the parapodia. The abdominal ganglion, which contains the siphon sensory and motor neurons, was partially desheathed, and the preparation was thoroughly washed with normal artificial seawater (40% of the seawater) before the experiment began.

19. Single-neuron electrophysiological techniques were used (7-9). Sensory neurons and motor neurons were impaled with single-barreled glass microelectrodes. The electrodes were filled with 2.5M potassium acetate and beveled to a resistance of 10 to 20 megohms. A Wheatstone bridge circuit was used for recordings. The electrodes were hyperpolarized to prevent it from firing, it was held at the same level while testing the PSP's from the two sensory neurons after training.


21. The sensory neurons are a cluster of about 24 cells that have similar properties and synaptic connections (7). These cells do not release l-dopa or dopamine, which are typical of the sensory neurons that innervate the tail. Instead, they release dopamine, which is known to inhibit the activity of the motor neurons. The dopamine released by these neurons is thought to inhibit the release of the excitatory neurotransmitter acetylcholine by the motor neurons, which results in the inhibition of the motor neurons and the suppression of the tail-flick reflex.

22. The summary statistics given are means and standard errors of the means. In all cases statistical comparisons are t-tests for correlated means. This is done to test for the significance of the differences in the experimental groups.

For within-cell comparisons, the control value was the pretreatment score, and for between-cell comparisons, the control value was the score for the cell in the other experimental condition. The EPSP's were tested for both the paired and unpaired EPSP's before training (4.2 ± 0.7 mV for the paired neurons and 4.9 ± 0.8 mV for the unpaired neurons). Typically each sensory neuron had a train of five potentials by intracellular injection of 40- to 50-msec depolarizing current pulses at 10 Hz. Currents were adjusted so that each of these potentials produced a single action potential. Two types of US were used: either a 1.5 seconds of 50 mV, 60- to 100-Hz train of current delivered to the tail through a bipolar capillary electrode, or a 1.5-second, 10-Hz train of 3-msec pulses delivered to the postero-lateral surface of a bipolar Ag-AgCl electrodes. The intensity of the tail or posterior-petal nerve stimulation was sufficient to produce a brisk firing in the postsynaptic neuron when it was not depolarized. Onset of the US coincided with injection of intracellular stimulation of the paired neuron and followed stimulation of the unpaired neuron by 2.5 minutes.

23. The degree of correlation between the cellular data and the behavioral data is better than might be expected since in one case we measured EPSP amplitude and in the other case we measured duration of siphon withdrawal. However, a word of caution is necessary since this type of data has also been observed in previous experiments for (for example, T. J. Carew and E. R. Kandel, Science (1973)) and could be explained by several possible mechanisms: (I) Frost et al. (20) identified interneurons in the siphon withdrawal pathway that had an extended train of spikes in response to brief excitatory synaptic input from the sensory neurons. An increase in the number of such potentials from sensory neurons could produce an increase in the duration of the resulting train of spikes in sensory neurons, which would produce an increase in the duration of siphon withdrawal. (ii) The same cellular change that underlies increased siphon withdrawal release from the sensory neurons (for example, a decrease in K" conductance) could also lead to repetitive firing of the sensory neurons in response to a brief excitatory stimulus. (iii) The activity-dependent change that occurs in the sensory neurons could occur in an all-or-none, and might produce an interneuron firing in a similar fashion. These possibilities are neither mutually exclusive nor exhaustive.

24. The abdominal ganglion was placed in a small well of the TEA solution while the rest of the preparation was perfused with normal seawater. The pleural-abdominal connectives were left intact so that enough grease seal was achieved at the walls of the well. Sensory neurons were assigned to be paired or unpaired in such a way as to balance the durations of their action potentials before training (71 ± 9 msec for the paired neurons and 77 ± 10 msec for the unpaired neurons). Instead, the paired neurons were electrically isolated by a single 5-msec depolarizing pulse. This typically produced a burst of sips in the sensory neuron, of which only the first was measured. The value of the spike duration for the pretreatment and each of the two trains of measurements taken at 5-minute intervals. The US was 1.5 seconds of 50 mV, 60- to 100-Hz a shock to the tail. Onset of US was followed by stimulation of the paired neuron by 500 mV and followed stimulation of the unpaired neuron by 2.5 minutes.

25. Continued broadening of the action potentials may have a number of possible explanations including (i) changes in the size and conductivity of the memory into a long-term form, (ii) recovery from spike narrowing caused by repeated stimulation of the sensory neurons during training, (iii) prolonged exposure of the neuron to TEA, or (iv) progressive deterioration of the preparation. Preliminary experiments indicate that US-alone training does not produce continued broadening, which suggests that it may be due to recovery from spike narrowing.


32. A synaptically induced long-lasting decrease in K" conductance in an interneuron or motor neuron would lead to (i) a long-lasting increase in the ease with which the neuron could be excited by the synaptic input (ii) an increase in the magnitude of the eventual EPSP produced by a single presynaptic action potential. A consequence of the mechanism we are proposing is that both of these consequences would be amplified if the neuron happened to be firing action potentials at the time of the modulatory synaptic input. This result would correspond formally to (and could underlie) an increase in the probability, rate, or frequency of a behavior by contingent reinforcement of that behavior.


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ASSOCIATIVE CONDITIONING OF SINGLE SENSORY NEURONS

SUGGESTS A CELLULAR MECHANISM FOR LEARNING

Abstract. A cellular analog of associative learning has been demonstrated in individual sensory neurons of the tail withdrawal reflex of Aplysia. Sensory cells activated by intracellular current injection shortly before a sensitizing shock to the animal's tail display significantly more facilitation of their monosynaptic connections to a tail motor neuron than cells trained either with intracellular stimulation unpaired to tail shock or with tail shock alone. This associative effect is acquired rapidly and is expressed as a temporally specific amplification of heterosynaptic facilitation. The results suggest that activity-dependent neuromodulation may be a mechanism underlying associative information storage and point to aspects of subcellular processes that might be involved in the formation of neural associations.

The use of intracellular techniques to investigate neuronal changes produced by classical and operant conditioning paradigms (J) encourages the belief that mechanisms of associative information storage can be analyzed on the cellular level. Because associative learning is usually quite sensitive to motivational and attentional factors, some psychologists have assumed that the formation of associations depends, in part, upon the contiguous activation of sensory “analyzers” and modulatory “arousal centers” (2). We have tested this general idea on the neuronal level in the mollusk Aplysia californica by examining the associative interaction of electrophysiological activity in individual sensory neurons with neuromodulatory concomitants of defensive arousal. Our results suggest a cellular mechanism for associative information storage, activity-dependent neuromodulation, that may be of general significance.

We applied a cellular analog of a different classical conditioning procedure simultaneously to three different neuronal populations—those innervating the tail (3)—in the left pleural ganglion of each animal. Because noxious tail stimulation produces defensive arousal and modulates (heterosynaptically facilitates) (3, 4) synaptic connections of these sensory neurons, we used tail shock as the reinforcing or unconditioned stimulus (US) (5). Training and testing were conducted in a reduced “split-foot” preparation (6). Associative and nonassociative effects of training were measured by testing the amplitude of single monosynaptic excit-
atory postsynaptic potentials (EPSP’s) evoked in a common postsynaptic tail motor neuron (3, 7) by brief intracellular stimulation of each sensory cell. These tests (8) were applied at 5-minute intervals before (pretest), during, and after (posttest) training.

After the pretest phase, the sensory neurons were assigned (9) to one of three training procedures: paired (CS+; N = 10), unpaired (CS−; N = 10), or sensitization (Sens; N = 9) (Fig. 1A). The paired and unpaired conditioned stimuli (CS+ and CS−) consisted of a brief suprathreshold train of nine depolarizing current pulses injected through the intracellular electrode (10). This spike train mimicked the response of these sensory neurons to moderate mechanical stimulation of the skin (3). The cells receiving Sens training were exposed only to the effects of the US. Each trial began with delivery of the CS+ to the paired sensory neuron (Fig. 1A); 600 msec later, the US was applied to the tail; and after 2 minutes the CS− was applied to the unpaired sensory neuron. After an additional 2 minutes (1 minute before the next trial), all three sensory neurons were tested with single depolarizing pulses (6) to elicit EPSP’s in the motor neuron. Five training trials were given (with four interpolated tests), followed by at least six additional tests.

Figure 1A illustrates the neural concomitants of each type of training, showing the sensory cell responses and the synaptic responses in the motor neuron to the CS+, CS−, and US. In this animal, CS+ produced more facilitation of the monosynaptic EPSP than did CS− or Sens training (Fig. 1, B and C).

Figure 2A (top) shows the pooled EPSP amplitudes in each group (ten animals and 29 cells) (11). A treatment-by-trials analysis of variance on the ten tests given during the training and posttest phases revealed overall significant effects of both type of training and number of trials (F2,26 = 3.77, P < .05; F9,234 = 27.64, P < .01). Subsequent pairwise comparisons at two selected tests were performed with the Newman-Keuls procedure. At test 5 (5 minutes after the last US) the CS+ cells showed significantly more synaptic facilitation than either the CS− or Sens cells (P < .05); there was no difference between the CS− and Sens cells. By contrast, at test 10 there were no significant differences among these groups.

The US alone produces nonspecific heterosynaptic facilitation of the sensory neurons (Sens, Fig. 2A, top). It seemed likely that variability in the amount of nonspecific facilitation produced by the US in different animals partially obscured the associative effect specific to the pairing of spike activity with the US. For example, on test 10 the mean EPSP amplitude of Sens cells in different animals ranged from 60 percent to 166 percent of their mean pretest levels, and similar variability was seen in CS+ (89 to 251 percent) and CS− (35 to 272 percent) EPSP’s. Nevertheless, nine of ten animals showed greater facilitation (or less depression) in CS+ cells than in CS− cells at test 10. To reduce the effects of variability among different animals in nonspecific facilitation, we used the Sens cell in each animal as an index of the amount of nonspecific facilitation in that animal and estimated the magnitude of the associative effect relative to the nonspecific facilitation on each test. Thus, for each cell we normalized all EPSP’s to the mean of the three baseline tests in the pretest phase. In each animal (N = 9), we then divided the normalized EPSP amplitudes of the CS+ and CS− cells on each test by the normalized Sens EPSP amplitude on that test to obtain CS+ and CS− facilitation ratios (Fig. 2A, bottom). A facilitation ratio of 1 indicates facilitation equal to that produced by sensitization alone, whereas facilitation ratios greater than 1 indicate synaptic facilitation greater than expected from sensitization alone.

A treatment-by-trials analysis of variance on the facilitation ratios of CS+ and CS− cells in the training and posttest phases revealed overall significant effects of type of training (F2,10 = 10.14, P < .01) but not of number of trials (F9,144 = 1.58). Subsequent Newman-Keuls tests revealed that CS+ test responses were significantly greater than CS− responses 5 minutes (test 5, P < .05) and 30 minutes after the last US (test 10, P < .01). In three of the animals, the sensory cells were held for over 75 minutes, and in each case, the CS+ cell showed more facilitation than the CS− cell. Thus the associative effect appears to be long-lasting. In addition, the nearly constant value of the CS+ facilitation ratio throughout the training and posttest phases (Fig. 2A, bottom) suggests that the associative process produces a nearly constant amplification of the nonspecific synaptic facilitation from the US on each trial.

The associative change is not due to a generalized change in the properties of
the postsynaptic cell since, in each animal, a single motor neuron manifested simultaneously the alterations produced by each of the three cellular training protocols. Alternatively, the association may occur within the modulatory system mediating the heterosynaptic facilitation. This would require nonoverlapping modulatory subsystems specific to each sensory neuron, an unlikely arrangement since focal stimulation of points on the tail (activating few tail sensory neurons) causes heterosynaptic facilitation of all the tail sensory neurons (3). Another possibility is that the associative locus is the sensory neuron itself. Support for this possibility comes from recent findings of changes in membrane potential and input resistance of the sensory cell soma produced by pairing (12).

These results suggest a cellular mechanism for storing associative information, namely, activity-dependent neuromodulation (Fig. 2B). We propose that a motivationally significant US causes the diffuse release (synaptic or humoral, or both) of substances that modulate the functional strength of various neurons. In the tail withdrawal reflex, this neuromodulation is expressed as heterosynaptic facilitation—and apparently an increase in excitability (3, 12, 13)—of the sensory neurons. Associative specificity occurs because the degree of neuromodulation depends on the timing of prior electrophysiological activity in the modulated cells, a dependence similar to the permissive effect of spike activity postulated by Kandel and Tauro (14). However, rather than acting permissively, spike activity immediately before a neuromodulatory signal from a US appears to cause a powerful amplification of the amplitude and duration of the modulatory effects, an amplification that does not occur if the same change in activity is separated in time from the modulatory signal.

Our proposed associative mechanism differs from many cellular models of associative learning in not being dependent on the concurrent activation of pre- and postsynaptic elements at particular synapses (15). One consequence is that this mechanism could be used flexibly for different associative effects in different neuronal systems. For example, in a sensory system, this mechanism could allow an organism to learn to attend selectively to previously insignificant stimuli without necessarily linking these stimuli to particular responses.

A subcellular mechanism for implementing the associative change is suggested by properties of mechanooaerent neurons in *Aplysia*. The modulatory effects of the US have been linked to the activity of adenosine 3',5'-monophosphate (cyclic AMP) in *Aplysia* siphon sensory neurons (16), and evidence now suggests a similar role in the tail sensory neurons (12, 17). Indirect evidence suggests an elevation of Ca\(^{2+}\) levels in tail sensory cells produced by the CS (18). Therefore, a simple, testable hypothesis is that one major intracellular messenger (Ca\(^{2+}\)) amplifies the effects of the other (cyclic AMP), perhaps through the activation of an adenylate cyclase by Ca\(^{2+}\) (19), or through synergistic effects of Ca\(^{2+}\)-dependent and cyclic AMP-dependent kinases (20) in the CS+ cell.

Because activity-dependent neuromodulation can selectively associate diffuse modulatory signals with functionally active target cells, this mechanism would seem to be useful for a variety of plastic processes. Its potential generality is further suggested by similar findings obtained independently in siphon sensory neurons of *Aplysia* (21) and by results consistent with activity-dependent and Ca\(^{2+}\)-dependent neuromodulation in mammalian cortical and hippocampal cells (22).

**EDGAR T. WALTERS  
JOHN H. BYRNE**

Department of Physiology and Cell Biology, University of Texas Medical School, Houston 77025

**References and Notes**


Methyl 4,6-Dichloro-4,6-Dideoxy-a-D-Galactopyranoside: An Inhibitor of Sweet Taste Responses in Gerbils

Abstract. The sugar methyl 4,6-dichloro-4,6-dideoxy-a-D-galactopyranoside (DiCl-gal) is a new type of inhibitor of the gerbil’s electrophysiological taste response to sucrose or saccharin. Saturated solutions of this compound alone barely stimulate the gerbil’s taste nerve. But, when mixed with sucrose or saccharin, DiCl-gal suppresses the gerbil’s taste response to these two sweeteners. In contrast, when mixed with sodium chloride or hydrochloric acid, DiCl-gal does not affect the taste responses to these compounds. However, unlike other inhibitors of sweet taste, the DiCl-gal taste suppression is short-lived and occurs only when the inhibitor is combined with the sweetener.

My colleagues and I have been investigating the electrophysiological taste responses of gerbils to sugars and artificial sweeteners (1-3), seeking inhibitors that would provide some insight into the mechanism of the sweet taste response. We discovered a new type of inhibitor when we were comparing the taste responses to sucrose with those to tetra-chloro-galacto-sucrose (4,6-dichloro-4,6-dideoxy-a-D-galactopyranosyl 1,6-dichloro-1,6-dideoxy-b-D-fructofuranoside). This chlorine-containing sucrose derivative is about 100 times as effective as sucrose in producing an electrophysiological response (2, 4). Therefore, in an attempt to produce a “super sweetener,” we synthesized methyl 4,6-dichloro-4,6-dideoxy-a-D-galactopyranoside (DiCl-gal), a monosaccharide that contains chlorine atoms and also closely resembles the chlorinated sucrose derivative. This sugar barely stimulated the gerbil’s taste receptors even at a concentration of 0.1M, a saturated solution. In our earlier research, whenever we found...