Genetics of Childhood Disorders: LIII. Learning and Memory, Part 6: Induction of Long-Term Potentiation

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The last several columns have discussed the organization of human memory. The discovery of different types of memory has come from studies of human neurological patients with deficits in some forms of memory while others are preserved. For example, the memory for a particular motor skill or the ability to learn a new motor task may persist even though an individual has lost the ability to learn new faces, names, or recent events.

A particularly striking example of this is the case of H.M., perhaps the most well-studied individual in the field of learning and memory. H.M. fell from a bicycle as a child and developed intractable seizures as an adolescent. In an effort to control his seizures, bilateral resections were made of his hippocampus and the medial aspect of his temporal lobes. Although his seizures abated, H.M. lost the ability to remember new information for more than a few minutes. However, H.M. was still capable of learning new motor tasks. As a result of these studies, neurobiologists came to the conclusion that the hippocampus is critically involved in translating short-term memories into long-term memories. A tremendous amount of research has been devoted to understanding the underlying molecular mechanisms that are responsible for this ability.

A prominent characteristic of synapses is their capacity for plasticity, reflected as increases or decreases in the efficiency of transmission. For nearly three decades, the effort to understand how synapses encode memories has focused mainly on one form of synaptic plasticity, *long-term potentiation* (LTP), with the majority of studies being conducted in the hippocampus. The persistent increase in synaptic strength observed in LTP has several characteristics that one would expect of a fundamental mnemonic process.

Before we look at an example of an experiment that examines LTP, we will review the neuroanatomy of the hippocampus. The hippocampus is a C-shaped structure that is tucked beneath the neocortex. It has a relatively simplified architecture and is called the *archicortex* to distinguish it from the more complex laminated structure found in the neocortex. The hippocampus is divided into two major parts: the *dentate gyrus* and the *CA fields*. CA stands for "cornu ammonis" or "Ammon's horn." The cells within the dentate gyrus are called granule cells and form a single layer of cells, whereas those of the CA fields are pyramidal neurons that also form a single monolayer.

The neuronal circuitry of the hippocampus is characterized by a trisynaptic pathway (Fig. 1A), with glutamate serving as the neurotransmitter at all three synapses. The first set of synapses is made by the incoming projections that originate in the entorhinal cortex and provide excitatory synaptic inputs to the granule cells in the dentate gyrus (*perforant pathway*). The granule cells then send their projecting, excitatory axons (*mossy fibers*) to make the second synaptic connections on the dendritic arbors of pyramidal neurons of the CA3 subregion. The CA3 neurons in turn send one branch of their excitatory projections to the pyramidal neurons of the CA1 subfield (*Schaffer collaterals*).

There are several reasons why this region is the most heavily studied in the field of LTP. The neuronal pathways within the hippocampus can be readily located and selectively activated, and the laminar organization facilitates recordings from either dendrites or cell bodies. All three synaptic projections exhibit LTP. Just as important is the fact that the hippocampus can be removed and cut into thin sections, termed slices. The acute *slice preparation* remains viable for as long as 12 hours, whereas the organotypic slice culture can be maintained in the laboratory dish with the appropriate buffers and nutrients for weeks. This is particularly useful for the types of electrophysiological experiments that are required to study LTP. The investigator must record electrical activity from the neurons that maintain their synaptic connections in the slices as well as be able to deliver either reagents or electrical stimuli to the slices.

Figure 1B summarizes an LTP experiment using the CA3—CA1 synapse in an acute slice of rat hippocampus. Here, synaptic strength is measured by recording the extracellular *excitatory postsynaptic potential* (EPSP). This is done by placing the recording electrode in close proximity to a group of neurons to record the summed response of multiple synapses within the postsynaptic neuron in response to a presynaptic stimulus. Following a baseline observation period, the synapses are activated with two trains of *high-frequency stimulation* (HFS; 100 Hz in this case) delivered to the presynaptic fibers. This intense stimulation produces an immediate and lasting increase in synaptic strength.

Several properties of LTP make it attractive as a potential cellular process subserving learning and memory. First, a brief synaptic event is encoded as a long-term physiological change in the neuron, a temporal arrangement that is reminiscent of experience-dependent learning. Second, LTP is input-selective in that the potentiation is restricted to the activated synapses, and possibly their near neighbors. Such selectivity is essential

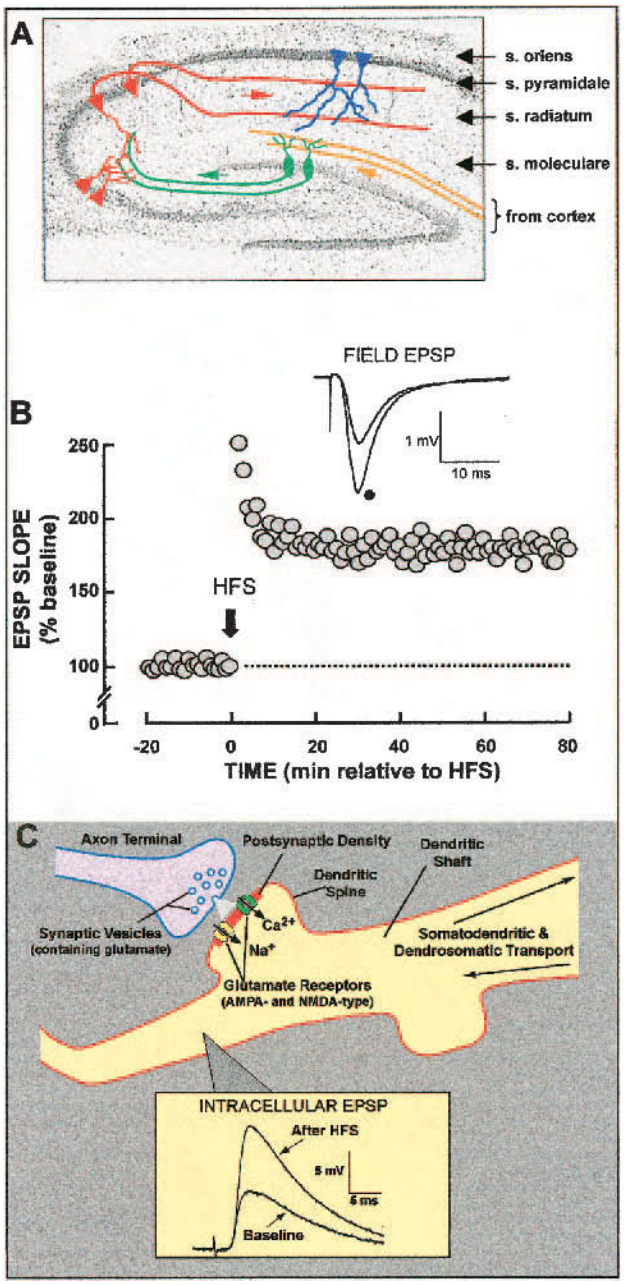

Fig. 1 A: The synaptic connections of the hippocampus. A Nissl-stained section of rat hippocampus is shown, with a superimposed cartoon of the primary neurons and their communicating processes. Note the sequence of three excitatory synaptic fields, referred to as the trisynaptic pathway. These synapses use glutamate as their neurotransmitter, and all exhibit LTP upon appropriate stimulation. The colored arrows indicate the direction of information flow. The perforant pathway, represented in yellow, originates in the entorhinal cortex and synapses in stratum moleculare on dendrites of the granule cells of the dentate gyrus (shown in green). These in turn project via the mossy fibers to the hippocampal pyramidal neurons in area CA3 (shown in red), where they terminate on dendrites in stratum radiatum. Finally, the Schaffer collaterals, which originate in the CA3 pyramidal cells, synapse on other pyramidal cells located in the CA1 region (blue). B: An example of an LTP experiment, performed in an acute rat hippocampal slice. An extracellular recording electrode was inserted into stratum radiatum of area CA1 to record synaptic potentials resulting from pulses delivered to the Schaffer collaterals with a stimulating electrode. The recordings are in the form of field excitatory postsynaptic potentials (EPSPs), shown in the traces, which are generated by the movement of Na^+ from the extracellular space into nearby dendrites. The efficiency of synaptic transmission is measured by the onset slope of the EPSP, and the slopes of a series of EPSPs (recorded every 30 seconds) have been plotted in the graph. After a stable baseline was established, the Schaffer collaterals were stimulated with a pattern of high-frequency stimulation (HFS), consisting of two trains of pulses, each 1-second long, separated by 20 seconds. During each train, stimuli were delivered at 100 Hz. Immediately following HFS (arrow), the EPSP was potentiated more than twofold above baseline. Following a brief period of decay, the EPSP stabilized at a new value, indicating an increase in synaptic efficiency of about 80% over the original baseline. The traces show an EPSP obtained before the delivery of HFS and one recorded 1 hour after HFS (marked by a dot). The brief downward deflection before the EPSPs is an artifact of the test stimulus. C: Summary of the mechanisms that contribute to LTP induction. The release of glutamate in response to a presynaptic action potential activates AMPA-type receptors and, if the membrane is sufficiently depolarized, NMDA-type receptors as well. The influx of Na^+ through AMPA channels depolarizes the dendrite, and the resulting EPSP is shown in the box as it would be recorded intracellularly (as opposed to the extracellular EPSP shown in B). An influx of Ca^{2+} through the NMDA channels is the triggering event in the induction of LTP, a process that is enabled during HFS by the concomitant release of glutamate along with AMPA receptor-mediated depolarization of the postsynaptic membrane. Following HFS, the EPSP is increased. Note that these traces were acquired when the membrane was in its resting, polarized state and thus represent only AMPA receptor-mediated potentials. To record NMDA receptor-mediated EPSPs, the membrane would have to be depolarized during the stimulating pulse. Dendrosomatic and somatodendritic transport refers to the movement of signaling molecules from the dendrites to the cell body (and nucleus) following HFS, and the subsequent transport of newly synthesized mRNAs and proteins to the dendrites. Such processes contribute to the maintenance of LTP.

for exploiting the huge mnemonic capacity of synapses, which can number 10,000 on a single hippocampal pyramidal cell. Finally, LTP is associative, in the sense that the activation of presynaptic fibers is effective only if the postsynaptic dendrite is properly prepared. The postsynaptic priming can be as simple as membrane depolarization or can involve the regulation of the postsynaptic signaling network. The discovery that LTP requires concomitant activity on both sides of the synapse was enthusiastically received by psychologists and neurophysiologists because it agreed well with the requirement for simultaneity that Hebb had proposed as the basis for the laying down of memory traces.

The physiological and biochemical events that trigger LTP are brief, compared with the duration of LTP expression. In exploring these underlying processes, it is convenient to distinguish between the brief *induction* phase of LTP and the *maintenance* phase. Our understanding of the underlying biochemical and physiological process has been progressing from induction to maintenance.

All three of the hippocampal pathways are excitatory and use glutamate as their neurotransmitter. There are two types of glu-

tamate receptors that will be discussed in this column. They are the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors and the *N*-methyl-D-aspartate (NMDA) receptors. One important difference between these receptors is in their responses after glutamate stimulation: AMPA receptors allow entry of Na^+ ions, while NMDA receptors are permeable to Ca^{2+} as well as Na^+.

The EPSP that follows a presynaptic stimulus reflects the activation of AMPA-type glutamate receptors as a result of the influx of Na^+ (Fig. 1C). However, the key initiating event for LTP involves a rise in the concentration of Ca^{2+} within the dendrite, as shown by studies in which LTP induction is prevented by the postsynaptic introduction of compounds that bind and remove calcium, called chelators. At the CA3—CA1 synapse and most others where LTP has been studied, the source of the Ca^{2+} is through the Ca^{2+}-conducting NMDA receptor. This channel has an interesting voltage dependence that explains the effectiveness of HFS as a conditioning protocol. When the postsynaptic membrane is at its resting voltage, the NMDA channel is blocked by a Mg^{2+} ion. This block is relieved upon membrane depolarization, so the NMDA receptor behaves as a coincidence detector: it operates only when there is both glutamate release from the presynaptic neuron and sufficient depolarization of the postsynaptic neuron. HFS provides the depolarization due to the summation of EPSPs. If the membrane is depolarized directly by injecting current into the postsynaptic neuron, even low-frequency stimulation can induce LTP.

The moments immediately following Ca^{2+} entry may be viewed as a *Sturm und Drang* period, when Ca^{2+} activates many signaling molecules, including protein kinases and protein phosphatases. Some of these enzymes have been shown to modulate the induction of LTP, but one of them has pride of place as a key component of the LTP pathway: Ca^{2+}/calmodulin-dependent protein kinase II (*CaMKII*). In the dendrites, CaMKII is abundant, particularly within the *postsynaptic density*, a structure at the synapse that contains anchoring proteins that position AMPA- and NMDA-type receptors, along with a variety of modulatory proteins, opposite the presynaptic terminal. Activated CaMKII phosphorylates AMPA-type receptors, increasing the conductance of their ion channels and consequently increasing the size of the EPSP.

A special feature of CaMKII is its ability to phosphorylate and activate other CaMKII molecules. CaMKII forms rosette-like clusters of 12 molecules, and an activated CaMKII molecule can interact and phosphorylate its immediate neighbor. In theory, if the Ca^{2+} influx is sufficient to activate enough of the CaMKII molecules in a cluster, the entire cluster will become persistently activated.

Experimentally, LTP-inducing stimulation results in the activation of CaMKII, and extensive research efforts have established a central role for CaMKII in the induction of LTP. In transgenic mice overexpressing a mutant form of CaMKII that lacks the CaMKII phosphorylation site, both LTP and spatial learning are impaired. When constitutively active CaMKII is introduced into hippocampal slices, synaptic efficiency is increased. As we shall see later, however, the maintenance of LTP, as opposed to its induction, may not require catalytically active CaMKII.

Even the turnover of individual members of the cluster need not compromise the cluster's active state because the new recruit will be promptly phosphorylated. However, the CaMKII cluster can be turned off by phosphatase activity. This mechanism comes into play in another form of synaptic plasticity: *long-term synaptic depression*.

As mentioned above, a rise in Ca^{2+} can activate phosphatases, the family of proteins that remove phosphate groups from proteins. Calcineurin is the phosphatase that is primarily responsible for the dephosphorylation of CaMKII. Indeed, calcineurin is more sensitive to Ca^{2+} than is CaMKII, so that a modest rise in dendritic Ca^{2+} can preferentially activate calcineurin, producing decreases in CaMKII activity, AMPA receptor conductance, and synaptic efficiency. This is precisely what happens when prolonged low-frequency stimulation is delivered to the CA3—CA1 synapse (1 Hz for 15 minutes is typical). If this form of stimulation is delivered on the heels of HFS, the resulting synaptic potentiation is reversed. However, within about 30 minutes after HFS, LTP becomes consolidated and can no longer be reversed by low-frequency stimulation. This is another example of the change from the induction phase of LTP to the maintenance phase and suggests that the source of the synaptic potentiation no longer resides in the catalytic activity of CaMKII and has shifted to some other molecule.

We can appreciate the intricate interplay that occurs between signaling molecules during the induction of LTP by adding cyclic adenosine monophosphate-dependent protein kinase (PKA) to the postsynaptic mix. PKA is activated by the ubiquitous second messenger cyclic adenosine monophosphate, which is generated in hippocampal neurons after Ca^{2+} influx during HFS. The induction of LTP by closely spaced trains of HFS, such as those used to generate the LTP shown in the figure, does not require the activation of PKA. However, if the multiple HFS trains are distributed over a period of 10 to 20 minutes, the resulting LTP is completely dependent on postsynaptic PKA activity. How do these LTPs differ? It is likely that only the more prolonged pattern stimulates calcineurin, which opposes the activation of CaMKII. The contribution of PKA is to inhibit calcineurin indirectly, thereby protecting CaMKII from dephosphorylation. This situation is complex enough without our mentioning that PKA also directly modulates AMPA and NMDA receptors and has transcriptional effects that promote the maintenance of LTP. Furthermore, we have neglected the role of numerous other proteins in LTP induction, including mitogen-activated protein kinase and protein kinase C. We will continue to review the underlying mech-

anism of LTP in the next column including how LTP is maintained over the prolonged periods of time associated with memory retention.

WEB SITES OF INTEREST

http://nootropics.com/memoryswitch/
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/L/LTP.html
http://synapses.bu.edu/learn/filo3D/howto.stm

ADDITIONAL READINGS

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