Progress on LTP at hippocampal synapses: a post-synaptic $Ca^{2+}$ trigger for memory storage?

Stephen J. Smith  
Howard Hughes Medical Institute,  
Section of Molecular Neurobiology, Yale University School of Medicine, New Haven, CT 06510, USA.

The strength of transmission at many synapses increases with repetitive use. When the increase in strength lasts for more than a few minutes, it is called long-term potentiation, or LTP. LTP has been studied most extensively at excitatory synapses onto principal neurons of the hippocampal formation, where it was first described by Lomo in 1966-1 to 3. Because hippocampal LTP can be triggered by less than one second of intense synaptic activity but lasts for hours or much longer, it has been recognized as a possible experimental model for memory storage. This short review will describe some recent observations that illuminate a novel mechanism that appears to couple synaptic activity to LTP triggering. A postsynaptic ion channel evidently detects temporal contiguity of pre- and post-synaptic activity and signals this contiguity by allowing post-synaptic entry of calcium ions. It may be significant that this mechanism embodies formal characteristics of a general mechanism postulated by Hebb in 1948 as a likely basis for memory storage and learning.

LTP processes, types, receptors and channels

Three criteria help to circumscribe the selective treatment of hippocampal LTP given here:

1. At least three different processes or aspects of LTP can be recognized, and correspondingly three types of questions can be asked: (A) triggering (how is use of a synapse transduced into an effective biochemical signal?); (B) expression (which step in synaptic transmission is potentiated?); and (C) maintenance (which molecules are responsible for the actual, long-term storage of information about past use?). The focus here will be mainly on new results pertinent to the triggering process.

2. It now appears that different mechanisms may underlie LTP at different synapses, even within the confines of the hippocampal formation. For instance, LTP at the mossy-fiber synapses onto the CA3 principal cells involves a mechanism distinctively different from that observed in CA1, dentate gyrus and other CA3 synapses. Here we shall consider mainly results obtained in the CA1 region, with some reference to results from apparently similar dentate region synapses. Unfortunately, it will not be possible here to treat any of the intriguing results from the mossy fiber–CA3 synapse (see for example Refs 5, 6).

3. The transmitter candidate at the CA1 excitatory synapse is the amino acid glutamate, and at least two types of glutamate receptor must be distinguished: the NMDA type (so named for N-methyl-D-aspartate, a selective agonist) and the kainate type (named for its own selective agonist). Both types of receptor open ion channels in response to glutamate and both are present postsynaptically.

The ion channels opened by the two different receptors have distinct permeation properties, so they can be designated NMDA channels and kainate channels. The kainate channel appears to carry most of the postsynaptic current during normal synaptic transmission, but the NMDA channel is of central interest here because of its possible role in triggering LTP.

Post-synaptic triggering of LTP

In principle, LTP could be triggered in response to either pre- or post-synaptic activity, and both models have been proposed. However, there has been increasing evidence that a crucial triggering step is indeed postsynaptic. Early indications of this came from interactions of multiple synaptic inputs that seemed most readily explained at the postsynaptic level. In addition, the NMDA channel blocker 2-amino-5-phosphonovalerate (APV) was found to block LTP triggering without blocking the expression of previously established LTP. Assumption that APV can only act postsynaptically, this finding also suggested that a postsynaptic step is necessary in LTP triggering, as well as immediately implicating NMDA receptors in the process. In addition, the finding that injection of the postsynaptic cells pressors LTP onset further implicates a postsynaptic component; in particular, one involving a transient $Ca^{2+}$ signal that can be suppressed by EGTA.

Important new insights about LTP triggering have recently been provided by electrophysiological studies from three groups. In these experiments, membrane potential in postsynaptic cells was manipulated independently of synaptic input through use of intracellular current-passing microelectrodes, in some cases using voltage-clamp circuitry. Normally, the synaptic inputs leading to LTP would by themselves produce a large postsynaptic depolarization, mainly due to opening of the kainate channels. However, when similar postsynaptic depolarizations were produced by microelectrode current (without synaptic input), LTP was not triggered. Nonetheless, postsynaptic membrane potential was found to have a profound effect on LTP triggering when synaptic inputs are present. Depolarization during intense synaptic input blocked triggering of LTP that otherwise would have occurred. Conversely, depolarization during less intense inputs promoted LTP triggering that otherwise would not have occurred with these weak inputs.

These findings constitute strong evidence for a postsynaptic triggering mechanism. The effect of postsynaptic potential would be very difficult to explain with any purely presynaptic model for LTP triggering. The ready reversibility of the experimental treatments (postsynaptic hyperpolarization or depolarization) permitted elegant controls, which reinforce all three sets of studies. In addition, these experiments demonstrate that it is not postsynaptic potential per se that triggers LTP but the precise coincidence of presynaptic activity (i.e. transmitter release) and postsynaptic activity depolarization.

Voltage-dependent $Ca^{2+}$ influx through NMDA channels

What kind of mechanism could the postsynaptic cell use to detect such coincidence of presynaptic transmitter release and its own depolarization? NMDA channels are the outstanding candidate. Pharmacological data noted above have already suggested that NMDA receptors are involved in LTP
triggering. In addition, NMDA channels have recently been shown to be strongly voltage dependent and to be highly permeable to Ca^{2+}. Combined with the evidence that a postsynaptic Ca^{2+} transient is a step in LTP triggering, these two recently discovered properties of NMDA channels suggest a very attractive hypothesis for LTP triggering. LTP may be triggered only when NMDA channels open and allow Ca^{2+} influx, and NMDA channels may open only when transmitter is bound and the postsynaptic membrane is sufficiently depolarized.

The voltage dependence of NMDA channel opening was first studied in spinal cord neurons, but it has also been shown in hippocampal NMDA channels. These ion channels are unique among channel characterized so far in that they combine strong voltage dependence with an absolute requirement for transmitter in order to open. Interestingly, a voltage-dependent block by extracellular Mg^{2+} ions appears to give rise to the voltage dependence of NMDA channel opening. At physiological Mg^{2+} concentrations, NMDA channels are strongly blocked at the resting potential, but unblock with depolarizations of the same magnitude that trigger LTP.

The first indications that an NMDA receptor might regulate Ca^{2+} permeability arose in studies of Ca^{2+} action potentials. Voltage-clamp observations on both spinal cord and hippocampal neurons now have shown that a sizable Ca^{2+} influx can occur directly through NMDA channels (but not through kainate channels), without involvement of the more familiar voltage-dependent Ca^{2+} channels. Though depolarization is necessary for Ca^{2+} influx through NMDA channels, this pathway differs from the voltage-dependent Ca^{2+} channel pathway in that transmitter is required in addition to depolarization. The NMDA channel is also much less selective for Ca^{2+} than the traditional voltage-dependent channels, so that Na^{+} and K^{+} normally carry much of the total current through NMDA channels.

Several observations favor NMDA channels over normal voltage-dependent Ca^{2+} channels as the pathway for the postsynaptic Ca^{2+} influx involved in CA1 LTP triggering. First, Ca^{2+} influx gated only by depolarization should not be very sensitive to NMDA channel blockage because the normal postsynaptic voltage change is dominated by kainate channels. As noted above, LTP triggering is blocked entirely by NMDA antagonists. Second, a purely voltage-dependent Ca^{2+} channel would not readily explain the observation that postsynaptic depolarization alone is not sufficient to trigger LTP onset. On the other hand, if NMDA channels were present in postsynaptic regions (e.g. a dendritic spine) that lacked other channels permeable to Ca^{2+}, one would expect Ca^{2+} influx to exhibit exactly the joint dependence on synaptic transmitter release and postsynaptic depolarization characteristic of LTP triggering in CA1.

Finally, it has been noted that a postsynaptic Ca^{2+} trigger involving NMDA channels provides a natural basis for two salient functional properties of LTP in the CA1 region, namely synapse specificity and associativity. LTP can be specific to individual synapses because Ca^{2+} can only enter where NMDA channels are activated by transmitter molecules. Since both Ca^{2+} and transmitter molecules have short diffusion ranges likely to confine them to individual synapses, only active synapses should be potentiated. Associativity refers to a cooperative action of multiple inputs in triggering LTP, where those inputs must occur in temporal contiguity. Since depolarization is required for NMDA channel opening, LTP triggering should be subject to associative action as long as the multiple inputs are close enough for electrotonic spread of depolarization, which should include a sizable fraction of the inputs to any given postsynaptic neuron.

How is the Ca^{2+} transient linked to LTP expression?

How might a postsynaptic Ca^{2+} signal lead to strengthening of a synapse? No definite answers are yet at hand, but intriguing conjectures and hints have not been lacking. In many cellular processes, Ca^{2+} signals act through Ca^{2+}-dependent protein kinases, which in turn regulate some other effector protein molecules by phosphorylation. Roles for two different Ca^{2+}-dependent kinases in LTP have been suggested. These are the Ca^{2+}-phospholipid-dependent kinase (C-kinase) and the type II Ca^{2+}-calmodulin-dependent kinase (Ca^{2+}/CaM kinase II). There is also evidence that a Ca^{2+}-dependent protease might transduce the postsynaptic Ca^{2+} signal.

Given the strong evidence for postsynaptic involvement in LTP triggering, there is one outstanding clue that directs attention to the Ca^{2+}/CaM kinase II. This kinase is believed to constitute a major fraction of the protein composing the postsynaptic density complex at the CNS synapses. These kinase molecules would thus be ideally situated to respond selectively to Ca^{2+} influx through postsynaptic NMDA channels. Although there is at present little evidence beyond this suggestive localization to implicate the Ca^{2+}/CaM kinase II in LTP, the possibility is especially intriguing following a discovery that activity of this enzyme can be regulated by autophosphorylation, at least in vitro conditions. This discovery has led to a suggestion that this kinase could account for the maintenance of LTP in addition to being a link in the triggering process. When this kinase phosphorylates itself, it becomes enzymatically active independent of the continued presence of Ca^{2+} or calmodulin. This property might permit the kinase to serve as a bistable molecular switch, in the manner envisioned by Lisman. That is, a transient Ca^{2+} increase could switch the enzyme from a stable, Ca^{2+}-dependent 'off' state to a stable 'on' state where the kinase is kept active by Ca^{2+}-independent autophosphorylation.

While circumstantial evidence may seem to implicate Ca^{2+}/CaM kinase II in LTP, there is actually stronger evidence for involvement of the C-kinase. For instance, activation of the C-kinase by phorbol esters appears to mimic LTP and occlude further potentiation by synaptic input. Whether the relevant phosphorylation target is pre- or post-synaptic is not definitely known. In any case, it could well be that both the C-kinase and Ca^{2+}/CaM kinase II are somehow involved in LTP processes, or that yet another Ca^{2+}-dependent
enzyme, such as a protease, mediates the triggering action of postsynaptic Ca\(^{2+}\) influx.

Regardless of the mechanism of postsynaptic Ca\(^{2+}\) action, it must be stressed that a postsynaptic locus for triggering does not necessarily constrain the expression or maintenance of LTP to reside at a postsynaptic locus. Indeed, the bases for both expression and maintenance of LTP remain uncertain. Some of the best evidence actually indicates that the presynaptic transmitter release mechanism may be the site for expression of LTP\(^{11,47}\). Thus, the postsynaptic mechanisms discussed above may act by triggering a change in the presynaptic terminal. This seems at first a puzzling notion. The synapses exhibiting LTP in CA1 do not have obvious structural features of a reciprocal synapse. A mechanism that could account for any such 'reversed' flow of information across the synapse has not yet been identified, but a number of possibilities have been suggested. These include: (1) neurochemical communication\(^{11}\), presumably via a structurally inconspicuous pathway (e.g. membrane-permeant arachidonic acid metabolites); (2) effects mediated by localized changes in ion concentration (e.g. a change in Ca\(^{2+}\) concentration within the synaptic cleft); and (3) structural changes in postsynaptic density complexes or dendritic spines (see for example Refs 48–52) that might exert a structural effect on the presynaptic release zone.

### A Hebbian memory storage mechanism?

Hebb suggested in 1948 that a synapse that increased in strength following simultaneous activity of pre- and post-synaptic neurons might provide a basic mechanism for memory storage\(^{30}\). Since LTP is a lasting increase of synaptic strength that appears to depend on coincidence of transmitter release (presynaptic activity) and postsynaptic depolarization (postsynaptic activity), it has been recognized as an instance of synaptic plasticity postulated by Hebb.\(^{12-17,20,21}\) We may therefore contemplate the possibility that mechanisms like those involved in LTP might underlie behaviorally significant forms of memory storage and learning. Indeed, it is possible that LTP itself could be a physiological component of memory functions commonly attributed to the hippocampus\(^{31,34}\).

NMDA channels are widely distributed in the vertebrate CNS. By virtue of their joint dependence on transmitter and membrane potential and their ability to transport Ca\(^{2+}\) ions, NMDA channels seem sufficiently suited to implement a triggering function in the regulatory scheme postulated by Hebb. We can therefore now ask whether an NMDA-channel/Ca\(^{2+}\)-trigger mechanism like that implicated in LTP triggering could be involved in actual conditioning and learning phenomena. The availability of drugs with specific actions on NMDA channels provides an opportunity to begin testing this hypothesis, and already there are intriguing hints of a positive outcome for such tests.\(^{34}\)

### Selected references

15. Levy, W. B. and Steward, U. (1979) Brain Res. 175, 233–245