Learning and Memory, Part II: Molecular Mechanisms of Synaptic Plasticity

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Learning and Memory, Part I, described how short-term memories are consolidated into long-term memories and the brain regions involved in this process.[1] As previously discussed, the hippocampus is required for the formation of declarative memories (conscious memories of events and facts). This type of memory was destroyed in the famous clinical case of H.M. after he sustained a bilateral hippocampal resection to treat his disabling seizures. Despite the severity of his memory deficits, H.M. retained his ability to form nondeclarative memories (unconscious habit learning), which requires brain structures outside the hippocampus. In fact, his performance on motor learning tasks, which require nondeclarative memory, was perfectly normal. One example of this sort of task is mirror writing; H.M. was asked to keep his pen within a narrow border along the edge of a star while observing the pen and his hand through a mirror, which reverses right and left. H.M.'s motor learning in this task improved for several days, although he recalled neither the individual administering the test nor the instructions from one day to the next.

The previous column highlighted the role of synaptic plasticity in consolidating both declarative and nondeclarative memories.[1] We also discussed studies of fear conditioning, another learning paradigm that has contributed to our understanding of how synaptic connections become strengthened as we learn. In the fear-conditioning paradigm, a form of associative learning takes place when a rodent hears a tone paired close in time with a mild foot shock. The rat or mouse quickly learns to fear the tone. This paradigm has relevance to human disorders such as panic attacks, posttraumatic stress disorder, and other anxiety disorders.

Consolidating fearful memories involves synaptic plasticity in the amygdala, a structure that lies deep in the temporal lobe just in front of the hippocampus. Afferents arising from thalamic nuclei that convey sensory information about the tone and other thalamic afferents representing the foot shock converge on dendrites of neurons in the lateral amygdala; the convergence of these inputs strengthens both sets of synapses, increasing their individual impact on the postsynaptic neuron. Evidence of this type of associative learning is observed during the testing phase 24 hours later when the animal freezes after hearing the tone alone. In other words, once the memory has been consolidated, synapses representing the tone alone are sufficient to trigger action potentials in the neurons of the lateral amygdala, although these same neurons were not previously responsive to the tone alone. The resulting action potential is passed along to the central nucleus of the amygdala and from there to deeper structures responsible for the freezing behavior, increased heart rate, and other autonomic responses associated with fear.

Exactly how synapses are "strengthened" has been the subject of intense investigation during the past 30 years and is the subject of this column. Three possible mechanisms were mentioned in the previous column. First, more glutamate may be released from the presynaptic neuron (glutamate is the neurotransmitter released in response to the tone), leading to stronger depolarization of the postsynaptic neurons. The second and third mechanisms are both postsynaptic. The number of glutamate receptors on the postsynaptic terminal may be increased, or the glutamate receptors could become more responsive to a given amount of glutamate, remaining open for longer periods and allowing the passage of more ions through their channels. Each of these mechanisms could mediate a stronger response to

Accepted September 23, 2008.

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DOI: 10.1097/CHI.0b013e318190c4b3

the tone alone. This column examines some of the molecular events responsible for strengthening synaptic connections. We first describe a laboratory preparation that allows investigators to test what actually happens in these neurons.

A major advance occurred in 1973 when Bliss and Lomo[2] discovered long-term potentiation (LTP), a physiological event now believed to reflect synaptic strengthening that occurs as a result of activity-dependent synaptic plasticity. Long-term potentiation has been studied most extensively in the hippocampus, although it is also observed in other brain regions that exhibit synaptic plasticity. The neuronal circuitry of the hippocampus consists of three major excitatory pathways that use glutamate as their neurotransmitter. These pathways are illustrated in Figure 1, which depicts two curved sheets of cells called the *dentate gyrus* and *Ammon horn*. First, the perforant pathway enters the hippocampus from the entorhinal cortex to make synaptic contact with granule cells of the dentate gyrus. Second, the granule cells assemble their axons into the "mossy fiber" pathway that projects to the dendritic fields of pyramidal neurons in the CA3 subfield of the Ammon horn in the hippocampus.

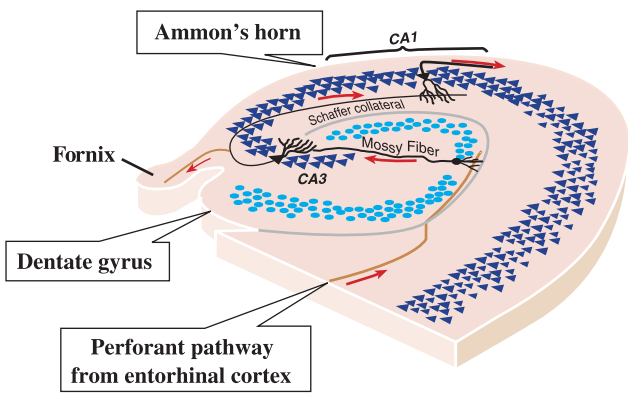

Fig. 1 Hippocampal anatomy and electrophysiological changes underlying long-term potentiation. The hippocampus, which lies deep on the medial surface of the temporal lobe, consists of two sheets of cells called *Ammon horn* and the *dentate gyrus* and three excitatory pathways as shown. Neurons in the entorhinal cortex, which is part of the hippocampal complex (not shown), project to cells in the dentate gyrus by way of fibers called the *perforant pathway*. Axons from the perforant pathway synapse on granule cells of the dentate gyrus. Granual cell axons project to the Ammon horn via "mossy fibers." Mossy fiber axons synapse on the dendrites of neurons of the CA3 division of the Ammon horn. Axons of the CA3 neurons project to the dendrites of neurons in the CA1 division of the Ammon horn by way of fibers called *Schaffer collaterals*. Reprinted with permission from Lippincott Williams & Wilkins. Figures adapted from Bear MF, Connors BW, and Paradiso MA. *Neuroscience, Exploring the Brain*. 2nd ed.; 2002, pp. 791–793.

Third, axons of the CA3 neurons form the "Schaffer collateral" pathway and synapse on pyramidal neurons in the CA1 subfield of the Ammon horn.

The hippocampus is used extensively in studies of synaptic plasticity, in part because it is possible to preserve these three synaptic pathways in hippocampal slices that can be removed from the brain and kept viable with in vitro techniques and investigated electrophysiologically. Horizontal slices, cut at a thickness of approximately 400 μm through the hippocampus, are placed in an oxygenated artificial cerebrospinal fluid, and a pair of electrodes is lowered into the slice. One is a stimulating electrode that delivers electric current to the Schaffer collateral fibers arising from CA3 neurons. The other is a recording electrode placed near neurons in the CA1 field to measure the frequency of action potentials fired by these neurons (Fig. 2a). This arrangement allows researchers to stimulate the Schaeffer collaterals electrically while recording from the pyramidal neurons in CA1. An initial series of mild stimulations is used to measure the baseline response of the CA1 neurons. A seminal discovery made in the hippocampal slice preparation showed that a burst of high-frequency electrical stimulation of Schaeffer collaterals is sufficient to strengthen the synaptic connections between these neurons. Note in Figure 2b how mild stimulation applied subsequently produces a much larger response in the postsynaptic neurons than it had during the baseline recordings. This increase in responsiveness is called *long-term potentiation* and may last for many hours or for as long as the slice preparation remains alive.

As mentioned earlier, glutamate is the neurotransmitter that activates CA1 neurons in the hippocampus. Several types of glutamate receptors (molecules that bind exclusively to glutamate) are present at the postsynaptic site, but only two of them are discussed here: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs) and *N*-methyl-D-aspartate receptors (NMDARs). There are important differences in the way these two receptor types respond to glutamate. The AMPARs are Na^+ channels that, when bound to glutamate, permit Na^+ ions to pass through the membrane into the postsynaptic neuron, leading to its depolarization. The summation of multiple excitatory postsynaptic potentials on the dendritic arbors of CA1 neurons leads to the generation of an action potential by these neurons that in turn travels down the axon toward the next neuron in the circuit.

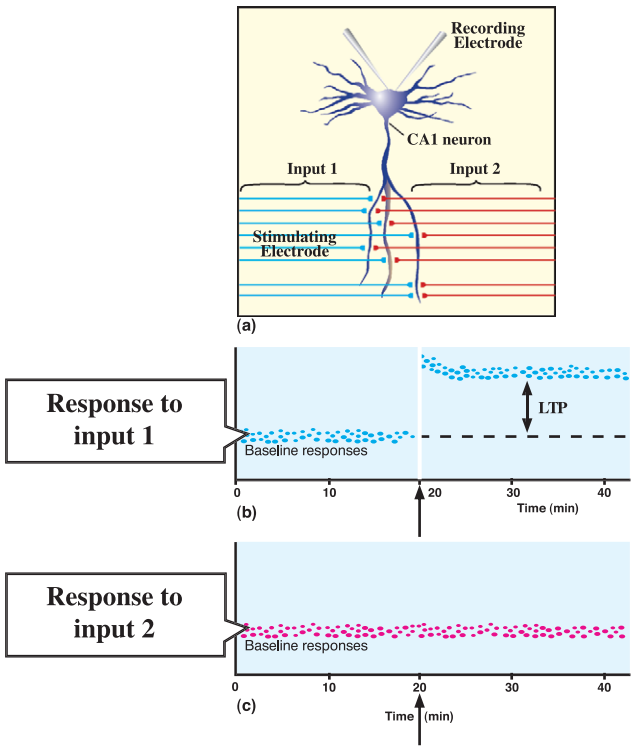

Fig. 2 The electrical activity in a neuron in the CA1 subfield of the Ammon horn is monitored with a recording electrode in response to inputs from two different sets of Shaffer collaterals (inputs 1 and 2) from CA3 of the Ammon horn (a). Input 1 is subjected to brief high-frequency electrical stimulation from a stimulating electrode (termed *tetanus*) after 20 minutes of recording the cell's baseline activity. Tetanus can be likened to a potent sensory event. Subsequently, the same set of axons produces a greatly amplified response in this neuron compared with baseline (b). The cell's response to axons from input 2 remains at baseline because they have not been subject to tetanus (c). The change in the cell's response to input 1 is an example of long-term potentiation because this change in responsiveness persists for as long as the preparation is viable. Reprinted with permission from Lippincott Williams & Wilkins. Figures adapted from Bear MF, Connors BW, and Paradiso MA. *Neuroscience, Exploring the Brain*. 2nd ed.; 2002, pp. 791–793.

The NMDAR is also an ion channel that opens when bound to glutamate, but its activation has some additional requirements. The NMDARs require that the postsynaptic terminal is strongly depolarized at the same time that glutamate is present. This is because NMDARs have a positively charged magnesium ion in the channel opening. The immediate region around the channel must be depolarized to remove the magnesium plug, or the channel remains closed. If this occurs within a narrow window of time during which glutamate is also present, then the channel opens. Like AMPARs, Na^+ ions pass through this channel when it opens, but unlike AMPARs, this channel also permits the rapid entry of Ca^{2+} as well.

Before describing the effects of Ca^{2+} influx through NMDARs, we must turn to a relatively new concept called *silent synapses*. The AMPAR do not remain in the synaptic membrane but must move into place in the membrane to be activated in the presence of glutamate. Silent synapses are thought to consist mostly of NMDARs, but here, we are faced with a dilemma. How is the second requirement, strong depolarization of the postsynaptic terminal, achieved in the absence of AMPARs in the postsynaptic membrane? Recent work has suggested that the depolarization required for NMDAR activation arises at adjacent synapses subjected to strong synaptic inputs, from modulatory neurotransmitters (see below), and that this depolarization spreads to nearby dendritic spines, depolarizing them and activating their NMDARs. This, in turn, leads to the rapid insertion of AMPARs into the active site where they, too, are responsive to glutamate.

Let us return now to the effect of Ca^{2+} influx within the postsynaptic terminal. Ca^{2+} ions activate several important signaling pathways, many of which include protein kinases and phosphatases. Kinases are enzymes that add a phosphate group to a target protein, whereas phosphatases have the opposite effect: they remove phosphates. The addition or removal of a phosphate group can have a dramatic effect on protein function. The presence of a bulky negatively charged phosphate group often causes a change in the structure of the protein. For example, the conformational change may expose a group of amino acids that had previously been buried deep within the protein's core. The newly exposed amino acid domain is then able to interact with another downstream substrate or may allow another site to be phosphorylated that, in turn, increases the protein's enzymatic activity.

One of the kinases activated by the influx of Ca^{2+} through NMDARs is Ca^{2+}/calmodulin–dependent protein kinase II (CaMKII—pronounced "cam-kinase-two"). The CaMKII is abundant within the postsynaptic terminal and rapidly translocates to the postsynaptic density with synaptic activity. The postsynaptic density is an electron-dense area where the postsynaptic receptors are concentrated. This area abuts the presynaptic density on the presynaptic terminal where neurotransmitter is released. Both AMPAR and NMDAR are enriched in the postsynaptic density and are anchored to the postsynaptic density by scaffolding proteins that tether them immediately below the synaptic cleft. The

CaMKII forms a petal-like structure of 12 neighboring molecules. The phosphorylation of one molecule leads to the phosphorylation of a neighboring molecule, and quickly, the enzyme becomes fully activated. An interesting feature of CaMKII that particularly excites investigators studying LTP is that, once activated by Ca^{2+}, CaMKII has a period of autonomous activity, even in the absence of Ca^{2+}. Here is an enzyme that is activated by Ca^{2+} and remains active for long periods even after Ca^{2+} levels return to normal. This is an important feature for an enzyme thought to be involved in the formation of long-term memories. The AMPARs are one of the substrates for CaMKII. Phosphorylation increases the conductance of this receptor, so more ions pass through and produce a larger excitatory postsynaptic potential.

Recent work has shown that CaMKII plays a central role in the induction of LTP. In one series of experiments, LTP was induced as previously described, and the activity of CaMKII was measured. The results showed that CaMKII rapidly increases in CA1 neurons after LTP is induced. Furthermore, LTP is blocked when CaMKII inhibitors are added to the bathing solution of the hippocampal slices. Other researchers produced transgenic mice that overexpress a mutated inactive version of CaMKII; this disrupted hippocampal LTP. More to the point, the transgenic mice were unable to consolidate certain types of memories known to require the hippocampus, which in this case was spatial learning. Finally, researchers tested a constitutively active variant of CaMKII, which means that it is always produced whether or not it is needed. Hippocampal slices of this variant display facilitated LTP, and learning is facilitated in behavioral studies of mice with constitutively active CaMKII.

CaMKII plays a critical role in initiating LTP. However, additional kinases are necessary to maintain LTP over longer periods. One of these kinases, termed *extracellular signal–regulated kinase* (ERK), is a member of the mitogen-activated protein family of kinases. It is now apparent that several aspects of ERK signaling are critical for memory consolidation, and two of them are discussed here. First, LTP requires local translation (at the synapse within the dendritic spines) of several proteins necessary for structural and enzymatic changes that underlie the synaptic plasticity characteristic of LTP. The ERK activity is required to initiate the local translation of messenger RNAs (mRNAs) that are present at spines into functional proteins.

A second function of ERK is its rapid translocation into the nucleus of the neuron where it phosphorylates several regulatory transcription factors. This leads to the transcription of several mRNAs that are transported along dendrites toward the spines and their synapses. Messenger RNA (mRNA) transcription is required for the later phases of LTP, as demonstrated in hippocampal slices. Researchers have added transcription inhibitors to the bathing solution of hippocampal slices before high-frequency stimulation. Inhibiting transcription does not disrupt the initiation of LTP because CaMKII activity is promoted by the influx of Ca^{2+} and is not affected by transcriptional inhibition. However, transcriptional inhibition prevents the later expression and maintenance of the persistent long-lasting phase of LTP, which has been implicated in consolidation.

Another mechanism of synaptic plasticity required for learning and memory involves AMPAR and NMDAR "trafficking." Trafficking refers to the movement of glutamate receptors into and out of the postsynaptic membrane at the postsynaptic density. This mechanism is believed to help regulate synaptic strength. Again, kinases and phosphatases are involved in this process. This is an area of active research, and many of the details are still being worked out. However, a model has emerged that implicates several kinases in receptor trafficking. Activation of these kinases leads to the phosphorylation of specific receptor subunits and the movement of the entire receptor complex into the postsynaptic membrane. One model has the receptors targeted to a region of the spine head adjacent to the active zone, a region termed the *extrasynaptic zone*. From there, another signal leads to the lateral movement of the receptors into the active site where they become anchored in place adjacent to the presynaptic terminal. According to this model, this mechanism can work both ways; that is, removing glutamate receptors from active sites can weaken synaptic strength, mediating another form of learning that is the opposite of LTP, a process termed *long-term depression*.

For the last several decades, it has become clear that other neurotransmitters may modulate LTP in slice preparations (serotonin, dopamine, acetylcholine, and noradrenaline). For example, LTP is facilitated by the addition of isoproterenol, an adrenergic agonist, into the bathing solution, implicating norepinephrine in LTP modulation. Similarly, LTP is facilitated by microinfusion of the second messenger, cyclic adenosine

monophosphate (cAMP), or compounds that increase cAMP concentrations within the target CA1 neurons. Conversely, inhibiting these compounds blocks or diminishes the ability of a high-frequency stimulation at CA3 fibers to initiate LTP. Each of the modulatory neurotransmitters uses cAMP as a second messenger as described next.

How do the modulatory neurotransmitters contribute to the process of memory consolidation? It is important to recognize that the receptors for these neurotransmitters are usually not ion channels themselves like AMPAR and NMDAR but are members of what are known as G-protein–coupled receptors. Rather than opening an ion channel directly, stimulating these receptors activates second-messenger systems. For example, when dopamine binds to dopamine D1 receptors, it activates adenylate cyclase, which increases the production of cAMP. cAMP is a potent protein kinase A activator, a kinase that leads to the phosphorylation of multiple target substrates, including glutamate receptor subunits. Phosphorylation of AMPAR by protein kinase A is a well-known mechanism that increases the probability that this channel will be open and helps target these receptors to the active site of synapses. The opposite occurs when dopamine binds to the dopamine D_2 receptor, which inhibits adenylate cyclase, lowers the production of cAMP, and makes it more difficult to establish LTP. Thus, a primary effect of the synaptic inputs from terminals containing acetylcholine, noradrenaline, serotonin, or dopamine is to modulate whether glutamate-mediated depolarization occurs.

We end our discussions by returning to fragile X syndrome (FXS) because this disorder exemplifies several concepts described here. The normal function of the fragile X mental retardation protein (FMRP) is to bind to mRNAs in the nucleus and to chaperone them as they are transported along dendrites to sites at the synapses where they will be translated. FMRP inhibits the translation of these messages until an appropriate synaptic signal arrives at the spine. In the absence of FMRP, translation is disrupted, and several mRNAs are inappropriately translated when they should in fact remain repressed. This leads to a dysregulation of the normal spine maturation. Spines in both humans with FXS and in animal models of the disorder are, as a consequence, long, thin, and immature in appearance compared with the more mushroom-like shape of normal mature spines.

One of the messages that normally associate with FMRP is a phosphatase called *striatal-enriched phosphatase* (STEP). We have shown that STEP is inappropriately upregulated in the absence of FMRP and that higher levels of this brain-specific phosphatase are found in transgenic models of FXS. STEP normally regulates glutamate receptor trafficking: it dephosphorylates specific receptor subunits, leading to the rapid removal of the receptors from the synapse. Thus, one of the effects of a mutation of the *Fmr1* gene is STEP overexpression and the inappropriate removal of glutamate receptors from spines, the sites of synaptic contact. The resulting decrease in glutamate receptors would be expected to weaken synaptic strength. This is precisely what is found in electrophysiological experiments of hippocampal slice preparations of *Fmr1* mice; they display an excess of long-term depression. The morphological disruption at spines in patients and animal models of FXS can now be better appreciated, as it reflects an inability to strengthen connections between neurons, impairing the consolidation of long-term memories. Other mutations that disrupt the plasticity at spines are sure to be discovered, and these studies will move forward our understanding of how we normally learn and how this ability may be disrupted.

Disclosure: The authors report no conflicts of interest.

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