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Publication Date

2013

DOI

10.1016/B978-0-12-415823-8.00017-4

Peer reviewed

Synaptic Mechanisms of Induction and Maintenance of Long-Term Sensitization Memory in *Aplysia*

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Key Words: *Aplysia*, withdrawal reflex, long-term memory, long-term sensitization, long-term facilitation, serotonin, PKM, reconsolidation, CPEB

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Abstract

The marine snail *Aplysia californica* exhibits a simple defensive withdrawal reflex that can undergo several forms of learning. In particular, the reflex can exhibit long-term sensitization (LTS), a form of nonassociative memory. LTS is mediated by long-term facilitation (LTF) of the monosynaptic connection between the sensory and motor neurons that mediate the withdrawal reflex. LTS and LTF represent one of the best-understood model systems of long-term memory extent. Furthermore, discoveries from work on this system have provided fundamental insights into the cellular and molecular mechanisms that mediate the induction and maintenance of long-term memory. The present chapter reviews this work; it concludes with a discussion of recent studies of the role of protein kinase M in the persistence of the long-term memory and of memory reconsolidation in *Aplysia*. It is suggested that the study of LTS and LTF can provide important mechanistic information these two intriguing memory phenomena.

Introduction

One of the more remarkable features of the brain is its ability to transform experience into memories that persist for a lifetime. That memories can remain more or less stable over the course of many decades despite the significant physical changes that occur in the brain with age is one of the grand mysteries of neuroscience. The last 30 years have witnessed rapid progress in our understanding of how long-term memories are induced in the brain; by comparison, we understand far less about how memories are maintained.

Much of what we know about the cell biology of long-term memory has come not from studies of the mammalian brain but, rather, from studies of the nervous systems of invertebrate organisms, the most influential of which, arguably, are those using the marine snail *Aplysia californica*. Why this relatively humble mollusk should have played such a key role in mechanistic studies of long-term memory requires some explanation. *Aplysia* has several important features that facilitate relating cellular and molecular changes to learned behavioral changes. First, its central nervous system (CNS) possesses only about 20,000 neurons [1], compared to, for example, about 21 million in the brain of a rat [2]. This greatly simplifies the task of identifying learning-related sites of change in the nervous system. The logic here is similar to that employed by the legendary Spanish neuroanatomist, Ramon y Cajal, who chose the embryos of animals such as dogs and cats for his initial investigations of the fine structure of the brain using the Golgi stain. In justifying this approach Cajal [3] stated, "Since the full grown forest [i.e., the adult brain] turns out to be impenetrable and indefinable, why not revert to the study of the young wood, in the nursery stage, as we might say?" Similarly, the simplicity of the *Aplysia* nervous system renders the specific pattern of cellular changes produced by a learning

experience more readily visible in *Aplysia* than in the relatively impenetrable neural thicket of the mammalian brain. Another major advantage of *Aplysia* is that neurons that mediate specific behaviors can be identified, and then individually dissociated and placed into dissociated cell culture. This means that behaviorally relevant neural circuits can be recreated in vitro. Importantly, these in vitro circuits have been shown to exhibit most, if not all, of the same forms of synaptic plasticity that they exhibit in vivo. This feature has been extensively exploited, most prominently by Eric Kandel and his colleagues, with the result that arguably more is known about the cell biology of learning and memory in *Aplysia* than in any other organism [4]. The monosynaptic connection between the sensory and motor neurons that mediate the defensive withdrawal reflex of the gill and siphon (the sensorimotor synapse) has been a particularly fruitful system for mechanistic analyses of learning. Among the major contributions of research on the sensorimotor synapse is an understanding of the critical role in long-term, learning-related synaptic plasticity of protein synthesis and gene transcription [5], particularly that stimulated by the transcription factor cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) [6-9]. Importantly, these processes have each been found to play a critical role in mammalian learning-related long-term synaptic plasticity as well [10-13], which indicates that there has been broad conservation of the cellular and molecular mechanisms of learning and memory over the course of evolution.

The present review will focus on the one specific form of long-term memory in *Aplysia*, the memory for long-term sensitization (LTS) of the defensive withdrawal reflex [14]. Arguably, knowledge regarding underlying mechanisms has advanced further for LTS than for any other form of long-term memory in *Aplysia*. The review will begin with a

description of the cellular and molecular mechanisms that mediate induction of LTS. It will then describe what is known about the mechanisms that underlie the maintenance of LTS. Finally, future directions toward an understanding of this form of nonassociative learning and memory will be discussed. Although sensitization in *Aplysia* involves nonsynaptic, particularly changes in neuronal excitability, this chapter will focus on the synaptic mechanisms of LTS. (For a recent review on nonsynaptic mechanisms of learning in *Aplysia* and other organisms, see ref. [15].)

Long-Term Sensitization in A*plysia*: Mechanisms of Induction

When an *Aplysia* receives a noxious stimulus, such as an electrical shock to its tail, the endogenous monoaminergic transmitter serotonin (5-HT) is released into the animal's CNS from a network of central serotonergic neurons [16-19]. This release of 5-HT triggers a number of cellular changes that lead to enhancement of the defensive withdrawal reflex [20]. Much of the analysis of these sensitization-related cellular changes has focused on the monosynaptic connections between the central sensory and motor neurons that mediate the reflex. Two such connections that have been particularly well studied with respect to sensitization have been that between the sensory and motor neurons that mediate contraction of the gill and siphon [21-23]; and the synaptic connection between the pleural sensory neurons and the pedal motor neurons, which mediates contraction of the tail [24,25]. The central sensory and motor neurons that mediate the gill- and siphon-withdrawal reflex are located in the adominal ganglion [26]. Tail withdrawal is mediated by sensory neurons in the paired pleural ganglia, whereas the motor neurons that mediate this reflex are located in the paired pedal ganglia [27].

Tail (or tail nerve) shock and 5-HT facilitate transmission at the sensorimotor synapse [25,28-32]. (Other endogenous transmitters can produce facilitation of the sensorimotor synapse, particularly the small cardioactive peptides [SCPs][30,33] and nitric oxide, released by the L29 facilitatory interneurons [34]; however, thus far only 5-HT has been shown to support long-term facilitation of the sensorimotor synapse.) The facilitation of the sensorimotor synapse plays a prominent role in both short-term [31,35] and long-term [36] sensitization.

A single tail shock, or a single, brief (≤ 5-minute) pulse of 5-HT will produce only short-term (< 30 minutes) behavioral and synaptic enhancement. However, several, spaced tail shocks, or repeated application of 5-HT, can result in long-term (≥ 24 hours) behavioral and synaptic changes in *Aplysia*. [5,14,36,37]. The long-term memory for sensitization differs in several fundamental respects from the short-term form. First, the long-term memory, unlike short-term form, depends on protein synthesis and gene transcription [5,7-9,38-48]. Second, LTS, unlike short-term sensitization (STS), typically—although not invariably [49]—involves significant structural reorganization, particularly the growth of new presynaptic varicosities [50-56].

Comment on cellular locus of inductive processes

A complication in describing the cellular and molecular mechanisms that underlie the induction—as well as the maintenance—of LTS in *Aplysia* is identifying the cellular site where the mechanisms operate. For decades it was assumed that the changes mediating the long-term behavioral and synaptic changes that characterized LTS were confined to the presynaptic sensory neuron [4,57]. Although, as summarized below, there are indeed

important presynaptic changes that contribute to the induction of the long-term memory for sensitization, it is now apparent that such changes are insufficient to account for the memory, and that postsynaptic changes are also critical (for review see ref. [58]). Furthermore, in at least some cases the changes, whether presynaptic or postsynaptic, are not cell autonomous, but require as yet unidentified transsynaptic signals; these signals may be retrograde [59-61] or anterograde [62]. The role of postsynaptic mechanisms and transsynaptic signals in long-term memory in *Aplysia* has been slighted (but see refs. [61,63-65]) prior to the last decade. There have been far more studies of presynaptic changes that underlie long-term memory than of postsynaptic changes; biochemical and molecular studies of LTS have been particularly weighted toward presynaptic mechanisms. A cursory survey of the literature on LTS in *Aplysia* might therefore lead the naïve reviewer to conclude that presynaptic mechanisms play a larger role than postsynaptic mechanisms in this form of learning. But the present prominence of presynaptic mechanisms in studies of long-term memory in *Aplysia* is unlikely to reflect any underlying biological reality [58], and the role of postsynaptic biochemical and molecular changes in LTS represents a rich, nearly untapped, vein of discovery for future investigation.

Biochemical and molecular mechanisms of induction

5-HT, released into the CNS of *Aplysia* by sensitizing stimuli, such as tail shock [17], activates the second messenger cAMP within sensory neurons [28,66-71]. Repeated or prolonged application of sensitizing stimuli, or of 5-HT, produces prolonged elevation of cAMP within the sensory neurons [70], resulting in persistent activity of protein kinase A (PKA) [72]. The persistent activity of PKA, a heterodimer with two regulatory and two

catalytic subunits, results, at least in part, from a selective downregulation of the regulatory subunits of the protein kinase [45]. The decrease in the ratio of regulatory to catalytic subunits requires protein synthesis [45] and is caused by selective proteolysis of the regulatory subunits [73].

The loss of PKA's regulatory subunits and its consequent persistent activation, produced by repeated stimulation with 5-HT, can lead to a translocation of the catalytic subunits from the cytoplasm of the sensory neuron to its nucleus [67]. The nuclear translocation of the PKA catalytic subunits, in turn, activates cyclic AMP response element binding (CREB) protein, a transcriptional activator [7,8,74]; the activation of CREB is a key step in the induction of long-term memory in *Aplysia*. CREB represents a family of transcription factors [75]. In *Aplysia* the activator isoform is termed CREB1. The activity of CREB1 is normally inhibited by a repressor CREB isoform, known as CREB2 [41]; stimuli that induce LTS or long-term facilitation (LTF) cause the derepression of CREB1 by CREB2, thereby leading to CRE-dependent gene expression [6]. It is believed that this derepression is mediated by extracellular signal-regulated kinase (ERK)/mitogen-activated kinase (MAPK); cytoplasmic MAPK is activated within sensory neurons by prolonged 5-HT treatment and translocates to the nucleus where it phosphorylates CREB2 [76-80]. Among the genes stimulated by CREB1 are the immediate-early gene ubiquitin-C hydrolase [81], which encodes an enzyme that enhances proteasome activity [82], as well as genes that encode two other transcription factors, *Aplysia* Activating Factor (ApAF) [83] and the CCAAT- box-enhanced binding protein (C/EBP) [84]. ApAF and C/EBP, together, possibly, with other as yet unidentified, trigger a second wave of gene activation; one of the consequences of this later wave of transcription is believed to be

the growth of new synaptic connections structural growth [53,85] (see below).

Presynaptic mechanisms of LTS and LTF

LTS is associated with facilitation of the monosynaptic connection between the central sensory and motor neurons that mediate the siphon- and tail-withdrawal reflex [36,37,86]. Because short-term facilitation (STF) of the sensorimotor synapse is largely, perhaps exclusively, the result of presynaptic changes [29,87-89], it was seductive to extend the presynaptic model to LTF. Unquestionably, LTF results partly from enhanced presynaptic release of glutamate, the neurotransmitter used by *Aplysia* sensory neurons [90-93]. Nonetheless, the idea that LTF results exclusively [4,57,87], or even predominately, from presynaptic mechanisms is no longer tenable [58,59,62,94].

A major advance in the mechanistic analysis of LTF in *Aplysia* was the demonstration of LTF in sensorimotor cocultures by Montarolo et al. in 1986 [5]. The methodology of Montarolo et al. has been used to make many of the most important discoveries regarding long-term memory in *Aplysia.* For in vitro studies of long-term synaptic plasticity identified sensory and motor neurons are individually dissociated from central ganglia of *Aplysia* and placed into cell culture together (**Figure 1B**). Under the right conditions the neurons will form chemical synaptic connections; the strength of these connections gradually increases over the first several days in vitro, and then reaches a stable value, typically after 3-5 days. (The precise time at which the synapses stabilize in strength stabilizes depends on factors such as the specific identities of the pre- and postsynaptic neurons—for example, whether the giant gill- and mantle- motor neuron L7 [1,5] or one of the small siphon motor neurons [23,95] is used for the postsynaptic neuron—and

the condition of the animals from which the neurons were dissociated or the cell culture medium used.) After the synaptic strength has stabilized, as indicated by measurements of the excitatory postsynaptic potential (EPSP), the experiment is begun. LTF is induced by five spaced pulses of 5-HT; each pulse is typically 5 min long and separated from the other pulses by a 15 min period during which the drug is washed out of the culture dish (**Figure 1B**). This training protocol, referred to as the 5X5-HT protocol, was originally designed to mimic the spaced delivery of tail shocks used to induce behavioral LTS. (If activation of the sensory neuron is paired with the 5-HT pulses the result is an associative form of LTF [96,97]. Activity-dependent LTF, which mediates so-called "site-specific" sensitization [98,99] and also plays a role in classical conditioning of the withdrawal reflex [100-103], differs somewhat mechanistically from activity-independent LTF [104].) Training with the 5X5-HT protocol can induce LTF that persists for 24 hr [5] to 72 hr [105,106]. Commonly, the 5-HT is simply added to the cell culture dish, but in some studies the 5-HT has been locally perfused in spaced bouts over a region of sensorimotor synaptic contact in order to induce synapse-specific LTF [105,107]. (Although the 5X5-HT delivery method, which uses a uniform interval between the pulses of 5-HT, is certainly effective, it has recently been shown that a protocol involving non-uniform interstimulus intervals results in enhanced LTF [108].)

LTF, like LTS itself, requires protein synthesis and CREB-dependent gene transcription [5,7,8,41,109]. Both presynaptic [45,105,107,110] and postsynaptic [59,62,111] protein synthesis are involved, and a critical component of this LTF-related protein synthesis is local [42,105,107,110-113]. Among the local mRNAs that are translated by prolonged 5-HT stimulation are those that encode the cytoskeletal proteins, α1-tubulin and β-thymosin, which may

play roles in synaptic growth, and ribosomal proteins [112]. The latter may increase the number of translationally competent ribosomes at the stimulated synapses. By thereby producing a localized site for translation, this mechanism could serve to restrict the products of learning-related transcription to stimulated synapses; such a mechanism may help to account for synapse-specificity during learning [42,107,114].

One important function of local protein synthesis during LTF is to stimulate a retrograde signal that travels to the nucleus and activates gene transcription. This phenomenon was first described by Martin et al. [107] in their study of synapse-specific LTF. In this study two sensory neurons were cocultured with, and synaptically connected to, a single L7 motor neuron. When one of the two regions of sensorimotor contact, but not the sensory neuron cell body, was given 5X5-HT training via local perfusion, the trained sensorimotor synapse exhibited LTF, whereas the second, untrained, synapse did not. By contrast, when local 5X5-HT treatment failed to induce LTF of the stimulated synapse when the protein synthesis inhibitor emetine was included in the 5-HT-containing solution. Martin and colleagues further showed that when a brief pulse of 5-HT—which, alone, produces only STF—was applied to one of the two regions of sensorimotor contact, and the other region of sensorimotor contact was given the 5X5-HT training, the result was that both synapses underwent LTF. This result implies that the 1X5-HT-trained synapse was able to "capture" the somal products required for LTF that are produced by the 5X5-HT treatment of the other synapse; in other words, the 1X5-HT treatment induces a "tag" of the synapse that is necessary, albeit not sufficient, for the synapse to undergo LTF. Induction of this tag does not depend on protein synthesis, because it is not prevented if the synaptic region is exposed to emetine (a long-lasting

protein synthesis inhibitor) together with the single pulse of 5-HT. Taken together, Martin et al.'s results indicate that local 5X5-HT training induces two processes required for LTF: a protein synthesis-dependent retrograde signal that activates gene transcription, and a protein synthesis-independent tag that permits the synapse to capture the somal proteins/mRNAs whose production results from the 5X5-HT stimulated transcription. (Note that similar results have been reported for long-term potentiation [LTP] in the mammalian hippocampus [115,116].) In a study of sensorimotor synapses in pleural-pedal ganglia, Sherff and Carew also found evidence for a protein synthesis-dependent retrograde signal that can be induced by relatively brief 5-HT treatment and that supports the induction of LTF [117].

One presynaptic protein that is locally synthesized in response to prolonged 5-HT stimulation is the neuropeptide sensorin [118]. Sensorin is peptide first identified in *Aplysia* where it found exclusively in sensory neurons [119]. When a sensorimotor coculture is stimulated with multiple pulses of 5-HT there is a rapid, protein synthesis-dependent increase in sensorin expression in the presynaptic varicosities of sensory neurons, as assessed with immunohistochemistry [120-122]. Interestingly, this increase in local translation of sensorin requires a postsynaptic signal; the increase does not occur if the motor neuron is injected with the rapid Ca^{2+} chelator 1,2-bis(o-aminophenoxy)ethane- N,N,N',N'-tetraacetic acid (BAPTA), or in the varicosities of isolated sensory neurons stimulated with 5X5-HT [59,60]. The enhanced local expression of sensorin is followed by release of the neuropeptide from the varicosities [122,123]; released sensorin binds to autoreceptors on the sensory neuron, thereby activating presynaptic MAPK, which then translocates to the sensory neuron's nucleus [123]. Release of sensorin is necessary for LTF: treatment of

sensorimotor cocultures with an anti-sensorin antibody blocks the induction of LTF [123]. Furthermore, release of sensorin requires PKA activity. (Note that it also requires protein kinase C activity. See ref. [104, and below].) This finding has led Schacher and colleagues to propose that sensorin release and binding to autoreceptors is a necessary intermediate step between the activation of PKA and the activation and nuclear translocation of MAPK [122,123] during LTF.

Besides inducing an increase in local synthesis of sensorin, multiple pulses of 5-HT cause an increase in transcription of sensorin [118]; the sensorin mRNAs are exported to synapses in sensorimotor cocultures, and this synaptic localization of the mRNAs requires an appropriate target motor neuron [118,124,125]. This result addresses a crucial general question for molecular models of long-term memory, namely, whether the products of nuclear transcription can support synapse specificity during learning-related long-term synaptic plasticity [126]. Evidence from studies of long-term potentiation in the mammalian hippocampus and hippocampal-dependent learning suggest that mRNAs can indeed be targeted to active synaptic regions [127-129]. Martin and colleagues have shown that the synaptic localization of sensorin reporter mRNAs in *Aplysia* sensorimotor cocultures is accomplished through cis-acting localization elements (LEs). LEs within the 3′ UTR are sufficient for localization of the reporter mRNA to distal sensory neurites, whereas LEs within the 5′ UTR are required for synaptic localization of the reporter mRNAs [125]. Recently this group has identified a 66 nucleotide-long element in the 5′ UTR of sensorin that is both necessary and sufficient for synaptic localization of the mRNA [130].

Structural changes underlying long-term sensitization memory

Early ultrastructural work in intact animals showed that LTS is associated with presynaptic morphological changes. In particular, the induction of LTS in *Aplysia* results in an increase in the number of presynaptic varicosities, the number of active zones associated with sensory neurons terminals, and in the number of vesicles per active zone [50,51,131]. Furthermore, generally, there is enhanced outgrowth of the neurites of sensory neurons during LTS [37,51,132], although some training protocols that induce LTS do not induce outgrowth of sensory neurites [49].

Glanzman et al. extended these results to the in vitro system; they demonstrated that LTF results in an increase in both the number of presynaptic varicosities and in the number of sensory neurites in sensorimotor cocultures [61]. Since then, much of the work on structural changes associated with long-term, sensitization-related memory in *Aplysia* has used the in vitro system. The increase in presynaptic growth in *Aplysia* sensory neurons due to LTF requires protein and RNA synthesis [133], can be induced by injecting cAMP into the sensory neuron, and is mediated by CREB1 [107]. Just as is the case for LTS, however, there are training protocols that can induce LTF that do not produce the presynaptic outgrowth [42], although the facilitation induced by these protocols does not persist for significantly longer than 24 hours.

One of the requirements for the structural changes that typically accompany LTS is the downregulation of an *Aplysia* neural cell adhesion molecule (ApCAM). 5X5-HT stimulation causes a downregulation of the ApCAM protein in sensory neurons; it also leads to a decrease in the number of ApCAM molecules on the surface of sensory neurites [134]. The decrease in the amount of surface ApCAM molecules results from their

endocytosis [54]. Furthermore, applying an antibody to ApCAM to isolated sensory neurons causes their neurites to defasciculate [134]; such defasciculation may well be a critical step in the 5HT-induced outgrowth of sensory neurites. The down-regulation of presynaptic ApCAM expression, and the internalization of surface ApCAM molecules on sensory neurites and subsequent neuritic defasciculation are likely to be initial steps in the process of structural change that accompanies LTS and LTS [135,136].

Besides playing a critical role in the restructuring of sensory neurons during long-term learning in *Aplysia*, internalization of apCAM also subserves retrograde synapse-to-nucleus signaling. Lee et al. [74] found that apCAM is associated with a transcriptional activator, CAM-associated protein (CAMAP). In its non-activated state CAMAP is bound to the cytoplasmic tail of apCAM molecules in the cell membrane of the sensory processes. Repeated pulses of 5-HT stimulate PKA, which phosphorylates CAMAP; phosphorylation of CAMAP, in turn, causes its dissociation from apCAM, an essential step in the internalization of the apCAM {Mayford, 1992 #42;Bailey, 1992 #4920}. The freed CAMAP then translocates to the nucleus where, it serves as a coactivator, together with CREB1, of the downstream transcription factor CCAAT enhancer-binding protein (C/EBP), an immediate gene whose activation is critical for the consolidation of LTF [84].

Whereas the majority of the work on structural changes related to long-term memory in *Aplysia* has focused on presynaptic changes, LTS also involves postsynaptic structural changes. In an electron microscopic study Bailey and Chen [63] quantified changes in the number of spine-like filapodia on the neurites of the identified motor neuron L7 that occurred in intact animals following LTS-inducing training. They observed an increase in both the number of small (< 0.5 μm), spine-like L7 processes.

Furthermore, they also saw an increase in the number of presynaptic contacts per square micrometer of L7's dendritic surface. These results suggest that LTS causes a coordinated growth of pre- and postsynaptic structures, resulting in increased synaptic contact between the sensory and motor neurons.

Postsynaptic and transsynaptic mechanisms

As pointed out above, persistent memory in *Aplysia* involves postsynaptic, as well as presynaptic changes. One such change is an increase in postsynaptic Ca^{2+}. Cai et al. [59] reported that LTF of the in vitro sensorimotor synapse is blocked by an injection of the rapid chelator BAPTA into the motor neuron prior to 5X5-HT stimulation. This result implies that multiple pulses of 5-HT cause a rise in postsynaptic intracellular Ca^{2+}. Similar results have been obtained if a postsynaptic injection of BAPTA is made prior to a 10-minute exposure to 5-HT [88,89], which causes intermediate-term facilitation (ITF), that is, facilitation that persists from 30 minutes to approximately 3 hours after the exposure to 5-HT [137]. The 5-HT-induced rise in postsynaptic Ca^{2+} results from release from intracellular stores, both inositol-1,4,5-trisphosphate (IP_3)-mediated and ryanodine receptor-mediated stores [88,138]. The increased in intracellular Ca^{2+} activates postsynaptic calcium-calmodulin II (CaMKII) [89]. The downstream effects of this pathway's activity remain to be determined, but are likely to involve at least some of those described below.

Prolonged 5-HT treatment in both the intact nervous system [65] and sensorimotor cocultures [64] produces functional upregulation of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-type glutamate receptors (AMPARs). The early evidence for this effect came from studies in which the effect of brief pulses of AMPAR agonists

delivered to *Aplysia* motor neurons was quantified electrophysiologically before and after prolonged exposure to 5-HT. It was found that this treatment resulted in a greater evoked potential in the motor neurons 24 hours later [64,65]. A 10-minute-long, ITF-inducing, exposure to 5-HT has a similar effect [88,138]. Together, these results imply that prolonged 5-HT stimulation triggers an increase in the number of postsynaptic AMPARs. Investigations of mammalian LTP and learning have shown long-term synaptic plasticity and learning are mediated, in part, by modulation of AMPAR trafficking [139,140]. In the case of the mammalian CNS, modulation of AMPAR receptor trafficking can be initiated by activation of postsynaptic *N*-methyl-D-aspartate receptors (NMDARs). The evidence from the work on *Aplysia* suggests that a monoamine can similarly initiate modulation of AMPAR trafficking. In mammals activation of NMDARs causes additional AMPARs to be inserted into dendrites, either at extrasynaptic sites or directly into the postsynaptic membrane [140]. The extrasynaptically inserted AMPARs then diffuse into the synapse [141]. Direct insertion of the AMPARs is believed to be mediated by exocytosis from a recycling pool of receptors [142,143].

The evidence from studies of ITF and LTF in *Aplysia* supports the following scheme: 5-HT exposure causes in increase in postsynaptic Ca^{2+} due to release from intracellular stores; the increased intracellular Ca^{2+} then drives insertion of additional AMPA into postsynaptic sites via an exocytotic mechanism [59,62,88,138]. This could involve direct synaptic insertion from a recycling pool of vesicles or lateral diffusion following exocytotic insertion into extrasynaptic sites. Unfortunately, at present there are no antibodies to *Aplysia* AMPARs available and, therefore, one cannot directly monitor possible movement of endogenous AMPARs into and out of postsynaptic sites. On the

other hand, several *Aplysia* AMPA-type receptors have been cloned [94,144], which has permitted experimenters to fabricate exogenous constructs of green fluorescent protein (GFP)-tagged *Aplysia* AMPA-type receptors and express these constructs in *Aplysia* motor neurons. Using this method, together with fluorescence confocal microscopy, the experimenters monitored the effect of 5-HT treatment on the exogenous AMPA-type receptors. The resulting data are consistent with the idea that LTF [94] and ITF [62] in *Aplysia* involve modulation of postsynaptic AMPAR trafficking. In the case of LTF the 5X5-HT training produced enhanced clustering of AMPA-type receptors in the motor neurons of sensorimotor cocultures [94], whereas in the case of ITF a 10-minute exposure to 5-HT caused a rapid increase in the number of GFP puncta in the postsynaptic cell membrane, reflecting receptor insertion.

Dopamine causes the insertion of new AMPARs into the dendrites of hippocampal neurons, and this insertion depends on local protein synthesis [145]. Postsynaptic protein synthesis may also play a role in the functional upregulation of AMPA-type receptors caused by prolonged 5-HT stimulation in *Aplysia*. Although it was originally concluded that postsynaptic protein synthesis was not mechanistically involved in LTF [65,107], recent work has established that proteins synthesized by the motor neuron are, in fact, critical for this form of synaptic plasticity [59,62]. Furthermore, at least some of these postsynaptic proteins result from local synthesis [111]. Besides mediating the increased number of postsynaptic AMPA-type receptors [65], postsynaptic protein synthesis probably subserves other important mechanistic roles in LTF. For example, it is likely to mediate the growth of new postsynaptic spines [63]. Another potential role for protein synthesis is in the regulation of postsynaptic protein kinase C (PKC). Recent work has

implicated the constitutively active protein kinase M (PKM) fragment of the atypical *Aplysia* PKC isoform, named PKM Apl III [146], in the maintenance of the memory for ITF [147,148], as well as LTF and LTS (below) [149]. In *Aplysia* PKM Apl III is formed by Ca^{2+}-dependent proteolytic cleavage of PKC Apl III, the atypical isoform [146]. Bougie et al. [148] used a Förster Resonance Energy Transfer (FRET) reporter construct to measure 5-HT-induced cleavage of PKC Apl III in *Aplysia* motor neurons. (Note that 5-HT does not induce cleavage of exogenous PKC Apl III in *Aplysia* sensory neurons [148].) They observed that a 10-minute exposure to 5-HT caused cleavage of the atypical PKC, and this cleavage depended on calpain and protein synthesis. (Interestingly, the mammalian homolog of PKM Apl III, PKMζ, is generated by local translation of the mRNA, which is formed by transcription from an alternate start site within the PKCζ gene [150].)

A fascinating theme to emerge from recent studies of learning-related persistent synaptic plasticity in *Aplysia* is the importance of transsynaptic mechanisms. The first evidence for a critical role for retrograde signaling in LTF came from the study of Glanzman et al. [61] of in vitro changes in presynaptic structure that mediate LTF. These investigators examined the effect of multiple pulses of 5-HT on the structure of isolated sensory neurons in culture and sensory neurons of sensorimotor cocultures. Whereas the 5-HT treatment triggered significant outgrowth of sensory processes and varicosities in the cocultures, it had not effect on the structure of isolated sensory neurons. These results suggest that a signal from the motor neuron is required for the 5-HT-stimulated growth of the sensory neuron. Later work established that LTF depended on postsynaptic Ca^{2+}, because it could be blocked by injecting BAPTA into the target motor neuron prior to the 5X5-HT treatment [59,62]. (Postsynaptic BAPTA also blocks the induction of ITF [88,89].) This

result implies that at least some of the presynaptic changes associated with LTF do not result exclusively from sensory neuron-autonomous processes, but require a retrograde signal for their expression. In support of this idea, one critical presynaptic mechanism that mediates LTF, the synaptic translation of the sensory neuron peptide sensorin, has been shown to require Ca^{2+} signaling in the motor neuron [59,60]. A retrograde signal implicated in LTF is an interaction between the synaptic adhesion molecules neurexin and neuroligin [151]. Choi et al. [152] identified *Aplysia* homologs of neurexin and neuroligin, ApNRX and ApNLG; ApNRX was found to be present in sensory neurons, whereas ApNLG was present in motor neurons. Recombinant versions of the two molecules were found to bind to each other. Furthermore, overexpression of ApNRX in sensory neurons and overexpression of ApNLG in motor neurons of sensorimotor cocultures produced a long-term increase in the strength of the sensorimotor synapse, while injection of antisense ApNRX in the presynaptic sensory neuron or antisense ApNLG in the postsynaptic motor neuron blocked the induction of LTF as well as the presynaptic structural changes associated with LTF. These results represent strong evidence for a role in LTF for transsynaptic signaling via a neurexin-neuroligin interaction; whether other transsynaptic pathways mediate retrograde signaling during LTF remains to be determined.

Recently, anterograde signaling has also been implicated in LTF in *Aplysia*. Jin et al. [153] reported that spontaneous release of transmitter from the sensory neuron was required for the induction of ITF and LTF. Additionally, this group found that spontaneous presynaptic release activates postsynaptic the group I metabotropic receptor mGluR5; activation of mGluR5, in turn, elevates postsynaptic Ca^{2+} through release from

IP_3-mediated stores [62]. This finding is consistent with previous evidence implicating release of Ca^{2+} from IP3-mediated postsynaptic stores in ITF in *Aplysia* [88,138]. Intriguingly, Jin et al. [62] found that injecting botulinum toxin, an exocytotic inhibitor, into presynaptic sensory neurons blocked the increase in AMPA-type receptors in the postsynaptic induced by a 10-minute application of 5-HT. These results suggest that modulation of postsynaptic AMPA-type receptor trafficking is regulated by an anterograde signal linked to enhanced presynaptic release.

The mechanistic model for LTS in *Aplysia* that has emerged within the previous decade is strikingly complex (**Figure 2**). Just a little over ten years ago it was widely believed that exclusively presynaptic mechanisms could adequately account for LTS and LTF [4,87]. Now, however, it is apparent that both pre- and postsynaptic changes are involved in the induction and stabilization of long-term memory; moreover, these changes are coordinated by retrograde and anterograde signaling. It is perhaps surprising that a simple, nonassociative form of learning in a relatively simple invertebrate organism has proved to involve such a rich repertoire of cellular and molecular processes. No doubt, there remain mediatory processes to be identified.

Maintenance of LTS Memory in *Aplysia*

Although significant progress has been made toward a mechanistic understanding of the induction and early stabilization of LTS (**Figure 2**), far less is understood about how the long-term memory persists within the nervous system of *Aplysia*. However, the previous decade has witnessed accelerated interest in memory maintenance in *Aplysia*, and we now have some insights into this fascinating problem.

CPEB and the prion hypothesis

One idea proposed to explain the persistence of the memory for LTF, and by extension, for LTS, centers on the translational activator cytoplasmic polyadenylation element binding protein (CPEB). CPEB mediates polyadenylation-induced translation and plays a key role in cell division during development [154]. A CPEB isoform has been identified in the nervous system of *Aplysia* (ApCPEB) [110]; this isoform has several properties that make it attractive as a memory maintenance molecule in *Aplysia*. mRNAs of ApCPEB are present in the neurites of sensory neurons and translation of ApCPEB mRNA is stimulated locally within the neurites by a prolonged application of 5-HT [110]. Another suggestive property of ApCPEB is that it can exist in two states, a monomeric state in which the protein is inactive, or acts as a repressor, and an active, multimeric state; in the multimeric state ApCPEB can recruit monomeric proteins to the multimer, and thereby become self-sustaining [155]. Thus, multimeric ApCPEB can act as a self-sustaining, local translational hub within sensory neurites, and thereby promote ongoing protein synthesis at the synapse. In this way ApCPEB could serve both as a synaptic marker and a mechanism for sustaining synapse-specific, long-term memories [107]. Consistent with this idea, 5X5-HT stimulation can induce mulimerization of ApCPEB in *Aplysia* sensory neurons, and injecting an antibody to multimeric ApCPEB into sensory neurons 24 hours after 5X5-HT treatment disrupts the maintenance of LTF [155]. Studies of long-term memory in *Drosophila* [156,157] provide further support for the involvement of CPEB in memory persistence.

The amyloid-like properties of ApCPEB in *Aplysia* have led to the suggestion that this molecule is a prion [155,158]. This is an intriguing idea, because it suggests a functional role for prions in the non-diseased brain, in contrast to its pathogenic role in devastating diseases such as Creutzfeldt-Jacob Disease [159]. Regardless of whether or not ApCPEB is a true prion, however, it is unlikely to mediate the maintenance of long-term memory in *Aplysia* beyond the first two days. Evidence for this assertion comes from a study by Miniaci et al. [105], who examined the effect on the memory for LTF of inhibiting protein synthesis at various times after treating sensorimotor cocultures with 5X5-HT. Consistent with a role for ApCPEB in the early maintenance of long-term synaptic memory, treatment with the protein synthesis inhibitor emetine at 24 hours or 48 hours after 5-HT training disrupted LTF, as indicated by tests at later times; by contrast, emetine treatment at 72 hours after 5-HT training did not affect LTF. Similar results were obtained from local application of an antisense ApCPEB oligonucleotide to sensorimotor synapses. Thus, by 72 hours after training, local translation by ApCPEB would appear not to be critical for the maintenance of LTF.

PKM and the maintenance of long-term memory in *Aplysia*

Work in mammals has implicated the constitutively active fragment of atypical PKCζ, PKMζ, in the persistence of long-term synaptic plasticity and memory [160-164]. As mentioned above, the nervous system of *Aplysia* contains an isoform of PKMζ, PKM Apl III. This kinase can be inhibited by the zeta inhibitory peptide (ZIP), which encodes the amino acid sequence of the pseudosubtrate region of the inhibitory domain of the atypical

PKC isoform, PKC Apl III [146], as well as, at micromolar concentrations, by the PKC inhibitor chelerythrine [147]. We have used these two compounds to test for a role for PKM Apl III in LTS and LTF in *Aplysia*. We have found that treatment with either ZIP or chelerythrine disrupts the maintenance of LTS [149]. (In our experiments the drugs were injected directly into the hemolymph of the animals at various times after sensitization training with tail shocks.) Zip or chelerythrine eliminated the memory for LTS, even when applied as late as 7 days after training; furthermore, the memory did not reappear for at least 2 days after the drug injection, nor could it be reinstated by a brief bout of sensitization training. Injection of the scrambled ZIP peptide did not affect the long-term memory. Both ZIP and chelerythrine were equally effective at disrupting the maintenance of long-term synaptic memory in *Aplysia*. In our experiments sensorimotor cocultures, after an initial test of synaptic strength, received 5X5-HT stimulation; 24 hours later some of the cocultures were incubated for 1 hour in either ZIP or chelerythrine. The synapses were then retested at 48 hours after training. LTF was reversed at 48 hours in cocultures treated with either ZIP or chelerythrine, whereas cocultures incubated at 24 hours in either scrambled ZIP or control perfusion medium exhibited significant facilitation. Similar results have been reported by Hu et al. [165] who trained cocultures with 5X5-HT for two consecutive days; such training yields LTF that persists for one week. These investigators found that treatment with chelerythrine on the second day of 5-HT treatment eliminated all LTF, causing a return of the sensorimotor EPSP to its baseline amplitude by Day 3 of the experiment; moreover, no facilitation was evident on Day 7. Treatment with the PKM inhibitor on the second day of 5-HT training also blocked the increase in presynaptic sensorin expression normally observed 24 hours

later. Taken together, the results of these two studies provide strong support for the notion that PKC activity, specifically, that of PKM Apl III, supports the long-term maintenance of memory in *Aplysia*.

Memory reconsolidation in *Aplysia*

One of the most intriguing of memory phenomena is so-called memory reconsolidation. Until relatively recently, the predominant idea regarding memory persistence has been that memories destined for long-term storage underwent a single, protein synthesis-dependent process of consolidation, after which they were stable, barring injury or disease [166]. Opposed to this idea have been data indicating that memories could become labile and subject to disruption upon reactivation [167-170]. Recent years have witnessed increasing evidence, from studies of both vertebrates and invertebrates, of the lability of reactivated long-term memories [171-180]. Two studies have now demonstrated reconsolidation of long-term synaptic and behavioral memory in *Aplysia*; specifically, both Cai et al. [106] and Lee et al. [181] showed that the memory for LTS and LTF can become disrupted by inhibition of protein synthesis following reactivation of the behavioral/synaptic memory. (A methodological difference between the two studies is that Cai and colleagues used brief sensitization training/5-HT stimulation to reactivate the memory for LTS/LTF, Lee et al. used test-type stimulation—weak tactile stimulation of the siphon in the case of LTS and brief homosynaptic activation of the synapse in the case of LTF—for memory reactivation.) The study by Hu et al. [165] provides additional support for the idea that the memory for LTF can undergo reconsolidation; they found that if a

protein synthesis inhibitor was applied to sensorimotor cocultures during the second day of 5-HT training (see above), both the new facilitation, resulting from training on the second day, and the persistent facilitation, resulting from the first day of 5-HT training, were disrupted.

Lee et al.'s [181] results implicate the ubiquitin-proteosome pathway in memory reconsolidation in *Aplysia*. These investigators found that the ubiquitin/proteasome inhibitor β-lactone blocked the disruption of long-term behavioral and synaptic memory when β-lactone was applied together with the protein synthesis inhibitor immediately following memory reactivation. These results suggest that when the long-term memory is reactivated it is destabilized by protein degradation via the ubiquitin/proteasome pathway; subsequently—barring inhibition of protein synthesis—the memory becomes restabilized via new translation see also refs. [182,183].

The data from the studies of memory "erasure" through inhibition of PKM and of memory reconsolidation in *Aplysia* have important implications. It has been contentious in the field of memory research whether, when long-term memories are apparently erased by inhibition of PKM or through disruption of reconsolidation, the memories are permanently eliminated or whether they are, instead, temporarily unable to be retrieved see, e.g., ref. [184]. Another issue of contention is the extent to which reconsolidation of memory resembles original consolidation [173,175,185]. These issues are difficult to resolve in the absence of a realistic synaptic model of memory erasure and reconsolidation. The recent studies in *Aplysia* provide such a model. The cellular and molecular consequences of inhibiting PKM Apl III, or of disrupting memory reconsolidation, can be examined over several days at a single synapse in cell culture, one amenable to rigorous

mechanistic analyses. Furthermore, the results from studies of the *Aplysia* in vitro sensorimotor synapse are likely to have significant ecological validity. The extensive body of prior work on the mechanisms underlying behavioral plasticity of the defensive withdrawal reflex, some of which has been reviewed here, has established that, not only can the findings from experiments on sensorimotor cocultures be extrapolated to the in vivo synapse, but they will also prove relevant to actual learning in the intact animal. One can therefore anticipate that future studies in *Aplysia* will provide valuable insights into the processes that underlie memory erasure and reconsolidation.

Summary

Over the last four decades sensitization of the defensive withdrawal reflex of *Aplysia* has served as a valuable model system for understanding the cellular and molecular mechanisms that mediate long-term memory. Importantly, discoveries from work on LTS in *Aplysia*, such as the importance of CREB-dependent transcription, have generalized to other systems, particularly mammalian systems. Moreover, I believe that the mine of mechanistic insights into long-term memory opened by work on learning and memory in *Aplysia* is far from being exhausted. As suggested by the recent work on PKM Apl III and on memory reconsolidation, in particular, one can anticipate that studies of LTS and LTF in *Aplysia* will be a source of fundamental knowledge about how the brain stores and maintains memories for many years to come.

Acknowledgments

I think Wayne Sossin for critically reading the manuscript and Diancai Cai for assistance with the figures.

References

1. Kandel ER. *Cellular basis of behavior: an introduction to behavioral biology*. San Francisco: W. H. Freeman; 1976.

2. Korbo L, Pakkenberg B, Ladefoged O, Gundersen HJG, Arlien-Søborg P, Pakkenberg H. An efficient method for estimating the total number of neurons in rat brain cortex. *J Neurosci Methods.* 1990;31(2):93-100.

3. Ramon y Cajal S. *Recollections of My Life*. Cambridge, MA: MIT Press; 1989.

4. Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. *Science.* 2001;294:1030–1038.

5. Montarolo PG, Goelet P, Castellucci VF, Morgan J, Kandel ER, Schacher S. A critical period for macromolecular synthesis in long-term heterosynaptic facilitation in *Aplysia*. *Science.* 1986;234:1249-1254.

6. Bartsch D, Ghirardi M, Skehel PA, et al. *Aplysia* CREB2 represses long-term facilitation: relief of repression converts transient facilitation into long-term functional and structural change. *Cell.* 1995;83:979-992.

7. Dash PK, Hochner B, Kandel ER. Injection of the cAMP-responsive element into the nucleus of *Aplysia* sensory neurons blocks long-term facilitation. *Nature.* 1990;345(6277):718-721.

8. Kaang BK, Kandel ER, Grant SG. Activation of cAMP-responsive genes by stimuli that produce long-term facilitation in *Aplysia* sensory neurons. *Neuron.* 1993;10(3):427-435.

9. Goelet P, Castellucci VF, Schacher S, Kandel ER. The long and the short of long-term memory—a molecular framework. *Nature.* 1986;322:419-422.

10. Frey U, Krug M, Reymann KG, Matthies H. Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. *Brain Res.* Jun 14 1988;452(1-2):57-65.

11. Impey S, Mark M, Villacres EC, Poser S, Chavkin C, Storm DR. Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. *Neuron.* 1996;16(5):973-982.

12. Frey U, Frey S, Schollmeier F, Krug M. Influence of actinomycin D, a RNA synthesis inhibitor, on long-term potentiation in rat hippocampal neurons in vivo and in vitro. *The Journal of Physiology.* February 1, 1996 1996;490(Pt 3):703-711.

13. Nguyen PV, Abel T, Kandel ER. Requirement of a critical period of transcription for induction of a late phase of LTP. *Science.* Aug 19 1994;265(5175):1104-1107.

14. Pinsker HM, Hening W, A., Carew TJ, Kandel ER. Long-term sensitization of a defensive withdrawal reflex in *Aplysia*. *Science.* 1973;182:1039-1042.

15. Mozzachiodi R, Byrne JH. More than synaptic plasticity: role of nonsynaptic plasticity in learning and memory. *Trends Neurosci.* Jan 2010;33(1):17-26.

16. Mackey SL, Kandel ER, Hawkins RD. Identified serotonergic neurons LCB1 and RCB1 in the cerebral ganglia of *Aplysia* produce presynaptic facilitation of siphon sensory neurons. *J. Neurosci.* 1989;9(12):4227-4235.

17. Marinesco S, Carew TJ. Serotonin release evoked by tail nerve stimulation in the CNS of *Aplysia*: characterization and relationship to heterosynaptic plasticity. *J. Neurosci.* Mar 15 2002;22(6):2299-2312.

18. Marinesco S, Kolkman KE, Carew TJ. Serotonergic modulation in *Aplysia*. I. Distributed serotonergic network persistently activated by sensitizing stimuli. *J Neurophysiol.* Oct 2004;92(4):2468-2486.
19. Hawkins RD. Localization of potential serotonergic facilitator neurons in *Aplysia* by glyoxylic acid histofluorescence combined with retrograde fluorescent labeling. *J Neurosci.* Dec 1989;9(12):4214-4226.
20. Glanzman DL, Mackey SL, Hawkins RD, Dyke AM, Lloyd PE, Kandel ER. Depletion of serotonin in the nervous system of *Aplysia* reduces the behavioral enhancement of gill withdrawal as well as the heterosynaptic facilitation produced by tail shock. *J. Neurosci.* Dec 1989;9(12):4200-4213.
21. Byrne JH, Castellucci VF, Carew TJ, Kandel ER. Stimulus-response relations and stability of mechanoreceptor and motor neurons mediating defensive gill-withdrawal reflex in *Aplysia*. *J. Neurophysiol.* Mar 1978;41(2):402-417.
22. Carew TJ, Castellucci VF, Byrne JH, Kandel ER. Quantitative analysis of relative contribution of central and peripheral neurons to gill-withdrawal reflex in Aplysia californica. *J Neurophysiol.* Mar 1979;42(2):497-509.
23. Frost WN, Clark GA, Kandel ER. Parallel processing of short-term memory for sensitization in *Aplysia*. *J. Neurobiol.* 1988;19:297-334.
24. Walters ET, Byrne JH, Carew TJ, Kandel ER. Mechanoafferent neurons innervating tail of *Aplysia*. I. Response properties and synaptic connections. *J. Neurophysiol.* 1983;50:1522-1542.
25. Walters ET, Byrne JH, Carew TJ, Kandel ER. Mechanoafferent neurons innervating tail of Aplysia. II. Modulation by sensitizing stimulation. *J Neurophysiol.* Dec 1983;50(6):1543-1559.
26. Kandel ER. *Behavioral biology of Aplysia: a contribution to the comparative study of opisthobranch molluscs*. San Francisco, CA: Freeman; 1979.
27. Cleary LJ, Byrne JH, Frost WN. Role of interneurons in defensive withdrawal reflexes in *Aplysia*. *Learn Mem.* May-Aug 1995;2(3-4):133-151.
28. Brunelli M, Castellucci V, Kandel ER. Synaptic facilitation and behavioral sensitization in *Aplysia*: possible role of serotonin and cyclic AMP. *Science.* 1976;194:1178-1181.
29. Castellucci V, Kandel ER. Presynaptic facilitation as a mechanism for behavioral sensitization in *Aplysia*. *Science.* 1976;194(4270):1176-1178.
30. Pieroni JP, Byrne JH. Differential effects of serotonin, FMRFamide, and small cardioactive peptide on multiple, distributed processes modulating sensorimotor synaptic transmission in Aplysia. *J. Neurosci.* Jul 1992;12(7):2633-2647.
31. Antonov I, Kandel ER, Hawkins RD. The contribution of facilitation of monosynaptic PSPs to dishabituation and sensitization of the *Aplysia* siphon withdrawal reflex. *J. Neurosci.* 1999;19(23):10438-10450.
32. Hochner B, Klein M, Schacher S, Kandel ER. Additional component in the cellular mechanism of presynaptic facilitation contributes to behavioral dishabituation in *Aplysia*. *Proc. Natl. Acad. Sci. USA.* Nov 1986;83(22):8794-8798.
33. Abrams TW, Castellucci VF, Camardo JS, Kandel ER, Lloyd PE. Two endogenous neuropeptides modulate the gill and siphon withdrawal reflex in *Aplysia* by presynaptic facilitation involving cAMP-dependent closure of a

serotonin-sensitive potassium channel. *Proc. Natl. Acad. Sci. USA.* Dec 1984;81(24):7956-7960.

34. Antonov I, Ha T, Antonova I, Moroz LL, Hawkins RD. Role of nitric oxide in classical conditioning of siphon withdrawal in Aplysia. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* Oct 10 2007;27(41):10993-11002.

35. Cohen TE, Kaplan SW, Kandel ER, Hawkins RD. A simplified preparation for relating cellular events to behavior: mechanisms contributing to habituation, dishabituation, and sensitization of the *Aplysia* gill-withdrawal reflex. *J. Neurosci.* 1997;17(8):2886-2899.

36. Frost WN, Castellucci VF, Hawkins RD, Kandel ER. Monosynaptic connections made by the sensory neurons of the gill- and siphon-withdrawal reflex in *Aplysia* participate in the storage of long-term memory for sensitization. *Proc. Natl. Acad. Sci. USA.* 1985;82:8266–8269.

37. Cleary LJ, Lee WL, Byrne JH. Cellular correlates of long-term sensitization in *Aplysia*. *J Neurosci.* Aug 1 1998;18(15):5988-5998.

38. Zwartjes RE, West H, Hattar S, et al. Identification of specific mRNAs affected by treatments producing long-term facilitation in Aplysia. *Learn. Mem.* 1998;4(6):478-495.

39. Castellucci VF, Blumenfeld H, Goelet P, Kandel ER. Inhibitor of protein synthesis blocks long-term behavioral sensitization in the isolated gill-withdrawal reflex of *Aplysia*. *J. Neurobiol.* 1989;20(1):1-9.

40. Cai D, Chen S, Fan X, Sossin WS, Glanzman DL. Postsynaptic inhibition of protein kinase C Apl III disrupts intermediate-term facilitation in *Aplysia* Paper presented at: Neuroscience 20112011; Washington, DC, USA.

41. Bartsch D, Casadio A, Karl KA, Serodio P, Kandel ER. CREB1 encodes a nuclear activator, a repressor, and a cytoplasmic modulator that form a regulatory unit critical for long-term facilitation. *Cell.* Oct 16 1998;95(2):211-223.

42. Casadio A, Martin KC, Giustetto M, et al. A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell.* Oct 15 1999;99(2):221-237.

43. Rajasethupathy P, Fiumara F, Sheridan R, et al. Characterization of small RNAs in *Aplysia* reveals a role for miR-124 in constraining synaptic plasticity through CREB. *Neuron.* 2009;63(6):803-817.

44. Liu R-Y, Shah S, Cleary LJ, Byrne JH. Serotonin- and training-induced dynamic regulation of CREB2 in Aplysia. *Learn. Mem.* April 1, 2011 2011;18(4):245-249.

45. Bergold PJ, Sweatt JD, Winicov I, Weiss KR, Kandel ER, Schwartz JH. Protein synthesis during acquisition of long-term facilitation is needed for the persistent loss of regulatory subunits of the Aplysia cAMP-dependent protein kinase. *Proc Natl Acad Sci U S A.* 1990;87(10):3788-3791.

46. Eskin A, Garcia KS, Byrne JH. Information storage in the nervous system of Aplysia: specific proteins affected by serotonin and cAMP. *Proc Natl Acad Sci U S A.* Apr 1989;86(7):2458-2462.

47. Noel F, Scholz KP, Eskin A, Byrne JH. Common set of proteins inAplysia sensory neurons affected by an in vitro analogue of long-term sensitization training, 5-HT and cAMP. *Brain Res.* 1991;568(1–2):67-75.

48. Liu Q-R, Hattar S, Endo S, et al. A Developmental Gene (Tolloid/BMP-1) Is Regulated inAplysia Neurons by Treatments that Induce Long-Term Sensitization. *The Journal of Neuroscience.* January 15, 1997 1997;17(2):755-764.
49. Wainwright ML, Zhang H, Byrne JH, Cleary LJ. Localized neuronal outgrowth induced by long-term sensitization training in *Aplysia*. *J Neurosci.* May 15 2002;22(10):4132-4141.
50. Bailey CH, Chen M. Morphological basis of long-term habituation and sensitization in Aplysia. *Science.* Apr 1 1983;220(4592):91-93.
51. Bailey CH, Chen M. Long–term memory in *Aplysia* modulates the total number of varicosities of single identified sensory neurons. *Proc. Natl. Acad. Sci. USA.* 1988;85:2373–2377.
52. Bailey CH, Kandel ER. Structural changes accompanying memory storage. *Annu. Rev. Physiol.* 1993;55:397-426.
53. Bailey CH, Kandel ER, Wayne S. Sossin J-CLVFC, Sylvie B. Synaptic remodeling, synaptic growth and the storage of long-term memory in *Aplysia*. *Progress in Brain Research.* Vol Volume 169: Elsevier; 2008:179-198.
54. Bailey CH, Chen M, Keller F, Kandel ER. Serotonin-mediated endocytosis of apCAM: an early step of learning-related synaptic growth in Aplysia. *Science.* May 1 1992;256(5057):645-649.
55. Kim JH, Udo H, Li HL, et al. Presynaptic activation of silent synapses and growth of new synapses contribute to intermediate and long-term facilitation in *Aplysia*. *Neuron.* Sep 25 2003;40(1):151-165.
56. Udo H, Jin I, Kim JH, et al. Serotonin-induced regulation of the actin network for learning-related synaptic growth requires Cdc42, N-WASP, and PAK in Aplysia sensory neurons. *Neuron.* Mar 24 2005;45(6):887-901.
57. Dale N, Schacher S, Kandel ER. Long-term facilitation in *Aplysia* involves increase in transmitter release. *Science.* 1988;239:282-285.
58. Glanzman DL. Common mechanisms of synaptic plasticity in vertebrates and invertebrates. *Curr. Biol.* Jan 12 2010;20(1):R31-R36.
59. Cai D, Chen S, Glanzman DL. Postsynaptic regulation of long-term facilitation in *Aplysia*. *Curr. Biol.* Jun 24 2008;18(12):920-925.
60. Wang DO, Kim SM, Zhao Y, et al. Synapse- and stimulus-specific local translation during long-term neuronal plasticity. *Science.* Jun 19 2009;324(5934):1536-1540.
61. Glanzman DL, Kandel ER, Schacher S. Target-dependent structural changes accompanying long-term synaptic facilitation in *Aplysia* neurons. *Science.* 1990;249(4970):799-802.
62. Jin I, Udo H, Rayman JB, Puthanveettil S, Kandel ER, Hawkins RD. Spontaneous transmitter release recruits postsynaptic mechanisms of long-term and intermediate-term facilitation in Aplysia. *Proc. Natl. Acad. Sci. USA.* 2012;109(23):9137-9142.
63. Bailey CH, Chen M. Long-term sensitization in Aplysia increases the number of presynaptic contacts onto the identified gill motor neuron L7. *Proc. Natl. Acad. Sci. USA.* Dec 1988;85(23):9356-9359.
64. Zhu H, Wu F, Schacher S. Site-specific and sensory neuron-dependent increases in postsynaptic glutamate sensitivity accompany serotonin-induced long-term

facilitation at *Aplysia* sensorimotor synapses. *J. Neurosci.* 1997;17(13):4976-4986.

65. Trudeau LE, Castellucci VF. Postsynaptic modifications in long-term facilitation in *Aplysia*: upregulation of excitatory amino acid receptors. *J. Neurosci.* 1995;15(2):1275-1284.

66. Ocorr KA, Byrne JH. Membrane responses and changes in cAMP levels in *Aplysia* sensory neurons produced by serotonin, tryptamine, FMRFamide and small cardioactive peptideB (SCPB). *Neurosci Lett.* Apr 9 1985;55(2):113-118.

67. Bacskai BJ, Hochner B, Mahaut SM, et al. Spatially resolved dynamics of cAMP and protein kinase A subunits in *Aplysia* sensory neurons. *Science.* 1993;260(5105):222-226.

68. Braha O, Edmonds B, Sacktor T, Kandel ER, Klein M. The contributions of protein kinase A and protein kinase C to the actions of 5-HT on the L-type Ca2+ current of the sensory neurons in Aplysia. *J. Neurosci.* 1993;13(5):1839-1851.

69. Kandel ER, Schwartz JH. Molecular biology of learning: modulation of transmitter release. *Science.* 1982;218:433-443.

70. Sweatt JD, Kandel ER. Persistent and transcriptionally-dependent increase in protein phosphorylation in long-term facilitation of *Aplysia* sensory neurons. *Nature.* 1989;339(6219):51-54.

71. Bernier L, Castellucci VF, Kandel ER, Schwartz JH. Facilitatory transmitter causes a selective and prolonged increase in adenosine 3':5'-monophosphate in sensory neurons mediating the gill and siphon withdrawal reflex in *Aplysia*. *J. Neurosci.* Dec 1982;2(12):1682-1691.

72. Greenberg SM, Castellucci VF, Bayley H, Schwartz JH. A molecular mechanism for long-term sensitization in *Aplysia*. *Nature.* 1987;329:62–65.

73. Bergold PJ, Beushausen SA, Sacktor TC, Cheley S, Bayley H, Schwartz JH. A regulatory subunit of the cAMP-dependent protein kinase down-regulated in aplysia sensory neurons during long-term sensitization. *Neuron.* Feb 1992;8(2):387-397.

74. Lee SH, Lim CS, Park H, et al. Nuclear translocation of CAM-associated protein activates transcription for long-term facilitation in Aplysia. *Cell.* May 18 2007;129(4):801-812.

75. Alberini CM. Transcription Factors in Long-Term Memory and Synaptic Plasticity. *Physiological Reviews.* January 2009 2009;89(1):121-145.

76. Ormond J, Hislop J, Zhao Y, et al. ApTrkl, a Trk-like receptor, mediates serotonin-dependent ERK activation and long-term facilitation in *Aplysia* sensory neurons. *Neuron.* Nov 18 2004;44(4):715-728.

77. Sharma SK, Carew TJ. The roles of MAPK cascades in synaptic plasticity and memory in Aplysia: facilitatory effects and inhibitory constraints. *Learn Mem.* Jul-Aug 2004;11(4):373-378.

78. Sharma SK, Sherff CM, Shobe J, Bagnall MW, Sutton MA, Carew TJ. Differential role of mitogen-activated protein kinase in three distinct phases of memory for sensitization in *Aplysia*. *J Neurosci.* May 1 2003;23(9):3899-3907.

79. Guan Z, Giustetto M, Lomvardas S, et al. Integration of long-term-memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. *Cell.* Nov 15 2002;111(4):483-493.

80. Martin KC, Michael D, Rose JC, et al. MAP kinase translocates into the nucleus of the presynaptic cell and is required for long-term facilitation in *Aplysia*. *Neuron.* Jun 1997;18(6):899-912.
81. Hegde AN, Inokuchi K, Pei W, et al. Ubiquitin C-terminal hydrolase is an immediate-early gene essential for long-term facilitation in Aplysia. *Cell.* 1997;89(1):115-126.
82. Zhao Y, Hegde AN, Martin KC. The ubiquitin proteasome system functions as an inhibitory constraint on synaptic strengthening. *Curr Biol.* May 27 2003;13(11):887-898.
83. Bartsch D, Ghirardi M, Casadio A, et al. Enhancement of memory-related long-term facilitation by ApAF, a novel transcription factor that acts downstream from both CREB1 and CREB2. *Cell.* Nov 10 2000;103(4):595-608.
84. Alberini CM, Ghirardl M, Metz R, Kandel ER. C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in Aplysia. *Cell.* 1994;76(6):1099-1114.
85. Bailey CH, Kandel ER, Si K. The persistence of long-term memory: a molecular approach to self-sustaining changes in learning-induced synaptic growth. *Neuron.* Sep 30 2004;44(1):49-57.
86. Zhang F, Goldsmith JR, Byrne JH. Neural analogue of long-term sensitization training produces long-term (24 and 48 h) facilitation of the sensory-to-motor neuron connection in Aplysia. *J Neurophysiol.* Aug 1994;72(2):778-784.
87. Byrne JH, Kandel ER. Presynaptic facilitation revisited: state and time dependence. *J. Neurosci.* 1996;16:425-435.
88. Li Q, Roberts AC, Glanzman DL. Synaptic facilitation and behavioral dishabituation in *Aplysia*: dependence upon release of Ca^{2+} from postsynaptic intracellular stores, postsynaptic exocytosis and modulation of postsynaptic AMPA receptor efficacy. *J. Neurosci.* 2005;25:5623-5637.
89. Jin I, Kandel ER, Hawkins RD. Whereas short-term facilitation is presynaptic, intermediate-term facilitation involves both presynaptic and postsynaptic protein kinases and protein synthesis. *Learn. Mem.* 2011;18(2):96-102.
90. Dale N, Kandel ER. L-glutamate may be the fast excitatory transmitter of *Aplysia* sensory neurons. *Proc. Natl. Acad. Sci. USA.* 1993;90(15):7163-7167.
91. Antzoulatos EG, Byrne JH. Learning insights transmitted by glutamate. *Trends Neurosci.* Sep 2004;27(9):555-560.
92. Levenson J, Endo S, Kategaya LS, et al. Long-term regulation of neuronal high-affinity glutamate and glutamine uptake in Aplysia. *Proc Natl Acad Sci U S A.* Nov 7 2000;97(23):12858-12863.
93. Levenson J, Sherry DM, Dryer L, Chin J, Byrne JH, Eskin A. Localization of glutamate and glutamate transporters in the sensory neurons of *Aplysia*. *J Comp Neurol.* 2000;423(1):121-131.
94. Li HL, Huang BS, Vishwasrao H, et al. Dscam mediates remodeling of glutamate receptors in Aplysia during de novo and learning-related synapse formation. *Neuron.* Feb 26 2009;61(4):527-540.
95. Lin XY, Glanzman DL. Long-term potentiation of *Aplysia* sensorimotor synapses in cell culture: regulation by postsynaptic voltage. *Proc. Biol. Sci.* 1994;255:113–118.

96. Schacher S, Wu F, Sun ZY. Pathway-specific synaptic plasticity: activity-dependent enhancement and suppression of long-term heterosynaptic facilitation at converging inputs on a single target. *J. Neurosci.* 1997;17(2):597-606.
97. Eliot LS, Hawkins RD, Kandel ER, Schacher S. Pairing-specific, activity-dependent presynaptic facilitation at *Aplysia* sensory-motor neuron synapses in isolated cell culture. *J. Neurosci.* 1994;14:368-383.
98. Walters ET. Multiple sensory neuronal correlates of site-specific sensitization in *Aplysia*. *J Neurosci.* Feb 1987;7(2):408-417.
99. Walters ET. Site-specific sensitization of defensive reflexes in *Aplysia*: a simple model of long-term hyperalgesia. *J Neurosci.* Feb 1987;7(2):400-407.
100. Antonov I, Antonova I, Kandel ER, Hawkins RD. Activity-dependent presynaptic facilitation and Hebbian LTP are both required and interact during classical conditioning in *Aplysia*. *Neuron.* Jan 9 2003;37(1):135-147.
101. Carew TJ, Hawkins RD, Kandel ER. Differential classical conditioning of a defensive withdrawal reflex in *Aplysia californica*. *Science.* 1983;219:397-400.
102. Hawkins RD, Abrams TW, Carew TJ, Kandel ER. A cellular mechanism of classical conditioning in *Aplysia*: activity-dependent amplification of presynaptic facilitation. *Science.* 1983;219:400-405.
103. Walters ET, Byrne JH. Associative conditioning of single sensory neurons suggests a cellular mechanism for learning. *Science.* 1983;219:405-408.
104. Hu JY, Chen Y, Schacher S. Protein kinase C regulates local synthesis and secretion of a neuropeptide required for activity-dependent long-term synaptic plasticity. *J Neurosci.* Aug 15 2007;27(33):8927-8939.
105. Miniaci MC, Kim JH, Puthanveettil SV, et al. Sustained CPEB-dependent local protein synthesis is required to stabilize synaptic growth for persistence of long-term facilitation in Aplysia. *Neuron.* Sep 25 2008;59(6):1024-1036.
106. Cai D, Pearce K, Chen S, Glanzman DL. Reconsolidation of long-term memory in *Aplysia*. *Curr. Biol.* 2012.
107. Martin KC, Casadio A, Zhu H, et al. Synapse-specific, long-term facilitation of *Aplysia* sensory to motor synapses: a function for local protein synthesis in memory storage. *Cell.* 1997;91(7):927-938.
108. Zhang Y, Liu R-Y, Heberton GA, et al. Computational design of enhanced learning protocols. *Nat Neurosci.* 2011;advance online publication.
109. Michael D, Martin KC, Seger R, Ning MM, Baston R, Kandel ER. Repeated pulses of serotonin required for long-term facilitation activate mitogen-activated protein kinase in sensory neurons of *Aplysia*. *Proc Natl Acad Sci U S A.* Feb 17 1998;95(4):1864-1869.
110. Si K, Giustetto M, Etkin A, et al. A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in *Aplysia*. *Cell.* Dec 26 2003;115(7):893-904.
111. Villareal G, Li Q, Cai D, Glanzman DL. The role of rapid, local postsynaptic protein synthesis in learning-related synaptic facilitation in *Aplysia*. *Curr. Biol.* 2007;17:2073-2080.
112. Moccia R, Chen D, Lyles V, et al. An unbiased cDNA library prepared from isolated Aplysia sensory neuron processes is enriched for cytoskeletal and translational mRNAs. *J Neurosci.* Oct 15 2003;23(28):9409-9417.

113. Martin KC, Barad M, Kandel ER. Local protein synthesis and its role in synapse-specific plasticity. *Curr Opin Neurobiol.* Oct 2000;10(5):587-592.
114. Martin KC, Kosik KS. Synaptic tagging–who's it? *Nat Rev Neurosci.* 2002;3(10):813-820.
115. Frey U, Morris RGM. Synaptic tagging and long-term potentiation. *Nature.* 1997;385:533–536.
116. Frey U, Morris RGM. Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. *Trends Neurosci.* 1998;21:181–188.
117. Sherff CM, Carew TJ. Coincident induction of long-term facilitation in *Aplysia*: cooperativity between cell bodies and remote synapses. *Science.* Sep 17 1999;285(5435):1911-1914.
118. Sun ZY, Wu F, Schacher S. Rapid bidirectional modulation of mRNA expression and export accompany long-term facilitation and depression of Aplysia synapses. *J. Neurobiol.* Jan 2001;46(1):41-47.
119. Brunet JF, Shapiro E, Foster SA, Kandel ER, Iino Y. Identification of a peptide specific for *Aplysia* sensory neurons by PCR-based differential screening. *Science.* 1991;252(5007):856-859.
120. Liu K, Hu JY, Wang D, Schacher S. Protein synthesis at synapse versus cell body: enhanced but transient expression of long-term facilitation at isolated synapses. *J Neurobiol.* Sep 5 2003;56(3):275-286.
121. Santarelli L, Montarolo P, Schacher S. Neuropeptide localization in varicosities of Aplysia sensory neurons is regulated by target and neuromodulators evoking long-term synaptic plasticity. *J. Neurobiol.* 1996;31(3):297-308.
122. Hu JY, Wu F, Schacher S. Two signaling pathways regulate the expression and secretion of a neuropeptide required for long-term facilitation in *Aplysia*. *J. Neurosci.* Jan 18 2006;26(3):1026-1035.
123. Hu JY, Glickman L, Wu F, Schacher S. Serotonin regulates the secretion and autocrine action of a neuropeptide to activate MAPK required for long-term facilitation in *Aplysia*. *Neuron.* Aug 5 2004;43(3):373-385.
124. Hu JY, Meng X, Schacher S. Target interaction regulates distribution and stability of specific mRNAs. *J Neurosci.* Apr 1 2002;22(7):2669-2678.
125. Lyles V, Zhao Y, Martin KC. Synapse formation and mRNA localization in cultured Aplysia neurons. *Neuron.* Feb 2 2006;49(3):349-356.
126. Lisman J. What does the nucleus know about memories? *Journal of NIH Research.* 1995;7:43–46.
127. Steward O, Wallace CS, Lyford GL, Worley PF. Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. *Neuron.* Oct 1998;21(4):741-751.
128. Steward O, Worley PF. Selective targeting of newly synthesized Arc mRNA to active synapses requires NMDA receptor activation. *Neuron.* Apr 2001;30(1):227-240.
129. Miller S, Yasuda M, Coats JK, Jones Y, Martone ME, Mayford M. Disruption of dendritic translation of CaMKIIα simpairs stabilization of synaptic plasticity and memory consolidation. *Neuron.* Oct 24 2002;36(3):507-519.

130. Meer EJ, Wang DO, Kim S, Barr I, Guo F, Martin KC. Identification of a cis-acting element that localizes mRNA to synapses. *Proc. Natl. Acad. Sci. USA.* 2012;109(12):4639-4644.
131. Bailey CH, Chen M. Time course of structural changes at identified sensory neuron synapses during long-term sensitization in Aplysia. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* May 1989;9(5):1774-1780.
132. Wainwright ML, Byrne JH, Cleary LJ. Dissociation of morphological and physiological changes associated with long-term memory in aplysia. *J Neurophysiol.* Oct 2004;92(4):2628-2632.
133. Bailey CH, Montarolo P, Chen M, Kandel ER, Schacher S. Inhibitors of protein and RNA synthesis block structural changes that accompany long-term heterosynaptic plasticity in Aplysia. *Neuron.* Oct 1992;9(4):749-758.
134. Mayford M, Barzilai A, Keller F, Schacher S, Kandel ER. Modulation of an NCAM-related adhesion molecule with long-term synaptic plasticity in *Aplysia*. *Science.* 1992;256(5057):638-644.
135. Zhu H, Wu F, Schacher S. *Aplysia* cell adhesion molecules and serotonin regulate sensory cell- motor cell interactions during early stages of synapse formation in vitro. *J. Neurosci.* November 1, 1994 1994;14(11):6886-6900.
136. Han J-H, Lim C-S, Lee Y-S, Kandel ER, Kaang B-K. Role of *Aplysia* cell adhesion molecules during 5-HT-induced long-term functional and structural changes. *Learn. Mem.* 2004;11(4):421-435.
137. Sutton MA, Carew TJ. Behavioral, cellular, and molecular analysis of memory in *Aplysia* I: intermediate-term memory. *Integ. Comp. Biol.* 2002;42:725-735.
138. Chitwood RA, Li Q, Glanzman DL. Serotonin facilitates AMPA-type responses in isolated siphon motor neurons of *Aplysia* in culture. *J. Physiol.* 2001;534:501–510.
139. Kessels HW, Malinow R. Synaptic AMPA receptor plasticity and behavior. *Neuron.* 2009;61(3):340-350.
140. Shepherd JD, Huganir RL. The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annual Review of Cell and Developmental Biology.* 2007/11/01 2007;23(1):613-643.
141. Makino H, Malinow R. AMPA receptor incorporation into synapses during LTP: the role of lateral movement and exocytosis. *Neuron.* 2009;64(3):381-390.
142. Lüscher C, Xia H, Beattie EC, et al. Role of AMPA receptor cycling in synaptic transmission and plasticity. *Neuron.* 1999;24:649–658.
143. Tao-Cheng J-H, Crocker VT, Winters CA, Azzam R, Chludzinski J, Reese TS. Trafficking of AMPA receptors at plasma membranes of hippocampal neurons. *The Journal of Neuroscience.* 2011;31(13):4834-4843.
144. Yung I, Chun S, Kapya E, et al. Cloning of glutamate receptors from the central nervous system of *Aplysia*. . *Soc. Neurosci. Abstr.* 2002;28:376.377.
145. Smith WB, Starck SR, Roberts RW, Schuman EM. Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. *Neuron.* Mar 3 2005;45(5):765-779.
146. Bougie JK, Lim T, Farah CA, et al. The atypical protein kinase C in *Aplysia* can form a protein kinase M by cleavage. *J. Neurochem.* 2009;109(4):1129-1143.

147. Villareal G, Li Q, Cai D, et al. Role of protein kinase C in the induction and maintenance of serotonin-dependent enhancement of the glutamate response in isolated siphon motor neurons of *Aplysia californica*. *J. Neurosci.* April 22, 2009 2009;29(16):5100-5107.
148. Bougie J, Cai D, Hastings M, et al. Serotonin-induced cleavage of the atypical protein kinase C Apl III in *Aplysia*. *J. Neurosci.* 2012.
149. Cai D, Pearce K, Chen S, Glanzman DL. Protein kinase M maintains long-term sensitization and long-term facilitation in *Aplysia*. *J. Neurosci.* 2011;31(17):6421-6431.
150. Hernandez AI, Blace N, Crary JF, et al. Protein kinase Mζ synthesis from a brain mRNA encoding an independent protein kinase Cζ catalytic domain. Implications for the molecular mechanism of memory. *J. Biol. Chem.* Oct 10 2003;278(41):40305-40316.
151. Lisé MF, El-Husseini A. The neuroligin and neurexin families: from structure to function at the synapse. *Cellular and molecular life sciences : CMLS.* 2006;63(16):1833-1849.
152. Choi Y-B, Li H-L, Kassabov SR, et al. Neurexin-neuroligin transsynaptic interaction mediates learning-related synaptic remodeling and long-term facilitation in *Aplysia*. *Neuron.* 2011;70(3):468-481.
153. Jin I, Puthanveettil S, Udo H, Karl K, Kandel ER, Hawkins RD. Spontaneous transmitter release is critical for the induction of long-term and intermediate-term facilitation in Aplysia. *Proc. Natl. Acad. Sci. USA.* 2012;109(23):9131-9136.
154. Richter JD. CPEB: a life in translation. *Trends in Biochemical Sciences.* 2007;32(6):279-285.
155. Si K, Choi YB, White-Grindley E, Majumdar A, Kandel ER. Aplysia CPEB can form prion-like multimers in sensory neurons that contribute to long-term facilitation. *Cell.* Feb 5 2010;140(3):421-435.
156. Keleman K, Kruttner S, Alenius M, Dickson BJ. Function of the *Drosophila* CPEB protein Orb2 in long-term courtship memory. *Nat. Neurosci.* 2007;10(12):1587-1593.
157. Majumdar A, Cesario Wanda C, White-Grindley E, et al. Critical role of amyloid-like oligomers of *Drosophila* Orb2 in the persistence of memory. *Cell.* 2012;148(3):515-529.
158. Si K, Lindquist S, Kandel ER. A neuronal isoform of the *Aplysia* CPEB has prion-like properties. *Cell.* Dec 26 2003;115(7):879-891.
159. Aguzzi A, Zhu C. Five questions on prion diseases. *PLoS Pathog.* 2012;8(5):e1002651.
160. Sacktor TC. How does PKMζ maintain long-term memory? *Nat. Rev. Neurosci.* 2011;12(1):9-15.
161. Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton AA, Sacktor TC. Storage of spatial information by the maintenance mechanism of LTP. *Science.* Aug 25 2006;313(5790):1141-1144.
162. Shema R, Haramati S, Ron S, et al. Enhancement of consolidated long-term memory by overexpression of protein kinase Mζ in the neocortex. *Science.* March 4, 2011 2011;331(6021):1207-1210.

163. Shema R, Sacktor TC, Dudai Y. Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKMζ. *Science.* August 17, 2007 2007;317(5840):951-953.
164. Ling DS, Benardo LS, Serrano PA, et al. Protein kinase Mζ is necessary and sufficient for LTP maintenance. *Nat. Neurosci.* Apr 2002;5(4):295-296.
165. Hu JY, Baussi O, Levine A, Chen Y, Schacher S. Persistent long-term synaptic plasticity requires activation of a new signaling pathway by additional stimuli. *J. Neurosci.* Jun 15 2011;31(24):8841-8850.
166. McGaugh JL. Memory--a century of consolidation. *Science.* Jan 14 2000;287(5451):248-251.
167. Bregman N, Nicholas T, Lewis DJ. Cue-dependent amnesia: permanence and memory return. *Physiol. Behav.* Aug 1976;17(2):267-270.
168. Misanin JR, Miller RR, Lewis DJ. Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. *Science.* May 3 1968;160(827):554-555.
169. Przybyslawski J, Sara SJ. Reconsolidation of memory after its reactivation. *Behav. Brain. Res.* Mar 1997;84(1-2):241-246.
170. Sara SJ, Roullet P, Przybyslawski J. Consolidation of memory for odor-reward association: beta-adrenergic receptor involvement in the late phase. *Learn Mem.* Mar-Apr 1999;6(2):88-96.
171. Debiec J, LeDoux JE, Nader K. Cellular and systems reconsolidation in the hippocampus. *Neuron.* Oct 24 2002;36(3):527-538.
172. Nader K, Schafe GE, Le Doux JE. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature.* Aug 17 2000;406(6797):722-726.
173. Dudai Y. The restless engram: consolidations never end. *Annu Rev Neurosci.* Mar 20 2012.
174. Eisenberg M, Kobilo T, Berman DE, Dudai Y. Stability of retrieved memory: inverse correlation with trace dominance. *Science.* Aug 22 2003;301(5636):1102-1104.
175. Taubenfeld SM, Milekic MH, Monti B, Alberini CM. The consolidation of new but not reactivated memory requires hippocampal C/EBPβ. *Nat. Neurosci.* 2001;4(8):813-818.
176. Alberini CM. The role of reconsolidation and the dynamic process of long-term memory formation and storage. *Front Behav Neurosci.* 2011;5:12.
177. Sangha S, Scheibenstock A, Lukowiak K. Reconsolidation of a long-term memory in Lymnaea requires new protein and RNA synthesis and the soma of right pedal dorsal 1. *J Neurosci.* Sep 3 2003;23(22):8034-8040.
178. Lagasse F, Devaud J-M, Mery F. A switch from cycloheximide-resistant consolidated memory to cycloheximide-sensitive reconsolidation and extinction in *Drosophila*. *J. Neurosci.* February 18, 2009 2009;29(7):2225-2230.
179. Nader K, Hardt O. A single standard for memory: the case for reconsolidation. *Nat Rev Neurosci.* 2009;10(3):224-234.
180. Rose JK, Rankin CH. Blocking memory reconsolidation reverses memory-associated changes in glutamate receptor expression. *J. Neurosci.* November 8, 2006 2006;26(45):11582-11587.

181. Lee S-H, Kwak C, Shim J, et al. A cellular model of memory reconsolidation involves reactivation-induced destabilization and restabilization at the sensorimotor synapse in Aplysia. *Proc. Natl. Acad. Sci. USA.* 2012;109(35):14200-14205.
182. Kaang BK, Choi JH. Protein degradation during reconsolidation as a mechanism for memory reorganization. *Front Behav Neurosci.* 2011;5:2.
183. Lee SH, Choi JH, Lee N, et al. Synaptic protein degradation underlies destabilization of retrieved fear memory. *Science.* Feb 29 2008;319(5867):1253-1256.
184. Lattal KM, Abel T. Behavioral impairments caused by injections of the protein synthesis inhibitor anisomycin after contextual retrieval reverse with time. *Proc Natl Acad Sci U S A.* Mar 30 2004;101(13):4667-4672.
185. Alberini CM. Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? *Trends Neurosci.* Jan 2005;28(1):51-56.

FIGURES

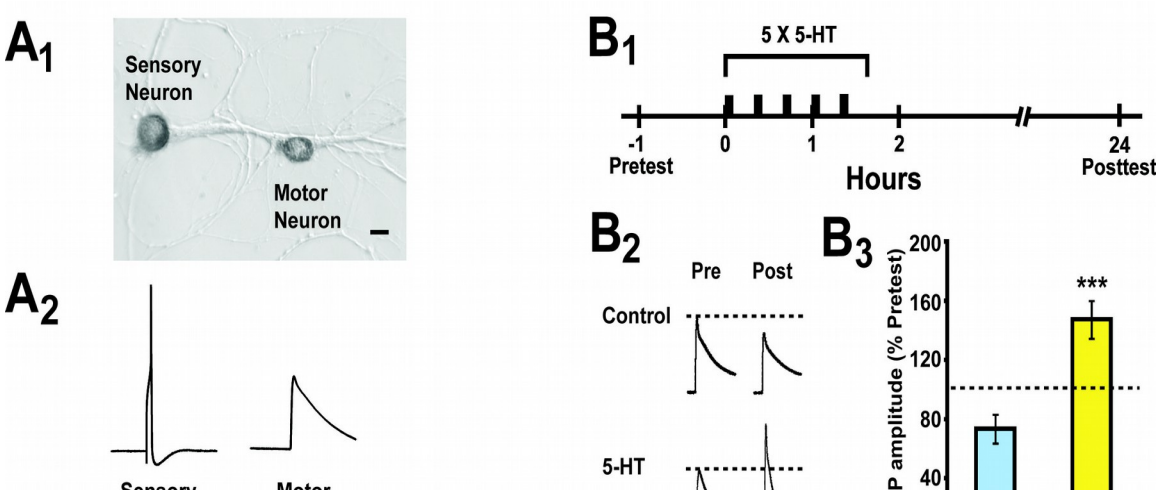

Figure 1. Long-term facilitation of the sensorimotor synapse *Aplysia* in dissociated cell culture. ***A₁***, Sensorimotor coculture. Here the presynaptic neuron is a pleural sensory neuron [24] and the postsynaptic neuron is a small siphon (LFS) motor neuron [23]. Scale bar, 20 □m. ***A₂***, Sample electrophysiological records from a pretest of a synapse. Scale bars, 20 mV and 200 ms. ***B₁***, Experimental protocol for the demonstration of 24 hr LTF. The coculture was treated with five spaced 5-minute applications of 5-HT (100 μM); the drug was washed out of the cell culture for 15 minutes between 5-HT pulses. ***B₂***, Sample EPSPs. Each pair of traces shows EPSPs recorded from the same sensorimotor synapse on Day 1 (Pre) and ~ 24 hr later (Post). Training with 5-HT produced LTF (lower traces). Scale bars, 10 mV and 80 ms. From ref. [149].

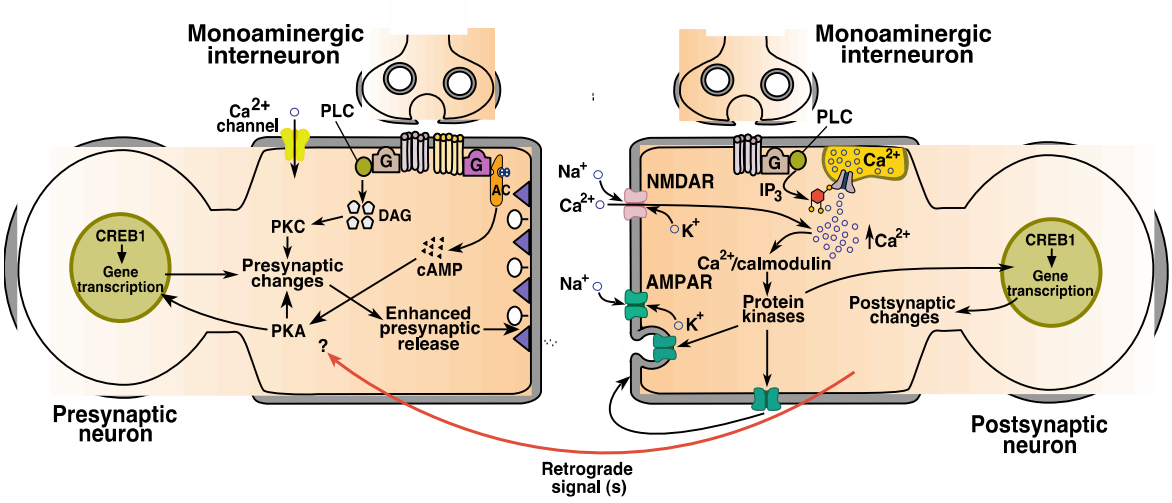

Figure 2. Cellular model for sensitization-related long-term facilitation. Prominent features of the model are postsynaptic modulatory input from monoaminergic interneurons and retrograde signaling. Prolonged stimulation with 5-HT causes enhanced release of glutamate, the presynaptic transmitter, and modulation of postsynaptic AMPA-type receptor trafficking; the latter process involves exocytotic insertion of AMPA receptors into the cell membrane of the motor neuron. This process is mediated by G protein-stimulated release of Ca^{2+} from intracellular stores and enhanced spontaneous presynaptic release (not shown). The elevated intracellular Ca^{2+} is also responsible, either directly or indirectly (perhaps through protein synthesis), for triggering the activation of one or more retrograde signals; the retrograde signals, in turn, contribute critically to presynaptic changes and enhanced presynaptic release. Not shown are potential anterograde signals [62] nor processes that mediate the maintenance of LTF [110,149,155]. Also, both LTS and LTF involve the growth of presynaptic processes and varicosities, as well as postsynaptic spines; these structural changes are not shown. From ref. [58]