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The Site of Expression of NMDA Receptor-Dependent LTP: New Fuel for an Old Fire

Review

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Few phenomena in neuroscience have attracted as much attention as long-term potentiation (LTP) of synaptic transmission in the mammalian cortex (Bliss and Collingridge, 1993). This is explained by the probable involvement of LTP both in the formation and storage of memories and in neuronal injury. Much of the interest in LTP also centers around a long-running debate about the nature of the enhancement in synaptic transmission: does LTP involve an increase in transmitter release or an enhanced postsynaptic response to neurotransmitter, or both? This review describes several recent attempts to resolve the locus of LTP, which also shed light on the basic mechanisms of synaptic transmission in the CNS.

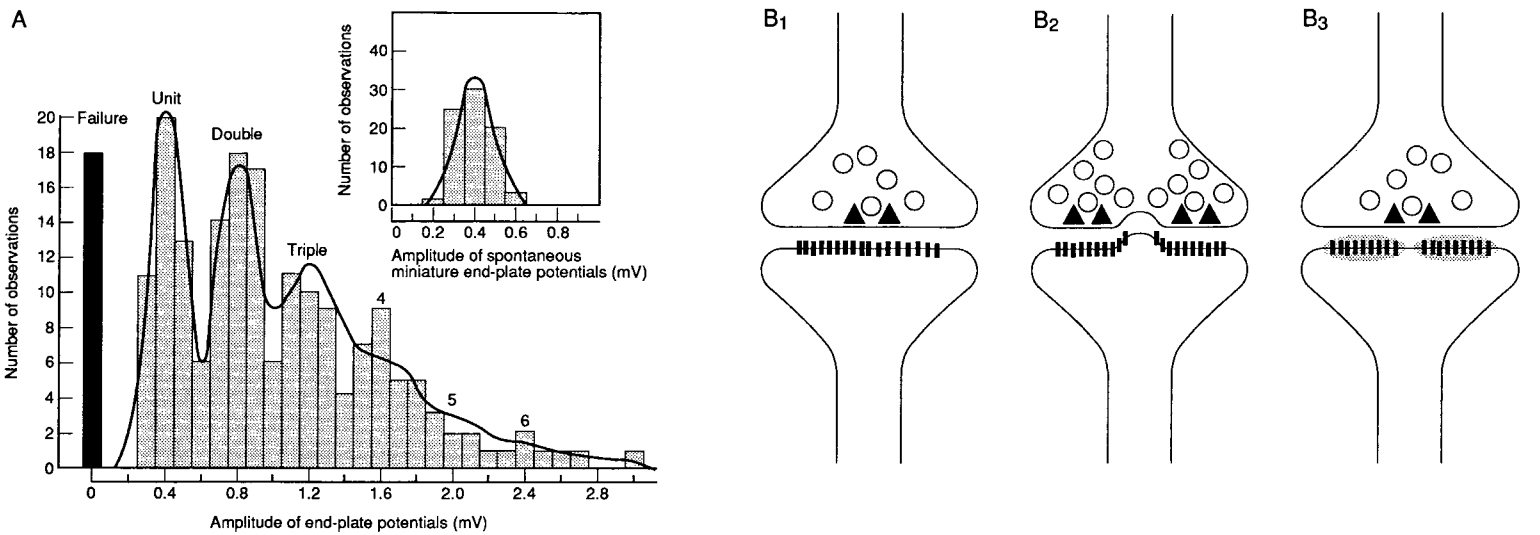
At the excitatory synapse between Schaffer collaterals and pyramidal cells in the CA1 region of the hippocampus, LTP is triggered by synchronous high frequency stimulation of many presynaptic fibers, sufficient to depolarize the postsynaptic cells. It can also be induced with low frequency stimulation of individual presynaptic fibers, as long as this is coupled with postsynaptic depolarization, which can be elicited by current injection through a microelectrode or by strong stimulation of an independent input converging on the same postsynaptic cells. There is general agreement that the induction of LTP at this and many other synapses requires Ca^{2+} entry into the postsynaptic dendritic spine via the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors. At the resting potential, NMDA receptor–linked channels are blocked by extracellular Mg^{2+}, but membrane depolarization relieves this block and allows Ca^{2+} influx. The NMDA receptors thus function as coincidence detectors, first postulated by Donald Hebb to allow the activity-dependent storage of information in neuronal networks. Events downstream from this, however, are less clear: protein kinases are involved, and possibly retrograde messengers transmitting a signal from the postsynaptic spine to the presynaptic terminal, but the locus of the resulting persistent modification is controversial (Bliss and Collingridge, 1993; Larkman and Jack, 1995; Nicoll and Malenka, 1995).

Pre- or Postsynaptic Expression?

At first sight, it would appear that the issue of whether the enhancement of synaptic transmission is presynaptic or postsynaptic could be resolved by looking for changes in glutamate release or postsynaptic sensitivity to glutamatergic agonists. Although both such changes have been reported (Bliss and Collingridge, 1993), the interpretation of the results is complicated by the fact that these approaches assay all synapses on a cell, whereas LTP is generally induced at only a very small proportion of these synapses. Some of the most compelling evidence in favor of either a pre- or postsynaptic site of expression has instead come from examining the pharmacological and quantal properties of an excitatory postsynaptic signal before and after induction of LTP. There have been disagreements here as well, but three landmarks stand out from extensive work using the in vitro hippocampal slice preparation.

First, LTP is not generally accompanied by a change in the degree of paired-pulse facilitation (PPF) of the postsynaptic signal. PPF occurs when two presynaptic stimuli are delivered within a brief interval (50–200 ms) and is thought to result from residual Ca^{2+} in the presynaptic terminal following the first stimulus, enhancing release during the second stimulus (see Manabe et al., 1993, and references therein; although also see references in Larkman and Jack, 1995). Many manipulations known to increase transmitter release do cause a decrease in the facilitation ratio, presumably because release is already enhanced to near saturation during the first stimulus. Thus, the lack of change of PPF argues against a presynaptic locus for LTP, and therefore in favor of a postsynaptic one. However, this result does not exclude the possibility that the target of LTP induction is a presynaptic rate-limiting step regulating exocytosis, separate from that responsible for facilitation. Moreover, PPF might not be altered if LTP selectively enhances release at synapses that start out with very low levels of release, and so do not approach saturation.

Second, the two components of the postsynaptic glutamatergic signal appear to be differentially increased in LTP. Glutamatergic transmission at most CNS synapses, including those in the CA1 region of the hippocampus, involves two types of ionotropic glutamate receptors: the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and the NMDA receptors. A number of studies have concluded that during LTP the AMPA receptor component of the excitatory postsynaptic current (EPSC) is selectively enhanced, with little or no change in the size of the component mediated by NMDA receptors (reviewed by Bliss and Collingridge, 1993; Larkman and Jack, 1995). Pharmacological modulation of presynaptic transmitter release, in contrast, alters both components equally, so this again argues for a selective postsynaptic alteration in either the density or properties of AMPA receptors (Asztely et al., 1992; Perkel and Nicoll, 1993). However, this line of reasoning has been undermined by the finding that NMDA receptors are down-regulated by Ca^{2+} influx (see Tong et al., 1995, and references therein). Since Ca^{2+} ions flow through the NMDA receptors during induction of LTP, this phenomenon could conceal an in-



Elements of Quantal Analysis

The debate surrounding LTP hinges on the assumptions underlying quantal analysis in the CNS.

(A) Quantal analysis at the neuromuscular junction. In the classical studies of Katz and colleagues, the endplate potential (EPP) was recorded from a skeletal muscle fiber in response to repeated stimulation of the presynaptic motor nerve in reduced extracellular Ca^{2+}. The graph shows a frequency histogram plotting the number of EPPs recorded versus EPP size (in millivolts). The first peak at 0 mV corresponds to failures of transmission. Successive peaks occur at equally spaced intervals of 0.4, 0.8, 1.2 mV, etc. Katz and colleagues proposed that transmitter was released in discrete packets called quanta and that the peaks in the histogram corresponded to EPPs composed of 0, 1, 2, 3, or more quanta released simultaneously. The EPP amplitude histogram followed a Poisson distribution (solid curve), which was consistent with the view that there are a large number of quanta (n) that can be released by an impulse, and that the probability that any given quantum is released (p) is very low. The Poisson distribution is a limiting case of the binomial distribution, where $n \rightarrow \infty$ and $p \rightarrow 0$, and is determined by the unique parameter $m = np$, which is the average number of quanta released in response to a presynaptic impulse. It is widely assumed that each quantum corresponds to the release of the contents from a single synaptic vesicle. The inset shows the amplitude distribution for spontaneous miniature EPPs (mEPPs), which occur in the absence of nerve stimulation and reflect the spontaneous release of single quanta. The mEPP distribution consists of a single peak at an amplitude equal to that of the unit peak in the evoked EPP histogram. The peak is fitted by a single Gaussian function whose standard deviation is determined, in part, by variations in transmitter content in vesicles, postsynaptic sensitivity of the membrane, and instrumentation noise. Data are from Boyd and Martin (1956).

(B) Models for the physical interpretation of n at central and peripheral synapses. (B_1) Several lines of evidence suggest that n corresponds to the number of release sites or active zones in a presynaptic terminal. At many neuronal synapses, a presynaptic bouton contains only a single release site (active zone, area indicated by closed triangles and open circles). One complication for quantal analysis at central synapses is the presence of multiple peaks or skewed distributions in some amplitude histograms recorded for spontaneous miniature synaptic responses. Different models for quantal release at central synapses have been invoked to explain the multiple peaks. (B_2) Neighboring active zones in a single presynaptic bouton may be coupled so that there is occasionally simultaneous spontaneous release from two or more active zones. In this case, n still corresponds to the number of release sites. The illustration shows the case of a presynaptic bouton with two active zones. (B_3) Postsynaptic receptors may be inserted in integral numbers of clusters (gray ovals) under presynaptic boutons. In this case, release of a single vesicle could produce a response corresponding to one or more depending on the presence of one or more clusters of receptors. In this case, n no longer refers to number of release sites but will depend on the total number of postsynaptic clusters of receptors.

crease in glutamate release at NMDA receptors. Another attack on this argument comes from the finding by some groups that the NMDA receptor–mediated signal can in fact increase with LTP (Asztely et al., 1992; Clark and Collingridge, 1995). The experimental conditions responsible for this discrepancy remain to be determined.

The third argument comes from quantal analysis and, in contrast to the first two, supports a presynaptic site of expression (see box for a brief overview of quantal analysis): LTP is associated with a decrease in the coefficient of variation (CV = standard deviation/mean) of the postsynaptic signal recorded from individual cells (see references in Bliss and Collingridge, 1993). That is, the trial to trial variability in the size of the postsynaptic signal, normalized by its average amplitude, is less after LTP induction than before. This is not expected from a postsynaptic site of expression, which in its simplest form should simply scale up the postsynaptic signal with no effect on the CV. It is, however, compatible with an increase in the average number of transmitter quanta released from the presynaptic terminals (or quantal content), as long as transmission is described by a simple probabilistic model such as the binomial or Poisson. The weakness of this approach is precisely that it has relied on untested probabilistic models, extrapolated from the neuromuscular junction. Several groups have examined the trial to trial fluctuation of very small postsynaptic signals elicited by activity in one or a few presynaptic fibers and have attempted to resolve individual quanta before and after LTP induction. They have generally found that the incidence of failures of transmission decreases, and that the quantal content increases, with LTP (see references in Kullmann, 1994; Stevens and Wang, 1994; Bolshakov and Siegelbaum, 1995; Stricker et al., 1995). This approach does not rely on a priori probabilistic models and, by extrapolation from the neuromuscular junction, argues for an increase in the average release probability, p, and therefore a presynaptic site of expression. Minimal stimulation experiments have, in addition, revealed an increase in quantal size, suggestive of a postsynaptic contribution to LTP (see references in Kullmann, 1994; Stricker et al., 1995), but this is not a universal finding (Stevens and Wang, 1994; Bolshakov and Siegelbaum, 1995).

There is thus a paradox: the first two arguments support a postsynaptic locus, while a relatively consistent result from quantal analysis is an increase in quantal content, implying a presynaptic locus. There is, however, an alternative interpretation for the increase in quantal content with LTP: rather than indicating an increase in the number of quanta of transmitter released, it reflects a postsynaptic uncovering of clusters of previously silent AMPA receptors (Edwards, 1991). If correct, this would reconcile all three of the above arguments in favor of a postsynaptic locus for LTP and also have extensive repercussions for the significance of quantal parameters in the brain.

Silent Synapses

Support for this hypothesis first came from a comparison of the probabilistic behavior of the two components of the excitatory signal: Kullmann (1994) recorded EPSCs, evoked with low frequency presynaptic stimulation, initially holding the cell at a negative potential, and subsequently at a positive potential in the presence of AMPA receptor blockers. This allowed the trial to trial amplitude fluctuations of the AMPA and NMDA receptor–mediated components of the EPSC to be recorded consecutively without altering presynaptic transmitter release. The CV of the AMPA component was consistently larger than that of the NMDA component. Since the CV is independent of the mean quantal amplitude, this implies that either n or p is larger for NMDA receptors than for AMPA receptors. AMPA and NMDA receptors appear to be colocalized, at least at some synapses (Bekkers and Stevens, 1989), so it is difficult to see how p could be different for the two receptor types. Instead, Kullmann (1994) proposed that there is a subset of synapses where NMDA receptors are present but AMPA receptors are either nonfunctional or absent. In terms of quantal analysis, n is smaller for the AMPA receptor–mediated component of the EPSC than for the NMDA receptor–mediated component. However, here the meaning of n is clearly different from that of classical quantal analysis: rather than reflecting solely the number of release sites, it now also depends on the number of postsynaptic sites containing active clusters of receptors. After LTP induction, the CV of the AMPA component fell, but there was little change in either the amplitude or the CV for the NMDA component when compared with the corresponding values measured in another pathway that acted as a control. The CV of the AMPA component thus became similar to, but never less than, the CV of the NMDA component. This was interpreted as resulting from activation of latent clusters of AMPA receptors at sites where only NMDA receptors were present under baseline conditions. In other words, LTP induction causes an increase in n for AMPA receptors, with little or no change in n for NMDA receptors (see Figure 1).

The CV is not exclusively determined by n and p, but also by quantal variability. Quantal variability describes both trial to trial variability in the peak amplitude of the quantal response at an individual release site and nonuniformity between sites (reflecting different synaptic properties and degrees of electrotonic attenuation). Although Kullmann (1994) argued that quantal variability could not account for the discrepancy between the CV of the AMPA and NMDA receptor–mediated components, an independent test of the hypothesis that did not rely on indirect estimates of quantal parameters was required. This was provided by Liao et al. (1995) and Isaac et al. (1995), who used minimal stimulation of presynaptic fibers to examine postsynaptic events arising from the release of very small numbers of quanta. Both groups showed that a very low intensity stimulus that failed to elicit AMPA receptor–dependent EPSCs at a negative holding potential could often elicit small NMDA receptor–mediated EPSCs at a positive holding potential. This implies that the stimulus was sufficient to elicit transmitter release, which activated NMDA receptors, but that no AMPA receptors were available to respond to the glutamate, and supports the hypothesis

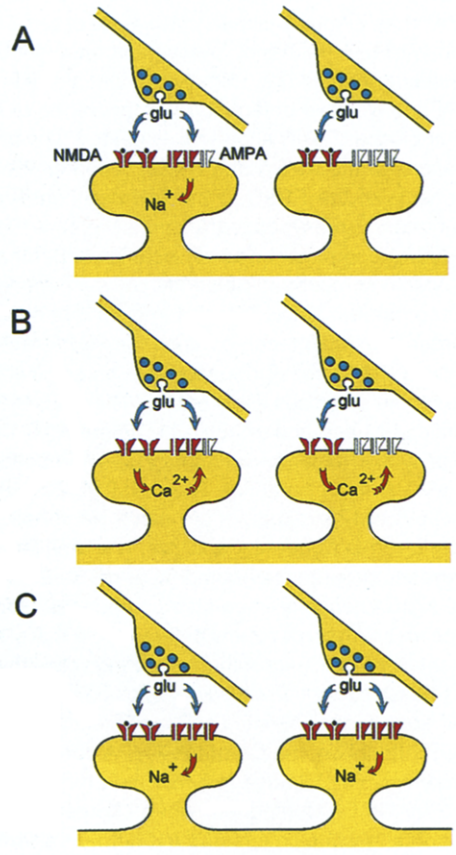


Figure 1. The Latent Cluster Hypothesis for NMDA Receptor–Dependent LTP

Two synapses on neighboring dendritic spines are shown, with two presynaptic terminals.

(A) Baseline transmission. Both synapses have NMDA receptors, but at one synapse the AMPA receptors are nonfunctional or absent (shown as open symbols). Low frequency presynaptic stimulation of both afferents (or stimulation when the postsynaptic cell is held at a negative potential) causes a synaptic current to flow only at the synapse at the left, where functional AMPA receptors (shown in red) are present. The NMDA receptor–linked ionophores are blocked by Mg^{2+}.

(B) Induction of LTP. Postsynaptic depolarization, elicited either by passing current through a postsynaptic electrode or by stimulating many presynaptic fibers at high frequency, relieves the voltage-dependent block of the NMDA receptor–linked ionophores. This allows Ca^{2+} influx into both dendritic spines, triggering the biochemical cascade necessary for LTP induction.

(C) Expression of LTP. A cluster of AMPA receptors is uncovered at the previously silent synapse (right), either by translocation from a site where they were not exposed to released glutamate or by phosphorylation. This synapse now responds to glutamate release when the postsynaptic cell is at a negative potential, giving an increase in quantal content. In addition, extra AMPA receptors are uncovered at the synapse at the left, increasing the quantal amplitude at this site.

that AMPA receptors are absent or nonfunctional at a subset of synapses where NMDA receptors are present.

Liao et al. (1995) and Isaac et al. (1995) induced LTP by pairing the low intensity presynaptic stimulation with postsynaptic depolarization to −10 mV, to maximize Ca^{2+} entry through NMDA receptor–linked ionophores. This caused AMPA receptor–mediated EPSCs to appear when the cell was returned to more negative potentials, suggesting that clusters of AMPA receptors were unmasked at previously silent synapses. In a further set of experiments, Liao et al. (1995) showed that the proportion of trials resulting in failures of transmission decreased after LTP induction when the EPSCs were measured at negative potentials (where the EPSC is mediated solely by AMPA receptors) but did not change when they were measured at positive potentials (where both AMPA and NMDA receptor components contribute to the EPSC). This was again compatible with a selective uncovering of latent clusters of AMPA receptors, with no change in NMDA receptors.

These results call for a novel interpretation for the increase in quantal content of AMPA receptor–mediated postsynaptic signals that accompany LTP: it reflects a change, not in p, the release probability, as was originally proposed, but in n, the number of sites at which transmission takes place. And in contrast to the neuromuscular junction, n is not simply the number of sites at which transmitter is released, since for AMPA receptors it is determined in part postsynaptically. The latent cluster hypothesis reconciles the three major sources of evidence on the locus of the synaptic modification listed above: no change in PPF is to be expected since the transmitter release probability is unchanged, and the phenomenon is selective for AMPA receptors. Activation of clusters of AMPA receptors at synapses where these were previously silent explains the increase in quantal content seen in LTP. Additional receptor clusters may also be activated at synapses where some AMPA receptors were already active before LTP, which would explain an increase in quantal amplitude. Nevertheless, there are some observations that are difficult to reconcile with this simple scheme.

Problems with the Silent Synapse Model

First, as mentioned above, some groups have reported that the NMDA receptor–mediated component of the postsynaptic signal does, in fact, increase with LTP, although the relative magnitude of this change varies from laboratory to laboratory. If the potentiation of NMDA receptor–mediated EPSCs is also accompanied by a change in quantal content, it will be important to determine whether this, too, reflects activation of latent clusters of NMDA receptors in parallel with AMPA receptors, or instead an additional increase in transmitter release.

Second, Malgaroli et al. (1995) have reported an increase in synaptic vesicle cycling in hippocampal cultures exposed to brief episodes of high extracellular glutamate with low Mg^{2+}, a stimulus that induces a long-lasting potentiation of transmission that shares many features with LTP. Vesicle kinetics were assayed by measuring the rate at which presynaptic terminals were labeled upon bath application of an antibody to the intraluminal domain of synaptotagmin, a synaptic vesicle membrane protein. Although this technique has the advantage of assaying presynaptic function directly, it remains to be determined whether similar changes in presynaptic function occur during conventional LTP studied in brain slices.

Third, there is a large, although far from unanimous, body of evidence implicating diffusible retrograde messengers, and by implication, a presynaptic target (Bliss and

Collingridge, 1993) and presynaptic second messengers (Arancio et al., 1995) in LTP. This topic is beyond the scope of this review, except to state that if diffusible messengers are involved, they may have either a postsynaptic target and/or a presynaptic one.

Finally, there is some disagreement about the precise change in quantal parameters in minimal stimulation experiments. Stevens and Wang (1994), in a careful examination of small, apparently single-quantum EPSCs elicited by minimal stimulation, reported only a decrease in failure rate with LTP. In contrast to several other reports, they found no change in the average amplitude of the postsynaptic signal once failures were excluded. If a latent cluster of AMPA receptors were uncovered, in addition to the one that was already active during the baseline period, one would expect to see occasional EPSCs resulting from the simultaneous activation of two clusters, and therefore an increase in the average amplitude of nonfailure EPSCs.

Bolshakov and Siegelbaum (1995) recently extended these results by stimulating individual presynaptic CA3 pyramidal neurons while recording the unitary EPSCs from single CA1 pyramidal neurons. At active synapses, stimulating a presynaptic neuron evoked responses with a relatively high failure rate, ranging from 0.5 to 0.8 among different cells. The distribution of the nonfailure EPSCs was composed of only a single quantal peak, implying that a typical CA3 neuron made at most a single functional synaptic contact onto a CA1 neuron. In agreement with Stevens and Wang (1994), there was a decrease in the fraction of failures in the first 30 min after induction of LTP, with no change in the size or shape of the quantal response peak. This result also argues that no new clusters of AMPA receptors were inserted at previously active synapses up to 30 min after induction of LTP.

The results of Stevens and Wang (1994) and Bolshakov and Siegelbaum (1995) might still be explained by the unmasking of latent receptors if the stochastic fluctuations in transmission reflected the dynamic switching on and off of a single AMPA receptor cluster. In this case, LTP would increase the fraction of time that a cluster was in the "on" state. Bolshakov and Siegelbaum, however, used two approaches known to affect release to demonstrate that most of the failures appear to be presynaptic in origin. First, during PPF the fraction of failures in response to the second stimulus was found to be much lower than the fraction of failures in response to the first stimulus. In one experiment, this decreased to 0.1, providing an upper limit to the fraction of failures that could be postsynaptic in origin. Although it might be argued that PPF also results from the unmasking of latent receptors, the biochemical mechanism would need to proceed to completion within 50 ms, the time between the two stimuli. In the second approach, the external Ca^{2+} concentration was increased from 2.5 to 5.0 mM, again causing the the fraction of failures to decrease to levels as low as 0.1. Since the whole-cell pipette solution contained a high concentration of a Ca^{2+} chelator (10 mM BAPTA), it is unlikely that the change in failure rate was due to a change in the postsynaptic CA1 neuron's intracellular Ca^{2+} levels.

Alternative Models

Could the data of Kullmann (1994), Liao et al. (1995), and Isaac et al. (1995) be reinterpreted by postulating a presynaptic locus for LTP? The simple answer is yes, but at the cost of greater complexity. NMDA receptors have a much higher affinity for glutamate than AMPA receptors, so it is conceivable that under baseline conditions, at some synapses, glutamate release is sufficient to activate NMDA receptors but not AMPA receptors. One way this could occur is if there were cross talk at synapses between two presynaptic boutons that originate from the same presynaptic neuron and terminate on neighboring postsynaptic cells. If one bouton had a high release probability and the other a negligible or zero release probability, glutamate released from the high probability terminal could diffuse to and activate NMDA receptors (but not AMPA receptors) under the low probability terminal. Recording from the cell postsynaptic to the low probability synapse would show only NMDA receptor–mediated signals, generated by spillover of glutamate from the neighboring synapse. If the probability of release at the low probability terminal were then enhanced with LTP, AMPA receptor–mediated EPSCs would appear, but NMDA receptor–mediated signals would undergo little change, in agreement with the results cited above. This hypothesis is difficult to reconcile with the finding that pharmacological enhancement of transmitter release alters NMDA and AMPA components of the EPSC equally (Asztely et al., 1992; Perkel and Nicoll, 1993), but this may be because such modulation operates differently from LTP: e.g., by failing to enhance release from low probability release sites. It may be difficult to test this hypothesis directly, short of resolving individual quantal events in neighboring cells, to establish whether cross talk occurs. While synchronous quantal signals have been resolved in pre- and postsynaptic GABAergic amacrine cells in culture (Frerking et al., 1995), achieving this in hippocampal slices presents formidable obstacles.

An alternative model is that a retrograde messenger travels to distant synapses with AMPA receptors but few if any NMDA receptors, where it potentiates presynaptic transmitter release. This raises the question of how such a diffusible message could travel from the site of induction while maintaining the specificity that is such a striking feature of LTP (although see Schuman and Madison, 1994).

Of course, it is possible that several mechanisms coexist to regulate synaptic strength, and that the relative importance of pre- and postsynaptic changes differs depending on the experimental protocol, the age of the animal, the mode of LTP induction, or the length of time that has elapsed after induction (see Edwards, 1995). For example, the studies of Stevens and Wang (1994) and Bolshakov and Siegelbaum (1995), which support a presynaptic site for LTP, necessarily selected for cell pairs that had a functional AMPA receptor–mediated synapse. Thus, their results do not exclude the possible recruitment of latent AMPA receptor clusters between cell pairs that start out with no active AMPA receptor–dependent synapses. Conversely, the experiments of Liao et al. (1995) and Isaac et al. (1995), demonstrating the recruitment of

latent AMPA receptor clusters after LTP, selected for cells that initially had no active AMPA receptor–mediated synapses. Thus, these results do not rule out the possibility of a presynaptic enhancement of release.

Where Next?

LTP has an early, protein synthesis–independent phase that lasts 1–2 hr, which is followed by a late, protein synthesis–dependent phase (Bliss and Collingridge, 1993). This late phase of LTP might be associated with the de novo growth of new synapses and/or the splitting of active zones into two or more synapses (Geinisman et al., 1993). Such changes would, of course, require coordinated pre- and postsynaptic modifications. Further studies are clearly needed to address the discrepancies listed above and to establish which details of experimental procedures might explain the discordant results. Refinements of electrophysiological and imaging methods to assess pre- and postsynaptic events would clearly help to resolve the outstanding issues. Whatever the outcome of the debate, it is clear that future progress in elucidating the locus of LTP will depend on a more thorough understanding of the basic mechanisms of synaptic transmission in the mammalian brain.

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