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Multiple Forms of Non-Associative Plasticity in Aplysia: A Behavioural, Cellular and Pharmacological Analysis

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Multiple forms of non-associative plasticity in *Aplysia*: a behavioural, cellular and pharmacological analysis

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SUMMARY

A complete understanding of the cellular mechanisms underlying the formation of associations between stimuli, as occurs during classical conditioning, requires an understanding of the non-associative effects of the individual stimuli. The siphon withdrawal reflex of *Aplysia* exhibits both non-associative and associative learning when a tactile stimulus to the siphon serves as a conditioned stimulus, and tail shock serves as an unconditioned stimulus. In this chapter we describe experiments which examine the non-associative effects of tail shock at three different levels of analysis.

At a behavioural level we found that the magnitude, and even the sign of reflex modulation induced by tail shock depended critically on three parameters: (i) the state of the reflex (habituated or non-habituated); (ii) the strength of the tail shock, and (iii) the time of testing after tail shock. Specifically, when non-habituated responses produced by water jet stimuli to the siphon were examined, tail shock produced transient inhibition 90 s later; facilitation of non-habituated responses (sensitization) only emerged after a considerable delay of 20–30 min. When habituated responses were examined, tail shock produced immediate facilitation (dishabituation); the amount of facilitation was inversely related to the strength of tail shock, with stronger shock producing no dishabituation.

At a cellular level it was found that the complex excitatory postsynaptic potential (EPSP) in siphon motor neurons produced by water jet stimuli to the siphon provides a reliable cellular correlate of several of the non-associative effects of tail shock that we observe behaviourally. When non-decremented complex EPSPs were examined, strong tail shock produced transient inhibition at a test 90 s after shock. When decremented complex EPSPs were examined, weak tail shock produced immediate facilitation whereas strong shock produced no facilitation. Moreover, in these experiments tail shock had differential effects on the complex and monosynaptic inputs to siphon motor neurons, suggesting that in addition to the well-studied monosynaptic input, other elements in the neural circuit for siphon withdrawal may contribute to the modulation induced by tail shock.

At a pharmacological level we found that the neuromodulator serotonin could reliably mimic some of the effects of tail shock. Specifically, brief application of serotonin produced transient inhibition of both the siphon withdrawal reflex and of nerve shock elicited complex EPSPs in siphon motor neurons. Interestingly, serotonin simultaneously produced facilitation of the monosynaptic connection from sensory to motor neurons. This dissociation in the effects of serotonin on complex and monosynaptic EPSPs suggests that serotonin may act at multiple synaptic loci to produce the net inhibition in complex synaptic input.

Taken collectively, these results suggest that the diverse behavioural effects of tail shock may be mediated by modulation at multiple sites in the neural circuit for siphon withdrawal. Understanding the cellular mechanisms that underlie these diverse non-associative effects of tail shock will be important in formulating comprehensive cellular models of associative learning in this reflex system.

1. INTRODUCTION

Learning theorists have often divided learning into two discrete classes, associative and non-associative (Mackintosh 1974; Rescorla 1988). The former refers to the formation of associations either between stimuli or between stimuli and responses, based on contingent relationships between the events, while the latter refers to behavioural change induced in a non-contingent manner, usually by the action of a single stimulus. However, recent evidence from a variety of systems suggests that there may be a mechanistic relationship between these two classes of learning. For example, in the analysis of long-term potentiation in the mammalian hippocampus (a leading candidate mechanism for information storage in the vertebrate brain (see Morris and Thompson, this symposium), it is clear that the same mechanism that gives rise to an increase in the efficacy of a single stimulus (a cellular analogue of non-associative learning) can also be modified to increase

the efficacy of temporally associated stimuli (a cellular analogue of associative learning; see, for example Brown *et al.* (1990)). A similar relationship between non-associative and associative learning has been elucidated by work in the marine mollusc *Aplysia*. This work shows that the formation of an association between a conditioned stimulus (CS) and an unconditioned stimulus (US) may be explained, at least in part, by an activity-dependent enhancement of the non-associative effects of the US (Hawkins *et al.* 1983; Walters & Byrne, 1983). Finally, from a theoretical perspective, many theories of associative learning have incorporated the non-associative effects of the CS and US as a requisite for successful modelling of the complex features of associative learning (Solomon & Corbit 1974; Wagner 1981; Mackintosh 1983). Thus from both experimental and theoretical perspectives, it is now clear that a complete understanding of the cellular mechanisms mediating the formation of associations will ultimately require a detailed mechanistic analysis of the non-associative effects of the individual stimuli. To this end, we have carried out behavioural and cellular analyses of non-associative learning in the defensive siphon withdrawal reflex of *Aplysia*.

The siphon withdrawal reflex in *Aplysia* offers several advantages for an analysis of the mechanisms mediating non-associative learning (Carew & Sahley 1986; Byrne 1987; Hawkins *et al.* 1987). First, at a behavioural level the reflex has been shown to exhibit several types of non-associative learning, including habituation, dishabituation and sensitization. Second, at the neuronal level, the behaviour is mediated by a relatively simple neural circuit, thus limiting the number of potential sites of modulation that might underlie the behavioural plasticity. Finally, the large size and identifiability of many of the neurons in the *Aplysia* central nervous system allow detailed subcellular analysis of the biophysical, biochemical and molecular events that mediate changes in synaptic efficacy.

In this chapter we will discuss recent experiments which we have carried out to further the analysis of non-associative learning in the siphon withdrawal reflex at three different levels. First, at a behavioural level we have characterized the effects of tail shock (a highly effective US in classical conditioning of siphon withdrawal) on reflex responding (produced by water jet stimuli to the siphon) in the intact, freely behaving animal, and have identified several novel forms of behavioural plasticity. Second, at a cellular level we have begun to identify sites of synaptic plasticity contributing to these different forms of reflex modulation by examining complex EPSPs elicited in siphon motor neurons by water jet stimuli to the siphon. Finally, at a pharmacological level we have begun to examine the potential role of modulatory neurotransmitters by asking whether their effects mimic the effects of tail shock on both reflex responding and on complex EPSPs in siphon motor neurons.

Three forms of non-associative learning, habituation, dishabituation and sensitization, are schematically illustrated in figure 1. Habituation is defined as a decrease in reflex response amplitude produced by repeated presentations of the eliciting stimulus. Dishabituation and sensitization both refer to a facilitation of response amplitude produced by the presentation of a strong or noxious stimulus. Dishabituation and sensitization differ, however, with respect to the state of the reflex before the facilitatory stimulus. Specifically, dishabituation refers to the facilitation of a *habituated* reflex response whereas sensitization refers to the facilitation of a *non-habituated* reflex response (a non-habituated baseline is typically established by delivering relatively few stimuli at a long inter-stimulus interval (ISI)).

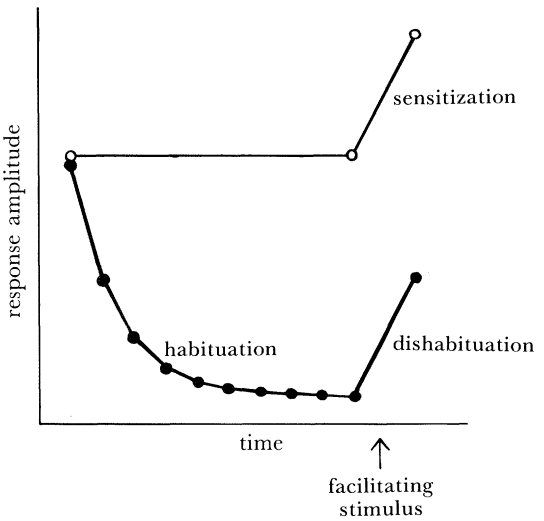

Figure 1. Schematic representation of three types of non-associative learning. Facilitation of a non-decremented reflex response (open circles) by presentation of a different stimulus (arrow) is referred to as sensitization. When the reflex is habituated (filled circles) by repeated presentation of the eliciting stimulus, the facilitation resulting from the modulatory stimulus is referred to as dishabituation.

2. BEHAVIOURAL ANALYSIS

We began our analysis of non-associative learning with a systematic investigation of the effects of tail shock on the siphon withdrawal reflex in the intact animal. Siphon withdrawal was elicited by delivering a jet of sea water of constant amplitude and duration to the siphon; the measure of reflex amplitude was the duration of siphon contraction. The modulatory stimulus consisted of electric shock to the tail, ranging in intensity from a single mild shock to a train of four strong shocks. Two behavioural paradigms were used, one to assess dishabituation and one to assess sensitization. In the dishabituation protocol, the siphon withdrawal reflex was first habituated by delivering 20 water jet stimuli to the siphon at a 30-second ISI; we then delivered a shock to the tail. Finally, we assessed the effects of tail shock by measuring the withdrawal response to the water jet 90 s after the tail shock and comparing it to the habituated baseline. The sensitization protocol was identical to the dishabituation protocol except that:

(i) the reflex was not habituated prior to the tail shock; rather it was tested by the water jet stimuli twice

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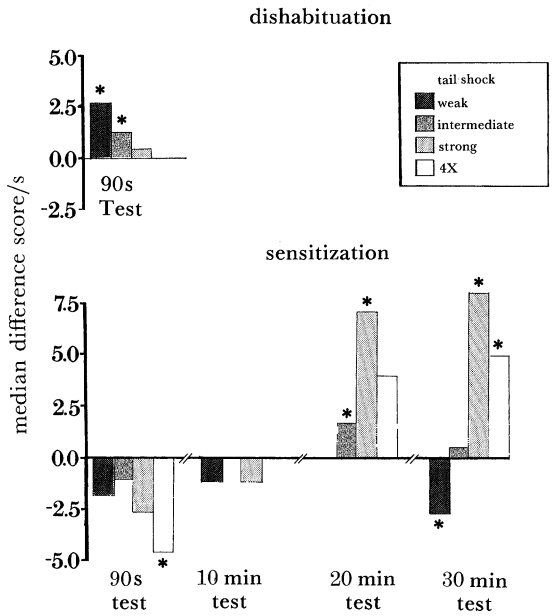

Figure 2. Effects of tail shock on the siphon withdrawal reflex of intact *Aplysia*. (*a*) Significant dishabituation (facilitation of decremented reflex responses) is produced at the 90 s test by weak and intermediate tail shock intensities. However, dishabituation is not produced by strong or 4X. (*b*) Non-habituated responses are not facilitated by any tail shock intensity at the 90 s test. In fact, the strongest intensity (4X) produces significant inhibition. Sensitization (facilitation of non-decremented reflex responses) is only observed after a considerable delay (20 and 30 min tests) and is produced only by stronger tail shock intensities. Data are expressed as difference score (post-shock test minus pre-shock baseline). Asterisks indicate statistical significance. The tail shock intensities used are indicated in the inset. (4X: a train of four shocks). Data from Marcus *et al.* (1988).

at a relatively long (10 min) ISI to establish baseline responding, and;

(ii) in addition to the 90-second post-shock test, the effects of tail shock were also assessed at 10, 20 and 30 min.

We found that the magnitude and even the sign of reflex modulation induced by tail shock were critically dependent on three parameters: (i) the state of the reflex (habituated or non-habituated); (ii) the strength of the tail shock, and (iii) the time of testing after tail shock (Marcus *et al.* 1988; figure 2). Specifically, when the reflex was previously habituated, tail shock produced immediate facilitation (dishabituation), measured 90 s after tail shock. Interestingly, the amount of facilitation was inversely proportional to the strength of the tail stimulus, with progressively stronger intensities producing progressively less dishabituation (figure 2*a*). In contrast, when the reflex was not habituated, tail shock produced inhibition of reflex amplitude at 90 s (figure 2*b*; similar tail shock induced inhibition of reflex responding has also recently been observed by Mackey *et al.* (1987) and Krontiris-Litowitz *et al.* (1987)).

Non-habituated reflexes were facilitated above baseline levels (i.e. sensitization was apparent) only after a delay of 20–30 min, a delay period considerably longer than had been previously described. Moreover, this delayed sensitization was not seen in response to weak stimuli, but only to strong stimuli. Thus, whereas facilitation of decremented responses has a rapid onset and is best produced by weaker modulatory stimuli, facilitation of non-decremented responses has a delayed onset and is best produced by stronger modulatory stimuli.

The diverse nature of the behavioural plasticity induced by tail shock has potentially important implications for the effects of tail shock as a US in classical conditioning paradigms. For example, studies of classical conditioning of the siphon and gill withdrawal reflex have often used tail or head shock as the US; our behavioural data suggest that the effectiveness of such a stimulus as a US may critically depend upon a number of variables, including the initial state of the reflex (habituated or non-habituated) and the magnitude of US intensity (see discussion).

3. CELLULAR ANALYSIS

The complex nature of the effects of tail shock on reflex responding suggests that these effects may be mediated by multiple and perhaps interactive neuromodulatory mechanisms. To begin to examine the cellular basis of this diverse behavioural plasticity, we explored the effects of tail shock on synaptic input to central motor neurons known to contribute to the siphon withdrawal reflex. The preparation we used is shown in figure 3. It consisted of the mantle organs (siphon, gill and mantle shelf) and the tail, left attached by peripheral nerves to the intact central nervous system. This preparation had three principal advantages:

(i) it allowed us to use the same stimuli that were

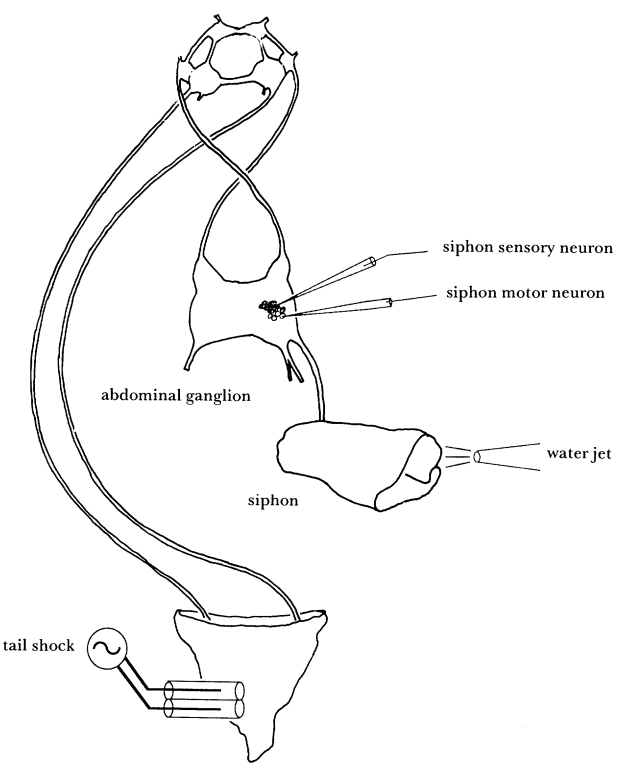

Figure 3. A reduced preparation used to analyse the neural correlates of tail shock induced reflex modulation.

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used in the behavioural experiments (water jet stimuli delivered to the siphon and electric shock to the tail);

(ii) it exhibited a siphon withdrawal reflex response, both to water jet stimuli and to tail shock, that was similar in form to the reflex in the intact animal, and

(iii) it permitted us to record intracellularly from identified siphon sensory and motor neurons at the same time that different forms of behavioural plasticity were induced by tail shock.

Several of the forms of tail shock induced reflex modulation which had been observed in the intact animal were reproduced in the behaviour of the reduced preparation (figure 4*a*). Specifically, when the reflex was not previously habituated, strong tail shock produced inhibition of reflex responding 90 s after the shock (figure 4*a*). This inhibition paralleled the onset and timecourse of the inhibition seen in the intact animal, recovering 10 min later. Moreover, when the reflex was first habituated by 20 repeated water jet stimuli, weak tail shock produced facilitation (dishabituation), while strong tail shock produced no facilitation. Thus the reduced preparation reflects two key aspects of tail shock induced modulation seen in the intact animal:

(i) the transient inhibition of non-habituated reflexes, and

(ii) the inverse relation of strength of tail shock and degree of facilitation of decremented reflexes. However, there was no evidence of delayed sensitization in this preparation (we are currently examining the factors that may contribute to the delayed onset of facilitation in this reflex).

Reasoning that the plasticity observed in the behaviour ought to be reflected in changes in the net synaptic input from sensory neurons, as well as interneurons, onto motor siphon neurons, we next used this preparation to examine the effects of tail shock on water jet elicited complex EPSPs in siphon motor neurons. We also examined one of the identified components of the siphon withdrawal circuit that is known to contribute to behavioural modification of this reflex, the monosynaptic connection between identified siphon sensory neurons, the LE neurons and siphon motor neurons.

As in the behavioural experiments, we used two stimulus protocols to examine the effect of tail shock on both non-decremented and decremented EPSPs. These protocols are the analogues of sensitization and dishabituation training, respectively. In examining non-decremented responses, we found that the complex EPSP in the motor neuron (elicited by a water jet stimulus to the siphon) was strongly inhibited 90 s after tail shock, and recovered 10 min later (figure 4*b*). In the same preparations, however, the direct monosynaptic connection to the same motor neuron was not inhibited by tail shock, but instead tended to be facilitated (Wright *et al.* 1988, 1989) (figure 4*c*).

In examining decremented responses, we found that

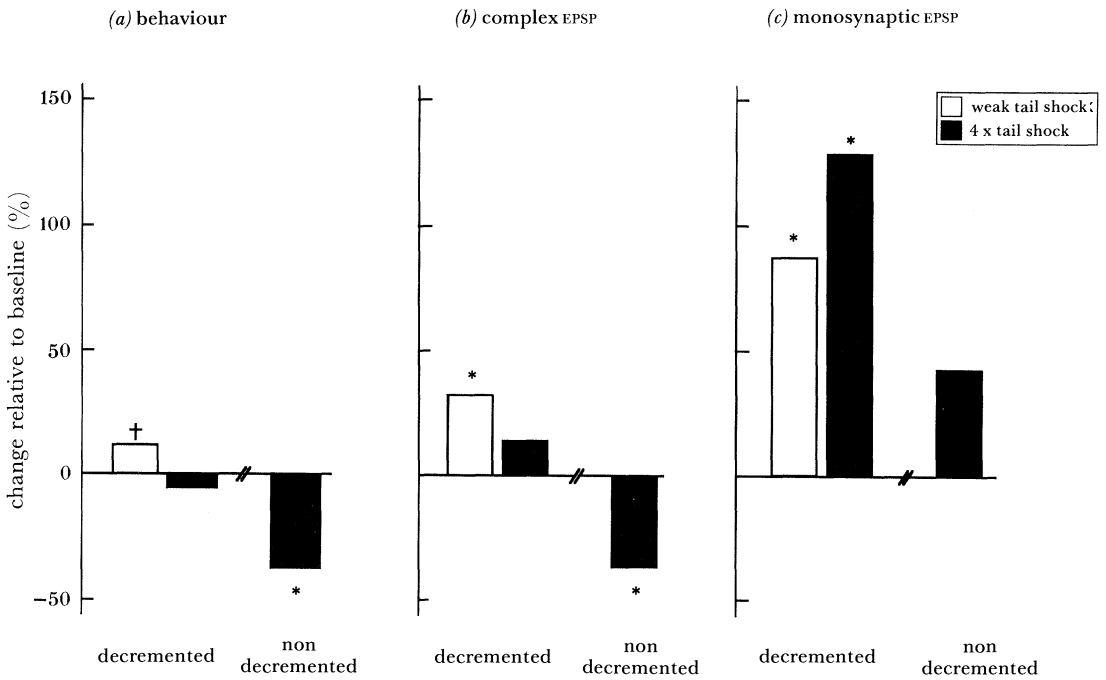

Figure 4. Effects of tail shock on siphon withdrawal and EPSPs in the reduced preparation. (*a*) Duration of siphon withdrawal reflex response. Weak shock (open bars) produces facilitation of decremented responses, while strong shock (filled bars) produces no facilitation. When the response is non-decremented, strong shock produces reflex inhibition. (*b*) Amplitude of complex EPSP elicited by water jet stimulation to the siphon. Consistent with the behaviour, weak shock produces significant facilitation of decremented EPSPs, while strong shock produces no significant facilitation. Also consistent with the behaviour, strong shock produces inhibition of non-decremented EPSPs. (*c*) Amplitude of monosynaptic EPSP elicited by intracellular stimulation of a sensory neuron. As in the habituated reflex and the decremented complex EPSP, the decremented monosynaptic EPSP is facilitated by weak tail shock. In contrast to the behaviour and the complex EPSP, (i) the decremented monosynaptic EPSP is facilitated after strong tail shock, and (ii) the non-decremented monosynaptic EPSP is not inhibited by strong tail shock. Data are expressed as mean percent change from baseline. Cross indicates $p < 0.05$ 1-tailed. Asterisks indicate at least $p < 0.05$, two-tailed. Data from Wright *et al.* (1988, 1989).

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complex EPSPs were significantly facilitated by weak tail shock, but were not significantly altered by strong tail shock (figure 4*b*). In the same preparations, the decremented monosynaptic connection (produced by repeatedly activating the sensory neuron with intracellular current pulses at a short ISI) was also significantly facilitated by weak tail shock. However, in contrast to the complex EPSP (and the behaviour), the monosynaptic EPSP was also facilitated by strong tail shock (figure 4*c*). Thus the complex EPSP in siphon motor neurons provides a reliable neural correlate of the siphon withdrawal reflex, exhibiting several of the forms of tail shock induced plasticity that we observe behaviourally, including some forms which are not reflected at the level of the monosynaptic EPSP.

4. PHARMACOLOGICAL ANALYSIS

Both the behavioural and cellular experiments described above indicate that tail shock can produce diverse forms of modulation in the neural circuit of the siphon withdrawal reflex. An important question that emerges from these observations is the nature of the neuromodulatory events that underlie these different forms of reflex plasticity. Several lines of evidence have implicated the neuromodulator serotonin as a mediator of some of the facilitatory effects of tail shock. Serotonin produces facilitation of the synapse from the LE siphon sensory neurons onto siphon and gill motor neurons (Brunelli *et al.* 1976). This synapse is also facilitated by tail shock, and it is thought that persistent enhancement of this monosynaptic component of the reflex underlies the behavioural change seen in sensitization (Castellucci & Kandel 1976). Moreover, there are serotonergic interneurons in the cerebral ganglia of *Aplysia* which respond to tail shock and which facilitate the siphon sensory-to-motor synapse (Mackey *et al.* 1989). Finally, depletion of serotonin in the intact animal has been shown to block behavioural facilitation (Glanzman *et al.* 1989).

As a first step in analysing the neuromodulatory mechanisms contributing to the diverse effects of tail shock, we asked whether serotonin might be involved in the inhibition of non-decremented responses produced by strong tail shock. We examined the effects of serotonin on the behavioural reflex, by using a reduced preparation which consisted of the siphon, gill and mantle shelf, connected to the abdominal ganglion via the siphon nerve. To establish a non-decremented baseline, a water jet was delivered to the siphon at 10 min intervals, and the duration of siphon withdrawal was measured as in the behavioural experiments in intact animals. Serotonin (50 μM) was then bath applied to the ganglion. It was found that brief application of serotonin produced significant inhibition of the reflex response (compared both to baseline responding and to control preparations that received no serotonin) when tested 90 s after serotonin administration (Fitzgerald & Carew 1989; figure 5*a*). The reflex recovered from inhibition within 10 min. Both the degree of inhibition and the time course of recovery paralleled that observed after tail shock in both behavioural and cellular experiments.

We next wished to determine where in the neural circuit for siphon withdrawal serotonin might be exerting its inhibitory influence. To explore this question the modulatory effects of serotonin on both monosynaptic and complex EPSPs in siphon motor

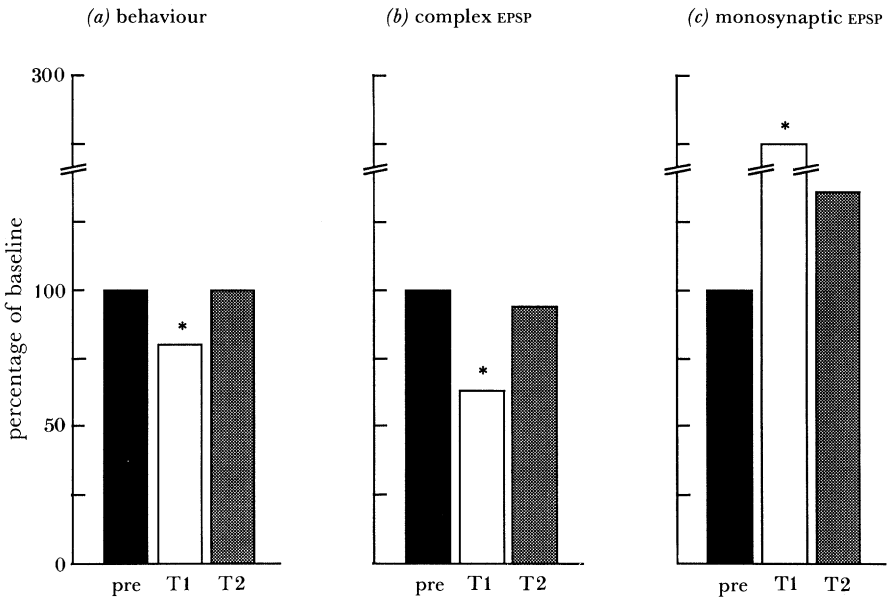

Figure 5. Effects of serotonin on siphon withdrawal and EPSPs in a reduced preparation. (*a*) Duration of siphon withdrawal response. Serotonin produces inhibition of the siphon withdrawal reflex, which recovers 10 min later (at test 2). This inhibition parallels that seen in the intact animal after tail shock. (*b*) Amplitude of complex EPSPs elicited by water jet stimulation of the siphon. Serotonin produces transient inhibition of the complex synaptic input to siphon motor neurons. (*c*) Amplitude of monosynaptic EPSPs elicited by intracellular stimulation of sensory neurons. In contrast to the behaviour and the complex EPSP, the monosynaptic EPSP shows facilitation after serotonin application. Data are expressed as mean percent of baseline. pre: average of two pre-test responses. T1: test 1, 30 s after the end of serotonin application. T2: test 2, 10 min later. Asterisks indicate statistical significance, at least $p < 0.05$, two-tailed. Data from Fitzgerald & Carew (1989, 1990).

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neurons were examined. Intracellular recordings were made from a siphon sensory neuron and a siphon motor neuron in the isolated abdominal ganglion. At 10 min intervals, both the monosynaptic EPSP (elicited by injection of depolarizing current in the sensory neuron) and the complex EPSP (elicited by a brief electric shock to the siphon nerve) were tested. After two baseline responses were obtained serotonin was briefly bath applied. Following application of serotonin (50 μM), the monosynaptic EPSP was significantly facilitated above baseline (figure 5*c*), an effect which has been described previously (Brunelli *et al.* 1976). At the same time, however, the complex EPSP showed significant inhibition, recovering 10 min later (Fitzgerald & Carew 1989; figure 5*b*). Thus, serotonin reliably mimics at least some of the effects of tail shock in producing transient inhibition of both the siphon withdrawal reflex and the complex synaptic input to siphon motor neurons. This inhibitory effect appears to be fairly specific to serotonin; another neuromodulator, the endogenous peptide SCP_B (which has facilitatory effects on the monosynaptic EPSP similar to those of serotonin; Abrams *et al.* (1984)) did not produce inhibition of the complex EPSP.

5. DISCUSSION

We have found that tail shock, which is a highly effective unconditioned stimulus in producing both non-associative and associative learning in *Aplysia*, can have diverse modulatory effects in the siphon withdrawal reflex. This chapter has focused primarily on two forms of modulation: (i) inhibition of non-decremented responses produced by strong tail shock, and (ii) facilitation of decremented responses (dishabituation) produced by weak but not strong tail shock. An important question that arises from these behavioural studies concerns the neuronal loci and cellular mechanisms that mediate the different forms of reflex modulation. Three broad classes of such possible loci that emerge from our cellular and pharmacological results are shown in figure 6. These include:

(*a*) changes in the monosynaptic connection from LE sensory neurons to motor neurons;

(*b*) changes in the synapses from another (novel) population of sensory neurons onto their follower cells;

(*c*) changes at the level of interneurons.

We will discuss each of these possibilities in turn.

Changes in the monosynaptic connection between LE siphon sensory and siphon motor neurons (figure 6*a*) parallel the facilitation of decremented responses to weak tail shock, since the monosynaptic EPSP is increased at the same time that the behaviour and the complex EPSP are increased. However, changes in the monosynaptic EPSP do not parallel changes in the behaviour or complex EPSP in two other conditions: (i) when decremented synapses are examined after strong shock (which produces less facilitation of the behaviour and complex EPSP but greater facilitation of the monosynaptic EPSP); and (ii) when non-decremented responses are examined after strong tail shock (which produces inhibition of the behaviour and complex EPSP, but facilitation of the monosynaptic EPSP). Thus there are clear instances of a dissociation between tail shock induced modulation of the monosynaptic connection on the one hand, and of both the complex EPSP and the behaviour on the other. It is possible that, if other responses (such as gill withdrawal) are examined and different stimuli are used to elicit or modulate the reflex, the monosynaptic connection may account for a greater amount of the reflex modulation (Mackey *et al.* 1988). Our results suggest, however, that in tail shock induced modulation of the siphon withdrawal reflex elicited by water jet stimuli, additional cellular loci are also important sites of change in producing the various forms of plasticity we observe.

An additional site of plasticity that could contribute to tail shock induced modulation is shown in figure 6*b*, which illustrates the possibility that another set of sensory neurons (as yet unidentified) may be modulated by tail shock in ways that would be consistent with the behavioural and synaptic changes that we observe. Recent work by Rosen *et al.* (1989) provides a precedent for differential effects of a common modulatory signal on sensory neurons. In their study, different classes of mechanoafferent neurons in the cerebral ganglion of *Aplysia* responded oppositely to the same modulatory input: nerve shock (or serotonin) produced spike broadening and enhanced synaptic transmission in some sensory neurons, and spike narrowing and diminished synaptic release in others. To examine the possibility that novel sensory neurons may play a role in reflex modulation, we are presently conducting a search for new sensory neurons that: (i) respond to water jet stimuli; (ii) make direct or indirect synapses onto siphon motor neurons; and (iii) are inhibited 90 s after a strong tail shock. Such neurons would be prime candidates to contribute to the effects of tail shock that we observe.

A third possibility is that plastic changes may be occurring at the level of interneurons that receive input from known or novel sensory neurons (figure 6*c*). Many interneurons mediating the siphon withdrawal

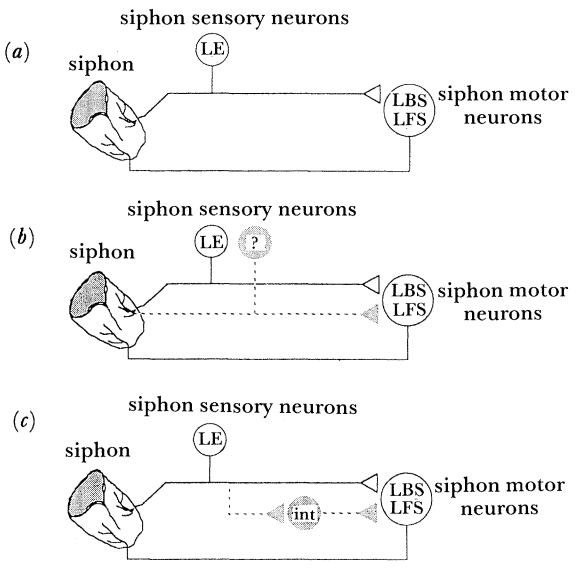

Figure 6. Schematic representation of possible sites of tail shock induced modulation in the neural circuit for siphon withdrawal (see text for details).

reflex (including inhibitory interneurons) have already been identified (Hawkins *et al.* 1981*a*, *b*; Frost *et al.* 1988). Any of these neurons, as well as other yet unidentified interneurons, may alter their excitatory or inhibitory contribution to the complex EPSP after strong tail shock in ways consistent with the behavioural plasticity. A related possibility is that an increase in transmitter release from LE sensory neurons after tail shock could also indirectly contribute to the diverse modulatory effects of tail shock. For example, if the LE neurons connect strongly to inhibitory interneurons, then facilitation of these synapse could in turn contribute to the inhibition of non-decremented responses that we observe. Thus there are several possible ways that tail shock induced plasticity at the level of interneurons, in conjunction with changes in monosynaptic input, could give rise to reflex modulation.

We should emphasize that the possibilities described above are neither exhaustive nor mutually exclusive. However, they do provide a set of testable hypotheses to explore mechanisms of plasticity that can account for the changes in reflex output produced by tail shock. In exploring these hypotheses it will be of interest to determine: (i) how subcellular and molecular mechanisms of plasticity in other elements in the neural circuit for siphon withdrawal will compare to the mechanisms that are known to modulate the monosynaptic connection; and (ii) how these other elements interact both with each other and with the monosynaptic connection to produce the net synaptic activity in the motor neurons that mediate the reflex and its modulation by tail shock.

Finally, it will be of considerable interest to determine the relationship of the non-associative processes in the siphon withdrawal reflex that we have described in this chapter to more complex associative learning in the same reflex. It is known that the siphon withdrawal reflex exhibits a variety of forms of associative learning, including differential classical conditioning as well as contingency and context effects, when electric shock is used as the US (Carew *et al.* 1983; Hawkins *et al.* 1986; Colwill *et al.* 1988). Now that we know that tail shock can have a variety of both inhibitory and facilitatory non-associative effects with different times of onset depending on shock intensity and the initial state of the reflex, it will be extremely interesting to examine the efficacy of different intensities of tail shock in classical conditioning of siphon withdrawal. For example, one interesting possible prediction from our non-associative behavioural data is that a weak tail shock may be a more effective US if the response to be conditioned is first decreased by repeated delivery of the CS (for example, siphon stimulation), whereas a strong shock might be more effective as a US if the response to be conditioned is not previously decremented. A further possible prediction is that the conditioned responses produced in these two conditions might have a differential onset, with the weak US condition (by analogy to our dishabituation results) giving rise to a rapid onset conditioned response and the strong US condition (by analogy to our sensitization results) giving rise to a delayed onset conditioned response.

Equally interesting questions can be explored at a cellular level. For example, activity-dependent presynaptic facilitation of sensory neuron output has been proposed as a possible cellular mechanism contributing to classical conditioning in *Aplysia* (Hawkins *et al.* 1983; Walters & Byrne 1983). However, the cellular data described in this chapter (Wright *et al.* 1988, 1989), as well as the recent results of Lukowiak and colleagues (Lukowiak 1986; Colebrook & Lukowiak 1988) suggest that additional sites of plasticity are also likely to be important in reflex modulation underlying different forms of learning in *Aplysia*. Moreover, additional mechanisms of modulation of the LE sensory neurons have recently been described (Hochner *et al.* 1986; Mackey *et al.* 1988; Belardetti *et al.* 1987; Small *et al.* 1989). Thus, as additional sites of plasticity are identified, it will be very interesting to see whether the same types of cellular mechanisms that are known to underlie non-associative and associative modulation at the identified monosynaptic connections from the sensory neurons are also used at these other sites of plasticity, and whether additional (perhaps novel) mechanisms are used. The ultimate goal of such experiments will be to try to account completely for the diverse forms of learning exhibited in this simple reflex at cellular and molecular levels of analysis.

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REFERENCES

Abrams, T. W., Castellucci, V. F., Camardo, J. S., Kandel, E. R. & Lloyd, P. E. 1984 Two endogenous neuropeptides modulate the gill and siphon withdrawal reflex in *Aplysia* by presynaptic facilitation involving cAMP-dependent closure of a serotonin-sensitive potassium channel. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7956–7960.

Belardetti, F., Kandel, E. R. & Siegelbaum, S. A. 1987 Neuronal inhibition by the peptide FMRFamide involves opening of S K^+ channels. *Nature, Lond.* **325**, 153–156.

Brown, T. H., Kairiss, E. W. & Keenan, C. L. 1990 Hebbian synapses: biophysical mechanisms and algorithms. *A. Rev. Neurosci.* (In the press.)

Brunelli, M., Castellucci, V. & Kandel, E. R. 1976 Synaptic facilitation and behavioral sensitization in *Aplysia*: possible role of serotonin and cyclic AMP. *Science, Wash.* **194**, 1178–1180.

Byrne, J. H. 1987 Cellular analysis of associative learning. *Physiol. Rev.* **67**, 329–439.

Carew, T. J., Hawkins, R. D. & Kandel, E. R. 1983 Differential classical conditioning of a defensive withdrawal reflex in *Aplysia californica*. *Science, Wash.* **219**, 397–400.

Carew, T. J. & Sahley, C. L. 1986 Invertebrate learning and memory: from behavior to molecules. *A. Rev. Neurosci.* **9**, 435–487.

Castellucci, V. & Kandel, E. R. 1976 Presynaptic facilitation as a mechanism for behavioral sensitization in *Aplysia*. *Science, Wash.* **194**, 1176–1178.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Colebrook, E. & Lukowiak, K. 1988 Learning by the *Aplysia* model system: Lack of correlation between gill and gill motor neurone responses. *J. exp. Biol.* **135**, 411–429.

Colwill, R. M., Absher, R. A. & Roberts, M. L. 1988 Conditional discrimination learning in *Aplysia californica*. *J. Neurosci.* **8**, 4440–4444.

Fitzgerald, K. & Carew, T. J. 1989 Serotonin differentially modulates monosynaptic and complex EPSPs in siphon motor neurons in *Aplysia*. *Soc. Neurosci. Abstr.* **15**, 1265.

Frost, W. N., Clark, G. A. & Kandel, E. R. 1988 Parallel processing of short-term memory for sensitization on *Aplysia*. *J. Neurobiol.* **19**, 297–334.

Glanzman, D. L., Mackey, S. L., Hawkins, R. D., Dyke, A. M., Lloyd, P. E. & Kandel, E. R. 1989 Depletion of serotonin in the nervous system of *Aplysia* reduces the behavioral enhancement of gill withdrawal as well as the heterosynaptic facilitation produced by tail shock. *J. Neurosci.* **9**, 4200–4213.

Hawkins, R. D., Abrams, T. W., Carew, T. J. & Kandel, E. R. 1983 A cellular mechanism of classical conditioning in *Aplysia*: activity-dependent amplification of presynaptic facilitation. *Science, Wash.* **219**, 400–405.

Hawkins, R. D., Carew, T. J. & Kandel, E. R. 1986 Effects of interstimulus interval and contingency on classical conditioning of the *Aplysia* siphon withdrawal reflex. *J. Neurosci.* **6**, 1695–1701.

Hawkins, R. D., Castellucci, V. F. & Kandel, E. R. 1981*a* Interneurons involved in mediation and modulation of gill-withdrawal reflex in *Aplysia*. I, identification and characterization. *J. Neurophysiol.* **45**, 304–314.

Hawkins, R. D., Castellucci, V. F. & Kandel, E. R. 1981*b* Interneurons involved in mediation and modulation of gill-withdrawal reflex in *Aplysia*. II, identified neurons produce heterosynaptic facilitation contributing to behavioral sensitization. *J. Neurophysiol.* **45**, 315–326.

Hawkins, R. D., Clark, G. A. & Kandel, E. R. 1987 Cell biological studies of learning in simple vertebrate and invertebrate systems. In *Handbook of physiology*, Part I, *higher functions of the nervous system* (ed. F. Plum), vol. 6, pp. 25–83. Bethesda: American Physiological Society.

Hochner, B., Klein, M., Schacher, S. & Kandel, E. R. 1986 Additional component in the cellular mechanism of presynaptic facilitation contributes to behavioral dishabituation in *Aplysia*. *Proc. natn. Acad. Sci. U.S.A.* **83**, 8794–8798.

Krontiris-Litowitz, J. K., Erickson, M. T. & Walters, E. T. 1987 Central suppression of defensive reflexes in *Aplysia* by noxious stimulation and by factors released from body wall. *Soc. Neurosci. Abstr.* **13**, 815.

Lukowiak, K. 1986 *In vitro* classical conditioning of a gill withdrawal reflex in *Aplysia*: neural correlates and possible neural mechanisms. *J. Neurobiol.* **17**, 83–101.

Mackey, S. L., Glanzman, D. L., Small, S. A., Dyke, A. M., Kandel, E. R. & Hawkins, R. D. 1987 Tail shock produces inhibition as well as sensitization of the siphon-withdrawal reflex of *Aplysia*: possible behavioral role for presynaptic inhibition mediated by the peptide Phe-Met-Arg-Phe-NH_2. *Proc. natn. Acad. Sci. U.S.A.* **84**, 8730–8734.

Mackey, S. L., Kandel, E. R. & Hawkins, R. D. 1989 Identified serotonergic neurons LCB1 and RCB1 in the cerebral ganglia of *Aplysia* produce presynaptic facilitation of siphon sensory neurons. *J. Neurosci.* **9**, 4227–4235.

Mackey, S. L., Lalevic, N., Hawkins, R. D. & Kandel, E. R. 1988 Comparison of dishabituation and sensitization of the gill-withdrawal reflex in *Aplysia*. *Soc. Neurosci. Abstr.* **14**, 842.

Mackintosh, N. J. 1974 In *The psychology of animal learning*. New York: Academic Press.

Mackintosh, N. J. 1983 *Conditioning and associative learning*. New York: Oxford University Press.

Marcus, E. A., Nolen, T. G., Rankin, C. H. & Carew, T. J. 1987 Behavioral dissociation of dishabituation, sensitization and inhibition in the siphon withdrawal reflex of adult *Aplysia*. *Soc. Neurosci. Abstr.* **13**, 816.

Marcus, E. A., Nolen, T. G., Rankin, C. H. & Carew, T. J. 1988 Behavioral dissociation of dishabituation, sensitization, and inhibition in *Aplysia*. *Science, Wash.* **241**, 210–213.

Rescorla, R. A. 1988 Behavioral studies of Pavolovian conditioning. *A. Rev. Neurosci.* **11**, 329–352.

Rosen, S. C., Susswein, A. J., Cropper, E. C., Weiss, K. R. & Kupferman, I. 1989 Selective modulation of spike duration by serotonin and the neuropeptides, FMRF-amide, SCP_B, buccalin and myomodulin in different classes of mechanoafferent neurons in the cerebral ganglion of *Aplysia*. *J. Neurosci.* **9**, 390–402.

Solomon, R. L. & Corbit, J. D. 1974 An opponent-process theory of motivation, I, temporal dynamics of affect. *Psychol. Rev.* **81**, 119–145.

Small, S. A., Kandel, E. R. & Hawkins, R. D. 1989 Activity-dependent enhancement of presynaptic inhibition in *Aplysia* sensory neurons. *Science, Wash.* **243**, 1603–1606.

Wagner, A. R. 1981 SOP: a model of automatic memory processing in animal behavior. In *Information processing in animals: memory mechanisms* (ed. N. E. Spear & R. R. Miller), pp. 5–47. New Jersey: Lawrence Erlbaum Associates.

Walters, E. T. & Byrne, J. H. 1983 Associative conditioning of single sensory neurons suggests a cellular mechanism for learning. *Science, Wash.* **219**, 405–408.

Wright, W. G., Marcus, E. A. & Carew, T. J. 1989 Dissociation of monosynaptic and polysynaptic contributions to dishabituation, sensitization and inhibition in *Aplysia*. *Soc. Neurosci. Abstr.* **15**, 1265.

Wright, W. G., Marcus, E. A., Thaker, H. & Carew, T. J. 1988 A cellular analysis of tail-shock induced inhibition in the siphon withdrawal reflex of *Aplysia*. *Soc. Neurosci. Abstr.* **14**, 841.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES