Slow depolarization produced by associative conditioning of Aplysia sensory neurons may enhance Ca\(^{2+}\) entry

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Sensory neurons activated by intracellular stimulation immediately before sensitizing tail shock displayed a slow depolarization after the shock. By contrast, sensory neurons exposed to the effects of tail shock alone or unpaired activation and tail shock showed a slow hyperpolarizing response to the shock. A voltage-sensitive Ca\(^{2+}\) conductance that is activated near the resting potential may be modulated by these opposite effects of associative and non-associative training.

We recently showed that a classical conditioning protocol applied directly to individual tail sensory neurons in Aplysia resulted in associative modification of monosynaptic connections to tail motor neurons\(^{14}\). Sensory neurons receiving a conditioned stimulus (CS, intracellular activation of the sensory neuron) immediately before the unconditioned stimulus (US, tail shock) showed significantly more synaptic facilitation than sensory neurons exposed to the US alone or to unpaired CS and US applications. It is of interest to examine changes in the cell soma during associative conditioning since these changes may parallel changes in the synapses\(^9\) and might also be important for signalling the genetic apparatus of the cell during learning\(^4\). We now report that associative conditioning of the tail sensory neurons is accompanied by a slow depolarization of the sensory neuron soma that contrasts strikingly with the slow hyperpolarizing responses to unpaired US application. In addition, we show that the changes in membrane potential produced by paired and unpaired conditioning may modulate voltage-dependent Ca\(^{2+}\) conductances in the sensory neuron. A preliminary report of some of these results has appeared in abstract form\(^{15}\).

The results described below on the modulation of membrane potential during conditioning were obtained by further analysis of the experiments on associative conditioning reported by Walters and Byrne\(^{14}\). Thus, the semi-intact tail withdrawal preparation\(^{14,15}\) was utilized, which allowed concurrent measurement of responses in the sensory and motor neurons. Details of the conditioning protocol have been described previously\(^{14}\). To examine the possible effect of changes in membrane potential on Ca\(^{2+}\) conductances in the sensory neuron soma we used a second preparation, the isolated pedal-pleural ganglion, in which the tail sensory neurons were axotomized near their somata by cutting across the root of the pleural-pedal connective\(^{15}\). This procedure eliminated axon spikes normally observed when voltage-clamping the intact neuron. Individual somata were voltage-clamped using techniques described below and by Byrne et al.\(^2\).

Examples of the long-lasting changes in membrane potential typically produced by the US (tail shock delivered outside of the excitatory receptive fields of the monitored cells) are illustrated by the responses of two tail sensory neurons recorded simultaneously (Fig. 1). The US alone produced a slow hyperpolarizing response that lasted nearly 5 min (Fig. 1A\(_1\), see also ref. 15). Similar hyperpolarizing responses were produced by the unpaired US (not shown). By contrast, the cell receiving a burst of 10 action potentials (20 Hz) immediately prior to (paired with) the same US exhibited a slow depolarization that persisted for at least 5 min (Fig. 1A\(_2\)).

A quantitative comparison of these effects was obtained by measuring the ‘peak depolarization’ during
A1

US ALONE

A2

PAIRED

20sec

4mV

B

PEAK DEPOLARIZATION (mV)

0

-1

1

2

3

1 5

UNPAIRED

US ALONE

PAIRED

1 5

1 5

Fig. 1. A: slow polarizing responses recorded simultaneously in two tail sensory neurons in response to the tail shock US. The arrow indicates the 'peak depolarization' (see text) in each record. The dashed line extends from the resting potential prior to the US. A2: prolonged hyperpolarization in the neuron exposed to the effects of the US alone. A2: slow depolarization produced by pairing intracellular activation (10 spikes) with the US. The spikes have been clipped to allow enlargement of the slow response. B: mean peak depolarizations (± standard error of the mean) in sensory neurons trained with paired, unpaired, and US-alone protocols (n = 10 cells in each group) after the first and fifth training trials.

a defined interval on the first and fifth training trials. This interval began 5 s after and ended 2 min after US application. We first determined the point of peak depolarization in each record (arrows in Fig. 1A) by finding the most depolarized (or least hyperpolarized) value in the interval. We then expressed this value as the difference from the resting potential recorded immediately before the US. Fig. 1B shows the mean peak depolarization (± S.E.) of the 3 groups of sensory neurons whose synaptic responses were previously analyzed. On both the first and fifth trials an analysis of variance revealed significant overall effects (P < 0.001), and on both trials the paired cells showed greater peak depolarizations than did cells exposed to the US alone or to unpaired CS and US presentations (P < 0.01 in each case, Newman–Keuls test). There were no significant differences within each group between responses on the first and fifth trials.

Although the function of the associative and non-associative modulations of membrane potential in the sensory neurons is not yet known, it seemed possible that these responses could contribute to cellular modifiability by altering Ca$^{2+}$ influx through voltage-dependent channels in the sensory neurons. To examine this possibility we voltage-clamped the axotomized sensory neuron soma under conditions in which all voltage-sensitive conductances except the Ca$^{2+}$ conductance should have been blocked or reduced. The ganglion was superfused with a Na$^+$ and Ca$^{2+}$ free solution containing 11 mM Ba$^{2+}$, 100 mM tetraethylammonium (TEA), 10 mM 4-aminopyridine (4-AP), and 10$^{-4}$ M serotonin (5-HT). Ba$^{2+}$ was used to replace Ca$^{2+}$ since Ba$^{2+}$ is thought to pass through Ca$^{2+}$ channels without activating Ca$^{2+}$-dependent K$^+$ currents. 5-HT was used to block the steady-state 5-HT-sensitive K$^+$ current. The possibility that residual K$^+$ currents remained cannot be entirely ruled out. Fig. 2A illustrates the currents produced by 2 s voltage-clamp steps from a holding potential of —55 mV. At —35 mV a clear steady state inward current was produced. The steady state current–voltage relationship for this cell (Fig. 2B) shows that even at —45 mV the current was less outward than predicted by extrapolating from the linear part of the curve. Since the normal resting potential of these cells ranges between —40 and —50 mV in the semi-intact preparation, these results suggest that a Ca$^{2+}$ conductance may be activated at or near the resting potential (assuming that Ca$^{2+}$ and Ba$^{2+}$ conductances have similar voltage dependencies). Therefore, this Ca$^{2+}$ conductance might be modulated by the slow changes in potential produced by associative and non-associative conditioning.

Previously we proposed that the associative conditioning of the tail sensory neurons might involve the amplification by Ca$^{2+}$ of cyclic AMP-mediated effects (see also refs. 8 and 11). The present results indicate that a voltage-dependent Ca$^{2+}$ conductance is
Fig. 2. Evidence for a voltage-dependent Ca\(^{2+}\) conductance in the tail sensory neuron soma that is activated near resting potential. The ganglion was superfused with a solution that blocks or reduces all currents except those flowing through Ca\(^{2+}\) channels (see text). A: currents in response to voltage clamp steps from a holding potential of \(-55\) mV. B: current-voltage relationship for the cell illustrated in A. The curve is non-linear for depolarizations of 10 mV or more from the holding potential (\(-55\) mV).

present in these cells and thus that Ca\(^{2+}\) probably enters during the brief burst of spikes that serves as the CS. Furthermore, the observation that this conductance can be activated at or near the resting potential suggests that the small but prolonged depolarization following each pairing of the CS with the US may result in additional Ca\(^{2+}\) influx. This additional influx might prolong the interaction of Ca\(^{2+}\) with the subcellular machinery underlying the associative changes in the soma and possibly also in the presynaptic terminals, and thus might be involved in the 'consolidation' of the associative memory. Conversely, the slow hyperpolarizing response to the unpaired US may reduce Ca\(^{2+}\) influx and thus decrease the degree of modifiability following the US. These considerations suggest that the opposite modulations of membrane potential by paired and unpaired stimulation may enhance the contrast between the associative and non-associative effects of arousing stimulation. Although these ideas are speculative, they are consistent with 4 general observations. First, long-lasting polarizing responses are being reported in an increasing number of systems. Second, long-lasting depolarization is produced by associative conditioning in at least one other preparation. Third, voltage-dependent Ca\(^{2+}\) conductances are activated at or near the resting potential in many neurons. Fourth, Ca\(^{2+}\) is a potent second messenger and has been implicated in many forms of cellular regulation.

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