REVIEWS

IS HETEROSYNAPTIC MODULATION ESSENTIAL FOR STABILIZING HEBBIAN PLASTICITY AND MEMORY?

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In 1894, Ramón y Cajal first proposed that memory is stored as an anatomical change in the strength of neuronal connections. For the following 60 years, little evidence was recruited in support of this idea. This situation changed in the middle of the twentieth century with the development of cellular techniques for the study of synaptic connections and the emergence of new formulations of synaptic plasticity that redefined Ramón y Cajal's idea, making it more suitable for testing. These formulations defined two categories of plasticity, referred to as homosynaptic or Hebbian activity-dependent, and heterosynaptic or modulatory input-dependent. Here we suggest that Hebbian mechanisms are used primarily for learning and for short-term memory but often cannot, by themselves, recruit the events required to maintain a long-term memory. In contrast, heterosynaptic plasticity commonly recruits long-term memory mechanisms that lead to transcription and to synaptic growth. When jointly recruited, homosynaptic mechanisms assure that learning is effectively established and heterosynaptic mechanisms ensure that memory is maintained.

SYNAPTIC PLASTICITY
A change in the functional properties of a synapse as a result of use.

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In the last decade of the nineteenth century, Santiago Ramón y Cajal introduced a theory of memory storage in which information is stored in the brain by anatomical changes in the connections between neurons[1]. This insight proved not only prescient but also premature. Over the next sixty years, few experiments were directed toward testing this idea. Indeed, during much of this period, Ramón y Cajal's suggestion was not even taken seriously by many scientists working on the brain because of opposition to the idea that the nervous system could be studied effectively at the level of individual cells and their connections[2–5].

This situation began to change in the late 1940s and early 1950s. Intracellular microelectrode recording methods were introduced for studying the synaptic actions of individual neurons and electron microscopy was applied to visualize the fine structure of synapses. These methodological advances allowed central synapses to be studied directly and led to a revival of interest in Ramón y Cajal's ideas. In addition, the ideas were refor-mulated in functional terms that made them more suitable to physiological and anatomical testing. These formulations defined two broad categories of SYNAPTIC PLASTICITY, generally referred to as homosynaptic and heterosynaptic plasticity. In this review we will focus on an extensively studied example of each type of synaptic plasticity: Hebbian homosynaptic potentiation in the hippocampus and heterosynaptic facilitation in *Aplysia*.

Homosynaptic and heterosynaptic rules

In 1949, Donald Hebb proposed a homosynaptic rule for long-term memory on the basis of the strengthening of synaptic connections: the events responsible for triggering synaptic strengthening occur at the same synapse as is being strengthened (FIG. 1a)[3]. Specifically, Hebb proposed that the strength of the connection between the two neurons is increased for a long period of time when the firing of the presynaptic and post-synaptic neuron are closely correlated in time. Subsequently, this synaptic strengthening has been
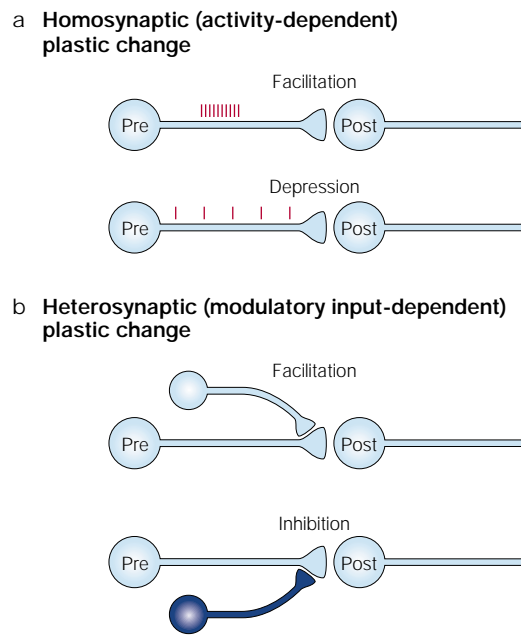
a Homosynaptic (activity-dependent) plastic change

Facilitation

Pre | Post

Depression

Pre | Post

b Heterosynaptic (modulatory input-dependent) plastic change

Facilitation

Pre | Post

Inhibition

Pre | Post

Figure 1 | **Homosynaptic and heterosynaptic mechanisms for long-term plasticity. a** | The plastic changes that underlie long-term memory follow a homosynaptic rule, that is, the events responsible for triggering synaptic strengthening occur at the same synapse as is being strengthened. These changes can result in an increase in synaptic strength (for example, homosynaptic facilitation), or a decrease in synaptic strength (for example, homosynaptic depression). **b** | Synaptic strengthening between a presynaptic and a postsynaptic cell can occur as a result of the firing of a third neuron, a modulatory interneuron, whose terminals end on and regulate the strength of the specific synapse. These changes can result in an increase (heterosynaptic, modulatory facilitation) or in a decrease (heterosynaptic inhibition) in synaptic strength.

SENSITIZATION
A strengthening of the response to a wide variety of neutral stimuli following an intense or noxious stimuli.

CLASSICAL CONDITIONING
Form of associative learning in which a subject learns the relationship between two stimuli.

HABITUATION
A decrease in the behavioural response to a repeated, benign stimulus.

could have one of two forms: non-associative or associative. The non-associative form is purely heterosynaptic, whereas associative, activity-dependent heterosynaptic modulation combines features of homosynaptic and heterosynaptic mechanisms. Here, the strengthening effect of the modulatory neuron is further enhanced if the firing of the modulatory input is associated in time with the firing of the presynaptic cell.

Studies in the mid 1960s and early 1970s exploring the long-term plastic capabilities of chemical synapses in the marine mollusc *Aplysia*, crayfish and other invertebrates, as well as in the spinal cord and the hippocampus of mammals, provided the initial evidence to support these two synaptic rules[4]. These studies also raised two questions to which experimental attention next turned: are such plastic changes actually induced by learning in a behavioural context? If so, is their time course adequate to subserve aspects of memory storage?

Synaptic plasticity and non-associative learning
In the first systematic attempt to address the two questions above, Kupfermann, Pinsker and their colleagues identified a circuit between specific nerve cells for the siphon and gill-withdrawal reflex in *Aplysia* that could be modified by two simple non-associative forms of learning: HABITUATION and sensitization[11,12]. This neural circuit consists of sensory neurons that innervate the siphon (later shown to be glutamate-mediated), stabilizing direct connections to motor cells and indirect connections through several groups of excitatory and inhibitory neurons[11,13–16]. In habituation, the repeated presentation of a novel stimulus leads to a gradual decrease in the response to the stimulus as the animal learns that the stimulus is innocuous. This decrease in behavioural response can be largely accounted for by the accompanying decrease in the strength of the glutamate-mediated synaptic connections between the sensory and motor neurons[17,18]. Moreover, repeated stimulation of even a single sensory neuron in the circuit produces a homosynaptic decrease in strength of the synaptic connection to the corresponding motor neurons[13]. This decrease in synaptic strength results from a decrease in transmitter release from the presynaptic sensory neuron[19]. In contrast, sensitization, a form of learned fear, consists of a generalized increase in response to neutral stimuli after presentation of a noxious stimulus to the head or tail[12,20,21]. This form of learning was found to be due, in part, to a heterosynaptic increase in synaptic strength at the same sets of connections between the sensory and motor neurons that were modified in the opposite direction by habituation[18,22].

During sensitization, the application of a noxious stimulus to the head or tail excites several classes of modulatory interneurons, the most important of which use serotonin (5-HT) as their transmitter[23]. The 5-HT released by these neurons activates G-protein-coupled receptors on the sensory neurons, including receptors on their presynaptic terminals. These receptors are of two types. One is positively coupled to adenylyl cyclase, leading to the activation of the cyclic AMP-dependent protein kinase (PKA). The second leads to the activation

termed associative, because it associates the firing of a postsynaptic neuron with that of a presynaptic neuron. After such an event, when the first of the two neurons is activated, the chance of the postsynaptic neuron firing is increased. In addition to its being homosynaptic and associative, Hebb implied that the synaptic strengthening is input-specific: when two neurons fire coincidentally the synapse between them is strengthened, but other synapses on either neuron remain unchanged. These three characteristics (homosynaptic plasticity, associativity and input specificity) form the modern definition of the Hebbian synapse[6,7].

Because behavioural learning processes such as CLASSICAL CONDITIONING and SENSITIZATION result from the consequences of one stimulus input on another, Kandel and Tauc proposed a second, heterosynaptic rule for strengthening synaptic connections[8,9]. Influenced by Dudel and Kuffler's finding of a transient form of presynaptic inhibition[10], and by their own finding of a more enduring presynaptic facilitation, Kandel and Tauc pointed out that a synapse could be strengthened or weakened without a requirement for activity of either the pre- or the postsynaptic neurons as a result of the firing of a third, modulatory interneuron (FIG. 1b). They further suggested that this heterosynaptic modulation

of protein kinase C (PKC). PKA and PKC then act on several substrates to enhance transmitter release[24–28].

The early efforts to examine the role of synaptic plasticity in memory storage concentrated on short-term synaptic changes and their relation to short-term memory processes lasting minutes to hours. In the late 1970s and early 1980s the focus shifted to the study of long-term events. It was found that memory in invertebrates has phases[29], much as had been shown earlier for vertebrates[30]. Whereas one noxious stimulus to the tail of *Aplysia* leads to short-term sensitization of the reflex that lasts minutes[12], five or more training trials spaced in time lead to long-term sensitization that lasts several days[21,31] and requires the synthesis of new proteins[32]. It seems that the distinction between memory phases evident on the behavioural level is also evident on the cellular level. As with behaviour, the heterosynaptic plasticity that mediates sensitization has both a transient and a persistent phase, and the persistent phase of heterosynaptic facilitation differs from the early phase in requiring new protein synthesis and transcription[29,33].

A model of the reflex and its plasticity can be created in a culture consisting of a single sensory neuron connected to a single motor neuron. Their synapses can then be modulated by a single 5-HT cell or even by pulses of 5-HT[33–35]. In culture, one brief pulse of 5-HT — the transmitter released by a sensitizing tail stimulus in the intact animal — produces short-term presynaptic facilitation that lasts minutes. This facilitation requires only covalent modifications of pre-existing proteins, induced largely by PKA and to a lesser degree by PKC[24,27,28,36–39]. With five spaced applications, 5-HT recruits PKA and mitogen-activated protein kinase (MAPK). They both translocate to the nucleus and activate the transcription factor CREB (the cAMP response element binding protein). CREB, in turn, activates a cascade of genes that leads to the growth of new synaptic contacts between the sensory and the motor neurons and to a facilitation of synaptic strength that persists for days[33,40–46]. These morphological changes *in vitro*[47,48] are similar to the synaptic growth associated with behavioural sensitization *in vivo*, where the memory lasts for weeks and the increase in the number of sensory neuron synapses parallels the retention of the memory[49–51].

These experiments, and parallel studies carried out with an inhibitory interneuron and its modulatory transmitter[48,52], showed that heterosynaptic plasticity is recruited during different forms of learning and that, with repetition, heterosynaptic modulation is both necessary and sufficient to activate transcription. This leads to the growth (or retraction) of synaptic connections, thereby producing persistent changes in synaptic strength that can contribute to long-term memory storage. But what about homosynaptic processes? Can they also persist?

Long-term potentiation in the hippocampus

In a groundbreaking study in 1973, Bliss and Lømo described a homosynaptic Hebbian form of plasticity in the mammalian brain[53]. They found that when the perforant path, a fibre pathway in the hippocampal formation, was repetitively stimulated at high frequency in an anaesthetized animal, the synapses between the perforant path and their target cells, the granule cells of the dentate gyrus, were strengthened. This phenomenon was subsequently termed long-term potentiation (LTP). The discovery of LTP was important for several reasons. First, it showed that long-term synaptic plasticity was possible in the adult mammalian brain. As the hippocampus had been implicated in human memory by the work of Penfield, and of Scoville and Milner, the finding of synaptic plasticity in this area of the brain was particularly intriguing[54]. Second, further investigation of this form of plasticity showed that in most cases it is induced by a homosynaptic associative mechanism resembling that described by Hebb. It depends on coincident pre- and postsynaptic firing and it is input-specific, at least to a first approximation[6,26,55,56].

With time, it has become clear that LTP is not a unitary phenomenon, but a family of processes that vary in their cellular and molecular mechanisms. The existence of these various forms of LTP was first found in the three main pathways of the hippocampus, but other variants were discovered in the cerebellum, in the lateral nucleus of the amygdala and in the prefrontal cortex[6,57–60]. Nevertheless, each variant of LTP examined so far has an early phase (E-LTP) and a late phase (L-LTP). For example, in the Schaffer collateral pathway, E-LTP induced by one weak train of stimuli lasts about 1–3 hours, whereas the L-LTP induced by four weak trains is stable beyond 24 hours. In each case, the late phase differs from the early phase in that it requires protein and messenger RNA synthesis[61–64]. Moreover, at all the synapses that have been studied so far, the induction of this late phase requires, at least in part, cAMP, PKA and MAPK (REFS 65–73).

The most detailed analysis has been obtained in the Schaffer collateral pathway between areas CA3 and CA1 in the hippocampus. Glutamate released from the axon terminals of the Schaffer collateral pathway acts on two types of postsynaptic receptors: AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA (*N*-methyl-D-aspartate) receptors[6,7]. Under normal circumstances, only the AMPA receptors are activated by glutamate, because magnesium blocks the pore of the NMDA receptor. The induction of LTP leads to the activation of the AMPA receptors, which depolarizes the postsynaptic cell, removes the magnesium block and leads to the activation of the NMDA receptor channel. Activation of the NMDA receptor is the critical trigger for this form of LTP — it leads to influx of calcium into the postsynaptic cell and results in the activation of several protein kinases including calcium/calmodulin-dependent protein kinase II (CaMKII). So to activate the NMDA receptor channel and to initiate LTP, two events need to occur simultaneously: glutamate needs to bind to the receptor; and the postsynaptic membrane needs to be depolarized sufficiently by the activation of the AMPA receptor to expel magnesium from the NMDA channel. Thus, the NMDA receptor is ideally suited to act as a molecular coincidence detector in Hebbian plasticity, where
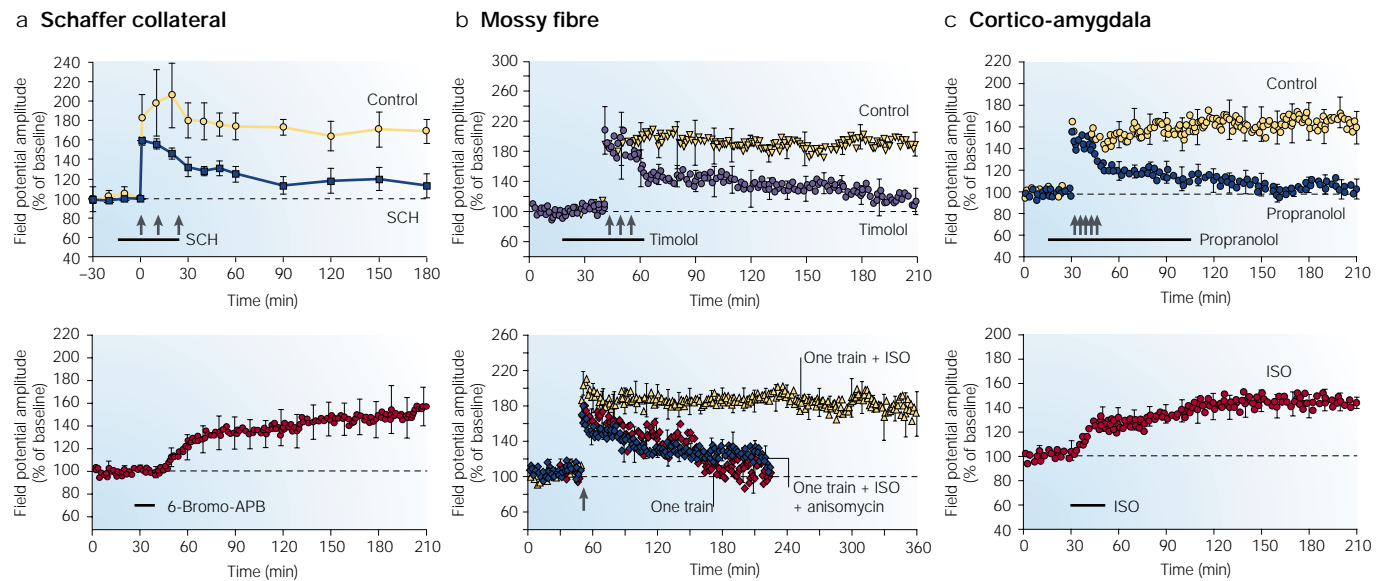
Figure 2 | **Modulatory transmitters enhance the duration of long-term potentiation.** Studies in the Schaffer collateral pathway, the mossy fibre hippocampal pathway and the cortico-amygdala pathway have shown that late-phase LTP (L-LTP) requires the participation of a heterosynaptically released modulatory transmitter. **a** | In the Schaffer collateral pathway, an inhibitor of the D1/D5 dopamine receptors (SCH23390, 0.1 µM) blocks L-LTP induced by three tetani (shown as arrows) without affecting the early phase (top panel) and the application of a D1/D5 agonist (6-Bromo-APB, 50–100 µM) induces L-LTP (bottom panel). **b** | In the mossy-fibre pathway, an inhibitor of the β-adrenergic receptor (timolol, 10 µM) can block L-LTP (top panel) and application of the β-adrenergic agonist isoproterenol (ISO, 10 µM) facilitates L-LTP (bottom panel). This effect of ISO is protein synthesis-dependent, as shown by the addition of anisomycin, an inhibitor of translation. **c** | In the cortico-amygdala pathway, blocking the β-adrenergic receptor (propranolol, 1 µM) also blocks L-LTP induced by five tetani (top panel), whereas β-adrenergic agonists (ISO, 15 µM) facilitate L-LTP (bottom panel). (Modified from REFS 71,90,93.)

synaptic strengthening can result from coincident firing of the pre- and postsynaptic neurons[6,55,56].

Whereas a single 100 Hz tetanus activates the kinases required for E-LTP (CaMKII, for instance), this phase typically does not persist for more than a few hours. To induce persistent L-LTP a very strong tetanus or multiple tetani are required[66,72,74]. The induction protocols for L-LTP not only recruit CaMKII, they also increase intracellular cAMP and recruit further kinases including PKA and MAPK, which activate CREB-mediated transcription[66,75]. The role of CREB in the transcription-dependent component of LTP was first suggested by Silva and colleagues, who found that LTP and hippocampus-dependent learning were disrupted in a mouse lacking the two most prevalent forms of CREB[76]. The role for CREB in LTP and memory storage has been further supported by studies in knockout and transgenic mice[77–79]. However, one study in mice did not find a role for CREB in L-LTP[80], perhaps because, in mice as in *Aplysia*, transcription factors other than CREB may also be required for the long-term process[81]. Nevertheless, the parallel between CREB activation and long-term memory seems quite good. Indeed, Daniel Storm and his colleagues found that a *lacZ* reporter gene activated by a CREB-responsive promoter is activated during the tetanic stimuli used for the induction of L-LTP *in vitro*[72,82], as well as by certain forms of hippocampus-dependent learning *in vivo*[75]. Finally, with one important exception[83], there has also been a good correlation between Hebbian LTP in the Schaffer collateral pathway and hippocampus-dependent memory in general, and L-LTP and long-term memory in particular[84].

The late phase of LTP

Is there any relation between the mechanisms of long-lasting heterosynaptic facilitation in *Aplysia* and homosynaptic LTP in the hippocampus? Studies of L-LTP in hippocampal pathways have led to the surprising finding that the late phase of LTP, which requires PKA and protein synthesis, has a further requirement for a heterosynaptic modulatory input that recruits the cAMP cascade[85–88].

So far the strongest evidence for the requirement of a modulatory input for L-LTP is pharmacological. For example, in the Schaffer collateral pathway, inhibiting the D1/D5 dopamine-mediated receptors, which are positively coupled to adenylyl cyclase, selectively blocks L-LTP without affecting E-LTP (FIG. 2a)[89–91]. Conversely, application of D1/D5 agonists increases cAMP levels and induces L-LTP (FIG. 2a)[90,92]. These findings indicate that L-LTP in this pathway requires the activation of a dopamine-mediated input that acts on D1/D5 receptors.

A similar requirement is present in the mossy-fibre pathway — inhibitors of the β-adrenergic receptors block L-LTP (FIG. 2b), and application of β-adrenergic agonists facilitates L-LTP (FIG. 2b)[87,93]. Finally, in the cortico-amygdala pathway, blocking of β-adrenergic receptors also blocks L-LTP (FIG. 2c)[71]. Although these modulatory transmitters have various other actions, including the modulation of components of E-LTP in some cases[93–96], they consistently seem to be required for L-LTP.

How these modulatory inputs are recruited is still not clear. The axons of the noradrenaline neurons from the LOCUS COERULEUS, those of the 5-HT neurons from the DORSAL RAPHE and those of the dopamine neurons from

LOCUS COERULEUS
Nucleus of the brainstem. The main supplier of noradrenaline to the brain.

DORSAL RAPHE
Nucleus of the brainstem. The main supplier of serotonin to the brain.

the VENTRAL TEGMENTAL AREAS, run along with the fibres of the Schaffer collateral, the mossy fibre, the perforant and the cortico-amygdala pathways stimulated for LTP[97,98]. The ascending dopamine fibres are highly concentrated in the pyramidal cell layer of the CA1 and CA3 regions, where they contact the soma and the proximal dendrites of pyramidal cells[99]. Similarly, there is an extensive noradrenaline projection from the locus coeruleus to the dentate gyrus and to the STRATUM LUCIDUM of the CA3 region where the glutamate-mediated mossy fibres terminate[100,101]. This overlapping distribution of dopamine axons and the Schaffer collateral terminals in the CA1 region, and the noradrenaline axons and the mossy fibre terminals in the CA3 region, provides an opportunity for heterosynaptic interaction between the two pathways in each case. Indeed, β-adrenergic receptors have been localized to both the dentate gyrus and the CA3 region[102,103], and activation of these β-adrenergic receptors increases the level of cAMP in the hippocampus[104,105].

Although there are several ways in which the glutamate pathways could activate the modulatory pathways, the nature of these interactions is not clear. The simplest possibility is that stimulating the Schaffer collateral or the perforant pathway also directly activates these modulatory axons. Alternatively, during high-frequency stimulation, the increased release of glutamate might lead to its spillover to presynaptic glutamate receptors on the terminals of the noradrenaline and dopamine axons, causing the release of these transmitters independently of activity in the modulatory axons themselves[106].

The repeated elicitation of homosynaptic LTP required to induce L-LTP seems to recruit a modulatory input whose action is required for the maintenance of the late phase. So can homosynaptic LTP by itself persist for days, or is heterosynaptic modulation an obligatory requirement for persistence? Pharmacological data indicate that Hebbian homosynaptic plasticity *per se* does not persist, and that the modulatory transmitters can produce persistent changes by themselves. But these findings in the hippocampus and amygdala are only suggestive because it is difficult to elicit homosynaptic LTP alone in any of these pathways without also activating fibres of passage from one or another modulatory system. However, the technical advantages of the gill- and siphon-withdrawal reflex in *Aplysia*, both in the intact animal and in culture, allow independent activation of the same synapses either homosynaptically, heterosynaptically or in combination. These studies support the findings of the pharmacological experiments in the hippocampus and raise the interesting possibility that homosynaptic action alone may not be sufficient to produce long-lasting plasticity.

Homosynaptic and heterosynaptic interactions
More direct insight into the nature of the interaction between homo- and heterosynaptic plasticity has come from studies of classical conditioning of the gill- and tail-withdrawal reflexes in *Aplysia*[107–111]. Classical conditioning resembles sensitization in that the response to a

stimulus to one pathway is enhanced by activity in another, but differs in requiring associative pairing of the two stimuli. When a weak stimulus to the siphon (conditioned stimulus, CS) is repeatedly paired with a shock to the tail (unconditioned stimulus, US), the withdrawal response to stimulation of the siphon is enhanced. The enhancement of the response to the CS in classical conditioning is greater than with sensitization, where the weak siphon stimulus and tail shock are not paired. For example, even a single pairing of the siphon stimulus and tail shock can produce conditioning that lasts more than 24 hours, whereas either stimulus by itself produces no long-term effect (FIG. 3a). Classical conditioning is reflected at the cellular level as an enhanced strengthening of the synaptic connections of the sensory neurons to their target cells, an enhancement that is also greater than that of the unpaired training[107–109,112].

In classical conditioning as in sensitization, the tail stimulus activates modulatory neurons that stimulate adenylyl cyclase and PKA, producing presynaptic facilitation of sensory neurons in the CS pathway. In classical conditioning, however, the CS is paired with the US, resulting in enhanced facilitation. This enhancement is due to the interaction of a homosynaptic process with a heterosynaptic one. When 5-HT released by the modulatory neurons of the US pathway acts on a sensory neuron that has just fired and undergone homosynaptic activity as a result of being activated by the CS, the calcium influx into the sensory neuron enhances the ability of 5-HT to activate adenylyl cyclase. So the temporal pairing of heterosynaptic facilitation and homosynaptic activity produces an enhanced increase in cAMP levels and in synaptic strength[26,111,113]. These increases are greater than the sum of those produced by the heterosynaptic and homosynaptic processes alone, so the combination can be considered a new category of plasticity.

In addition to contributing to this activity-dependent presynaptic facilitation, the US also depolarizes the motor neurons, activating postsynaptic NMDA receptors[114–116]. The calcium influx through the NMDA receptor channels in turn activates a signalling cascade in the postsynaptic cell that seems to generate a retrograde signal that further facilitates the presynaptic enhancement of transmitter release[117,118]. So facilitation of the connections between the sensory and motor neurons that occurs with classical conditioning superimposes on the activity-dependent heterosynaptic facilitation, a homosynaptic, Hebbian component that requires calcium influx into the postsynaptic cell. These synapses combine the two associative mechanisms proposed earlier by Hebb, and by Kandel and Tauc, resulting in a hybrid mechanism. This example illustrates the general idea that the basic forms of plasticity may constitute an alphabet of elementary mechanisms that can be combined in various ways for different functional purposes. As we shall discuss below, such a combinatorial mechanism may serve both to extend the duration of the plasticity and to provide greater synapse specificity.

Stabilizing transient homosynaptic plasticity

From the perspective of the presynaptic neuron, homosynaptic plasticity is cell-wide, whereas heterosynaptic modulation can be restricted to a single synapse[35,81]. How do these two processes interact at the level of the single synapse? Martin *et al.*[35] have developed a culture preparation in *Aplysia*, consisting of a single bifurcated sensory neuron that forms independent synaptic connections with each of two motor neurons (FIG. 3a). This culture has allowed a new approach to the study of how homosynaptic and heterosynaptic mechanisms interact at the level of individual synaptic connections[119].

A single homosynaptic tetanus applied to the glutamate-releasing sensory neuron produces not only post-tetanic potentiation (PTP) that is evident in the first few minutes after the tetanus, but also a subsequent

homosynaptic Hebbian potentiation that has properties similar to LTP. As with LTP in the Schaffer collateral pathway, this form of LTP is blocked by injecting calcium chelators into the postsynaptic cell and, when repeated, is also blocked by NMDA receptor antagonists[114,116,120]. This homosynaptic potentiation is both cell-wide and transient; it is evident at all the connections of the sensory neuron and it lasts around one hour. Even when a series of four spaced tetani are given, the resulting potentiation is not enhanced in its duration and also lasts only about one hour. So in this culture system, we can see directly what could only be inferred in the hippocampus and in the amygdala: a homosynaptic, Hebbian type of LTP by itself is not maintained and lasts only about 1–2 hours. However, if a single homosynaptic train of activity in the presynaptic sensory neuron is combined with even a single pulse of
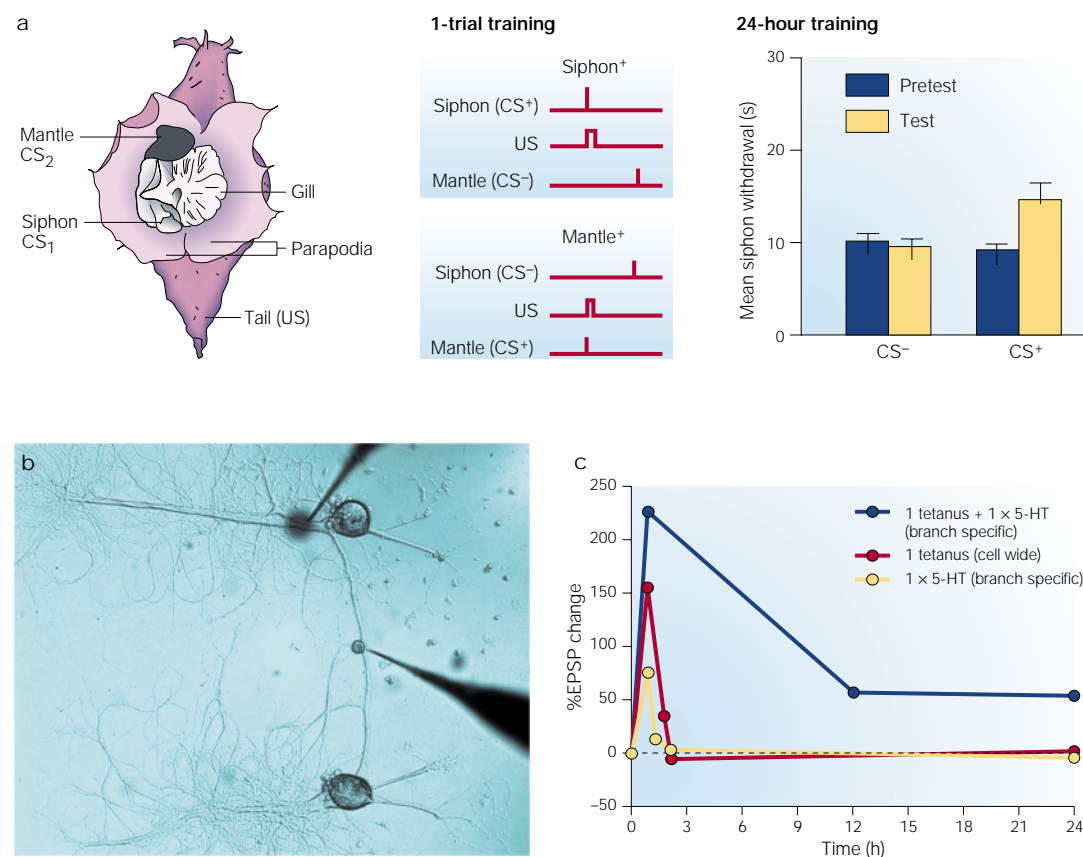

Figure 3 | **Comparison of homosynaptic facilitation with paired homo- and heterosynaptic facilitation. a** | In *Aplysia*, a single pairing of a stimulation to the siphon (CS$_1$) or mantle (CS$_2$) and a tail shock (US) can produce behavioural conditioning that lasts more than 24 hours. Left panel: experimental preparation. Centre panel: Differential training protocol. Right panel: Duration of withdrawal 24 hours after pairing. Pretest values were obtained before conditioning. Only the CS paired with the shock continued to elicit increased withdrawal 24 hours after pairing. **b** |The bifurcated sensory neuron–two motor neuron culture. The bifurcated sensory neuron makes synaptic contacts with two spatially separated L7 motor neurons. Homosynaptic activation was applied by delivering electrical stimulation to the cell body of the sensory neuron. Heterosynaptic modulation was achieved by delivering a single pulse of 5-HT at a specific sensory-motor synapse. **c** | Time course of homosynaptic and paired homo- and heterosynaptic facilitation. A single homosynaptic tetanus (20 Hz for 2 seconds) applied to the cell body of the bifurcated presynaptic sensory neuron produces short-term facilitation of the excitatory postsynaptic potential (EPSP) at both of its connections with the motor neurons, which lasts between 1–2 hours (red symbols). A single heterosynaptic stimulus (one pulse of 5-HT) applied to one branch of the sensory neuron produces short-term facilitation at that branch which lasts 10–20 minutes (yellow symbols). However, when a single homosynaptic train of spike activity in the sensory neuron is paired with a single pulse of 5-HT to one of the two branches of the bifurcated culture (blue symbols), the synaptic strength of only that branch is selectively enhanced for more than 24 hours. (Modified from **REFS 107, 119**.)

5-HT to one of the two branches of the bifurcated culture, the synaptic strength of that branch, and only that branch, is selectively enhanced for more than a day. So a single heterosynaptic pulse of 5-HT is sufficient to greatly extend — about 20-fold — the duration of the homosynaptic potentiation (FIG. 3b)[120,121].

These results lead to three conclusions. First, like LTP in the hippocampus, homosynaptic potentiation in *Aplysia* is transient in its response to both a single train or to repeated trains. Second, the combination of this homosynaptic potentiation (lasting only 1–2 hours) with a single heterosynaptic stimulus that, by itself, results in facilitation that lasts for 10–20 minutes, produces effects that are more than just additive — a facilitation that lasts more than 24 hours (FIG. 4). Third, whereas Hebbian homosynaptic plasticity can invariably provide some specificity and short-term synaptic changes, it does not seem to ensure the persistence necessary for the storage of long-term memory. In contrast, heterosynaptic facilitation can provide persistence — repeated application of modulatory transmitters readily leads to persistent changes. So what might be the functional significance of this dichotomy between homo- and heterosynaptic facilitation?

Modulatory transmitters and memory

The noradrenaline-mediated and the dopamine-mediated modulatory projections important for different variants of hippocampal L-LTP are thought to be relevant for modulating memory storage, especially of emotionally charged material, but also during the normal heightened arousal that accompanies attentive learning[122–127].

Important insights into the functional significance of modulatory transmitters have come from studies of how memory storage is modulated in rodents and people. When a rat or a mouse hears a tone paired with a mild electrical shock, after a few pairings the animal responds to the tone as if it is afraid. This form of conditioned fear is dependent on the amygdala[128]. Studies pairing a neutral tone with a loud noise have shown that the amygdala plays a similar role in the implicit learning of fear in humans[129]. Using positron emission tomography, Larry Cahill, James McGaugh and colleagues found that activity in the amygdala at the time of learning coincides with the long-term storage of those explicit memories with emotional content. Blockade of β-adrenergic receptors interferes with the formation of emotional memory in humans and, conversely, local infusion of β-adrenergic agonists into the amygdala in animals enhances memory consolidation[130]. Similarly, dopamine D1/D5 receptor antagonists impair learning, and D1/D5 agonists enhance learning and L-LTP in both young[131] and old[92,132] animals. Because β-adrenergic receptors and D1/D5 receptors, like the 5-HT receptor in *Aplysia*, are G-protein-coupled receptors that activate cAMP production, these studies indicate that the cAMP pathway is probably important for the storage of certain types of memory in mammals, much as it is important for memory in invertebrates[133].

a Homosynaptic activation
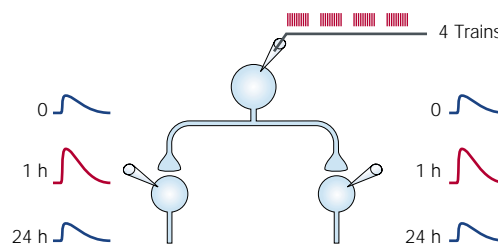
b A single heterosynaptic stimulus
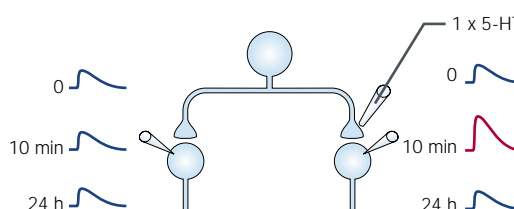
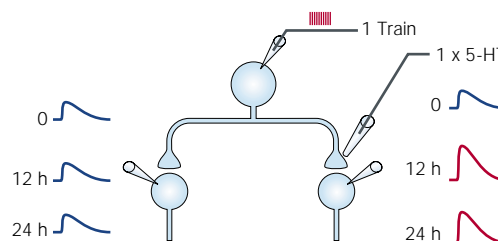**c** Pairing homosynaptic activation with heterosynaptic modulation

Figure 4 | **Non-additive interaction of homo- and heterosynaptic plasticity. a** | The homosynaptic stimulation of the presynaptic cell produces short-term facilitation of each sensory–motor neuron synapse that lasts about 1 hour. **b** | The application of a single pulse of 5-HT to one synapse of the bifurcated culture produces short-term facilitation of only that synapse, which lasts 10–20 minutes. **c** | Pairing a single homosynaptic train of activity in the presynaptic sensory neuron with a single pulse of 5-HT to one of the two branches of the bifurcated culture produces a selective increase in synaptic strength of only that branch that now persists for at least 24 hours. So the combination of homosynaptic LTP with a single heterosynaptic stimulus produces more than an additive effect.

The findings that blockade of modulatory neurotransmitters in intact animals and people can interfere with the formation of emotionally charged memories indicate that heterosynaptic plasticity induced by modulatory pathways may provide the attentional and motivational significance for long-term storage of a memory in the brain. Dopamine and noradrenaline neurons, as well as acetylcholine neurons that project widely to other brain areas, have been found to fire in relation to the expectation of reward[134–136]. Activation of these modulatory systems could serve a 'now save' function for long-term memory formation in brain areas that are active at the same time as the rewarding input.

a Homosynaptic activation

b A single heterosynaptic stimulus

c Pairing homosynaptic activation with heterosynaptic modulation (expressed presynaptically)

d Pairing homosynaptic activation with heterosynaptic modulation (expressed postsynaptically)
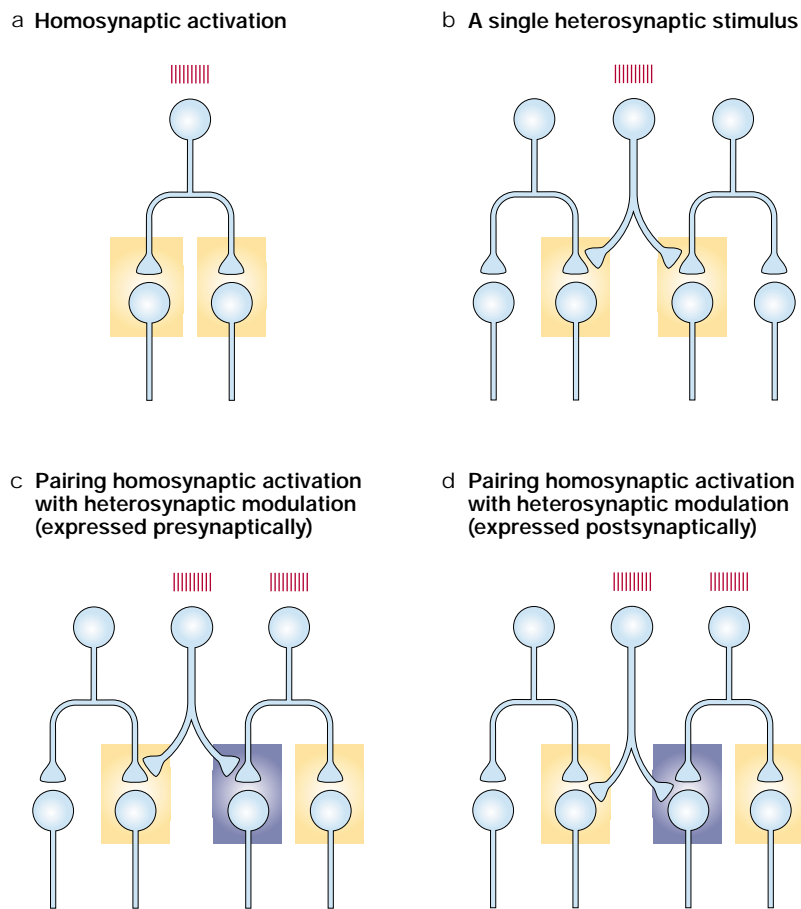
Figure 5 | **Interaction of homo- and heterosynaptic mechanisms sharpens long-term synapse specificity. a** | Although Hebbian plasticity ensures specificity on a given postsynaptic target, the active presynaptic neuron can, in principle, induce a synaptic change on all of its follower cells. **b** | As with homosynaptic activation, in heterosynaptic plasticity there is no obligatory restriction to a single set of synapses because the modulatory neuron can equally affect a number of potential targets. **c**, **d** | When homo- and heterosynaptic mechanisms are paired, the spatial distribution of the combinatorial effect now becomes restricted to their point of overlap (blue shading), resulting in a sharpening of long-term synapse specificity. This enhancement in synapse-specificity can be expressed presynaptically (**c**), postsynaptically (**d**), or both.

The combinatorial power of synaptic rules

We have reviewed recent studies and current thinking on two main rules for learning-related synaptic plasticity — homosynaptic and heterosynaptic plasticity — and have considered the evidence indicating that these forms of synaptic plasticity have different properties and serve different functions[108,111,137]. The homosynaptic Hebbian plasticity mechanism of LTP persists for one or more hours but there is no clear evidence that, when initiated by itself, LTP can persist for longer periods of time at most synapses (FIG. 2). So the Hebbian mechanism may be used primarily for learning and for short-term memory, and it may not be able to recruit the signalling pathways and transcriptional events required for synaptic growth and for the maintenance of stable long-term memory. In contrast, heterosynaptic mechanisms when presented repeatedly can readily and by themselves recruit long-term memory mechanisms that lead to transcription and to the growth of new synaptic connections.

When homosynaptic Hebbian and heterosynaptic modulatory mechanisms are recruited together, as we propose to occur under many experimental and naturally occurring behavioural situations (FIG. 2), the combination can result in new categories of synaptic plasticity that are more than the sum of the individual components (FIG. 3). These combinations have at least two novel properties. First, the combined mechanisms enhance the duration of the plastic change in a non-additive way (FIG. 4). Second, the combined mechanisms sharpen the synapse specificity of the plastic change by restricting the long-term plasticity to a smaller set of synaptic connections than either mechanism alone (FIG. 5). Homosynaptic Hebbian plasticity is not restricted presynaptically, but only in relation to other inputs on a given postsynaptic cell. Any presynaptic neuron that becomes recruited by learning will end on many postsynaptic target cells, any one of which might also be facilitated. With heterosynaptic facilitation, there is no obligatory restriction to a single synapse either, as the modulatory neuron that is recruited by a reinforcing stimulus can affect a number of potential targets. When homo- and heterosynaptic mechanisms are combined, however, the spatial distribution of the combined effects now becomes restricted to their point of overlap, resulting in a greater level of synapse specificity. These examples illustrate the idea that the elementary forms of homo- and heterosynaptic plasticity may form an alphabet that can be combined in various ways to produce new types of plasticity with new properties, expanding the capabilities of the nervous system for encoding information.

The ability in *Aplysia* to stimulate selectively either the homosynaptic glutamate synapses of the intrinsic circuitry, or the 5-HT-mediated modulatory inputs of the heterosynaptic extrinsic circuitry, has allowed the induction of either homosynaptic or heterosynaptic plasticity alone or in combination. This has aided the analysis of each category of synaptic change and the contribution that each makes to the combined, interactive facilitation. We have proposed here that a similar interaction is required in the mammalian brain to cause the stabilization of Hebbian homosynaptic plasticity and the maintenance of long-term memory. To make a rigorous comparison of the relative contributions of homosynaptic Hebbian and heterosynaptic modulatory mechanisms in the mammalian brain, it will be necessary to develop methods for selectively activating homosynaptic and heterosynaptic processes similar to those that have served well in *Aplysia*.

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DATABASE LINKS Apylsia database project
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ENCYCLOPEDIA OF LIFE SCIENCES Long-term potentiation | Molluscan nervous systems | Heterosynaptic modulation of synaptic efficacy | Protein phosphorylation and long-term synaptic plasticity | Learning and memory

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