The cellular basis of classical conditioning in *Aplysia californica* – it's less simple than you think

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Classical conditioning of the withdrawal reflex of the marine snail *Aplysia californica* can be used as an important model system for investigating the neurobiology of associative learning. It results when weak tactile stimulation of the snail's mantle shelf or siphon is repeatedly paired with strong electrical shocks to the animal's tail. This learned behavioral change is thought to be mediated by a presynaptic neuronal mechanism – activity-dependent presynaptic facilitation of the connections between sensory and motor neurons in the CNS of *Aplysia*. Recent evidence suggests, however, that another type of synaptic plasticity – Hebbian potentiation of the sensorimotor connections – might contribute to classical conditioning in *Aplysia*. Additional evidence indicates that this relatively simple form of learning is likely to be mediated by multiple neuronal mechanisms.

SEVERAL YEARS AGO I attended a symposium in which neurobiologists, cognitive psychologists and computer scientists were brought together to evaluate connectionist models of brain function. One of the speakers was a neural-network researcher who discussed the relative advantages of various models of classical conditioning. Among the models the speaker discussed was that for classical conditioning of the defensive gill- and siphon-withdrawal reflex of *Aplysia californica*, simultaneously proposed in 1983 by Hawkins and colleagues[1] and by Walters and Byrne[2]. The speaker argued that there were necessary limitations in the learning capabilities of *Aplysia*, whose neuronal circuitry for classical conditioning comprised, according to the speaker, 'just five neurons'. When it was pointed out during the subsequent question-and-answer period that the CNS of *Aplysia* contains approximately 20 000 neurons and that hundreds, if not thousands, of these neurons are probably active during classical conditioning of *Aplysia*'s gill- and siphon-withdrawal reflex, the speaker expressed surprise: 'But', he protested, 'all the reviews of classical conditioning in *Aplysia* that I've read show only five neurons!'

Evidently the speaker failed to realize that the symbols used to illustrate the neuronal model of classical conditioning in *Aplysia* represent classes of neurons rather than individual neurons. But the point of this anecdote is not to suggest that neural-network theorists should be more concerned with the often messy facts of biology. Rather, it is to preface the argument that will be advanced here, namely that the current cellular model of classical conditioning in *Aplysia* is too simple, although perhaps not for the reasons the neural-network researcher supposed. Classical conditioning of *Aplysia*'s defensive withdrawal reflex is generally believed to be mediated by a single presynaptic cellular mechanism known as activity-dependent

presynaptic facilitation[1] (ADPF) or activity-dependent neuromodulation[2]. This presynaptic form of plasticity is thought to be induced during conditioning at the synapses between central sensory and motor neurons of the defensive withdrawal circuit. However, recent evidence from my laboratory[3,4] suggests that a postsynaptic mechanism, Hebbian[5] potentiation of the sensorimotor synapses, might also mediate classical conditioning of *Aplysia*'s withdrawal reflex. Moreover, data from several laboratories[6–14], including mine (see below), indicate that plasticity at sites other than central monosynaptic sensorimotor connections, including peripheral sites, probably contributes to this learned behavioral change also.

The current cellular model of classical conditioning in *Aplysia*: a presynaptic associative process

Weak tactile stimulation of the siphon or mantle shelf of *Aplysia* (the conditioned stimulus or CS) paired repeatedly with strong electrical shocks applied to the animal's tail (the unconditioned stimulus or US) produces a prolongation of the animal's withdrawal reflex to subsequent presentations of the CS (Refs 15 and 16). This behavioral change represents associative learning because various control training conditions, such as unpaired or randomly paired presentation of the CS and US, or presentation of the US alone, produce significantly less enhancement of the withdrawal reflex than does paired presentation of the CS and US. According to the current cellular model[1,2], paired presentation of the CS and US during conditioning results in ADPF of the sensorimotor connections (Fig. 1). Evidence for this model comes from experiments involving cellular analogs of differential classical conditioning of the withdrawal reflex[16]. These cellular analogs use so-called 'reduced preparations' of *Aplysia*. Two types

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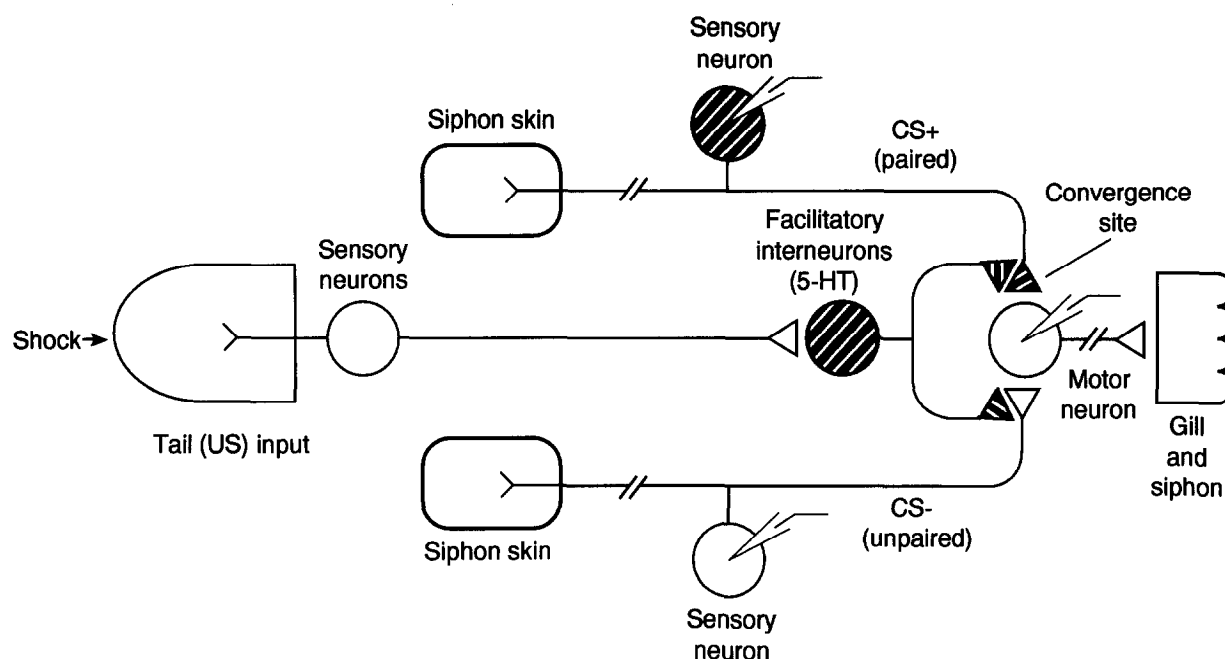
Fig. 1. *Schematic diagram of the basic experimental design of Hawkins and colleagues[1]. The preparation consisted of the* Aplysia *CNS dissected away from all of the animal's body except the tail, to which it was left connected by peripheral nerves. A single siphon (or gill and siphon) motor neuron, together with two siphon sensory neurons monosynaptically connected to the motor neuron, were impaled with intracellular electrodes. One of the sensory neurons was used for CS+ (conditioned stimulus) training which consisted of brief, intracellular activation (five spikes at 10 Hz) of the sensory neuron paired with trains of electrical shocks delivered to the tail (or to peripheral nerves connecting the tail to the CNS). The second sensory neuron was used for CS– training which consisted of intracellular activation of the sensory neuron separated from the unconditioned stimulus (US) by 2.5 min. The figure also illustrates the presynaptic model of classical conditioning[1,2]. According to the model, activity-dependent presynaptic facilitation (ADPF) is induced by the conjoint firing (as indicated by the blue-and-white stripes) of sensory neurons of the CS+ pathway, and the facilitatory interneurons. (At least some of the facilitatory interneurons contain 5-HT[17,18].) The diagram indicates that the US induces non-associative enhancement (presynaptic facilitation) of the CS– sensorimotor connections. Note that the site of associative convergence of the CS and US signals is presumed to be presynaptic – the terminals of the CS+ sensory neurons. In addition, the model assumes that the only cellular effect of the US that is significant for classical conditioning is the activation of the facilitatory interneurons. In reality, tail shocks activate not only facilitatory interneurons, but also a variety of inhibitory and excitatory interneurons[6,11,19,20]. Activation of these other interneurons is also likely to play crucial roles in classical conditioning.*

of preparations were used in the original experiments: (1) the entire CNS, dissected away from all of the animal's body excluding the tail, to which it was left attached by posterior pedal nerves[1]; and (2) a 'split-foot' preparation in which the animal's body, excluding the tail, was split in half, all of the ganglia were removed, excluding the pleural and pedal ganglia on one side, and all the peripheral nerves were transected, excluding a single posterior pedal nerve connecting the tail to the remaining pedal ganglion (see Ref. 21). The experiments assessed the effects of differential conditioning on the strength of monosynaptic connections between mechanosensory and motor neurons in either the abdominal ganglion, whose sensorimotor connections mediate the gill- and siphon-withdrawal reflex[22], or the pleural–pedal ganglia, whose sensorimotor connections mediate the tail-withdrawal reflex[21]. (Although general mechanisms of classical conditioning of *Aplysia*'s withdrawal reflexes have been inferred from experiments on pleural–pedal sensorimotor connections, as yet, there has been only a preliminary report of classical conditioning of tail withdrawal[23].) Brief trains of action potentials elicited in the sensory neurons by intracellular depolarization served as the CS, and electrical shocks delivered either to the tail of the preparation, or to peripheral pedal nerves connecting the tail to the central ganglia, served as the US. Differential conditioning was carried out using two or three different sensory

neurons, which were monosynaptically connected to the same motor neuron. During training, spike activity elicited intracellularly in one of the sensory neurons was paired repeatedly with the US (the CS+ condition), whereas spike activity in another sensory neuron was unpaired with the US (the CS– condition) (Fig. 1). (In some experiments there was a sensitization or US-alone condition in which the sensory neuron was not activated during training.) The main result was that the strength of the synapse between the CS+ sensory neuron and the motor neuron was significantly enhanced following training, whereas the strength of the synapse between the CS– (or US alone) sensory neuron and the motor neuron was not.

What is the evidence that ADPF mediates the enhancement of the CS+-sensorimotor connection? First, tail shock, which produces behavioral sensitization of *Aplysia*'s withdrawal reflexes[21] (W.N. Frost, PhD Thesis, University of Columbia, 1987), also produces presynaptic facilitation of sensorimotor connections in both the abdominal[17] and pleural–pedal[24] ganglia. Therefore, it seems likely that the conditioning protocol would cause presynaptic facilitation of sensorimotor connections also (although see Ref. 25). Second, evidence from additional experiments by Hawkins and colleagues[1] suggest that CS+ and CS– training have different effects on presynaptic transmitter release. In these experiments, the amount of broadening of the

sensory neuron action potential following CS+ was compared with that following CS– training. Both types of training resulted in prolongation of the sensory-neuron action potential, but CS+ training produced significantly greater prolongation than did CS– training. This finding has been taken as support for the idea that one consequence of the CS+ training is enhanced presynaptic facilitation of sensorimotor connections because broadening of the sensory-neuron action potential is associated with, and has been thought to contribute to, presynaptic facilitation of these connections[26–29] (but see Ref. 30 and below). Experiments by Walters and Byrne[31] provide further evidence that the differential conditioning results are a result of different presynaptic effects. They found that tail shock produces slow depolarization of the membrane of sensory neurons in the CS+ condition, whereas sensory neurons in the CS– and US-alone conditions exhibit slow hyperpolarization in response to the US. They suggest that these results reflect differential modulation of a voltage-dependent Ca^{2+} conductance in the sensory neurons. Thus, the prolonged depolarization of the sensory-neuron cell membrane produced by the US in the CS+ condition might result in an additional influx of Ca^{2+} into the sensory neurons; this additional influx of Ca^{2+} might, in turn, contribute to the associative enhancement of the CS+ sensorimotor EPSP. Finally, recent experiments on isolated sensorimotor synapses in cell culture[32] support the theory that the CS+ conditioning produces ADPF of sensorimotor connections. Eliot and colleagues[32] found that pairing tetanic stimulation of a sensory neuron with application of 5-HT, an endogenous facilitatory transmitter which mediates behavioral sensitization and whose release is stimulated by tail shock[17,18], produces significantly greater enhancement of sensorimotor connections *in vitro* than does tetanus alone, 5-HT application alone, or unpaired presentation of the tetanus and 5-HT.

How does paired training result in greater enhancement of the sensorimotor EPSP than does unpaired training? The basis of ADPF is thought to be the amplification of production of cAMP in the sensory neurons as a result of sensory-neuron activity[33,34]. Sensitizing stimuli, such as tail shock, have been shown to increase concentrations of cAMP in *Aplysia* sensory neurons[35–37]; this increase, in turn, contributes to presynaptic facilitation of the sensorimotor synapse[38,39]. It is hypothesized that during paired training, the influx of Ca^{2+} into the sensory neuron (as a result of the CS) just before the onset of the US causes an enhanced activation of adenylate cyclase via Ca^{2+} and calmodulin[33,34]. This hypothesis is supported by the finding that exposure of *Aplysia* sensory neurons to a biochemical analogue of classical conditioning, high-K^+ artificial seawater (which depolarizes the neurons) paired with 5-HT, produces greater enhancement of cAMP in the sensory neurons than does unpaired treatment with high-K^+ seawater and 5-HT (Ref. 36). Furthermore, an adenylate cyclase has been identified in the CNS of *Aplysia* that is activated dually by Ca^{2+} and calmodulin and by 5-HT (Ref. 40).

Despite evidence that suggests a role for ADPF in classical conditioning of *Aplysia*'s withdrawal reflex, there is still no direct experimental link between ADPF and classical conditioning in the behaving animal. For example, it has not been demonstrated that ADPF of sensorimotor connections in the CNS of *Aplysia* occurs during behavioral classical conditioning. Nor has it been demonstrated that disrupting ADPF of sensorimotor connections (for example, by depleting 5-HT in the *Aplysia* nervous system[17]) interferes with either behavioral conditioning of the withdrawal reflex or the associative enhancement of the sensorimotor EPSP that is observed in the cellular conditioning analogue. Furthermore, data from experiments by Colebrook and Lukowiak[25] raise questions about the original model of classical conditioning in *Aplysia*. Using reduced preparations of *Aplysia*, they compared quantitatively the enhancement of the gill-withdrawal reflex, following classical conditioning training, to the facilitation of the CS (siphon tap)-elicited EPSP in identified central gill motor neurons. The comparisons between alterations in the reflex and in the EPSP were made in the same preparations. Colebrook and Lukowiak found that although the mean size of the reflex and that of the complex EPSP in the conditioned group were both enhanced 30 min following classical conditioning training, the two phenomena were dissociative in certain respects. Thus, less than half (10 out of 22) of the preparations that received paired CS–US (tail shock) training exhibited both significant facilitation of the EPSP and enhancement of the reflex. In another seven preparations, Colebrook and Lukowiak observed facilitation of the synaptic response in gill motor neurons without an increase in the reflex; conversely, in one preparation they observed significant enhancement of the reflex but no facilitation of the EPSP. Moreover, in those preparations that exhibited both facilitation of the EPSP and enhancement of the reflex after associative conditioning, the physiological and behavioral increases were disjunct temporally: facilitation of the EPSP was apparent during training, whereas the enhancement of the reflex did not appear until 30 min after the last training trial. These observations imply that while strengthening of central sensorimotor synapses might contribute to classical conditioning of *Aplysia*'s withdrawal reflex, this form of associative learning must be mediated by other physiological mechanisms also. Finally, the original evidence that supports the hypothesis that the strengthening of sensorimotor synapses produced during classical conditioning results from ADPF is also somewhat problematic. Although Hawkins and colleagues[1] found that a paired conditioning protocol produced significantly greater broadening of the sensory-neuron action potential than did an unpaired protocol, recent data from experiments on *Aplysia* sensorimotor synapses in cell culture[30] indicate that such broadening might contribute little to facilitation of sensorimotor synapses.

Possible involvement of a postsynaptic mechanism in classical conditioning in *Aplysia*

Recent experiments by Xiang Y. Lin and myself[3,4] suggest that the strengthening of central sensorimotor synapses observed during cellular analogues of classical conditioning of *Aplysia*'s withdrawal reflex[1,2] might involve another type of synaptic plasticity. It has been found that sensorimotor

Fig. 2. (Right.) *Hebbian induction of LTP of Aplysia sensorimotor synapses and its blockade by the NMDA-receptor antagonist APV.* (A) *Sample EPSPs to test stimuli from experiments of long-term potentiation (LTP) using isolated sensorimotor synapses in cell culture. Each synapse was composed of one or two pleural sensory neurons co-cultured with a single small siphon (LFS) motor neuron (see Ref. 3 for details). During the experiments, a presynaptic sensory neuron was stimulated once every 10 min with an extracellular electrode, and the resulting EPSPs were recorded in the motor neuron with an intracellular electrode. There was a total of ten test trials. After the second (0 min) test trial some synapses received experimental stimulation. Shown here are test EPSPs from experiments in which synapses received: only the test stimuli (test alone); a single bout of 25 Hz presynaptic stimulation paired with strong postsynaptic depolarization (single pairing); a single bout of 25 Hz presynaptic stimulation alone (single tetanus); or a single bout of presynaptic stimulation paired with postsynaptic depolarization in the presence of the N-methyl-D-aspartate (NMDA) antagonist D,L-2-amino-5-phosphonovalerate (APV; 50 μM) (single pairing + APV). The test-alone EPSPs exhibit the normal homosynaptic depression characteristic of Aplysia sensorimotor synapses[41]. (B) Group data from the LTP experiments. For each experiment, the EPSP values have been normalized to the size of the EPSP on the 0-min trial. Each point represents the group mean ± SEM. A single pairing of presynaptic activity and postsynaptic depolarization resulted in prolonged enhancement of the EPSP relative to the size of the EPSP for synapses receiving only the test stimuli. Thus, the mean single-pairing EPSP was significantly larger than the corresponding test-alone EPSP for each test trial from 10–80 min. By contrast, a single bout of presynaptic stimulation alone produced only short-term synaptic enhancement (single-tetanus EPSPs), as did a single pairing of pre- and postsynaptic stimulation in the presence of APV (single pairing + APV EPSPs). The arrow indicates the occurrence of pairing and presynaptic stimulation. Reproduced, with permission, from Ref. 4.*
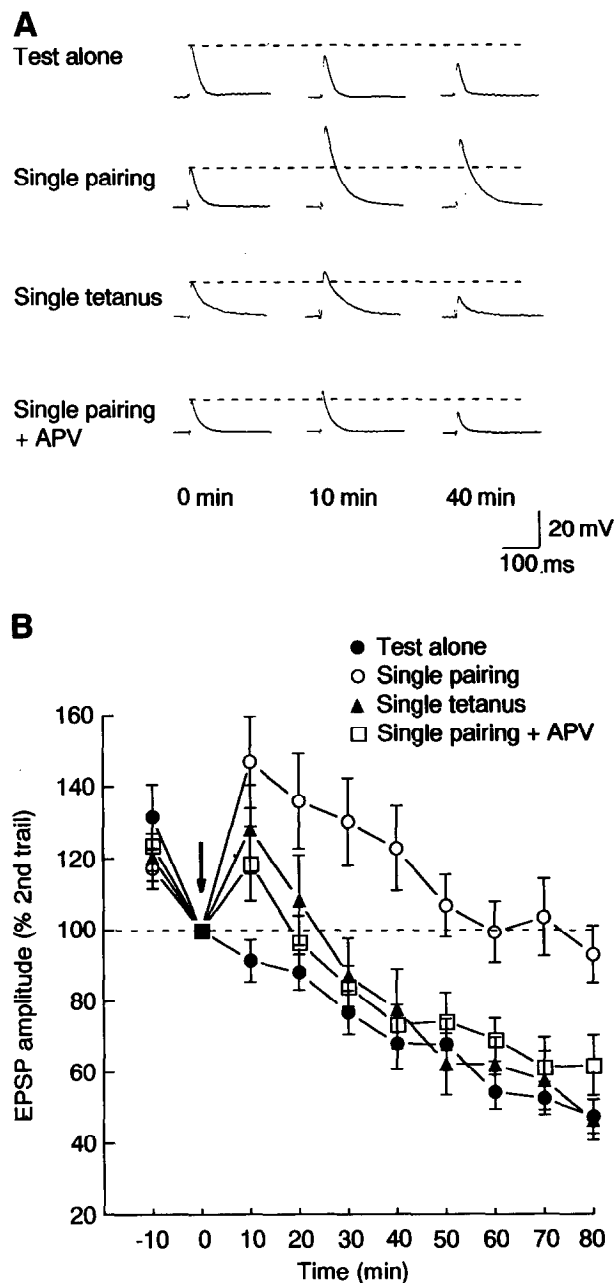
synapses of *Aplysia* in primary cell culture exhibit a form of long-term potentiation (LTP) whose induction appears to be regulated by the voltage of the postsynaptic motor neuron. Thus, the induction of this form of LTP can be blocked by strong hyperpolarization of the motor neuron[3]. Furthermore, LTP of sensorimotor synapses *in vitro* can be induced by pairing a single, brief bout of high-frequency stimulation of the presynaptic sensory neuron, which, by itself, is insufficient to induce LTP, with strong depolarization of the motor neuron[4] (Fig. 2). These findings resemble those reported previously for LTP of synapses in the CA1 region of the mammalian hippocampus[42-44]. Moreover, as is the case for LTP of CA1 synapses[45,46], induction of LTP of *Aplysia* sensorimotor synapses can be blocked by the specific N-methyl-D-aspartate (NMDA)-receptor antagonist D,L-2-amino-5-phosphonovalerate (APV) (Fig. 2), or by the presence of a chelator of intracellular Ca^{2+} in the postsynaptic neuron[4]. The results from my laboratory implicate a postsynaptic NMDA, or NMDA-related, receptor in the induction of LTP of *Aplysia* sensorimotor synapses (see Ref. 47). Pharmacological studies[48,49] of neurotransmission at sensorimotor synapses provide additional support for the involvement of an NMDA-related postsynaptic receptor in LTP in *Aplysia*. These studies indicate that the sensory-neuron transmitter might be glutamate or another excitatory amino acid. Furthermore, voltage-clamp data show that the excitatory postsynaptic current in the motor neurons has a nonlinear current–voltage relation with a plateau region between −40 mV and −70 mV; this plateau region is a result of voltage-dependent blockade of the receptor-

channel by Mg^{2+} (Ref. 48). *Aplysia* siphon motor neurons, therefore, appear to possess a postsynaptic receptor that is similar in some respects to the vertebrate NMDA receptor[47].

Induction of LTP of *Aplysia* sensorimotor synapses in cell culture appears to require coincident presynaptic activation and postsynaptic depolarization, conditions reminiscent of the neurophysiological rule for learning proposed by Hebb[5]. Does Hebbian modulation of central sensorimotor synapses play a role in learning in *Aplysia*? A potentially important clue to this question's answer is the observation that tail shock not only activates facilitatory interneurons within the CNS of *Aplysia*[18], but also strongly depolarizes many siphon and tail motor neurons[6,24]. Thus, the paired presentation of the CS and US during classical conditioning of *Aplysia*'s withdrawal would be expected to result in moderate firing of sensory neurons together with strong depolarization of the motor neurons of the withdrawal circuit – a pattern of neuronal activity similar to that which induces LTP of sensorimotor synapses *in vitro*[4].
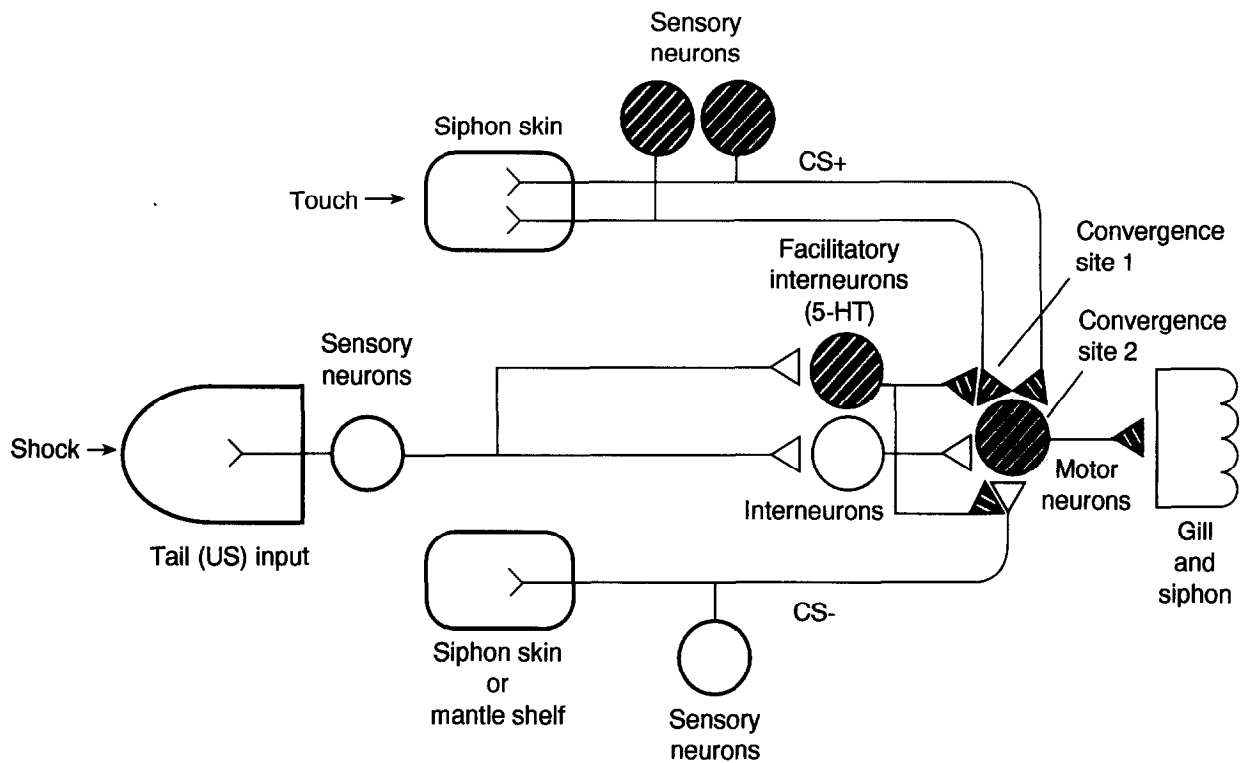
Fig. 3. *Neuronal model for differential classical conditioning of* Aplysia's *defensive withdrawal reflex[16] which incorporates Hebbian potentiation of* Aplysia *sensorimotor connections[4]. The model only specifies changes in the monosynaptic component of the reflex and is therefore almost certainly incomplete. Classical conditioning of* Aplysia *probably involves changes in polysynaptic and in peripheral components of the reflex also (see Refs 6–14 and 25). According to the model, classical conditioning induces two types of associative enhancement of the monosynaptic connections. The presynaptic associative component, activity-dependent presynaptic facilitation[1,2] (ADPF), is indicated in blue; the postsynaptic associative component, Hebbian potentiation[4], is indicated in red. The stripes indicate which two neurons must be coactive in order to induce each associative synaptic change. Hebbian potentiation is presumed to be induced by the conjoint firing of sensory neurons of the CS+ pathway, and the firing, or strong depolarization, of the motor neurons for the reflex produced by the unconditioned stimulus (US; see Ref. 6). The site of convergence of the CS and US signals for Hebbian potentiation is the dendrites of the motor neurons. For the sake of clarity, ADPF and Hebbian potentiation are depicted in the model as operating at separate sensorimotor synapses; however, it is possible that both types of associative processes occur at the same synapses. Abbreviation: CS, conditioned stimulus. Adapted from Ref. 51.*

The hypothesis that a Hebbian mechanism might mediate classical conditioning in *Aplysia* has been tested previously and rejected[50]. However, this hypothesis was possibly rejected prematurely. A potential source of uncertainty in this previous study is whether the intrasomally injected current used for the tests of Hebb's postulate sufficiently polarized the cell membrane in dendrites of the motor neurons (for further discussion, see Ref. 51).

Arguments for a multiprocess model of classical conditioning

Under certain circumstances, classical conditioning of siphon withdrawal can result in a change in the form of the reflex as well as in its prolongation[52,53]. As has been pointed out previously[52,53], ADPF cannot account for such 'response specificity' of the conditioned reflex if the ADPF is assumed to be cell-wide; in other words, if all of the synaptic connections made by a given sensory neuron are facilitated equally. Other non-Hebbian mechanisms, among them branch-specific facilitation[52] and concatenation of sensory- and motor-neuron facilitation[53], have been appended to the basic model in an attempt to explain response specificity. However, these hypothetical mechanisms are somewhat problematic. For example, if response specificity of *Aplysia*'s conditioned withdrawal reflex was mediated by branch-specific facilitation, then specialized facilitatory interneurons would be

required for each learned response. But, although different identified facilitatory interneurons in the CNS of *Aplysia* do exhibit some variety in their receptive fields and stimulus selectivity, their responses also exhibit considerable overlap with respect to these properties[18,54]. A major advantage of Hebbian plast-icity as a mechanism of associative learning is that it provides a parsimonious solution to the problem of response specificity. I suggest that classical conditioning of *Aplysia*'s defensive withdrawal reflex can best be explained by a model in which the monosynaptic connections between the sensory and motor neurons are enhanced by two modulatory processes: ADPF and Hebbian potentiation (Fig. 3).

Experiments on the shortening reflex of the leech provide general support for such a model of invertebrate classical conditioning. Sahley and her colleagues have shown that depletion of 5-HT within the leech nervous system by the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) disrupts sensitization and dishabituation of the shortening reflex[55]. (This effect of depletion of 5-HT on non-associative learning in the leech is similar to that obtained in analogous experiments with *Aplysia*[17].) Interestingly, although 5,7-DHT completely eliminates sensitization of the leech shortening reflex, Sahley[56] has recently found that it only partially blocks classical conditioning of this reflex. This result implies that classical conditioning in the leech involves both

5-HT-dependent mechanisms, such as ADPF, and 5-HT-independent mechanisms, such as Hebbian potentiation.

The potential role of polysynaptic pathways in classical conditioning

The *terra incognita* of our current knowledge about the cellular basis of classical conditioning in *Aplysia* is the polysynaptic component of the withdrawal reflexes. However, work on the sensitization of gill and siphon withdrawal[6,8–10,12,13] and of tail withdrawal[11,14] in recent years has begun to reveal that plasticity at polysynaptic, in addition to monosynaptic, central sites, as well as plasticity at peripheral sites, contribute importantly to this type of non-associative learning (see also Ref. 7). The cellular basis of associative learning in *Aplysia* is unlikely to prove less complex than that for non-associative learning. Indeed, evidence from experiments in my laboratory indicates that changes in the polysynaptic pathway between the siphon sensory and motor neurons might play a more significant role in classical conditioning of withdrawal than thought previously. These experiments involved a cellular analogue of classical conditioning of siphon withdrawal similar to that of Hawkins and colleagues (Fig. 1). Geoffrey Murphy and I have found that whereas before CS+ training a single action potential elicited in a sensory neuron can elicit an apparently monosynaptic EPSP in a siphon motor neuron, after training a single sensory neuron action potential frequently elicits an apparently polysynaptic EPSP in the motor neuron (G.G. Murphy and D.L. Glanzman, unpublished observations). It therefore appears that one consequence of classical conditioning is the recruitment by the CS of interneuronal input to the motor neurons. This might result from changes at synapses between the sensory neurons and interneurons, at synapses between interneurons and motor neurons, or at synapses between different classes of interneurons (for example, see Ref. 57).

Why study invertebrate learning?

Some researchers might question the value of persisting with work on the cellular basis of learning and memory in invertebrates believing, on the one hand, that the major intellectual problems in invertebrate learning have been solved and, on the other hand, that with the advent of modern cellular, computational, and molecular techniques, we will soon understand the specific neuronal changes that mediate various forms of vertebrate learning. However, the data reviewed here suggest that we are far from having achieved a complete understanding of one prominent example of invertebrate learning – classical conditioning of *Aplysia*'s defensive withdrawal reflex. Indeed, our knowledge about this relatively simple form of learning might just scratch the surface of its neurobiological complexity. If so, then one wonders just how long it will be before we have a realistic cellular model of one of the intensively studied forms of mammalian associative learning, for example, spatial learning[58]. Regardless, cellular work on invertebrate learning is likely to continue to make important contributions to a general neurobiological understanding of learning and memory. This is because currently it is only in the nervous systems of certain invertebrates that whether changes at specific synapses actually contribute to the expression of a specific learned behavior can be tested rigorously (see Ref. 59). This situation is unlikely to change in the foreseeable future.

Note added in proof

Geoffrey Murphy and David Glanzman have found recently that infusing the postsynaptic motor neuron with the Ca^{2+} chelator BAPTA blocks the cellular analogue of classical conditioning of the withdrawal reflex[60]. This is the first direct experimental evidence that a postsynaptic mechanism plays a critical role in classical conditioning in *Aplysia*.

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Compensatory plasticity and sensory substitution in the cerebral cortex

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Cats deprived visually from birth show few overt impairments in their natural behavior. Therefore, they seem well suited as an animal model for the study of compensatory plasticity after early vision loss. It can be demonstrated that binocularly deprived cats show improved abilities of auditory localization, and at least equal tactile behavior compared to normal controls. Within the anterior ectosylvian cortex of binocularly deprived cats, where different sensory modalities come together, the anterior ectosylvian visual area is completely taken over by auditory and somatosensory inputs. Furthermore, the auditory spatial tuning of single units in this cortical region is sharpened significantly as a result of visual deprivation. Somatosensory compensation for early loss of vision can be demonstrated by a hypertrophy of the facial vibrissae, and a corresponding expansion of their central representation in the somatosensory cortex of binocularly deprived animals. The compensatory changes in the cortex can be explained by a reorganization of sensory representations under the guidance of sensorimotor feedback rather than by instruction through an extraneous 'supervisory' signal. These processes might form the neural basis of sensory substitution in blind humans.

D O BLIND PEOPLE develop capacities of their remaining senses that exceed those of sighted individuals? This has been a question of debate for a long time[1]. Anecdotal evidence in favor of this hypothesis abounds. There are many examples of brilliant, blind musicians, including Louis Braille himself who, blinded at the age of three, later developed a system for reading and writing using tactile cues. Obviously, this system was based on the assumption that the blind have heightened sensitivity in their finger tips. A number of systematic studies have provided experimental evidence for compensatory plasticity in blind humans[2–7].

By contrast, empiricist scholars have argued often that blind individuals should have perceptual and learning disabilities in their other senses also, because vision is needed to 'instruct' them[8–10]. Without vision, the argument goes, neither a sense of space nor real knowledge of gestalt can be developed. Auditory space per se, it is asserted, does not exist, but has to be calibrated by vision, and visual-

ization is needed for auditory- or tactile-form perception. This hypothesis receives support from an almost equal number of studies as the other hypothesis[11–13].

Thus, the question of whether intermodal plasticity exists has remained one of the most vexing problems in cognitive neuroscience[14–16]. One approach to solving the puzzle is to reduce it to the neural level, and develop an animal model. This would then enable the neural mechanisms underlying possible structural and functional changes in compensatory plasticity to be elucidated. An understanding of the neural mechanisms is also a necessary requirement for possible treatment, including the development of effective neural prostheses.

An animal model for human blindness, which has been used in neurobiological studies first by Wiesel and Hubel[17], is the binocularly lid-sutured cat. While some diffuse light can still reach the retina through the closed lids, all pattern vision is prevented, and the animals can, in effect, be regarded 'blind'. Lid suture can have physiological consequences that are

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