

Synapses and Memory Storage

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The synapse is the functional unit of the brain. During the last several decades we have acquired a great deal of information on its structure, molecular components, and physiological function. It is clear that synapses are morphologically and molecularly diverse and that this diversity is recruited to different functions. One of the most intriguing findings is that the size of the synaptic response is not invariant, but can be altered by a variety of homo- and heterosynaptic factors such as past patterns of use or modulatory neurotransmitters. Perhaps the most difficult challenge in neuroscience is to design experiments that reveal how these basic building blocks of the brain are put together and how they are regulated to mediate the information flow through neural circuits that is necessary to produce complex behaviors and store memories. In this review we will focus on studies that attempt to uncover the role of synaptic plasticity in the regulation of whole-animal behavior by learning and memory.

The idea that learning results from changes in the strength of the synapse was first suggested by Santiago Ramon y Cajal (1894) based on insights from his anatomical studies. That modulation of synaptic connectivity is a critical mechanism of learning was incorporated into more refined models by Hebb in the 1940s and 1950s. The experimental investigation of these intriguing conjectures required the development of behavioral systems in which one could examine changes in the neuronal components of a specific behavior during or after the modification of that behavior with learning (Kandel and Spencer 1968).

SYNAPTIC CHANGES WITH LEARNING

Procedural Memory in Simple Systems

The first attempts to identify neuronal changes that underlie learning and memory used simple forms of procedural memory such as habituation, sensitization, and classical conditioning. From 1969 to 1979 several useful model systems emerged: the flexion reflex of cats (Spencer et al. 1966); the eye-blink response of rabbits (Thompson et al. 1983); and a variety of reflex behaviors in invertebrate systems, including the gill-withdrawal reflex of *Aplysia* (Kandel and Tauc 1963), the escape reflex of *Tritonia*

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(Willows and Hoyle 1969), and various behavioral modifications in *Hermisenda* (Alkon 1974), *Pleurobranchaea* (Mpitsof and Davis 1973), *Limax* (Gelperin 1975), crayfish (Krasne 1969), and honeybees (Menzel and Erber 1978). These preparations were chosen for the limited number of neurons (or neuronal types) that participated in the behavior. This reductionist approach allowed the specific circuitry that controlled the behavior to be defined and examined for modification following learning. The studies were aimed at pinpointing the sites within a neural circuit that are modified by learning and used for memory storage, and for identifying the cellular basis for those changes.

By allowing electrophysiological recording from individual neurons that are readily identifiable from animal to animal and that form part of a simple behavioral circuit, these systems provided the first experimental insight into the cellular mechanisms of memory. One mechanism for learning and short-term memory, evident in both the gill-withdrawal reflex of *Aplysia* and in the tail-flick response of crayfish, is a change in synaptic strength brought about by modulating the release of transmitter. A decrease in transmitter release is associated with short-term habituation, whereas an increase in transmitter release occurs during short-term dishabituation and sensitization (Castellucci et al. 1970, 1974,

1976; Zucker et al. 1971; for early reviews, see Kandel 1976; Carew and Sahley 1986). The plasticity occurred at the sensory neuron inputs onto the motor neurons that control the reflex response and thus directly modulate its magnitude. These studies provided the first evidence for the idea that behavioral memory is mediated by plasticity in the synaptic connections between neurons that participate in the behavior.

Cell biological studies of the connections between the sensory and motor neurons of the gill-withdrawal reflex in *Aplysia* revealed a biochemical mechanism for the short-term increase in transmitter release produced by sensitization (Fig. 1) (Kandel 2001). A single noxious (sensitizing) stimulus to the tail leads to the activation of three known classes of modulatory neurons. The most important releases serotonin, which acts to increase the level of cAMP in the sensory neurons. This in turn activates the cAMP-dependent protein kinase (PKA), which enhances synaptic transmission. Injecting cAMP or the catalytic subunit of PKA directly into the sensory neurons is sufficient to enhance learning-related transmitter release (Brunelli et al. 1976; Castellucci et al. 1976).

Studies of the gill-withdrawal reflex also revealed that even elementary forms of learning have distinct short- and long-term stages of memory storage. Whereas one training trial

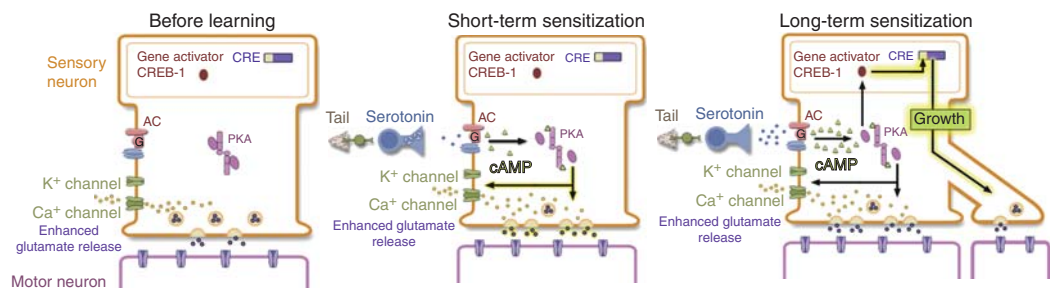


Figure 1. Short-term and long-term sensitization of the gill-withdrawal reflex in *Aplysia* involves posttranslational modifications and alterations in protein synthesis. (*Left*) The gill-withdrawal circuit. A tactile stimulus to the siphon causes a sensory neuron to release glutamate to excite a motor neuron. (*Center*) A shock to the tail causes serotonin release from interneurons. This activates a stimulatory G protein (G), which activates adenylyl cyclase (AC), leading to production of cAMP and PKA-dependent phosphorylation of different substrates, including K^+ and Ca^{2+} channels, which enhances glutamate release from the sensory neuron terminals. (*Right*) Repeated shocks to the tail elicit a persistent increase in cAMP, leading to altered gene transcription and protein synthesis. This leads to growth of new synapses.



gives rise to a short-term memory lasting minutes, repeated spaced training gives rise to long-term memory lasting days to weeks (Carew et al. 1972; Pinsky et al. 1973). These behavioral stages have a parallel in the stages of the underlying synaptic plasticity—a short-term form lasting minutes to hours and a long-term form lasting days to weeks (Carew et al. 1972; Castellucci et al. 1978). In addition to the immediate and short-term changes in synaptic function with learning, profound structural changes accompany the storage of long-term memory in both habituation and sensitization of the gill-withdrawal reflex. The sensory neurons from habituated animals retract some of their presynaptic terminals so that they make fewer connections with motor neurons and interneurons than do sensory neurons from control animals (Bailey and Chen 1983, 1988a). In contrast, following long-term sensitization the number of presynaptic terminals of the sensory neurons increases more than twofold (Bailey and Chen 1983, 1988a). This learning-induced synaptic growth is not limited to sensory neurons. The dendrites of the postsynaptic motor neurons also grow and remodel to accommodate the additional sensory input (Bailey and Chen 1988b). These results show that clear structural changes in both the pre- and postsynaptic cells can accompany even elementary forms of learning and memory in *Aplysia* and serve to increase or decrease the total number of functional synaptic connections critically involved in the behavioral modification.

Together, these early cellular studies of simple behaviors provided direct evidence supporting Ramon y Cajal's suggestion that synaptic connections between neurons are not immutable but can be modified by learning, and that those anatomical modifications serve as elementary components of memory storage (Bailey and Kandel 1993). In the gill-withdrawal reflex, changes in synaptic strength occurred not only in the connections between sensory neurons and their motor cells but also in the connections between the sensory neurons and the interneurons (Hawkins et al. 1981; Frost and Kandel 1995). Thus, memory storage, even for elementary procedural memories, is distributed

among multiple sites. The studies showed further that a single synaptic connection is capable of being modified in opposite ways by different forms of learning, and for different periods of time ranging from minutes to weeks for different stages of memory.

Studies of memory in invertebrates also delineated a family of psychological concepts (Hawkins and Kandel 1984) paralleling those first described in vertebrates by the classical behaviorists (Pavlov and Thorndike) and their modern counterparts (Kamin, Rescorla, and Wagner). These concepts include the distinction between various forms of associative and non-associative learning and the insight that contingency—that the conditioned stimulus (CS), in associative learning, is predictive of the unconditional stimulus (US)—is more critical for learning than mere contiguity; this is the CS that must precede the US by a short interval of time (see, for example, Rescorla and Wagner 1972). Such psychological concepts, which had been inferred from purely behavioral studies, could be explained in terms of their underlying cellular and molecular mechanisms. For example, the finding that the same sensory-to-motor neuron synapses that mediate the gill-withdrawal reflex are the cellular substrates of learning and memory illustrates that procedural memory storage does not depend on specialized, superimposed memory neurons whose only function is to store rather than process information. Rather, the capability for simple procedural memory storage is built into the neural architecture of the reflex pathway (Castellucci and Kandel 1976).

Declarative Memory and the Hippocampus

In the mammalian brain two major early threads of research were critical in moving the study of memory forward to the point that the question of cellular and synaptic mechanisms could begin to be addressed. The first thread was anatomical and revealed a remarkable localization of memory function in the mammalian brain. Studies of patients with damage to the medial temporal lobes revealed that there are two major memory systems in the brain: declarative

(explicit) and procedural (implicit or nondeclarative). Declarative memory, a memory for facts and events—for people, places, and objects—requires the medial temporal lobe and in particular the hippocampus (Scoville and Milner 1957; Squire 1992; Schacter and Tulving 1994). In contrast, procedural memory, a memory for perceptual and motor skills also evident in invertebrate animals, involves a number of brain systems depending on the specific type of learning and in the most elementary instances uses simple reflex pathways themselves, as discussed above for simple systems. Although these seminal studies identified important classifications of memory and helped to solidify the notion of functional localization at a broad anatomical level, they did not provide insight into the mechanisms acting at the finer level of the individual neuron or synapse. This next step forward was made in rodent studies, in which the hippocampus plays a similar role in declarative types of memory, such as memory for place.

In 1971 recordings of single unit firing in the hippocampus of awake, freely moving rats revealed that neurons in the hippocampus register information not about a single sensory modality—sight, sound, touch, or pain—but about the space surrounding the animal, a feat that depends on information from several senses (O’Keefe and Dostrovsky 1971). These cells, referred to as “place cells,” fire selectively when an animal enters a particular area of the spatial environment. Based on these findings, O’Keefe and Nadel (1978) suggested that the hippocampus contains a cognitive map of the external environment that the animal uses to navigate. These studies helped to define the role of the hippocampus in declarative memory as a multimodal integrator and mapmaker. Although spatial location is a strong component of this hippocampal map, the human lesion studies and work from Howard Eichenbaum in rodents suggest that it may be a more general integrator of memory-specific associations (Squire and Stark 2004)

Nearly contemporaneous with the discovery of place cells, the synaptic responses in the hippocampus were found to display plasticity

with several features advantageous for memory storage (Bliss and Lømo 1973). Stimulation with a high-frequency train of action potentials was shown to produce a prolonged strengthening of synaptic transmission in all three of the major hippocampal pathways. This long-term potentiation (LTP), which is discussed in detail in the article by Lüscher and Malenka (2012) in this collection, has several forms that differ in molecular mechanism and duration (Fig. 2). In both the perforant path synapses from entorhinal cortex to dentate gyrus and Schaffer collateral synapses from CA3 to CA1 pyramidal neurons, LTP follows learning rules first postulated by Hebb. It requires that presynaptic activity be closely followed by postsynaptic activity. In the mossy fiber pathway, LTP does not follow Hebb’s rules; it requires only presynaptic activity with no coincident postsynaptic activity (Bliss and Collingridge 1993).

The Hebbian form of LTP has been the focus of intense interest as it became clear that it possessed many other features useful for a synaptic mechanism for certain forms of learning. These critical features of LTP are synapse specificity, cooperativity, and associativity. LTP is synapse specific in that it is only induced at synapses that are activated by the tetanic stimulation; neighboring synapses that are not active do not undergo potentiation. LTP is cooperative because multiple inputs must be activated simultaneously to produce sufficient postsynaptic depolarization to induce LTP. Finally, LTP is associative because when a weak input that is normally insufficient to induce LTP is paired with a strong input, the weak input will now become potentiated. These features can largely be explained by the behavior of the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor, whose activation is critical for the induction of the Hebbian form of LTP (see Lüscher and Malenka 2012). Unlike most neurotransmitter receptors that respond simply to the presence or absence of their cognate transmitter in the synaptic cleft, the NMDA receptor is also sensitive to the state of the postsynaptic membrane in which it resides. Under normal resting conditions a Mg^{2+} ion present in the NMDA receptor pore blocks ion flux. However, this Mg^{2+}

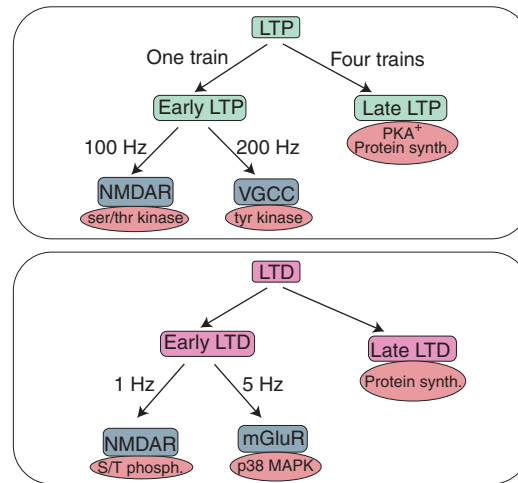


Figure 2. Multiple forms of long-term synaptic plasticity may contribute to learning and memory. (*Top*) A single train of high-frequency tetanic synaptic stimulation can produce forms of long-term potentiation (LTP) of synaptic transmission lasting 1–2 h. This early LTP (E-LTP) does not require changes in transcription or translation. Different patterns of tetanic stimulation can recruit distinct forms of E-LTP that differ in molecular mechanism and site of expression. Multiple trains of tetanic synaptic stimulation can induce a more persistent form of synaptic plasticity that can persist for many hours. This late LTP (L-LTP) requires altered gene expression, activation of PKA, and new protein synthesis. (*Bottom*) Weaker forms of synaptic activity can lead to a long-term depression of synaptic transmission (LTD). Different patterns of synaptic stimulation recruit distinct forms of LTD through differing signaling pathways.

blockade can be relieved by depolarization of the postsynaptic membrane, which expels the blocking ion through electrostatic repulsion. Thus, NMDA receptors are only active when there is both glutamate present because of presynaptic activity and the postsynaptic neuron is substantially depolarized. LTP is therefore produced only at synapses from a given presynaptic neuron that are active (leading to glutamate release) and impinge on a postsynaptic neuron that receives sufficient concurrently active inputs (associativity and cooperativity) to produce enough depolarization to relieve Mg^{2+} blockade of the NMDA receptor. As a result, LTP conforms to the type of learning rule Hebb suggested: “When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased.”

Activation of NMDA receptors produces an influx of postsynaptic Ca^{2+} that is critical to the

induction of LTP (Lynch et al. 1983; Malenka et al. 1988). In addition to LTP, which is routinely induced at hippocampal synapses by brief high-frequency (100-Hz) stimuli, hippocampal synapses also show a form of long-lasting synaptic depression, or LTD, with prolonged low-frequency stimulation. Surprisingly, this form of plasticity also requires activation of NMDA receptors and postsynaptic Ca^{2+} . The direction of the synaptic change likely depends on the magnitude and dynamics of the postsynaptic Ca^{2+} signal, as well as on the current state of the synapse, for example, whether it has been recently potentiated.

LTP and LTD were identified and have been studied primarily using artificial electrical stimulation that activates large numbers of fibers in a manner that is unlikely to occur naturally. The discovery of a spike-time-dependent form of synaptic plasticity (STDP) (Markram et al. 1997; Magee and Johnston 1997) was an important advance in providing a physiologically plausible manipulation that induced synaptic

plasticity. In STDP a synapse becomes potentiated if a postsynaptic spike follows presynaptic release, whereas the synapse becomes depressed if the postsynaptic spike precedes release. The plasticity occurs within a tight time window such that release and spike activity offset by more than 40 ms produce no synaptic change. STDP is also NMDA receptor dependent and is likely to incorporate some of the molecular mechanisms of LTP and LTD studied using less physiological stimuli, although there are clearly some important differences, as we shall see below.

Although the properties of NMDA-dependent synaptic plasticity are intriguing and provide a straightforward cellular mechanism for forming learned associations, it has been significantly more difficult to provide direct experimental links between LTP and behavior. In 1986 pharmacological studies made the first connection of LTP to spatial memory by showing that NMDA receptors must be activated to form this type of memory in the rat. When NMDA receptors are blocked pharmacologically, not only is LTP blocked, but the animal can no longer form spatial memories that are dependent on the hippocampus. This was shown using the Morris water maze, in which an animal must integrate multiple visual cues to form a spatial memory of the location of a platform submerged below the surface of a water bath (Morris et al. 1986). Importantly, blockade of the NMDA receptors does not impair the ability of an animal to learn to swim to the platform when it is visible, a task that does not require the hippocampus.

Similarly, the pharmacological blockade of NMDA receptors has been found to alter the stability of the place fields of hippocampal place cells (Kentros et al. 1998). When animals are placed in the same environment two days in a row, they activate substantially the same ensemble of hippocampal place cells whose place fields are similar in position and size on each day. This is consistent with the idea that these cells encode a stable map of the environment. However, if an NMDA blocker is administered during the formation of this place map, then the place-cell-firing pattern is not stable over 24 h.

These results provide a causal link between NMDA receptor function, long-term place cell

stability, and long-term spatial memory. It is tempting to think that these effects are linked by a causal requirement for LTP or LTD in these processes; however, a number of caveats should be kept in mind. For example, certain genetic manipulations that disrupt hippocampal LTP do not impair forms of memory believed to require the hippocampus (e.g., Zamanillo et al. 1999). Conversely, manipulations that do not alter hippocampal LTP can disrupt spatial learning (Shimshak et al. 2006).

One of the most difficult problems in linking synaptic plasticity mechanisms to behavior in declarative memory is the sparse and distributed nature of the circuits. How the various neural representations of the environment are modified with learning, the location of the critical sites of plasticity, and how these modified circuits are recruited to alter motor behavior in a memory test are still largely unclear. This makes interpreting the effects of a single type of pharmacological manipulation quite difficult. One approach that has been taken in both simple and complex models is to apply molecular and genetic approaches to provide a richer understanding of the underlying mechanisms of plasticity and to use the tools generated to probe the links to behavior.

Procedural Fear Memory in the Mammalian Amygdala

Some of the strongest evidence linking learning and memory to LTP comes from experiments focused on the amygdala, which is essential for both instinctive and learned fear (Davis et al. 1994; LeDoux 1995, 1996). When an animal is given a shock to the foot paired with a tone—a classical conditioning paradigm—the animal exhibits a learned fear response by freezing in response to the tone alone. This form of learning is known to involve the amygdala, a region of the brain that receives direct auditory information from the thalamus and processed information from neocortex, and which provides an output to the hypothalamus that regulates autonomic fear responses. Neurons in isolated slices of amygdala undergo LTP in response to tetanic stimulation protocols similar to those

used in the hippocampus. Importantly, behavioral pairing of a tone and shock, which induces fear learning, also potentiates synaptic responses in the amygdala to auditory stimuli in vivo (Rogan et al. 1997). Following fear learning the synaptic response to electrical stimulation of auditory inputs in isolated amygdala slices is also enhanced (McKernan and Shinnick-Gallagher 1997). Moreover, this synaptic enhancement must be closely related to LTP because behavioral fear conditioning occludes the ability to induce LTP in isolated amygdala slices in response to high-frequency stimulation (Tvsetkov et al. 2002).

SYNAPTIC AND MOLECULAR MECHANISMS OF PLASTICITY AND LEARNING

Procedural Memory in Invertebrates

Molecular biology revealed a remarkable conservation of mechanism underlying short-term memory among different animals. In 1974 Seymour Benzer and his students discovered that *Drosophila* can learn fear and that mutations in single genes interfere with short-term memory. Flies with such mutations do not respond to classical conditioning of fear or to sensitization, suggesting that the two types of learning have some genes in common (Quinn et al. 1974; Dudai et al. 1976). Identification of the causal mutations in the fly revealed that they represented one or another component of the cAMP pathway, the same pathway underlying sensitization and classical conditioning in *Aplysia* (Byers et al. 1981). These early studies that pointed to the central role of cAMP signaling examined short-term memory lasting only a few minutes. The next step was to ask how the short-term changes become stabilized to last days or years.

The first clue to how short-term memory is switched to long-term memory came when Louis Flexner observed that the formation of long-term memory requires the synthesis of new proteins (Flexner et al. 1963). Subsequent work in *Aplysia* (Dash et al. 1990; Bacsikai et al. 1993; Martin et al. 1997; Alberini et al. 1994; Hegde et al. 1997; for review, see Kandel 2001) and *Drosophila* (Dudai et al. 1976; Duerr and

Quinn 1982; Drain et al. 1991; for review, see Waddell and Quinn 2001) showed that with repeated training PKA moves from the synapse to the nucleus of the cell, where it activates the transcription factor cAMP response element binding protein-1 (CREB-1). CREB-1 acts on downstream genes to activate the synthesis of protein and stimulate the growth of new synaptic connections.

Further studies in *Aplysia* and in the fly revealed the surprising finding that the switch to long-term synaptic change and the growth of new synaptic connections is constrained by memory suppressor genes (see Abel et al. 1998). One important constraint on the growth of new synaptic connections is CREB-2 (Yin et al. 1994; Bartsch et al. 1995), which when overexpressed blocks long-term synaptic facilitation in *Aplysia*. When CREB-2 is removed, a single exposure to serotonin, which normally produces an increase in synaptic strength lasting only minutes, will increase synaptic strength for days and induce the growth of new synaptic connections.

Declarative Memory

Molecular and pharmacological studies in hippocampal slice preparations have identified some of the key signaling events that are triggered by Ca^{2+} influx through the NMDA receptors during the induction of LTP (Fig. 3). The initial Ca^{2+} signal activates directly or indirectly at least three critical protein kinases in the post-synaptic neuron: (1) calcium calmodulin-dependent kinase II (CaMKII) (Malenka et al. 1989; Malinow et al. 1989); (2) protein kinase C (PKC) (Routenberg 1986; Malinow et al. 1988); and (3) the tyrosine kinase Fyn (O'Dell et al. 1991; Grant et al. 1992). These findings provided a catalog of targets for manipulation using advances in mouse genetics that allowed for the selective deletion or knockout of individual genes in the whole animal. The general strategy was to delete a gene critical for LTP and to then examine both synaptic plasticity and behavioral learning and memory in the same animal to test the idea that LTP is a key molecular event important for memory storage.

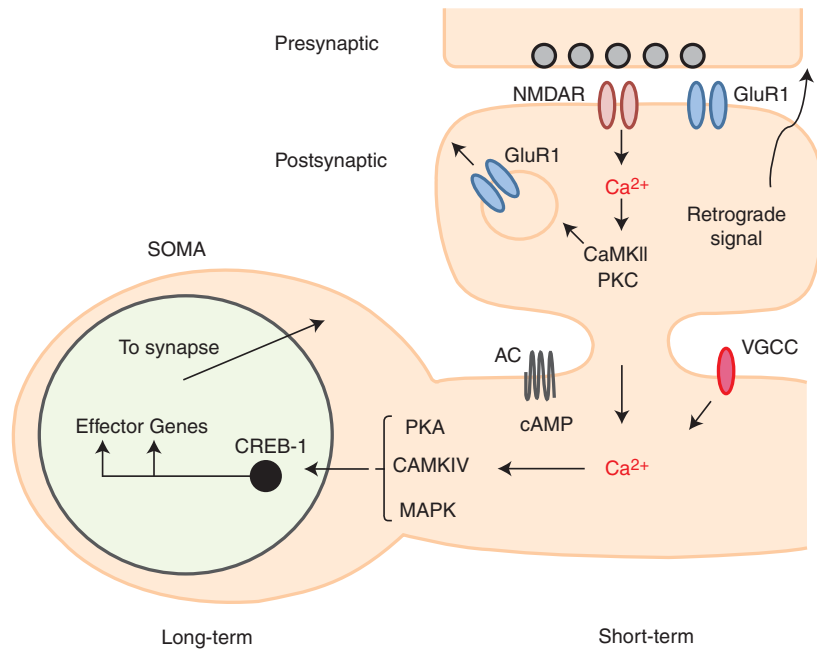


Figure 3. Molecular mechanisms for induction of LTP and memory. These basic signaling pathways have been implicated in both NMDAR-dependent LTP and behavioral learning. Short-term plasticity (lasting several hours) is produced by NMDAR-dependent Ca^{2+} signaling to protein kinases and the recruitment of new glutamate receptors to the synapse. Long-term plasticity (lasting days) requires CREB-dependent gene activation in the nucleus by the action of multiple protein kinases. Long-term plasticity and memory also requires synthesis of a constitutively active isoform of PKC.

The first set of studies applied these techniques to examine the importance of the α subunit of CaMKII and the tyrosine kinase Fyn. Genetically altered mice lacking either of these kinases were viable and survived to adulthood. However, in each line of mutant mice hippocampal LTP and spatial memory were impaired. These results extended the link between hippocampal synaptic plasticity and memory, first established pharmacologically with NMDA receptor blockers, and outlined a genetic approach for exploring the mechanisms of synaptic and behavioral plasticity. Genetics is the gold standard for determining molecular function in modern biology, and its application to complex questions of neurophysiology and behavior in the mammalian brain opened up a myriad of molecular questions that were previously inaccessible. However, it soon became clear that the approach suffered a number of drawbacks for the study of circuits and behavior that have

stimulated several important technical refinements.

A major question regarding the molecular mechanism of LTP is whether it is expressed presynaptically, involving an increase in glutamate release; postsynaptically, depending on an increase in the postsynaptic response to glutamate; or through a coordinated change in presynaptic and postsynaptic properties. A number of apparently contradictory results have been reported in the literature over the past 30 years. However, in our view much of the controversy results from the fact that neurons can express multiple forms of LTP that may differ in their synaptic locus, molecular mechanisms, time-scale, and role in learning and memory (Fig. 2).

The controversy dates back to some of the earliest studies of LTP. Measurements of radio-labeled glutamate binding to hippocampal membranes indicated that LTP was postsynaptic, caused by an increase in the number of

postsynaptic glutamate receptors (Lynch et al. 1982). At the same time another study concluded that LTP was presynaptic, based on the finding of increased levels of extracellular glutamate following induction of LTP (Dolphin et al. 1982). The interpretation of such early studies was complicated by the fact that they did not directly assess synaptic function. Rather, it was the finding that LTP in the Schaffer collateral pathway is associated with a selective increase in the AMPA-type receptor component of the excitatory postsynaptic potential with little change in the NMDA-type receptor component that provided the first evidence that LTP at this synapse is both initiated and expressed postsynaptically (Kauer et al. 1988). Later it was found that the increase in response of the AMPA receptors to glutamate during LTP is attributable to the rapid insertion of new clusters of receptors in the postsynaptic membrane from a pool of intracellular receptors stored in recycling endosomes (Carroll et al. 1999; Shi et al. 1999; Park et al. 2004; Nicoll et al. 2006). Other studies, however, have implicated additional presynaptic changes following induction of Schaffer collateral LTP (Bolshakov and Siegelbaum 1994; Zakharenko et al. 2001; Emptage et al. 2003; Enoki et al. 2009). As nearly all studies of Schaffer collateral LTP indicate that its induction requires Ca^{2+} influx into the postsynaptic cell, the finding that LTP may involve an increase in transmitter release implicates the need for one or more retrograde messengers that are released from the postsynaptic cell to enhance release from the presynaptic cell. As discussed below, whether LTP is presynaptic or postsynaptic (or both) likely depends on the frequency or pattern of stimulation used to induce plasticity.

Changes in presynaptic function associated with LTP can be assayed selectively by measuring the rate of release of a fluorescent dye, FM 1-43, from synaptic vesicles in the presynaptic terminals when the vesicles release their contents by exocytosis in response to presynaptic stimulation (Ryan et al. 1996). When LTP is induced using a 50-Hz tetanic stimulation, there is no change in the rate of dye release in response to presynaptic action potentials, suggesting that the expression of 50-Hz LTP is purely postsyn-

aptic. However, when LTP is induced using stronger 200-Hz or theta burst stimulation protocols, there is a marked increase in the rate of dye release, suggesting an enhanced presynaptic function (Zakharenko et al. 2001) that appears to develop more slowly than the enhancement in AMPA receptor response (Bayazitov et al. 2007). The presynaptic and postsynaptic forms of LTP also show a differential dependence on NMDA receptors. Whereas 50-Hz LTP is fully blocked by the NMDA receptor antagonist APV, 200-Hz and theta burst LTP recruit an NMDA receptor-independent form of LTP that requires activation of L-type voltage-gated Ca^{2+} channels (Grover and Teyler 1990; Zakharenko et al. 2001). Genetic evidence for the existence of distinct presynaptic and postsynaptic loci of LTP has been provided by studies of AMPA receptor- and brain-derived neurotrophic factor (BDNF)-knockout mice.

The GluR1 (GluRA) AMPA receptor subunit is important for the activity-dependent postsynaptic insertion of AMPA receptors with LTP. Knockout mice carrying a deletion in GluR1 show a severe deficit in “standard” NMDA-dependent LTP induced by high-frequency stimulation, as might be expected from previous studies. Surprisingly, these mice were not impaired in long-term spatial memory as seen with previous knockouts or NMDA receptor antagonists that blocked LTP (Zamanillo et al. 1999). Subsequent studies showed that not all forms of LTP were impaired in these mice; for example, a component of theta burst LTP was spared (Hoffman et al. 2002). Moreover, not all spatial learning was intact; a short-term form of spatial working memory was impaired in the knockout animals (Reisel et al. 2002).

A line of mice in which the BDNF gene was selectively deleted in the forebrain shows relatively normal LTP in response to a 50-Hz tetanic stimulus (Zakharenko et al. 2003). However, these mice have a significant reduction in LTP in response to 200-Hz or theta burst tetanic stimulation. Moreover, the ability of these strong LTP protocols to produce a presynaptic enhancement in the rate of FM 1-43 release is completely blocked in the mutant mice. These data support the view for separable components

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of presynaptic and postsynaptic LTP that can be differentially recruited by distinct patterns of physiological activity. Interestingly, whereas a reduction in NMDA receptor–dependent LTP is observed as animals age, and is correlated with an age-related decline in memory, older animals show an increase in L-type Ca^{2+} channel–dependent LTP (Shankar et al. 1998). Moreover, L-type Ca^{2+} channel antagonists improve memory in older animals (Deyo et al. 1989).

Clearly the relationship between LTP and memory is more complex than a simple 1:1 correspondence. Different forms of LTP have different underlying molecular mechanisms and different roles in behavior that we are just beginning to unravel (e.g., Woodside et al. 2004).

Long-Term Memory

Procedural and declarative memories differ dramatically. They use a different logic (unconscious vs. conscious recall) and they are stored in different areas of the brain. Nevertheless, these two disparate memory processes share several molecular steps and an overall molecular logic. Both are created in at least two stages: one that does not require the synthesis of new proteins and one that does. In both, short-term memory involves covalent modification of preexisting proteins and changes in the strength of preexisting synaptic connections, whereas long-term memory requires the synthesis of new proteins and the growth of new connections. Moreover, both forms of memory use PKA, mitogen-activated protein kinase (MAPK), CREB-1, and CREB-2 signaling pathways to convert short-term to long-term memory. Finally, both forms appear to use morphological changes at synapses to stabilize long-term memory (Bailey et al. 2008).

Long-term potentiation in the hippocampus proved to have both early and late phases, much as long-term synaptic facilitation in *Aplysia* does. One train of stimuli produces the early phase (E-LTP), which lasts 1–3 h and does not require protein synthesis. Four or more trains induce the late phase (L-LTP), which lasts at least 24 h, requires protein synthesis, and is activated by PKA (Frey et al. 1993; Abel et al. 1997). The role of this L-LTP has been investi-

gated genetically using mice that express a mutant gene that blocks the catalytic subunit of PKA, or that carry an inhibitory mutation in the CREB-1 gene. Both lines of mice have a serious defect in long-term spatial memory and both have roughly similar defects in LTP. The early phase is normal but the late phase is blocked, providing evidence linking the phases of LTP to the phases of memory storage (Silva et al. 1992a,b; Bourtchouladze et al. 1994; Huang et al. 1995; Abel et al. 1997). Finally, an intermediate phase of LTP that requires PKA but not new protein synthesis can be induced by two trains of stimuli (Winder et al. 1998). Theoretical studies indicate that these multiple components of plasticity are necessary to generate long-lasting memories in the face of ongoing synaptic plasticity (Fusi et al. 2005).

Procedural Memory in Vertebrates

We now have a good understanding of the neural circuit and molecular mechanisms underlying learned fear and of the role of synaptic plasticity in fear memory, thanks to the work of Joseph LeDoux, Michael Davis, Michael Fanselow, and James McGaugh. Pairing of a tone with a foot shock leads to a conditioned fear response to the tone alone, which elicits freezing behavior in the conditioned animal. This conditioned fear response depends on the long-term potentiation of the auditory response in neurons of the amygdala (Johansen et al. 2011). Both the synaptic changes and the persistence of the memory for learned fear require PKA, MAPKs, and the activation of CREB (Won and Silva 2008). Moreover, similar to mechanisms of NMDA receptor–dependent LTP, learned fear requires the enhanced trafficking of AMPA receptors to the synapses of amygdala neurons (Rumpel et al. 2005). In contrast to learned fear, when a tone predicts a period of safety when an animal is protected from the foot shock, there is a long-term depression of the auditory inputs to the amygdala (Rogan et al. 2005). Thus, learned fear and learned safety involve opposing changes in synaptic strength.

Eye-blink conditioning is produced by pairing a tone (the CS) with an aversive air puff to

the eye (the US), resulting in a learned eye blink that is appropriately timed to the paired US (Thompson et al. 1983). Theoretical and experimental studies have proposed that the conditioning involves a relatively simple cerebellar circuit. Prior to learning, activation of cerebellar Purkinje neurons in response to the CS leads to an inhibition of neurons in the interpositus nucleus (one of the deep nuclei of the cerebellum), thereby inhibiting motor output. With conditioning there is a decrease in the Purkinje cell activity in response to the CS, resulting in disinhibition of the neurons of the interpositus nucleus, leading to eye blink. This model is consistent with findings that Purkinje cell activity can be reduced as a result of LTD at the excitatory parallel fiber synaptic input onto the Purkinje neurons (Ito 2001). This decrease in the strength of the parallel fibers occurs when the climbing fiber inputs to the cerebellum are activated in appropriate temporal proximity to parallel fiber activity. Thus, the Purkinje cells become less responsive to input, as a result of a down-regulation of AMPA receptors at the parallel fiber to Purkinje cell synapse.

The similarity between the changes in synaptic function and both procedural and declarative learning in the mammalian and invertebrate central nervous systems supports the view that alterations in synaptic strength represent an evolutionarily conserved general mechanism of memory formation. Moreover, studies of fear learning, eye-blink conditioning, and modifications of the vestibular–ocular reflex (Lisberger et al. 1987; Boyden et al. 2006), as well as habituation in *Aplysia* and crayfish, provide support for the role of both synaptic potentiation and synaptic depression as parallel mechanisms for memory storage.

Synapse-Specific Local Protein Synthesis and Learning Networks

There is now significant evidence from various forms of learning in a number of different species that the critical changes that store information in the brain occur at specific synapses within a circuit. The finding that long-term memory and synaptic plasticity involve changes in gene

expression and therefore the nucleus of the cell—which is shared by all the synapses of the neuron—raises the question of how the gene products required for long-term memory influence the specific synapses that were altered to produce the immediate and short-term memory. Studies in both *Aplysia* (Martin et al. 1997) and the hippocampus (Frey and Morris 1997) suggest that the synaptic modifications associated with short-term plasticity leave a molecular mark or “tag” on the synapses that were modified. The presence of the synaptic tag allows those synapses to specifically capture and use newly synthesized gene products arriving from the nucleus to stabilize the initial changes produced with learning.

How is a synapse marked? Two distinct components of marking have been identified in *Aplysia*, one that requires PKA and initiates long-term synaptic plasticity and growth, and one that stabilizes long-term functional and structural changes at the synapse and requires (in addition to protein synthesis in the cell body) local protein synthesis at the synapse (Martin et al. 1997). Because mRNAs are made in the cell body, the need for the local translation of some mRNAs suggests that these mRNAs may be dormant before they reach the activated synapse. If that were true, one way of activating protein synthesis at the synapse would be to recruit a regulator of translation at the activated synapse that is capable of activating dormant mRNA. In *Xenopus* oocytes, maternal RNA is silent until activated by the cytoplasmic polyadenylation element binding protein (CPEB) (Richter 1999). In *Aplysia* a new isoform of CPEB (ApCPEB) with novel properties was found in neurons. Blocking this isoform at a marked (active) synapse prevented the maintenance but not the initiation of long-term synaptic facilitation (Si et al. 2003a,b). Indeed, blocking ApCPEB blocks memory days after it is formed. An interesting feature of this isoform of *Aplysia* CPEB is that its amino terminus resembles the prion domain of yeast prion proteins and endows it with similar self-sustaining properties. But unlike other prions, which are pathogenic, ApCPEB appears to be a functional prion. The active self-perpetuating form of the

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protein does not kill cells but rather has the important physiological function of maintaining long-term synaptic facilitation.

THE EMERGENCE OF A SYSTEMS APPROACH TO MEMORY STORAGE

The application of molecular and genetic tools and the use of simple systems have allowed us to test some of the foundational theories of Cajal and Hebb regarding the cellular mechanisms of learning. There are a number of commonalities that have been established across multiple species, such as:

1. Synaptic change is elicited by patterns of neuronal activity at critical points within a behavioral circuit.
2. Both increases and decreases in synaptic strength can contribute to behavioral plasticity.
3. Synaptic plasticity has similar temporal and molecular properties to behavioral learning, e.g., short- and long-term phases dependent on discrete signaling pathways.
4. Apparently different forms of learning use similar underlying cellular and molecular mechanisms.

One of the remaining challenges in rigorously testing some ideas in the mammalian nervous system is the problem of sparse encoding in distributed networks and the identification of the engram, the physical or functional memory trace present in a neural network posited by Lashley (1950). Although the fine-tuning of the hippocampal representation of the world and the sensitization of the gill-withdrawal reflex in *Aplysia* may recruit some of the same underlying molecular mechanisms, the changes in the hippocampus are dispersed throughout a large structure with no clear anatomical segregation. And although the hippocampus plays a critical role in memory, the formation of complex associations must involve activity in multiple brain areas. How can the dispersed circuits for a given specific memory or complex representation be

isolated and functionally probed in the same manner as a reflex circuit in a simple system?

Several advances in the optical and molecular toolbox for circuit analysis in mammals are laying the foundation to answer this type of question. Advances in in vivo optical imaging techniques have enabled the visualization of plastic changes in neuronal properties associated with learning and memory (Hübener and Bonhoeffer 2010). Such changes can involve alterations in morphology of preexisting synapses, including the enlargement of dendritic spines (the sites of a pyramidal neuron's excitatory input) during LTP and spine shrinkage during LTD (Kasai et al. 2010). Additional structural changes may involve the growth of new synaptic connections, implied by the appearance of new dendritic spines following induction of synaptic plasticity. That such structural changes may contribute to learning and memory is supported by a recent study that reported the growth of new dendritic spines in motor cortex neurons following motor learning (Xu et al. 2009).

One area of rapid progress is in the introduction of genetically encoded molecules that allow the selective activation or suppression of the neurons in which they are expressed with light (Zhang et al. 2007a,b; Zhao et al. 2008; Airan et al. 2009). A great advantage of light-regulated channelrhodopsin or halorhodopsin is that they allow precise millisecond temporal control over action potential firing such that a genetically tagged group of neurons can be fired in a controlled pattern simply by patterning the light pulses. Also effective for turning neurons on or off are ligand-gated proteins with customized binding sites, such as the G-protein-coupled designer receptor (Alexander et al. 2009) and a chimeric ligand-gated ion channel, which are activated by an inert ligand (Magnus et al. 2011). These systems are allowing sparse but genetically defined neural cell types (e.g., inhibitory neurons) to be manipulated selectively within a background of many other functionally distinct cell types.

What defines a circuit in the mammalian brain? At one level there is a clear, developmentally controlled pattern of connectivity, for

example, the hippocampal trisynaptic circuit or a cortical column. Although this canonical connectivity is clearly an important constraint on function, what is remarkable is that these circuits can represent so many different external events or encode a wide range of memories. Clearly, any individual neuron can participate in many different representations or memories, and at a deeper level a neural circuit is defined by what it represents. That is, we could define a circuit as all the neurons that are recruited during the recognition of an individual's home, or during the recollection of one's last holiday. How many neurons does it take to form a specific memory? How predetermined are these circuits? How are they modified during learning and differentially recruited during recall? And how can a new memory be formed through altered synaptic strength without overwriting a preexisting memory encoded in a neuron's synapses? Some new genetic techniques are beginning to probe these questions.

Competition between neurons is necessary for refining neural circuitry, but does it play a role in encoding memories in the adult brain? In studies of fear conditioning it was found that the introduction of excess or constitutively active CREB into a sparse subset of amygdala neurons caused those neurons to be specifically recruited to encode the memory to which the animals were subsequently trained (Han et al. 2007). Conversely, if such neurons are deleted after learning, that specific fear memory is blocked while other fear associations stay intact (Han et al. 2009). This study reveals that there is great flexibility in the particular group of neurons recruited to any given memory, at least in the amygdala, and that the resting state of the neuron governs the probability that it will be recruited.

Long-term memory requires transcriptional activity, and genes such as *cfos*, *zif268*, and *arc* are rapidly and transiently induced by high-frequency neural activity and have been used for many years to map brain activity patterns in rodents. By providing a genetic readout of patterns of neural activity, these genes provide the potential to obtain direct molecular control over ensembles of neurons based on their re-

sponse to a given experience. In one study the *cfos* promoter was combined with elements of the TET regulatory system in transgenic mice to allow the introduction of a *lacZ* marker into neurons activated with fear conditioning (Reijmers et al. 2007). The marker provided a long-lasting record of brain activity during learning that could be compared to activity during recall. A partial reactivation of the neurons active during learning occurred, and the strength of the recalled memory was correlated with the degree of circuit reactivation. More importantly, this approach provides an opportunity to introduce any genetically encoded effector molecule into neurons based on their recent activity, providing the potential to study circuits based on the specific memory they encode.

Why are there so many forms of synaptic plasticity that differ in their mechanism of induction, time of persistence, and synaptic locus (Fig. 2)? An interesting insight into this question was provided by a theoretical study that approached the question of how a memory can persist in the face of the barrage of synaptic inputs and synaptic plasticity that a neuron experiences during an individual's lifetime. Although it was impossible to encode robust memories with a single form of plasticity, multiple forms of plasticity with distinct timescales of induction and persistence were able to yield persistent memory storage (Fusi et al. 2005). A challenge in the future will be to examine how the diverse array of plasticity mechanisms may indeed cooperate and interact to yield a unified mechanism of long-lasting yet ongoing memory storage.

SUMMARY

We now understand in considerable molecular detail the mechanisms underlying long-term synaptic plasticity and the importance that such plastic changes play in memory storage, across a broad range of species and forms of memory. One surprising finding is the remarkable degree of conservation of memory mechanisms in different brain regions within a species and across species widely separated by evolution. However, although it is now clear

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that long-term synaptic plasticity is a key step in memory storage, it is important to note that a simple enhancement in the efficacy of a synapse is not sufficient to store a complex memory. Rather, changes in synaptic function must occur within the context of an ensemble of neurons to produce a specific alteration in information flow through a neural circuit. With the recent development of powerful genetic tools, it may soon be possible to meet the daunting challenge of visualizing and manipulating such changes in neural circuitry. A second important challenge is to understand how the basic processes of memory storage are altered with age or disease, including Alzheimer's disease. There is now compelling evidence that defects in memory storage result from pathological changes in the fundamental mechanisms underlying long-term synaptic plasticity. Thus, it becomes of critical importance to understand in sufficient detail both the basic mechanisms of memory storage and the changes that take place in disease to design specific compounds that can be used to restore cognitive function.

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