Genetics of Childhood Disorders: LIV. Learning and Memory, Part 7: Maintenance of Long-Term Potentiation

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Before moving on to the processes that contribute to the persistence of long-term potentiation (LTP), we need to address an apparently straightforward issue: which side of the synapse expresses LTP? We have seen that the main events of LTP induction occur in postsynaptic signaling pathways. Does this mean that the locus of LTP expression is also postsynaptic? At the CA3–CA1 synapse, the preponderance of electrophysiological evidence favors the postsynaptic side of the synapse, but this has been a contentious area of research and some data support the hypothesis of increased transmitter release in LTP. The problem of the site of LTP expression has been approached using several different strategies.

One method is to determine the rate of glutamate release. The efflux of glutamate from a hippocampal slice can be directly measured, and early work showed an elevation during LTP. However, synaptic release represents only a fraction of total glutamate efflux, and metabolic changes could be largely responsible for the observed increases. A more sophisticated experimental approach exploits the active uptake of synaptic glutamate by glial cells, resulting in graded glial transporter currents that can be recorded. These currents are not increased during LTP, suggesting that the locus of the potentiation is postsynaptic.

The hypothesis that LTP expression is restricted to the postsynaptic cell has been challenged by experiments in which vesicle recycling at the CA3–CA1 synapse was monitored using the presynaptic loading of a fluorescent dye. An increase in vesicle recycling would indicate that glutamate-containing vesicles were being turned over at a higher rate, presumably due to increased synaptic release. Such an effect occurred following LTP-inducing stimulation, supporting a presynaptic contribution to the expression of LTP.

A second approach to identifying the locus of LTP exploits the voltage-dependent block of *N*-methyl-D-aspartate (NMDA) receptors described in the previous column, a property that is not shared by (AMPA)-type receptors. This difference can be used to separate the synaptic response into AMPA and NMDA components because the contribution of the NMDA-mediated current will depend upon the postsynaptic membrane voltage. If an increase in glutamate release is responsible for LTP, then the AMPA and NMDA receptor-mediated responses should increase equally. But most studies show that only the AMPA component is potentiated during LTP, consistent with a postsynaptic change that is restricted to AMPA receptors.

The case for such a postsynaptic change was strengthened by an elegant set of experiments on LTP in organotypic hippocampal slice cultures. It is possible in the slice preparation to introduce foreign cDNAs that are taken up by the neurons. This process is termed transfection and permits the investigator to introduce cDNAs that will be transcribed into message and translated into protein. Neurons were transfected with a subtype of AMPA receptors with electrophysiological characteristics distinct from those normally expressed in these cells. These introduced receptors did not contribute to synaptic transmission until the neurons received an LTP-inducing stimulation. These results suggested that synaptic activity delivered the new receptors to the synapse. Remarkably, the expression of constitutively active CaMKII mimicked the effect of synaptic stimulation, but not by phosphorylating the AMPA receptor. Instead, CaMKII may promote the insertion of the receptor at the synapse.

A final strategy, the use of quantal analysis methods, has generated heated debate. Transmitter molecules are released in packets referred to as quanta, which correspond anatomically to the transmitter vesicles seen in the nerve terminal. Assuming that the amount of transmitter molecules per quantum is consistent at all synapses, it is theoretically possible to derive the presynaptic and postsynaptic parameters that determine the efficiency of synaptic transmission by observing a series of very small synaptic events. Typically, the measured event is the electrical current that flows across the cell membrane of an individual postsynaptic neuron when synaptic AMPA channels are activated, termed an excitatory postsynaptic current (EPSC). One way to obtain sufficiently small EPSCs is to deliver single weak presynaptic stimuli that are near the threshold for evoking a synaptic response, so that the stimulus often fails to elicit any EPSC.

Under these conditions, the probability of presynaptic release is so low that EPSCs, when they occur, represent the response to no more than a few quanta of neurotransmitter. The degree of variation in the quantal EPSC in this preparation is substantial, so the quantal nature of the recorded events is not immediately obvious upon examination of the current traces. Instead, the results of many trials are analyzed by plotting the frequency distribution for different EPSC amplitudes, revealing a series of frequency peaks that correspond to integral multiples of the quantal EPSC. The samples clustered around zero current represent the trials where stimulation failed to evoke any response at all.

How can this information be used to decide if the locus of LTP expression is presynaptic or postsynaptic? If an enhanced postsynaptic response is entirely responsible for LTP, then the frequency distribution should look the same except that each peak in the EPSC frequency distribution will correspond to a larger current value. Alternatively, an increase in the presynaptic release probability will leave the peak values unchanged but will decrease the failure rate and selectively increase the frequency of the larger EPSCs. Many of the quantal analysis studies at the CA3–CA1 synapse seem to favor a presynaptic contribution to the LTP expression: failure rates decline after stimulation, or a related measure of synaptic "potency" increases. However, an alternative, postsynaptic explanation for these data is now widely accepted, based on the phenomenon of "silent synapses". Some glutamatergic synapses possess functional NMDA-type receptors, but not AMPA-type receptors, and under baseline conditions they do not generate EPSCs in response to presynaptic stimulation. If the membrane is depolarized directly by injecting current into the postsynaptic neuron, even low-frequency stimulation can induce LTP, a procedure referred to as pairing. When the pairing procedure is used at these synapses, functional AMPA receptors are introduced in an NMDA receptor-dependent manner and EPSCs are seen. At the level of quantal analysis, the result is indistinguishable from an increase in the probability of release because there are new functional synapses to reduce the likelihood of failure on any given trial and to skew the frequency distribution of EPSCs toward the larger values.

If low-frequency stimulation is delivered on the heels of a high-frequency train, the resulting synaptic potentiation is reversed. However, within about 30 minutes after high-frequency stimulation, LTP becomes consolidated and can no longer be reversed by low-frequency stimulation. The mechanism that underlies consolidated LTP must differ in some way from that responsible for LTP induction. As discussed in last month's column, the activity of CaMKII that is so central to induction seems not to be a player in maintenance. Attempts to reverse established LTP by inhibiting numerous signaling enzymes that participate in induction have generally been unsuccessful, suggesting that the synaptic potentiation is maintained by some process other than sustained catalytic activity.

A growing body of evidence indicates that the long-term expression of LTP involves changes in gene expression, at the level of transcription as well as protein synthesis. LTP cannot survive for more than a few hours in slices treated with inhibitors of transcription or translation. This dependence is consistent with mammalian learning studies, showing that memory retention requires protein synthesis. These findings must be interpreted cautiously because they do not prove that an increase in protein synthesis is necessary for the persistence of LTP or memory. Instead, the interruption of protein synthesis might deplete the neuron of proteins that play a supporting role in the maintenance of plasticity (e.g., a transport protein whose expression is not regulated by synaptic stimulation but participates in the insertion of newly functional AMPA receptors).

However, recent work has shown that genes are transcribed and proteins synthesized following LTP-inducing stimulation. In addition, these studies have afforded insights into the neuronal repertoire for providing activated synapses with newly synthesized plasticity-related proteins. At the transcriptional level, attention has focused on the cyclic AMP response element binding protein (CREB). This transcription factor is rapidly activated following the induction of LTP and is also activated in the hippocampus of animals during learning.

Transgenic mice that are deficient in CREB are impaired in tests of long-term memory. Moreover, hippocampal slices taken from these animals exhibit only brief potentiation following stimulation that normally produces robust LTP responses. These findings are open to the same criticism as the synthesis inhibitor results: there could be a loss of proteins that serve only a supporting role in LTP.

The prospect of transcriptional regulation in LTP raises important issues. One is the identity of those newly expressed proteins that contribute to LTP. This is an area of active research. Another question relates to the input specificity of LTP, and here the neuron would seem to be faced with a daunting task: how to target the new proteins only to the properly stimulated synapses. One solution to this problem was demonstrated in hippocampal slices by stimulating two independent sets of synapses: one with weak stimulation that normally produces only transient potentiation and the other with strong, LTP-inducing stimulation. Remarkably, the transient potentiation was converted to a persistent LTP when the two sets of synapses were stimulated within a critical interval. In another experiment, strong stimulation was delivered to one set of synapses in the presence of a protein synthesis inhibitor. Normally, this procedure gives only transient potentiation; however, if there had been prior strong stimulation of the other set of synapses in the absence of the inhibitor, both sets of synapses expressed a full-blown LTP. It seems that even weak synaptic stimulation can "tag" the synapse, enabling the capture of the new proteins that are being distributed throughout the neuron. The tag itself does not require protein synthesis because it is generated in the presence of the translation inhibitor.

Another cellular solution to the problem of input specificity in the face of a requirement for protein synthesis is conceptually more simple: just manufacture the proteins locally. The conventional view of the cell body as the exclusive domain for protein synthesis in neurons has been toppled by the recognition that dendrites contain many components of the translation apparatus. These include polyribosomes, messenger RNAs (mRNAs), initiation factors, and structures similar to endoplasmic reticulum. This machinery is functional in hippocampal neurons; moreover, it is activated by stimulation that produces

long-lasting synaptic potentiation. Regulated dendritic translation could provide the neuron with tremendous flexibility and specificity in shaping the timing and distribution of protein synthesis. For example, resident mRNAs could be rapidly translated to establish an early stage of consolidated potentiation, which would be maintained only upon the arrival of additional plasticity-related transcripts. Some of the mRNAs that have been identified in dendrites code for components of the translational apparatus itself, so the first wave of translation may actually serve to increase local translational capacity. In this case, newly transcribed mRNAs coming from the cell body could be preferentially translated only at the stimulated synapses. This is one way that LTP-related increases in translation and gene transcription could come together to create unique patterns of protein expression at synapses destined for LTP.

LTP is unlikely to be the only cellular basis for memory formation and storage, and dissociations between LTP and memory have been reported. For example, transgenic approaches have identified modifications that prevent LTP in hippocampal slices while sparing hippocampus-based spatial memory. For some forms of learning, such as taste aversion in which a specific association must be made between events that are separated by hours, the conventional LTP paradigm seems inappropriate. Certain memory-related processes may not even be based on synaptic plasticity, as suggested by the demonstration of experience-dependent neurogenesis in the dentate nucleus of adult rats, although such mechanisms may serve to increase mnemonic capacity rather than to establish specific memories. However, although LTP clearly has limitations as a cellular model of memory, the principles of neuronal plasticity derived from the effort to understand this phenomenon will continue to yield insights into the brain's capacity to encode experiences in an enduring way.

WEB SITES OF INTEREST

http://www.benbest.com/science/anatmind/anatmd3.html
http://thalamus.wustl.edu/course/limbic.html

ADDITIONAL READINGS

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Accepted March 12, 2003.

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0890-8567/03/4209–1131©2003 by the American Academy of Child and Adolescent Psychiatry.

DOI: 10.1097/01.CHI.0000070243.24125.31