown to occur following the generation of LTP (*21*) (see discussion below).

Several other protein kinases, including protein kinase C (PKC), cyclic adeonosine 3′,5′-monophosphate (cAMP)–dependent protein kinase (PKA), the tyrosine kinase Src, and mitogen-activated protein kinase (MAPK), have also been suggested to contribute to LTP (*2*). The evidence in support of critical roles for these kinases is, however, considerably weaker than that for CaMKII. PKC has been suggested to play a role analogous to that of CaMKII, because PKC inhibitors have been reported to block LTP and because increasing postsynaptic PKC activity can enhance synaptic transmission (*23*). However, it remains to be determined whether the synaptic enhancement due to increasing PKC activity uses the same mechanisms as LTP. PKA has been suggested to boost CaMKII activity indirectly by decreasing competing protein phosphatase activity by means of phosphorylation of inhibitor-1, an endogenous protein phosphatase inhibitor (*24*). Src may participate in a more indirect way by enhancing NMDA receptor function during LTP induction (*25*). The specific function of MAPK in LTP remains unknown (*26*).

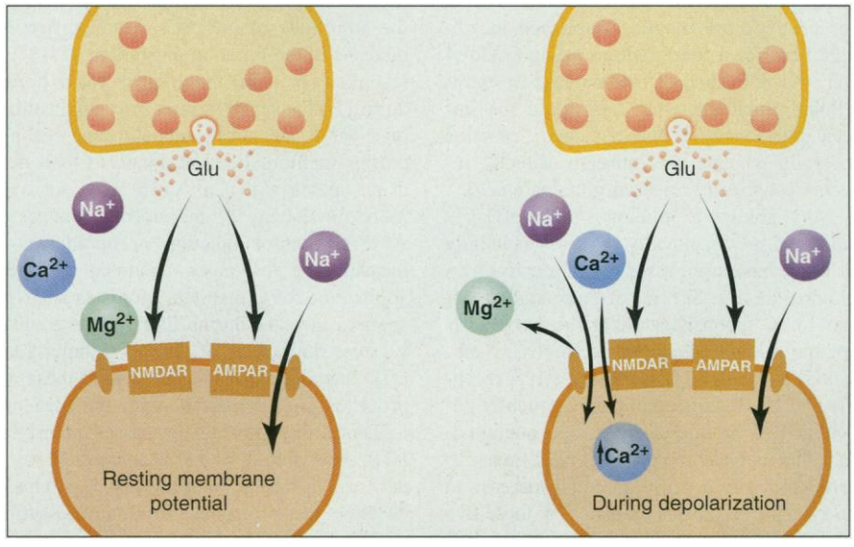

Fig. 1. Model for the induction of LTP. During normal synaptic transmission, glutamate (Glu) is released from the presynaptic bouton and acts on both AMPA receptors (AMPARs) and NMDA receptors (NMDARs). However, Na^+ flows only through the AMPA receptor, but not the NMDA receptor, because Mg^{2+} blocks the channel of the NMDA receptor. Depolarization of the postsynaptic cell relieves the Mg^{2+} block of the NMDA receptor channel, allowing Na^+ and Ca^{2+} to flow into the dendritic spine by means of the NMDA receptor. The resultant rise in Ca^{2+} within the dendritic spine is the critical trigger for LTP.

Members of another class of signal transduction molecules related to LTP are the postulated retrograde messengers. If presynaptic changes in transmitter release contribute to LTP, a retrograde messenger must be released from the postsynaptic cell to modify presynaptic function, because the initial triggering of LTP clearly resides in the postsynaptic cell. Molecules that have received the most attention as possible retrograde messengers include nitric oxide (NO), carbon monoxide, arachidonic acid, and platelet-activating factor (*2, 27*). Currently it is difficult to conclude that any of these molecules are absolutely required for the generation of LTP (*28*).

Expression Mechanisms

No question concerning LTP has generated more debate and confusion over the last two decades than the seemingly simple question of whether the increase in synaptic strength is due primarily to a pre- or postsynaptic modification. Great technical difficulties are inherent in examining the changes at individual synapses that are embedded in a network in which each individual cell receives about 10,000 to 30,000 synapses. Most neurobiologists agree that the simplest postsynaptic change that could cause LTP would be a modification in AMPA receptor function or number (or both), whereas the simplest presynaptic change would be an increase in the probability of neurotransmitter release.

In early attempts to address this issue, an increase in extracellular glutamate after the induction of LTP, consistent with a presynaptic change, was measured (*2*). The finding shortly thereafter that the induction of LTP was clearly postsynaptic established the requirement for a retrograde messenger. However, the relevance of the increase in extracellular glutamate after LTP induction has been questioned, in part because it remains uncertain whether this measure directly reflects synaptically released glutamate (*29*).

Most studies examining whether LTP is expressed presynaptically or postsynaptically (or both) have used a variety of electrophysiological assays. Several of these studies appear to be inconsistent with the hypothesis that the release of glutamate increases substantially during LTP. Because AMPA receptors and NMDA receptors are frequently colocalized at individual synapses, manipulations that increase the probability of transmitter release would cause an equal increase in the synaptic responses mediated by these two subtypes of receptor. Most investigators find, however, that LTP increases the AMPA receptor–mediated excitatory postsynaptic current (EPSC) to a greater extent than the NMDA receptor–mediated EPSC (*30, 31*). Changes in transmitter release probability dramatically influence various forms of short-lasting (0.5- to 3-s) presynaptic plasticity such as paired-pulse facilitation, yet these phenomena are minimally affected by LTP (*32*). In addition, if LTP involved an increase in release probability, it should be possible to occlude LTP by increasing this probability to its maximum, but this has no effect on LTP (*33*).

To measure glutamate release more directly, two additional approaches have been taken. One utilized use-dependent antagonists of the NMDA receptor or a mutant AMPA receptor that lacks the specific subunit GluR2. These antagonists decrease the EPSC at a rate that is directly proportional to the probability of transmitter release (*34*). The other approach took advantage of the fact that glial cells tightly ensheath synapses and respond to synaptically released glutamate by activation of electrogenic transporters, which generate a current that is directly proportional to the amount of glutamate released (*35*). Manipulations known to increase transmitter release affected all of these measures as predicted, but LTP had no discernible effect.

In addition to these negative findings, the case for postsynaptic modifications accounting for LTP is made much stronger by a number of recent electrophysiological and biochemical measures that were found to increase during LTP. Measurement of miniature EPSCs (mEPSCs) is a classic method of determining the locus of any synaptic change. These postsynaptic events are due to the spontaneous exocytosis of individual presynaptic vesicles, each containing multimolecular packets of transmitter termed quanta. If one assumes that the amount of glutamate in each vesicle is relatively fixed, an increase in the amplitude of mEPSCs would reflect an increase in the function or number of AMPA receptors (or both). Such an increase occurs during LTP, as well as after brief applications of NMDA or strong depolarizing voltage pulses, manipulations designed to load dendritic spines with Ca^{2+} (*36, 37*). An even more direct way of monitoring changes in AMPA receptor function or number is to measure the responses generated by direct application of glutamate agonists; such responses increase during LTP, albeit gradually, over the course of tens of minutes (*38*). LTD has also been examined with these approaches and, consistent with the idea that LTD is a reversal of the processes underlying LTP, was found to be accompanied by a decrease in mEPSC amplitude (*37, 39*) and a decrease in the response to glutamate (*40*).

What is the mechanism of this change in AMPA receptor responsiveness? Over the last few years, much evidence has accumulated that phosphorylation of the AMPA receptor subunit GluR1 is critically important for this change in responsiveness. The AMPA receptor in CA1 pyramidal cells is a heteromer composed primarily of GluR1 and GluR2 subunits. Both in native hippocampal cells and expression systems, GluR1 can be phosphorylated on Ser^{831} by CaMKII and PKC, whereas PKA phosphorylates Ser^{845} (*21, 41*). The induction of LTP specifically increases the phosphorylation of Ser^{831}, an effect that is blocked by a CaMKII inhibitor (*21*). This phosphorylation event increases the single-channel conductance of homomeric GluR1 AMPA receptors (*42*). Because an increase in AMPA receptor single-channel conductance also occurs during LTP (*43*), it appears likely that one mechanism underlying LTP is CaMKII-mediated phosphorylation of the AMPA receptor subunit GluR1. Further support for this idea comes from the recent report that genetic deletion of GluR1 prevents the generation of LTP in CA1 pyramidal cells (*44*). It is interesting that LTD is accompanied by a dephosphorylation of Ser^{845}, a site that appears to be constitutively phosphorylated under basal conditions (*45*).

Quantal Synaptic Transmission

The evidence presented thus far may make the reader wonder why there has been any debate about the locus of expression of LTP. The source of this intense discussion derives almost entirely from experiments that took advantage of the fact that the action potential–dependent release of quanta is a probabilistic and rather infrequent event at individual synapses, such that release occurs only 10 to 40% of the time. Therefore, if a single or very small number of synapses are activated once every few seconds, a mixture of so-called failures and successes (small quantal EPSCs) is recorded. An extensively replicated finding is that after the generation of LTP, the proportion of synaptic failures decreases (*2, 46*). Because these failures have been assumed to be due to failures of neurotransmitter release, it was concluded that LTP involved an increase in the probability of neurotransmitter release. Consistent with this conclusion was the observation that the variation around the mean of the EPSCs decreased during LTP (*46*). This coefficient of variation (CV; SD/mean) is thought to be inversely proportional to the quantal content (the average number of synapses that are activated and release neurotransmitter with each stimulation). If the probability of release increases during LTP, then on average the quantal content will increase and the coefficient of variation will decrease.

These results posed a serious dilemma. How could all the results that argued against an increase in transmitter release probability and for a postsynaptic change during LTP be reconciled with this decrease in failure rate and the CV? The first important clue came from the observation that the CV of AMPA receptor–mediated EPSCs was greater than

the CV of NMDA receptor–mediated EPSCs (*46*). This suggested that with any given stimulus, synaptically released glutamate activated more NMDA receptor–containing synapses than AMPA receptor–containing synapses. A simple explanation for this would be that some excitatory synapses express only NMDA receptors (but not AMPA receptors), whereas others express both NMDA receptors and AMPA receptors. Such NMDA receptor–only synapses would be functionally silent at hyperpolarized membrane potentials (because of the strong voltage dependence of the NMDA receptor) and thus, even when transmitter is released, would not yield a response. Furthermore, if the induction of LTP caused the rapid conversion of these functionally silent, NMDA receptor–only synapses to synapses that also expressed AMPA receptors, the change in failure rate and CV during LTP could be explained without invoking a change in neurotransmitter release probability.

There is now reasonably strong electrophysiological and anatomical evidence that supports this model of a rapid and selective upregulation of AMPA receptors after the induction of LTP. First, several groups have demonstrated that it is possible to record EPSCs that are mediated solely by NMDA receptors and that an LTP induction protocol causes the rapid appearance of AMPA receptor–mediated EPSCs (*31, 47*). Second, immunocytochemical analysis has shown undetectable levels of AMPA receptors in a substantial proportion of synapses in native hippocampal tissue and hippocampal cultures, although all synapses appear to contain NMDA receptors (*48, 49*). It is interesting that synapses that do not express NMDA receptor–dependent LTP always appear to contain a substantial number of AMPA receptors (*49*). Third, AMPA receptors and NMDA receptors interact with different putative clustering proteins at the synapse, a finding that might explain the differential redistribution of synaptic AMPA receptors and NMDA receptors after prolonged (hours or days) increases or decreases in neuronal activity (*48, 50*). Fourth, an NMDA receptor–dependent increase in fluorescence occurs in dendritic spines of cells expressing a green fluorescent protein–GluR1 fusion protein (*51*). Conversely, NMDA receptor–dependent LTD in hippocampal cultures caused a loss of synaptic AMPA receptors but had no effect on synaptic NMDA receptors (*39, 52*). Fifth, proteins involved in membrane fusion can interact with AMPA receptors and thereby provide a mechanism for rapidly changing the number of AMPA receptors at the synapse (*53*). Consistent with this hypothesis, interference with membrane fusion in the postsynaptic cell impairs LTP (*54*).

The demonstration that AMPA receptors and NMDA receptors can be independently regulated by activity at individual synapses suggests a model that can account for most of the experimental results that have been obtained from the study of LTP (Fig. 2). According to this model, LTP is induced by the synaptic activation of NMDA receptors during strong postsynaptic depolarization. Because NMDA receptors are highly permeable to Ca^{2+}, this results in an increase in Ca^{2+} concentration within the activated dendritic spines that in turn causes local activation of CaMKII (and perhaps other protein kinases), the final step in the induction of LTP. The expression of LTP, at least initially, is caused by both phosphorylation of AMPA receptors and the delivery or clustering of AMPA receptors within the synaptic plasma membrane. These events would occur both at synapses that already contain functional AMPA receptors and at synapses that do not express surface synaptic AMPA receptors. The phosphorylation causes an increase in AMPA receptor single-channel conductance, whereas the delivery or insertion of AMPA receptors at synapses that previously did not contain surface synaptic AMPA receptors explains the change in failure rate that is observed during LTP. Because of its simplicity, an attractive addition to this model would be that phosphorylation of the AMPA receptor itself, or adjacent synaptic proteins, influences the subsynaptic localization of the AMPA receptors. LTD could be explained simply by a dephosphorylation of AMPA receptors and their movement away from the synaptic plasma membrane, both of which have been shown to occur during LTD (*39, 45*).

One experimental observation that is not consistent with this model is that LTP can be entirely explained by the decrease in the failure rate, and thus the averaged size of the EPSCs when they occur is unaltered (*55*). This observation is inconsistent with the model, because the conversion of silent to functional synapses during LTP would cause more synapses, on average, to contribute to the EPSC, which therefore should increase. These findings, however, have not been universally agreed upon (*43, 56*).

The hypothesis that the movement of AMPA receptors at the synapse contributes to LTP is reminiscent of a proposal made 15 years ago (*57*). In the ensuing frenzy of activity, however, the simple idea that the number of functional AMPA receptors at individual synapses can be rapidly modified by activity was largely ignored. As was the case then, much more experimental work remains to be done to test this idea and to provide the molecular mechanisms by which movement of AMPA receptors is accomplished (*58*).

Conclusions

The long-standing debate regarding the mechanisms of NMDA receptor–dependent LTP may be close to resolution. A valuable outcome of the intense work on this problem has been that a number of fundamental properties of pre- and postsynaptic function in the mammalian brain have been revealed. There remain a few, relatively isolated experimental results that are difficult to explain by the proposed mechanisms (*59*). Furthermore, there are reports of some novel properties of LTP that we have not discussed here because little is known about their mechanisms (*60*) or meaning (*61*). Despite these caveats, we think that the evidence is now sufficiently strong to indicate that the initial increase during LTP involves postsynaptic modifications of AMPA receptor function and localization. This conclusion does not preclude the occurrence of substantial presynaptic changes. The synapse is a structural unit and, as do many in the field, we would predict that if long-lasting synaptic modifications are in fact a mechanism by which experiences are translated into memories, then pre- and postsynaptic structural alterations are likely to occur. According to the current model, these would be triggered initially in the postsynaptic cell and would involve modifications in dendritic spine morphology that

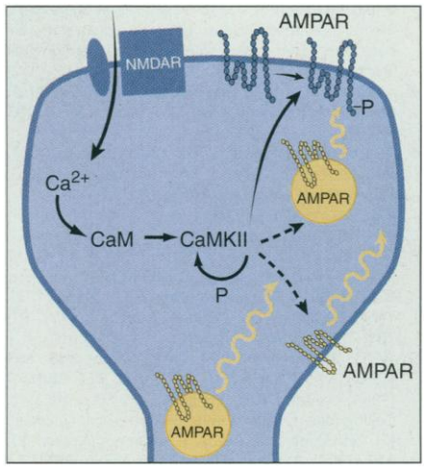

Fig. 2. Simplified model for the expression of LTP. An increase in Ca^{2+} within the dendritic spine binds to calmodulin (CaM) to activate CaMKII, which undergoes autophosphorylation, thus maintaining its activity after Ca^{2+} returns to basal levels. CaMKII phosphorylates AMPA receptors (AMPARs) already present in the synaptic plasma membrane, thus increasing their single-channel conductance. CaMKII is also postulated to influence the subsynaptic localization of AMPA receptors such that more AMPA receptors are delivered to the synaptic plasma membrane. The localization of these "reserve" AMPA receptors is unclear, and thus they are shown in three different possible locations. Before the triggering of LTP, some synapses may be functionally silent in that they contain no AMPA receptors in the synaptic plasma membrane. Nevertheless, the same expression mechanisms would apply.

in turn might cause functional and structural presynaptic alterations.

Although the molecular machinery responsible for LTP will likely turn out to be much more complex than that presented here, progress may be facilitated by keeping the molecular model as simple as reasonably possible.

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28. Criteria that provide strong support for a specific retrograde messenger in LTP include the following: (i) it is produced in CA1 pyramidal cell dendrites in response to NMDA receptor activation, (ii) LTP is blocked when its production is inhibited, and (iii) application of the retrograde messenger during afferent stimulation and in the presence of an NMDA receptor antagonist causes a synaptic enhancement that occludes LTP. In our opinion, the consensus in the field is that none of the postulated retrograde messengers have adequately met these criteria.
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52. Although these data (*31*, *47–51*) provide strong evidence for the existence of a population of synapses without functional AMPA receptors, under some conditions, the spillover of glutamate onto neighboring synapses could contribute to EPSCs mediated only by NMDA receptors [see D. M. Kullmann and F. Asztely, *Trends Neurosci.* **21**, 8 (1998)].
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58. Where are the reserve AMPA receptors that are proposed to be recruited to the postsynaptic density during LTP? AMPA receptors could be recruited from membrane adjacent to the postsynaptic density, from within the cytoplasm of the dendritic spine, or from the dendritic shaft at the base of the spine. Current anatomical data are most consistent with the last possibility (*48*, *49*).
59. In hippocampal cultures, direct measures of vesicle exocytosis have been reported to be increased in an NMDA receptor–dependent manner [A. Malgaroli *et al.*, *Science* **268**, 1624 (1995); T. A. Ryan, N. E. Ziv, S. J. Smith, *Neuron* **17**, 125 (1996)], and a role for NO as a retrograde messenger that activates presynaptic guanylate cyclase has been proposed (*27*). However, the mechanisms responsible for the reported changes in vesicle exocytosis are unknown, and the relation of these changes to the NMDA receptor–dependent LTP routinely studied in slices is unclear.
60. It has been suggested that the induction of LTP at one set of synapses may cause LTP at adjacent, unactivated synapses [E. M. Schuman and D. V. Madison, *Science* **263**, 532 (1994); F. Engert and T. Bonhoeffer, *Nature* **388**, 279 (1997)]. Because very little is known about the mechanisms underlying this phenomenon, we think it is premature to incorporate it into current models of LTP.
61. Repetitive activation of excitatory afferents can cause an NMDA receptor–dependent outgrowth of spine-like structures from dendrites [M. Maletic-Savatic, R. Malinow, K. Svoboda, *Science* **283**, 1923 (1999); F. Engert and T. Bonhoeffer, *Nature* **399**, 66 (1999)]. It is unknown whether these changes represent new functional synapses that contribute to LTP.

62. R.C.M. is supported by grants from NIH, the Human Frontier Science Program, and the McKnight Endowment Fund for Neuroscience. R.A.N. is supported by grants from NIH and Bristol-Myers Squibb and is a member of the Keck Center for Integrative Neuroscience and the Silvio Conte Center for Neuroscience Research. We thank the members of our laboratories for many stimulating discussions and M. Tessier-Lavigne for critical reading of the manuscript.