Learning in *Aplysia*: looking at synaptic plasticity from both sides

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Until recently, learning and memory in invertebrate organisms was believed to be mediated by relatively simple presynaptic mechanisms. By contrast, learning and memory in vertebrate organisms is generally thought to be mediated, at least in part, by postsynaptic mechanisms. But new experimental evidence from research using a model invertebrate organism, the marine snail *Aplysia*, indicates that this apparent distinction between invertebrate and vertebrate synaptic mechanisms of learning is invalid: learning in *Aplysia* cannot be explained in terms of exclusively presynaptic mechanisms. NMDA-receptor-dependent LTP appears to be necessary for classical conditioning in *Aplysia*. Furthermore, modulation of trafficking of postsynaptic ionotropic glutamate receptors underlies behavioral sensitization in this snail. Exclusively presynaptic processes appear to support only relatively brief memory in *Aplysia*. More persistent memory is likely to be mediated by postsynaptic processes, or by presynaptic processes whose expression depends upon retrograde signals.

Approximately a decade ago it was widely accepted that simple forms of invertebrate learning, such as habituation, sensitization and classical conditioning, could be explained in terms of comparatively simple forms of synaptic plasticity. As epitomized by the marine snail *Aplysia*, learning-related synaptic plasticity in invertebrates was thought to involve exclusively presynaptic cellular changes, including presynaptic facilitation and presynaptic depression [1–3]. By contrast, vertebrate learning is believed to depend on more complex forms of synaptic plasticity that involve postsynaptic changes. Most prominent among the candidate cellular mechanisms of learning in vertebrates is NMDA-receptor-dependent long-term potentiation (LTP) [4,5] – also known as Hebbian LTP [6,7]. NMDA-receptor-dependent synaptic plasticity was not believed to exist in the nervous systems of invertebrates [8], which seemed to provide a partial explanation for their more primitive learning capabilities.

Although many remain skeptical of the solidity of the experimental link between Hebbian LTP and vertebrate learning [9–11], convergent results from a broad array of studies, many of which have used transgenic mice [12], have convinced significant numbers of neuroscientists that Hebbian LTP does indeed underlie certain forms of learning and memory in vertebrates [13]. Meanwhile, knowledge of the cellular mechanisms of learning in invertebrates has also progressed. Postsynaptic mechanisms, including NMDA-receptor-dependent plasticity, have been shown to play crucial roles in learning in *Aplysia*. Here, we review recent data that indicate the importance of postsynaptic mechanisms in both classical conditioning and sensitization in *Aplysia*, and discuss their implications for a general understanding of the synaptic mechanisms of learning and memory.

Contributions of presynaptic and postsynaptic mechanisms to classical conditioning in *Aplysia*

Classical conditioning, a form of learning originally described in dogs by the Russian physiologist and psychologist Ivan Pavlov [14], occurs when a more-or-less behaviorally neutral stimulus (the conditioned stimulus or CS) is presented to an animal together with a reinforcing stimulus (the unconditioned stimulus or US). Before training, presentation of the US evokes a reflexive response in the animal (the unconditioned response or UCR), whereas the CS does not. However, as a result of paired stimulation with the CS and US, the CS alone becomes able to evoke a response in the animal (the conditioned response or CR) that resembles the reflexive UCR [15]. Most experimental investigations of classical conditioning have used mammals but such conditioning has also been demonstrated in a variety of invertebrate organisms, including mollusks and arthropods [16]. Classical conditioning of a simple defensive withdrawal reflex in the marine snail *Aplysia* was first described in 1981 by Eric Kandel and colleagues [17]. This form of invertebrate learning was originally hypothesized to be due to an exclusively presynaptic mechanism, known as activity-dependent presynaptic facilitation (ADPF; Box 1). However, the discovery that sensorimotor synapses of *Aplysia* possess the capacity for NMDA-receptor-dependent LTP [18–20] led to an alternative hypothesis: that classical conditioning might depend, in part, on Hebbian LTP [21]. Support for this hypothesis has come mostly from studies that used so-called 'cellular analogs' of classical conditioning, which involve reduced preparations or synapses in dissociated cell culture [22–25]. But a new study by Antonov *et al.* [26], who used a reduced preparation of

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Box 1. LTP and classical conditioning in *Aplysia*: a brief history

Three influential papers published together in 1983 in *Science* [98–100] demonstrated differential classical conditioning of the withdrawal reflex of *Aplysia*, and proposed a simple synaptic mechanism to account for this form of invertebrate learning. The mechanism, activity-dependent presynaptic facilitation (ADPF), involves a presynaptic interaction between elevated intracellular Ca^{2+} levels and serotonin (5-HT). The elevated presynaptic Ca^{2+} concentration results from the conditioned stimulus (CS) – weak tactile stimulation of the siphon – whereas 5-HT is released onto the sensory neurons in response to the unconditioned stimulus (US) – strong electrical shock of the tail [40]. In ADPF there is greater activation of presynaptic adenylyl cyclase, and consequently greater synthesis of cAMP [37,38], than in standard presynaptic facilitation in *Aplysia*. The enhanced synthesis of presynaptic cAMP results in enhanced downstream modulatory actions [41] (Figure 3a of main text), and greater transmitter release from the presynaptic terminals.

A 1984 study [101] compared ADPF with a Hebbian [6] mechanism as a synaptic explanation for classical conditioning in *Aplysia*. Hebbian plasticity depends on the conjunctive occurrence of presynaptic activity and strong postsynaptic depolarization [5,7]. A cellular analog of classical conditioning was used to test for the involvement of Hebbian plasticity. Here, direct activation of a sensory neuron via a microelectrode serves as the CS, and electrical shock of the pedal (tail) nerves serves as the US. Successful conditioning is represented by associative enhancement of the sensorimotor excitatory postsynaptic potential (EPSP). Two tests were performed. In the first, firing of the postsynaptic motor neuron with a microelectrode was used as the US rather than nerve shock. Paired stimulation using postsynaptic stimulation for the US did not enhance the sensorimotor EPSP. In the second test, the motor neuron soma was hyperpolarized to prevent it from firing during the US (tail-nerve shock). Postsynaptic hyperpolarization during the US did not block associative enhancement of the EPSP. Based on these results, it was concluded that classical conditioning in *Aplysia* does not involve a Hebbian mechanism.

A decade later, whether *Aplysia* sensorimotor synapses can express Hebbian LTP was re-investigated. Lin and Glanzman [19,20] demonstrated unambiguously Hebbian LTP at synapses in dissociated cell culture. Like LTP of CA1 hippocampal synapses [5], this invertebrate LTP depends on postsynaptic depolarization, activation of NMDA receptors [47] and elevated postsynaptic Ca^{2+} concentration. The demonstration of Hebbian LTP of sensorimotor synapses raised the question of whether LTP mediates learning in *Aplysia*. Murphy and Glanzman used a cellular analog of classical conditioning to re-examine whether Hebbian LTP was involved. They found that injecting the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*-*N*′,*N*′-tetraacetic acid (BAPTA) into the motor neuron before training blocked synaptic enhancement [23]. Murphy and Glanzman further showed that the NMDA-receptor-antagonist Dl-2-amino-5-phosphonovalerate (APV) blocked associative synaptic enhancement due to paired CS–US stimulation. By contrast, APV did not affect non-associative enhancement of the synapse due to unpaired delivery of the CS and US [22]. A study by Bao *et al.* [102] found that postsynaptic BAPTA blocked associative enhancement of sensorimotor synapses *in vitro*. Finally, new evidence from a study by Antonov *et al.* [26] supports a role for NMDA-receptor-dependent LTP in behavioral classical conditioning.

How was the contribution of Hebbian plasticity to classical conditioning originally missed [101]? It seems probable that the hyperpolarization and depolarization of the motor neuron were insufficient. The postsynaptic hyperpolarizing current used might have declined at distal postsynaptic sites such that the postsynaptic membrane was only weakly hyperpolarized, if at all [103]. (Interestingly, a similar mistake appears to have led to an early, erroneous conclusion that postsynaptic depolarization is not required for the induction of LTP at the Shaffer-collateral-to-CA1-neuron synapse [104,105].) Furthermore, action potentials generated within the motor neuron soma by postsynaptic depolarization might have failed to backpropogate to the crucial postsynaptic regions, possibly owing to the distribution of voltage-dependent K^+ or Na^+ channels in distal dendrites [106,107].

Aplysia that permits simultaneous electrophysiological and behavioral investigations, provides the strongest evidence to date that Hebbian LTP actually mediates classical conditioning in *Aplysia*. Antonov and colleagues found that Dl-2-amino-5-phosphonovalerate (APV), an antagonist of NMDA receptors in both mammals [27] and mollusks [19,26,28], blocks the associative enhancement of the siphon-withdrawal reflex caused by paired stimulation with a siphon tap (the CS) and tail shock (the US). These investigators also succeeded in blocking behavioral conditioning of the reflex by injecting several siphon motor neurons with the rapid Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*-*N*′,*N*′-tetraacetic acid (BAPTA) before behavioral training. The results of Antonov *et al.*, together with those of the earlier studies [22–25], represent forceful evidence for a role for Hebbian LTP in classical conditioning in *Aplysia*. Indeed, it could be argued that the experimental link between LTP and learning is now more compelling for classical conditioning in *Aplysia* than it is for any form of mammalian learning. This is because the connection between changes at specific synapses within the hippocampus (the part of the brain in which LTP has been most intensively studied) and any learned behavioral change in a rat or mouse, such as learning to navigate a water maze [29], is necessarily weak; this is largely due to the complexity of neural pathways – not to mention the vast number of neurons – engaged in hippocampus-dependent behaviors. (The proposed relationship between LTP of synapses in the basolateral nucleus of the amygdala and classical conditioning of fear is more convincing [30], owing to the relative simplicity of the neural pathways involved.) By contrast, LTP in *Aplysia* has been demonstrated at synapses – the siphon sensorimotor synapses – that directly control a specific reflexive behavior in *Aplysia* [26,31,32]. Because these synapses are potentiated during classical conditioning in the snail [33], it is highly likely that the withdrawal reflex will also be enhanced. Antonov *et al.* have now confirmed that enhancement of the reflex due to conditioning requires NMDA receptor activation. Skeptics could point to the lack of molecular data regarding NMDA receptors in *Aplysia*. However, NMDA receptors have recently been cloned and sequenced from the *Aplysia* CNS (GenBank accession numbers AY163562, AY234809 and AY315153). Moreover, NMDA receptors have been cloned and sequenced from the CNS of other invertebrates [34,35]. Thus, the NMDA receptor, like classical conditioning itself, is not unique to vertebrates.

How can the idea that LTP plays a crucial role in classical conditioning in *Aplysia* be reconciled with the original presynaptic model of this form of learning [2]? Lechner and Byrne [36] and Antonov *et al.* [26] have proposed a revised model of associative enhancement of the sensorimotor synapse during classical conditioning. According to this model (the hybrid model), there are two coincidence detectors, one presynaptic (ADPF) and the other postsynaptic (NMDA-receptor-dependent LTP). To accommodate the finding that postsynaptic BAPTA blocks

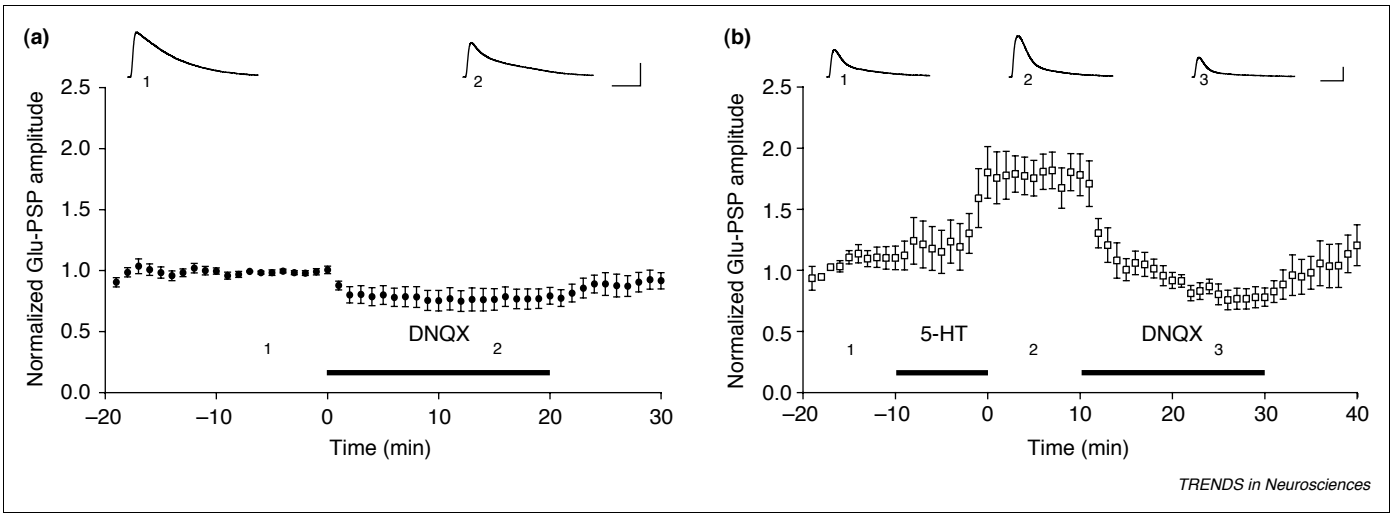

Figure 1. Serotonin facilitates the response of AMPA receptors in isolated *Aplysia* motor neurons. **(a)** Effect of the AMPA receptor antagonist DNQX on the non-facilitated glutamate response. Bath application of DNQX (100 μM) produced significant, but modest, inhibition of the glutamate-mediated postsynaptic potential (Glu-PSP; $n = 8$). Each trace at the top of the graph is the average of five consecutive Glu-PSPs from one experiment, recorded at the time in the experiment indicated by the number. **(b)** Effect of DNQX on the facilitated glutamate response. In these experiments, serotonin (5-HT) was first applied for 10 min and then washed out. After the Glu-PSP had reached an asymptotic level of facilitation, DNQX was applied for 20 min. The DNQX reversed the facilitation ($n = 6$). Note that the residual glutamate response in DNQX after 5-HT treatment was the same magnitude as the glutamate response in DNQX without 5-HT treatment. Each trace at the top of the graph is the average of five consecutive Glu-PSPs from one experiment, recorded at the time in the experiment indicated by the number. Scale bars for traces in both panels: 4 mV and 200 ms. Reproduced, with permission, from Ref. [45].

associative enhancement of the sensorimotor synapse [23,25], as well as the associative behavioral change [26], the hybrid model proposes that the rise in postsynaptic Ca^{2+} levels due to LTP induction activates a retrograde signal; this retrograde signal somehow interacts with the signaling pathways involved in ADPF, thereby gating or amplifying the presynaptic associative mechanism.

The hybrid model can account for the significant experimental evidence that presynaptic pathways, particularly the cAMP-dependent protein kinase (PKA) pathway [25,26,37,38], are important in conditioning-related enhancement of the sensorimotor synapse. However, the hybrid model does not incorporate recent experimental date that indicate that serotonin (5-HT) – which is released within the CNS of *Aplysia* by sensitizing stimuli, such as tail shock [39,40] – can enhance the responsiveness of the postsynaptic motor neuron to the presynaptic transmitter independently of any presynaptic actions (discussed in the section on the role of elevated postsynaptic Ca^{2+} levels and modulation of postsynaptic AMPA receptor trafficking in sensitization). These new findings suggest that, although processes that are autonomous to the presynaptic terminal can support short-term memory, persistent enhancement of the sensorimotor synapse (lasting $>5–10$ min) is due primarily to postsynaptic changes, including a persistent increase in the glutamate sensitivity of the motor neuron, as well as to presynaptic changes that are initiated by trans-synaptic signals.

Role of elevated postsynaptic Ca^{2+} levels and modulation of postsynaptic AMPA receptor trafficking in synaptic facilitation and behavioral sensitization in *Aplysia*

Evidence that elevated postsynaptic Ca^{2+} is crucial for sensitization-related, non-associative enhancement of the *Aplysia* sensorimotor synapse, as well as for associative enhancement, came initially from Murphy and Glanzman [23]. An unexpected finding in that study was the lack of residual facilitation during training in preparations that received conditioning-type stimulation with BAPTA present in the motor neuron (fig. 4 of Ref. [23]). If non-associative facilitation evoked by the nerve shock were due predominately to presynaptic processes [41], then some residual synaptic enhancement should have been apparent in these preparations despite the presence of the Ca^{2+} chelator in the motor neuron. The absence of nerve-shock-evoked facilitation in Murphy and Glanzman's data implies that any persistent facilitation of the sensorimotor synapse – whether associative or non-associative – depends crucially on elevated postsynaptic Ca^{2+} levels. Bao *et al.* [25] reported that postsynaptic BAPTA did not block 5-HT-induced facilitation of the sensorimotor synapse *in vitro*. But these investigators used a brief application of 5-HT in their experiments, which produced relatively weak, short-lived facilitation (fig. 4c of Ref. [25]).

What effect might elevated postsynaptic Ca^{2+} levels have on the sensorimotor synapse? An important clue came from studies of long-term facilitation (LTF). Long-term (≥ 24 h) facilitation of the sensorimotor synapse can be induced by repeated or prolonged exposure to 5-HT [42]. Two groups reported that LTF was accompanied by a long-term increase in the sensitivity of AMPA-type receptors in the motor neuron [43,44]. However, the cellular mechanisms for this increased sensitivity of the postsynaptic glutamate receptors during LTF are not known.

Our laboratory [45] has found that a relatively brief treatment with 5-HT can also facilitate the AMPA-receptor-mediated response in isolated *Aplysia* motor neurons. After a delay of a few minutes, 5-HT caused persistent enhancement of the response of isolated motor neurons in cell culture to brief applications of glutamate, the sensory neuron transmitter [46,47]. The 5-HT-induced

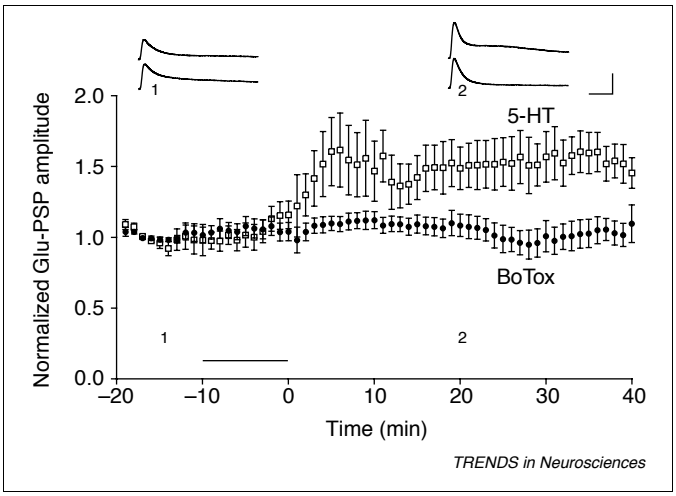

Figure 2. Serotonin-induced facilitation of the AMPA receptor-mediated response in isolated *Aplysia* motor neurons depends on exocytosis. Data from control experiments (open squares, $n = 6$) are shown together with data from experiments in which the exocytotic inhibitor botulinum toxin (BoTox) was injected into the motor neurons before the start of the experiment (closed circles, $n = 5$). The intracellular BoTox blocked the serotonin (5-HT)-induced facilitation of the glutamate-evoked postsynaptic potentials (Glu-PSPs). Each trace at the top of the graph is the average of five consecutive Glu-PSPs from one experiment, recorded at the time in the experiment indicated by the number. Scale bars for traces: 4 mV and 200 ms. Reproduced, with permission, from Ref. [45].

facilitation was blocked by prior injection of BAPTA into the motor neuron. Furthermore, facilitation of the glutamate response depended on modulation of AMPA receptors, as demonstrated by an experiment in which application of 5-HT, to facilitate the glutamate-evoked postsynaptic potential (Glu-PSP), was followed by application of DNQX, an AMPA receptor antagonist [47]. The DNQX reversed the 5-HT-induced enhancement of the Glu-PSP (Figure 1). This result indicates that the facilitatory action of 5-HT is specific for the AMPA-receptor-mediated component of the response. How might 5-HT modulate the response of AMPA receptors in the motor neuron? One possibility is that 5-HT causes additional AMPA receptors to be inserted into the motor neuron cell membrane via an exocytotic process. In support of this idea, prior injection of botulinum toxin, a selective inhibitor of vesicle exocytosis [48], into the motor neuron blocks 5-HT-induced facilitation of the glutamate response (Figure 2).

These results have now been extended to facilitation of the sensorimotor synaptic response. Both elevated postsynaptic Ca^{2+} levels and postsynaptic exocytosis are required for persistent facilitation of sensorimotor connections due to application of either 5-HT [49] or sensitizing-type stimuli (tail nerve shock) to the nervous system [50]. How is intracellular Ca^{2+} concentration elevated in the motor neuron by 5-HT or sensitizing stimuli? 5-HT can activate inositol-1,4,5-trisphosphate [Ins(1,4,5)P_3] receptors in *Aplysia* [51]. If the motor neurons contain Ins(1,4,5)P_3 receptors that are activated by 5-HT, application of 5-HT would cause release of Ca^{2+} from intracellular stores within the motor neurons. In support of this idea, prior injection of heparin, an inhibitor of Ins(1,4,5)P_3 receptors, into motor neurons blocks facilitation of sensorimotor synapses by 5-HT or sensitizing stimuli [52,53]. How might Ca^{2+} release from postsynaptic intracellular stores lead to the possible insertion of additional AMPA receptors into the postsynaptic membrane? We do not yet know, but recent experimental evidence points to a central role for protein kinase C (PKC) in this process. Chelerythrine, a specific inhibitor of PKC, blocks 5-HT-induced enhancement of the glutamate response in siphon motor neurons [54]. Although previous studies have implicated PKC in synaptic facilitation in *Aplysia* [41], it is commonly believed that it is presynaptic PKC that is crucial [41,55]. Our results suggest a role for postsynaptic PKC in sensitization-related facilitation as well.

That some forms of synaptic plasticity in *Aplysia* depend on modulation of AMPA receptor function is reminiscent of findings from studies of synaptic plasticity in mammals. NMDA-receptor-dependent LTP of synapses in the CA1 region of the hippocampus [56,57] and in the cortex [58,59] depends, in part, on upregulation of AMPA receptors, which converts so-called 'silent synapses' into active ones. Furthermore, this upregulation of AMPA receptor function appears to be due to the delivery of additional receptors to the postsynaptic membrane, possibly via exocytosis [60–62]. Monoamines can also modulate AMPA receptor function in the mammalian CNS. A mammalian parallel to the *Aplysia* findings has been provided by Zhuo and colleagues, who showed that 5-HT enhances AMPA receptor function in neurons of the spinal cord dorsal horn [63]. This action of 5-HT within the spinal cord is mediated by postsynaptic PKC and appears to involve an interaction between specific AMPA receptor subunits and PDZ-domain proteins [64], scaffolding proteins that structurally organize synapses [65].

Classical conditioning in *Aplysia*: contribution of an interaction between postsynaptic 5-HT-mediated modulation and NMDA-receptor-dependent plasticity

Previous cellular models of classical conditioning in *Aplysia* have assumed that 5-HT acts exclusively presynaptically [2,26,36,37,66,67] (Figure 3a). However, postsynaptic actions of 5-HT could be crucial to synaptic facilitation during classical conditioning. In particular, postsynaptic pathways activated by 5-HT and those activated by stimulation of postsynaptic NMDA receptors might interact. The likely cellular locus for the interaction is postsynaptic intracellular Ca^{2+}. Both 5-HT and activation of NMDA receptors appear to cause increases in intracellular Ca^{2+} concentration within siphon motor neurons (Figure 3b). These two sources of intracellular Ca^{2+} elevation might sum or interact synergistically. For example, certain isoforms of the Ins(1,4,5)P_3 receptor exhibit significant Ca^{2+} dependency [68,69]. Possibly, the Ca^{2+} influx through open NMDA channels enhances the release of Ca^{2+} from Ins(1,4,5)P_3-receptor-mediated stores within the motor neuron, stimulated by 5-HT. The effect of this postsynaptic interaction between the two sources of elevated intracellular Ca^{2+} might be to prolong or enhance the associative synaptic plasticity induced during classical conditioning.

What is the relationship between the postsynaptic and presynaptic actions of 5-HT [25,26] during classical conditioning in *Aplysia*? Antonov *et al.* [26] found that during classical conditioning there was an increase in the

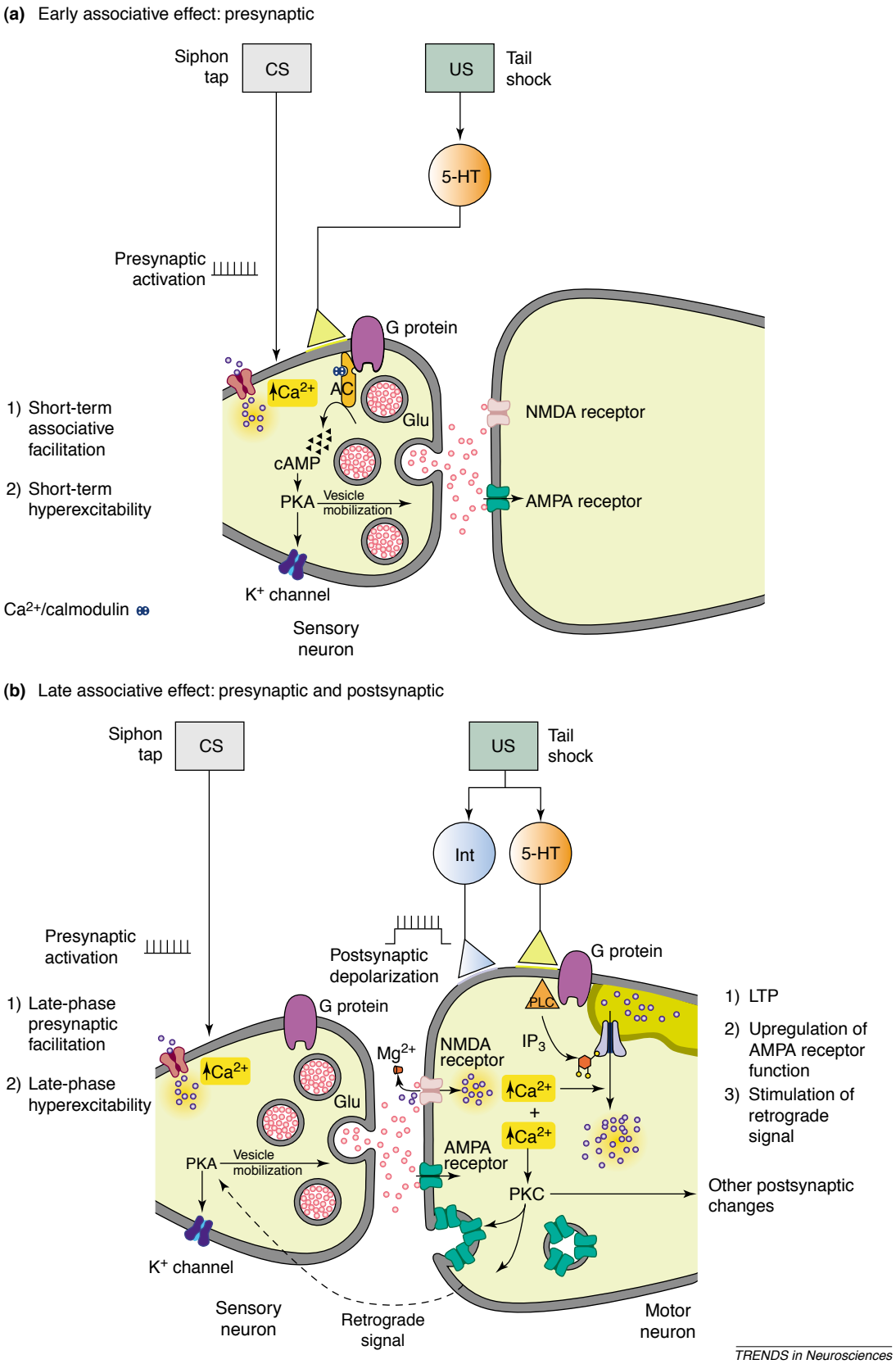

Figure 3. Model for associative enhancement of the sensorimotor synapse during classical conditioning in *Aplysia*. **(a)** Paired stimulation with the conditioned stimulus (CS) and unconditioned stimulus (US) leads to short-term associative enhancement of transmission and excitability in the sensory neuron. These changes are due to autonomous mechanisms in the sensory neuron and are mediated by an associative interaction between elevated intracellular Ca^{2+} levels (due to the CS) and serotonin (5-HT; due to the US). This interaction is mediated by an adenylyl cyclase (AC) that is stimulated by both 5-HT and Ca^{2+}/calmodulin [37,38,66,94]. The consequent activation of cAMP-dependent protein kinase (PKA) leads to downstream changes that produce short-term increases in transmitter release and neuronal excitability. In the absence of a signal from the postsynaptic neuron, however, these changes do not persist. **(b)** Paired stimulation also leads to activation of postsynaptic processes that mediate

excitability of sensory neurons that were activated by the CS. This unambiguously presynaptic change, however, was blocked by the presence of BAPTA in the postsynaptic neuron. This result argues, as Antonov *et al.* pointed out, that the prolonged change in the excitability of the presynaptic sensory neuron that was induced during classical conditioning must be somehow regulated by a rise in postsynaptic Ca^{2+} levels, probably via a retrograde signal. If so, the persistent associative increase in presynaptic excitability could be triggered by a trans-synaptic signal, rather than by presynaptic actions of 5-HT. In particular, persistent activation of presynaptic PKA – previously ascribed entirely or predominately to presynaptic actions of 5-HT [36,41] – might be due to a retrograde signal stimulated by elevated levels of postsynaptic Ca^{2+} (Figure 3b). Recent data from a study of mossy fiber LTP in the CA3 region of the hippocampus provide support for a parallel scheme in a form of mammalian synaptic plasticity [70].

Are autonomous processes in sensory neurons sufficient for persistent memory in *Aplysia*?

The preceding argument implies that stimulation of sensory neurons by 5-HT cannot by itself support persistent memory in *Aplysia*. This idea is admittedly contrarian. It goes against standard cellular models of learning and memory in *Aplysia*, according to which sensory neuron autonomous processes can support intermediate-term memory (30 min to 3 h) [71,72] and long-term memory (persisting $\geq$24 h) [42,73]. A large body of literature supports the current belief that sensory neuron processes are sufficient for persistent memory in *Aplysia* [3,41]. However, several of the key findings that buttress that belief now appear less compelling than they did a decade ago. For example, an early quantal analysis of sensitization-related facilitation of the sensorimotor synapse determined that the facilitation occurred via exclusively presynaptic changes [74]. However – as the recent controversy regarding quantal analyses of LTP in the hippocampus [75,76] illustrates – there are many pitfalls involved in the use of this statistical technique, which was developed for the neuromuscular junction, for analyzing changes at central synapses. It has also been reported that repeated applications of 5-HT to isolated sensory neurons in dissociated cell culture produce a long-term increase in their excitability [77]. But a recent study was unable to replicate this result [78]. Long-term morphological changes in *Aplysia* sensory neurons (specifically, an increase in the number of branches and the number of varicosities on the neurites of sensory neurons) have been observed during both LTF [79–81] and long-term sensitization [82]. But these structural changes require the presence of a postsynaptic motor neuron for their expression [79]. Isolated neurites of sensory neurons in dissociated cell culture exhibit significant protein synthesis after prolonged treatment with 5-HT [81]. However, it has not been shown that this synthesis of sensory neuron proteins is sufficient for persistent memory in *Aplysia*. (A similar point can be made regarding the long-term decrease in the regulatory subunits of PKA observed in isolated sensory neurons after repeated applications of 5-HT [83].) In summary, memory-related cellular changes in the *Aplysia* sensory neuron might not persist in the absence of signals initiated in the motor neuron.

Long-lasting synaptic plasticity in *Aplysia* depends on coordinated interaction between presynaptic and postsynaptic mechanisms

Assuming that persistent memory in *Aplysia* does require activation of postsynaptic mechanisms, why should this be the case? One possibility is that exclusively presynaptic mechanisms have evolved as a kind of cellular working memory: they retain the memory of the occurrence of a learning-related stimulus until more persistent processes, which depend on postsynaptic mechanisms, can take over. The postsynaptic mechanisms, such as release of Ca^{2+} from intracellular stores, appear to have a relatively long intrinsic latency of onset, in contrast to exclusively presynaptic mechanisms, the onset of which can be rapid. In the case of classical conditioning in *Aplysia*, associative presynaptic facilitation, besides subserving working memory, might also increase the probability that the paired CS–US stimulation will induce LTP at the sensorimotor synapse, and thereby increase the likelihood that the CS–US association will be retained by the animal.

An interesting question is whether the above proposal, based on studies of *Aplysia*, is applicable to learning-related synaptic plasticity in the mammalian CNS. It is generally agreed that short-term facilitation in the mammalian CNS is mediated by exclusively presynaptic mechanisms [84]. But whether prolonged synaptic enhancement, such as LTP, can be mediated via exclusively presynaptic mechanisms is controversial. It has been claimed that mossy fiber LTP is both presynaptically induced [85,86] and presynaptically expressed [87–89]. However, some evidence indicates that mossy fiber LTP, although NMDA-receptor-independent, is induced postsynaptically by a rise in intracellular Ca^{2+} levels [90,91].

persistent associative presynaptic and postsynaptic changes. The paired CS–US stimulation activates postsynaptic NMDA receptors [18,19,22,26]. This is because the CS causes the presynaptic release of glutamate (Glu) – the sensory neuron transmitter [46,47] – and the US activates excitatory interneurons (Int) to cause strong depolarization of the motor neuron [95,96]. The US also activates phospholipase C (PLC) within the motor neuron via a G protein, owing to 5-HT released from facilitatory interneurons in response to the US [51]. Activation of NMDA receptors leads to an influx of Ca^{2+} through open NMDA receptor channels, whereas activation of PLC leads to release of Ca^{2+} from inositol-1,4,5-trisphosphate [Ins(1,4,5)P_3]-receptor-mediated intracellular stores. These two sources of Ca^{2+} could interact synergistically, as a rise in intracellular Ca^{2+} levels enhances Ins(1,4,5)P_3-receptor-mediated release of Ca^{2+} from intracellular stores [68,69]. [It is also possible that the Ca^{2+} influx due to postsynaptic action potentials generated by the US enhances release of Ca^{2+} from Ins(1,4,5)P_3-sensitive stores [97].] The consequent prolonged rise in postsynaptic intracellular Ca^{2+} concentration leads to several downstream actions, including activation of protein kinase C (PKC) [54], upregulation of AMPA receptor function (possibly through exocytotic insertion of additional AMPA receptors into the postsynaptic membrane [45]) and stimulation of a retrograde signal. This retrograde signal triggers the persistent presynaptic cellular changes that accompany classical conditioning, including the persistent increase in excitability of the sensory neuron [26]. According to this model, the persistent associative changes are induced entirely postsynaptically but are expressed, in part, presynaptically via trans-synaptic activation of PKA within the sensory neuron. Note that the short-term processes in (a) and the more persistent processes in (b) are assumed to overlap temporally somewhat, even though they are shown here as temporally separate.

A recent study argues that mossy fiber LTP is induced postsynaptically, and expressed presynaptically via a retrograde signal [70]. This retrograde signal, according to this study, involves an interaction between Eph receptor tyrosine kinases and presynaptic ephrins, and results in activation of PKA within the presynaptic terminals of the mossy fibers. Such a scheme is similar to the one we propose for enhancement of the sensorimotor synapse during classical conditioning in *Aplysia* (Figure 3).

Some of the ideas presented here are speculative – we have as yet no candidates for retrograde signaling molecules that might contribute to synaptic plasticity in *Aplysia* – and potentially controversial. However, the past underestimation of the contribution of postsynaptic mechanisms to learning in *Aplysia* might have encouraged some to ignore the neurobiological work on learning in *Aplysia* and other invertebrates. Invertebrates show many of the same basic forms of learning that vertebrates do, including classical conditioning [16] and operant conditioning [92,93]. Until recently, it has been possible to suppose that disparate neuronal mechanisms might underlie vertebrate and invertebrate learning. But the evidence summarized here suggests that the phylogenetic universality of the mechanisms of learning and memory might be far greater than has previously been apparent.

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