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Distributed Input to the Tail-Siphon Withdrawal Circuit in *Aplysia* from Neurons in the J Cluster of the Cerebral Ganglion

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Plasticity in the circuits for the withdrawal reflexes has been correlated with several simple forms of nonassociative and associative learning in *Aplysia*, and biochemical, biophysical, and molecular mechanisms of plasticity in these circuits have been described. In order to examine network features of this plasticity, we identified and characterized a component of the modulatory circuitry for the tail-siphon withdrawal circuit. Activation of mechanoafferent neurons in the J cluster of the cerebral ganglion produced strong and distributed input to the tail-siphon withdrawal circuit. Stimulation of the J cells led to excitatory and inhibitory effects in the sensory neurons in the pleural ganglion, the tail motor neurons in the pedal ganglion, and several classes of interneurons in the pleural ganglion, including the multifunctional neuron LPl17. Activation of the J cells produced both fast and slow postsynaptic potentials in neurons of the tail-siphon withdrawal circuit. Of particular interest was the ability of the J cells to produce slow EPSPs in the pleural sensory neurons. These slow EPSPs were associated with an increase in the excitability of the sensory neurons, but no effect of the J cells on spike duration in the sensory neurons was observed. The J cells appear to mediate both sensory and modulatory inputs to the circuit for tail withdrawal.

[Key words: excitability, facilitation, invertebrate, network, plasticity, sensory neuron]

The neural mechanisms of several simple defensive behaviors and their modification during learning have been studied extensively in the marine mollusk *Aplysia*. Research has focused in particular on the ability of stimuli delivered to the tail or siphon to elicit defensive behaviors. Mechanical or electrical stimulation of the tail elicits the tail-siphon withdrawal reflex, a coordinated set of defensive responses, which involves withdrawal of the tail and siphon as well as contraction of the gill and, with very intense stimuli, the release of ink and opaline (Hening et al., 1979; Carew et al., 1983; Mackey and Carew, 1983; Rayport et al., 1983; Walters et al., 1983a; Walters and Erickson, 1986; Scholz and Byrne, 1987; Denny, 1989; Byrne et al., 1991). Similarly, stimulation of the siphon elicits the siphon-gill withdrawal reflex, which involves withdrawal of the siphon and gill (Kupfermann and Kandel, 1969). Elements of the neural circuitry for these defensive reflexes have been identified, including sensory neurons, motor neurons, and interneurons (Kandel et al., 1967; Kupfermann and Kandel, 1969; Peretz, 1969; Castellucci et al., 1970; Byrne et al., 1974, 1978; Carew et al., 1974; Kupfermann et al., 1974; Carew and Kandel, 1977; Kanz et al., 1979; Perlman, 1979; Tritt and Byrne, 1980; Byrne, 1981, 1983; Hawkins et al., 1981a,b; Walters et al., 1983a; Cleary and Byrne, 1985, 1986, 1993; Mackey et al., 1987, 1989; Frost et al., 1988; Wright et al., 1991; Xu et al., 1991; Buonomano et al., 1992).

The circuits for the two reflexes can be modified at multiple cellular and subcellular sites, and different forms of plasticity in the circuits have been correlated with different forms of learning (Castellucci et al., 1970; Kupfermann et al., 1970; Carew et al., 1971; Castellucci and Kandel, 1974, 1976; Hawkins et al., 1981a,b, 1983; Byrne, 1982; Walters and Byrne, 1983, 1984, 1985; Walters et al., 1983a,b; Frost et al., 1988; Wright et al., 1991; Xu et al., 1992). The sensory neurons in the pleural and abdominal ganglia, which mediate the tail-siphon and siphon-gill withdrawal reflexes, respectively, are important sites of plasticity for several forms of learning. Therefore, detailed biophysical, biochemical, and molecular studies have been made of these sensory neurons and their synaptic connections to motor neurons that produce the withdrawal responses. By studying these synapses in isolation, much has been learned about the neural mechanisms of synaptic plasticity (for reviews, see Hawkins and Kandel, 1984; Byrne et al., 1991, 1993; Hawkins et al., 1993). Several recent studies, however, have begun to focus on the coordinated modulation of multiple behaviors and the integration of individual behaviors into the overall behavioral pattern in *Aplysia* (Cleary and Byrne, 1985, 1986, 1993; Teyke et al., 1990, 1991; Botzer et al., 1991; Ziv et al., 1991). These studies highlight the importance of examining a synapse or a simple circuit in the context of the larger neural network in which it is embedded. Hence, a more complete understanding of the simple defensive reflexes in *Aplysia* and their modulation awaits further elucidation of the neural circuitry that contributes to the mediation and modulation of the reflexes.

In the present study, we begin to do this by examining inputs to the circuit for the tail-siphon withdrawal reflex. Previously, most studies of this reflex have focused on the modulation of the pleural sensory neurons and the pleural sensory neuron-to-pedal motor neuron synapses produced by sensitizing stimuli delivered to posterior parts of the animal or by electrical stim-

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ulation of peripheral nerves that exit the pedal ganglion and that innervate the tail and posterior parts of the animal (Byrne et al., 1991, 1993). Several interneurons that may mediate some of the effects of tail stimulation on the tail-siphon withdrawal circuit have been identified. These include LPl17, RPl4, and RPl5 (Cleary and Byrne, 1985, 1986, 1993; Xu et al., 1991, 1994; Buonomano et al., 1992). In addition to these inputs from the tail, there is evidence that stimuli to the head and anterior parts of the animal can also modulate the tail-siphon withdrawal reflex and the circuit that mediates it. Stimuli to the head produce activity-dependent enhancement of the reflex (Walters, 1987) and can elicit tail and siphon withdrawal (J. L. Raymond and J. H. Byrne, unpublished observations). In addition, mechanical stimulation of most regions of the body, including the head, affects the membrane potential of the sensory neurons in the pleural ganglion (Walters et al., 1983a). Finally, stimulation of peripheral nerves C1–C3 (the upper labial, anterior tentacular, and lower labial nerves), which innervate the head and anterior regions of the animal, can produce depolarizing and hyperpolarizing membrane responses and an increase in the excitability of the pleural sensory neurons (Raymond and Byrne, unpublished observations).

As a first step in the identification of the neural circuitry that mediates these inputs from the head to the tail-siphon withdrawal circuit, the effects of neurons in the J cluster of the cerebral ganglion on several classes of neurons in the tail-siphon withdrawal circuit are described. The bilateral J clusters, overlying the trunk of the cerebral–pleural connective on the ventral surface of the cerebral ganglion, each contain a population of 50–100 primary mechanoafferent neurons. These neurons innervate the lips, anterior tentacles, dorsal portion of the head, neck, and perioral zone through axons in peripheral nerves C1–C3. Similar to other mechanoafferents in *Aplysia,* these neurons have no spontaneous action potentials, have little or no spontaneous input, and exhibit spike frequency accommodation in response to a sustained stimulus. The J cells are a heterogeneous population of cells; at least three classes of J cells have been distinguished based on their synaptic connections and their response to modulatory transmitters such as 5-HT (Rosen et al., 1979, 1982, 1989).

The J neurons affected all of the elements of the tail-withdrawal circuit that were examined. Stimulation of the J cells produced several types of excitatory and inhibitory effects in the sensory neurons of the pleural ganglion, the tail motor neurons in the pedal ganglion, and several classes of interneurons in the pleural ganglion, including the multifunctional neuron LPl17. Thus, the J neurons appear to be part of a circuit for complex and distributed input from the head to the tail-siphon withdrawal reflex.

Materials and Methods

Aplysia californica were maintained in closed artificial seawater aquaria at 15°C. The animals were fed seaweed, and maintained on a 12 hr light, 12 hr dark cycle. For the experimental procedures animals were anesthetized by the injection of a volume of isotonic $MgCl_2$ equal to about one-half of the volume of the animal. Our experimental preparation consisted of surgically isolated cerebral and left pleural-pedal ganglia. The pleural and pedal ganglia contain sensory neurons, interneurons, and motor neurons that mediate the tail-siphon withdrawal reflex. The isolated ganglia were transferred to a Sylgard-coated (Dow-Corning) recording chamber, and the sensory neurons and interneurons in the pleural ganglion and the J cells in the cerebral ganglion were exposed by surgically removing the surrounding connective tissue. For experiments that involved recording from the motor neurons, the connective tissue overlying those cells in the pedal ganglion was also removed. The preparation was maintained in a static bath (approximate volume, 3 ml) of artificial seawater (Instant Ocean) buffered to a pH of 7.6 with 10 mM Tris (Sigma).

Intracellular recordings in all preparations were performed using glass microelectrodes (4–10 MΩ) filled with 3 M potassium acetate. Individual sensory neurons, interneurons, and motor neurons were impaled with microelectrodes, and recordings were made from these neurons using conventional current-clamp techniques. Data were recorded on an FM tape recorder and a Gould pen recorder and were in some cases digitized for subsequent analysis. All experiments were performed at room temperature (approximately 20°C).

Sensory neurons in the pleural ganglion, tail motor neurons in the pedal ganglion, and mechanoafferent neurons in the J cluster of the cerebral ganglion were identified by their location, biophysical properties, and synaptic connections (Rosen et al., 1979; Walters et al., 1983a; Cleary and Byrne, 1985, 1986, 1993). LPl17 neurons were identified by their location, biophysical properties, and ability to produce a slow EPSP in a tail motor neuron; other interneurons in the pleural ganglion were identified by their effects on tail motor neurons (Cleary and Byrne, 1985, 1993). In experiments that examined excitability in the sensory neurons, excitability was tested at 60 sec intervals by passing 1 sec, depolarizing constant current pulses into the sensory neurons and recording the number of spikes elicited. In experiments that examined the duration of action potentials in the sensory neurons, single spikes were elicited in the sensory neurons at 30 sec intervals with 3 msec depolarizing constant current pulses. The duration of the action potential was measured as the time between the peak of the spike and the point of the repolarizing phase at which the membrane potential was 10% of the peak amplitude (Sugita et al., 1992a). Single action potentials were elicited in the J cells with single 50 msec intracellular current pulses just above spike threshold. Bursts of action potentials were elicited in the J cells with 10 Hz trains of 50 msec superthreshold depolarizing current pulses that were 50 msec to 34 sec in duration. This rate and duration of stimulation is comparable to the firing pattern of neurons in the J cluster in response to mechanical stimulation of the skin. The rate of firing in the J cells in response to a sustained mechanical stimulus initially can be higher than 10 Hz, and this slowly adapts over tens of seconds (Rosen et al., 1979). For statistical tests, a p value < 0.05 was considered significant.

Results

The J neurons produced EPSPs and IPSPs in sensory neurons in the pleural ganglion

A population of mechanoafferent neurons in the paired J clusters of the cerebral ganglion was found to have functional input to the sensory neurons in the pleural ganglion, which mediate the tail-siphon withdrawal reflex. Stimulation of the J neurons produced several types of PSPs in the pleural sensory neurons. In an initial survey of J cell effects on pleural sensory neurons in 107 preparations, recordings were made from 122 J cell–sensory neuron pairs in which stimulation of the J cell elicited a slow PSP in the sensory neuron. J cell–induced slow EPSPs were observed in 82 sensory neurons, IPSPs were observed in 12 sensory neurons, and multicomponent responses (E-I or I-E) were observed in 28 sensory neurons. An example of each of these three types of PSPs is shown in Figure 1*A–C*. J cells were activated with trains of 10 Hz depolarizing current pulses, but much less intense activation was often sufficient to elicit a slow PSP. Thirty-five of the J cells were also activated with a single 50 msec pulse that produced only one or two spikes, and in 24 cases the one or two spikes were sufficient to produce a slow PSP in the sensory neuron. An example is shown in Figure 1*D* of a multicomponent PSP produced in a sensory neuron by a single spike in a J cell.

In order to gain insight into the membrane currents that might mediate the slow effects of the J cells on the membrane potential of the sensory neurons, changes in membrane input conductance associated with 25 PSPs (17 EPSPs, four IPSPs, four multicom-

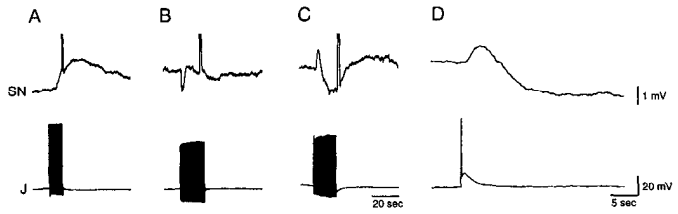

Figure 1. PSPs produced in sensory neurons by intracellular stimulation of J cells. *Each pair of traces* represents a different sensory neuron (*SN*) and J cell (*J*) that were recorded from simultaneously. *A*, A burst of spikes in a J cell produced a slow EPSP in a sensory neuron, lasting >1 min. *B*, A burst of spikes in a J cell produced an IPSP in a sensory neuron. *C*, A burst of spikes in a J cell produced a response in a sensory neuron consisting of more than one component, in this case depolarization followed by a longer-lasting hyperpolarization. The large upward deflections in each sensory neuron trace represent excitability tests (see Results), which were clipped by the pen recorder. *D*, A single spike in a J cell produced a multicomponent response in a sensory neuron, consisting of depolarization followed by hyperpolarization.

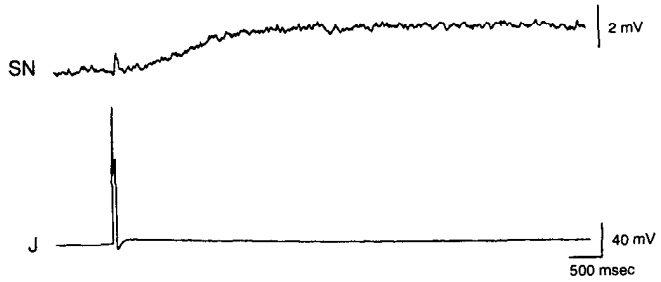

Figure 2. Fast and slow EPSPs produced in a sensory neuron (*SN*) by a single action potential in a J neuron (*J*).

ponent PSPs) and the reversal potential of five PSPs (two IPSPs, three EPSPs) were measured. Twenty-four of the 25 PSPs examined were not associated with any clear change in membrane input conductance. The one remaining PSP was an IPSP associated with an increase in membrane conductance. The reversal potentials of five PSPs were examined. Two IPSPs examined reversed near −55 mV. The amplitudes of three EPSPs examined were reduced but not reversed by hyperpolarizing the sensory neurons to approximately −90 mV.

In addition to the relatively slow effects, the J neurons produced conventional, fast EPSPs and IPSPs in the sensory neurons (Figs. 2, 3). Discrete fast PSPs were observed much less frequently than the slow PSPs. Fast EPSPs were observed in four of 107 preparations, and fast IPSPs were observed in four of 107 preparations. In one preparation (Fig. 2), fast EPSPs in a sensory neuron followed spikes in a J cell at a short and constant latency (15 msec), suggesting that they may have been monosynaptic. The other J cell–triggered fast PSPs appeared to be polysynaptic, since they did not follow spikes in the J neurons in a one-to-one manner.

In pilot experiments, application of a bathing solution containing high concentrations of divalent cations (Byrne et al., 1978) blocked the J cell–triggered PSPs (data not shown), suggesting a polysynaptic connection. However, the traditional interpretation of the high divalent cation test may not be appropriate for testing the connections of the J cells in the cerebral ganglion to sensory neurons in the pleural ganglion, because the long axonal distances are likely to cause a conduction block that would eliminate monosynaptic as well as polysynaptic connections in the solution of high divalent cations (Waxman, 1971). Since the results of this test were inconclusive, we did not persist in applying it to the connections of the J cells.

Depression of a sensory neuron's response to J cell stimulation was frequently observed in successive stimulations of a J cell. One functional consequence of having multiple components of the J cell–to–follower cell connections in combination with differential synaptic depression was that the overall effect of a J cell on a follower cell could change with successive stimulations. This is illustrated in Figure 3, which shows the response of a single sensory neuron to four successive stimulations of the same J cell, delivered several minutes apart. Initially, fast IPSPs were a major component of the input from the J cell to the sensory neuron. During subsequent stimulations, the fast input appeared to depress, and a slow depolarization was the principal effect of the J cell on the sensory neuron. Such effects could represent synaptic depression in any monosynaptic components of the J cell's effects that may exist, or they may represent the recruitment or dropout of intercalated interneurons.

J cell–induced slow EPSPs were associated with an increase in excitability in the sensory neurons

Sensitizing stimuli such as tail stimulation and pedal nerve stimulation produce depolarizing and hyperpolarizing membrane responses in the pleural sensory neurons and an increase in the duration of action potentials and the excitability of those neurons (Walters et al., 1983b; Walters and Byrne, 1985; Mercer et al., 1991). Since the J cells produced hyperpolarizing and depolarizing responses in the sensory neurons, their ability to produce additional effects on excitability and spike duration was examined.

In particular, the ability of J cells that produced a slow EPSP in a sensory neuron to also effect changes in the excitability of the sensory neuron was examined. For 59 of the sensory neurons in which a J cell produced a slow EPSP, excitability tests were delivered to the sensory neuron at 1 min intervals and excitability was plotted as the number of spikes elicited by a 1 sec depolarizing current pulse. Stimulation of the J cells produced a large increase in the excitability of these sensory neurons (Fig. 4). The excitability of the sensory neurons immediately after stimulation of the J cells (Post 1) was 180% of the excitability immediately before stimulation of the J cells (Pre 2) [ANOVA $F_{4,232} = 21.2$, $p < 0.01$; planned comparison between Pre 2 and Post 1, 3.5 ± 0.2 vs 6.3 ± 0.6 spikes (mean ± SEM), $F_{1,58} = 25.7$, $p < 0.01$]. Although the change in excitability generally lasted less than 2 min (Post 2 = 3.8 ± 0.3 spikes), in some cases (24 of 59) it did outlast the slow EPSP, suggesting that the modulation of excitability was not a direct consequence of depolarization of the sensory neurons. Furthermore, in nine additional experiments, an increase in the excitability of a sensory neuron was observed in response to the stimulation of a J neuron in the absence of a PSP; also, a direct depolarization of the sensory neurons of comparable amplitude with intracellular injection of current does not produce a comparable increase in the excitability of the sensory neurons (Raymond and Byrne, unpublished observations).

The J cell–induced hyperpolarizations were accompanied by either an increase or no change in the excitability of the sensory neuron, with no significant overall effect (4.0 ± 0.4 spikes before vs 5.8 ± 0.8 spikes during J cell–induced hyperpolarization; $F_{1,4} = 6.0$).

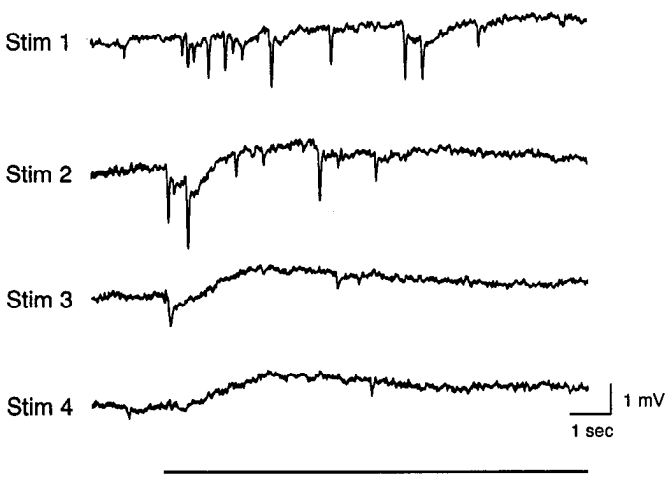

Figure 3. Changing responses of a sensory neuron to repetitive stimulation of a J cell. The four *traces* represent the response of a single sensory neuron to successive 50 Hz stimulations of a J neuron (indicated by the *horizontal bar*), delivered several minutes apart. Initially, fast IPSPs were a major component of the input from the J cell to the sensory neuron. During subsequent stimulations, the fast input seemed to depress, and slow depolarization was the principal effect on the sensory neuron.

There was no change in spike duration in the sensory neurons associated with J cell–induced slow EPSPs

Another modulatory effect of sensitizing stimuli is an increase in the duration of action potentials in the sensory neurons (Walters et al., 1983b; Mercer et al., 1991). The ability of the J cells to modulate this property of the sensory neurons was examined also. Individual action potentials were elicited with 3 msec depolarizing current pulses before and during the activation of a J cell. A representative example is illustrated in Figure 5A_1. Although the J cell produced a slow EPSP in the sensory neuron, it did not produce a change in the duration of the action potential (Fig. 5A_2). Similar results were obtained in five preparations in which spike duration was measured in a sensory neuron before and during a J cell–triggered slow EPSP (Fig. 5*B*). There was no change in spike duration associated with the slow EPSPs (ANOVA $F_{4,16} = 1.34$).

Connectivity

Neurons in both the ipsilateral and contralateral J clusters produced PSPs in the pleural sensory neurons, and more than one J neuron could produce PSPs in a particular sensory neuron. Sensory neurons that responded to a particular J cell were located throughout the sensory cluster of the pleural ganglion, but each of the J cells produced PSPs in only a subset of the pleural sensory neurons. Typically, simultaneous recordings were made from two sensory neurons during the stimulation of each J cell. In some cases the J cell produced PSPs in both sensory neurons, but in other cases the J cell produced a PSP in one but not the other sensory neuron. Thus, each J neuron appears to connect either monosynaptically or polysynaptically to a subpopulation of the pleural sensory neurons, and there appears to be substantial divergence and convergence in these projections.

The variety of responses produced in the pleural sensory neurons by J neurons suggested that there might be distinct subclasses of J neurons that could be distinguished based on these effects. However, by recording from two or more sensory neurons during stimulation of the J cells, it was found that a single J cell could simultaneously produce different types of PSPs in different sensory neuron targets. The J cell in Figure 6, for example, produced a triphasic hyperpolarization-depolarization–hyperpolarization response in one sensory neuron (SN1), while it produced a biphasic depolarization-hyperpolarization response in another sensory neuron (SN2), with no initial hyperpolarizing component. Thus, the J cells could not be classified solely based on their effects on the pleural sensory neurons. In addition, no systematic differences were observed in the electrophysiological properties of the multiple J cells recorded in

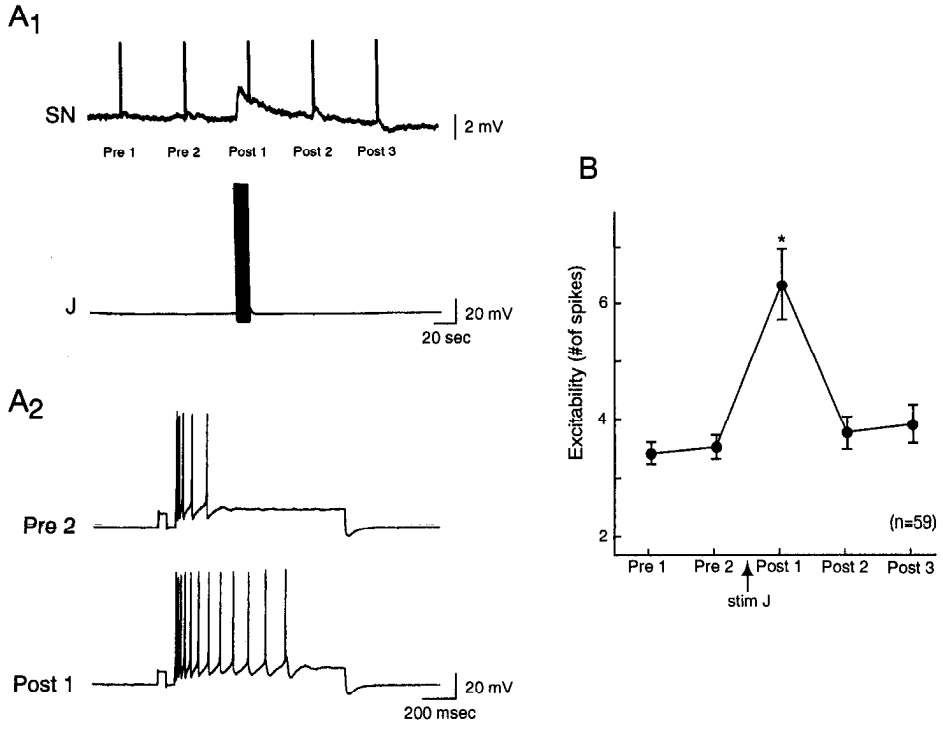

Figure 4. Increase in the excitability of the sensory neurons associated with the J cell–induced slow EPSPs. A_1, Excitability tests were delivered to the sensory neuron (*SN*) every 60 sec. A burst of spikes in a J cell (*J*) produced a slow EPSP in the sensory neuron. A_2, The same excitability test that initially elicited five spikes in the sensory neuron (*Pre 2*) elicited 12 spikes in the sensory neuron after stimulation of the J cell (*Post 1*). (A calibration pulse precedes the spikes in A_2.) *B*, Summary data for 59 sensory neurons in which a slow EPSP was produced by a J cell. Excitability was plotted as the number of spikes elicited in the sensory neuron. Stimulation of the J cells (*arrow*) produced a large increase in the excitability of the sensory neurons (*Post 1*) that returned to baseline within 2 min of J cell stimulation (*Post 2*).

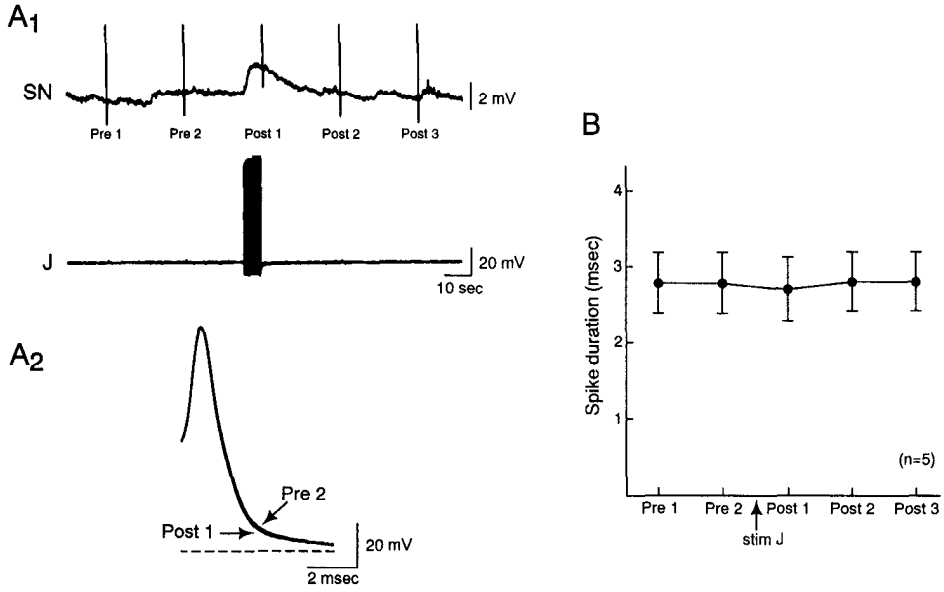

Figure 5. Duration of action potentials in sensory neurons before and during J cell–induced slow EPSPs. A_1, Action potentials were elicited in a sensory neuron (*SN*) every 30 sec. A burst of spikes in the J cell (*J*) produced a slow EPSP in the sensory neuron. Spikes in the sensory neuron were clipped by the pen recorder. A_2, The spikes elicited in the sensory neuron just before (*Pre*) and during (*Post*) the slow EPSP are shown on an expanded time scale. *B*, Group data for five sensory neurons in which a slow EPSP was produced by a J cell. No spike broadening was observed.

an individual preparation. All had no spontaneous action potentials, showed little or no spontaneous input, and exhibited rapid spike frequency accommodation. We did not attempt to classify individual J cells based on their pharmacological and morphological properties (Rosen et al., 1979, 1982, 1989). It may be that subclasses of the J cells have specific effects on subclasses of the pleural sensory neurons based on some as yet unidentified organizational principle.

The J neurons had parallel effects on motor neurons of the tail-siphon withdrawal circuit

Sensitizing stimuli have been shown to produce parallel changes at multiple loci in addition to the sensory neurons in the circuits for the withdrawal reflexes (Walters et al., 1983b; Frost et al., 1988). In particular, changes have been reported in the tail motor neurons in response to sensitizing stimuli (Walters et al., 1983b). Therefore, given their functional input to the pleural sensory neurons, an interesting question was whether the J cells also made connections to the tail motor neurons.

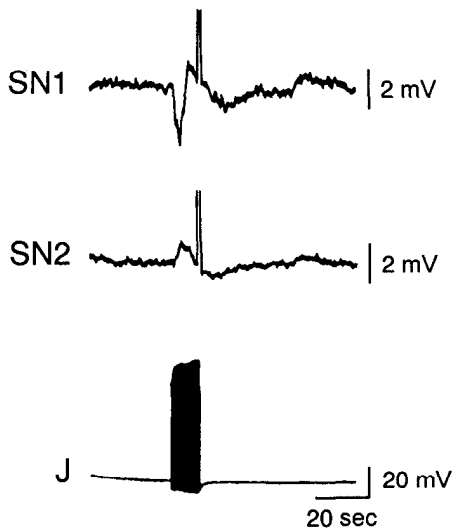

Figure 6. Responses in different sensory neurons to stimulation of a single J cell. Simultaneous recordings were made from two sensory neurons (*SN1* and *SN2*) during intracellular stimulation of a J cell (*J*). Stimulation of the J cell produced a triphasic response in SN1 and a biphasic response in SN2. The large upward deflections in the *two upper traces* represent excitability tests, which were clipped by the pen recorder.

Our experiments revealed functional connections between neurons in the J cluster and tail motor neurons in the pedal ganglion. Recordings were made from J neurons that produced PSPs in a motor neuron in 20 preparations. In 17 of the 20 preparations more than one neuron in the J cluster was found to project to a single motor neuron. As many as 11 J cells were observed to produce PSPs in a particular motor neuron. We did not attempt to record from more than one motor neuron in a single preparation to determine whether a particular J cell produced a response in all of the identified tail motor neurons or just a subset thereof. Most of the PSPs in the motor neurons were fast EPSPs and fast IPSPs (Fig. 7*A,B*), but slow PSPs were also observed (Fig. 7*C*). Based on the variable latency that was observed in the response to single spikes, most of the J cells seemed to be connected to the motor neurons via polysynaptic connections. In three preparations, however, fast PSPs in a motor neuron did follow spikes in a J neuron at a short and constant latency, suggesting that they may have been monosynaptic.

Some of the actions of the J neurons may be mediated by connections to interneurons in the pleural ganglion

Cleary and Byrne (1985, 1986, 1993) described several classes of interneurons on the ventral surface of the pleural ganglion that produce IPSPs and EPSPs in tail motor neurons as well as IPSPs in the pleural sensory neurons. These neurons are activated by pedal nerve stimulation and may therefore contribute to the modulation of the tail-siphon withdrawal reflex produced by tail stimulation. In order to determine whether these same interneurons may also be activated by the J cells, and whether they may mediate some of the effects of the J cells on the sensory neurons and motor neurons, recordings were made from several classes of interneurons in the pleural ganglion.

LPl17 is an interneuron in the pleural ganglion that is characterized by its ability to produce both fast and slow EPSPs in

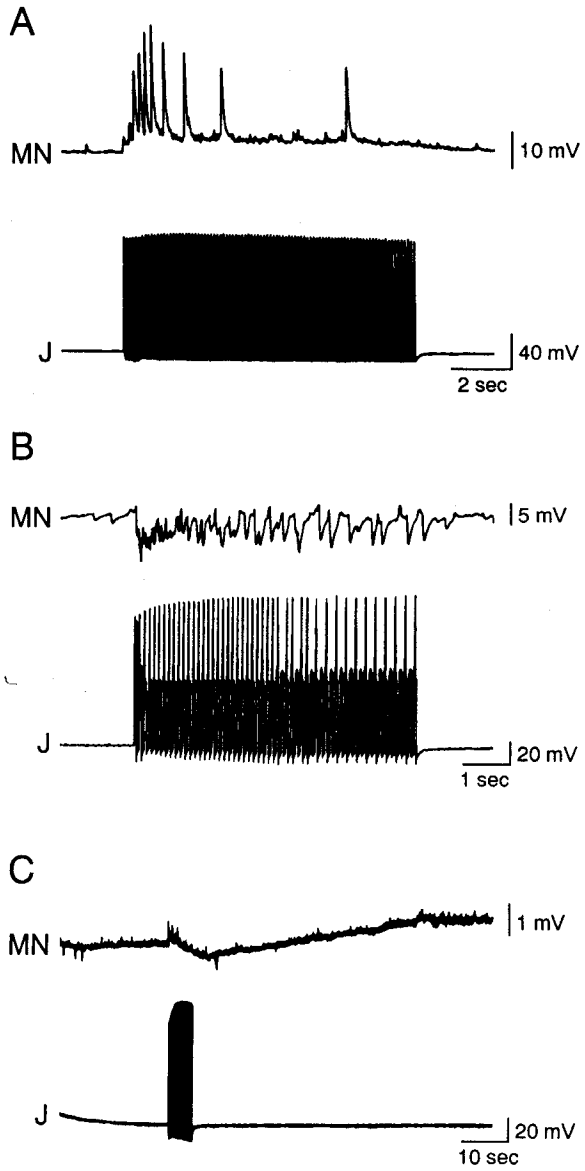

Figure 7. Three types of PSPs produced in the tail motor neurons by the stimulation of J cells. *Each pair of traces* represents a different J cell (*J*) and motor neuron (*MN*) that were recorded from simultaneously. *A,* A burst of spikes in the J cell produced a burst of fast EPSPs in the motor neuron. *B,* A burst of spikes in the J cell produced a burst of fast IPSPs in the motor neuron. *C,* A burst of spikes in the J cell produced a response in the motor neuron consisting of more than one component, in this case relatively fast EPSPs followed by a slower IPSP.

tail motor neurons (Cleary and Byrne, 1985, 1993; Fig. 8*A*). It receives excitatory input from pleural sensory neurons that appears to be monosynaptic (Cleary and Byrne, 1993). Recordings were made from LPl17 neurons in four preparations. In two preparations, LPl17 produced, in addition to its effects on a motor neuron, inhibition of a pleural sensory neuron which projected to the motor neuron (e.g., Fig. 8*A*). LPl17 received extensive input from J cell activation. Each of the LPl17 neurons received excitatory input from activation of at least one J cell (Fig. 8*B*), and one LPl17 received excitatory input from activation of nine different J cells. The input from individual J cells was strong enough to elicit spikes in two of the LPl17 neurons. Both fast and slow EPSPs were observed, and no inhibitory J cell–to–LPl17 connections were observed.

In addition to LPl17, the effects of the J cells on a less well described population of interneurons in the pleural ganglion (Cleary and Byrne, 1985, 1993) were examined. This is a population of lightly pigmented, small (40–80 μm diameter) cells located on the ventral surface of the pleural ganglion. This population includes interneurons that produce IPSPs, fast EPSPs, or mixed effects in the pedal motor neurons. In 13 preparations, recordings were made from eight excitatory interneurons, seven inhibitory interneurons, and one interneuron with mixed effects on a motor neuron.

Each type of interneuron received extensive excitatory and inhibitory input from activation of the J cells. For some individual interneurons only excitatory or only inhibitory J cell inputs were observed, but other interneurons received both excitatory and inhibitory J cell input. The interneuron in Figure 9*A* received mixed excitatory and inhibitory input from activation of a single J cell. As with the sensory neurons and motor neurons, both fast (Fig. 9*A,B*) and slow PSPs (Fig. 9*A*) were produced in the interneurons by stimulation of the J cells. Three of the interneurons received excitatory input from activation of a J cell that was sufficient to elicit spikes (Fig. 9*B*). Thus, the previously identified interneurons for the tail-siphon withdrawal circuit may mediate some of the PSPs observed in response to stimulation of the J cells.

Discussion

The J cells have multiple excitatory and inhibitory effects on the sensory neurons of the tail-withdrawal circuit

The mechanoafferent neurons in the J cluster of the cerebral ganglion make extensive connections to the circuit for the tail-siphon withdrawal reflex. The J cells produced several different effects on the sensory neurons in the pleural ganglion, and this is the first report of facilitatory effects produced in the pleural

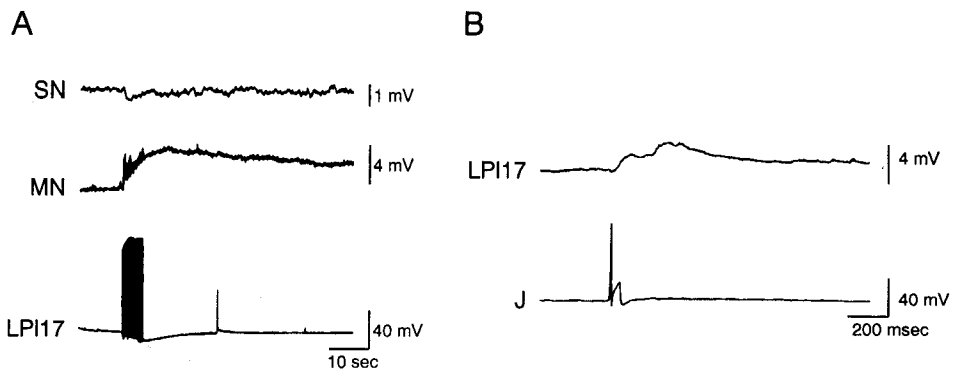

Figure 8. J cell connections to the multifunctional neuron LPl17. *A,* LPl17 produced fast and slow EPSPs in a tail motor neuron (*MN*) and a small IPSP in a sensory neuron (*SN*). *B,* A single spike in a J cell produced a short-latency EPSP in the LPl17 neuron shown in *A* and also recruited inputs with longer latencies. Eight additional J neurons in this preparation also produced EPSPs in LPl17.

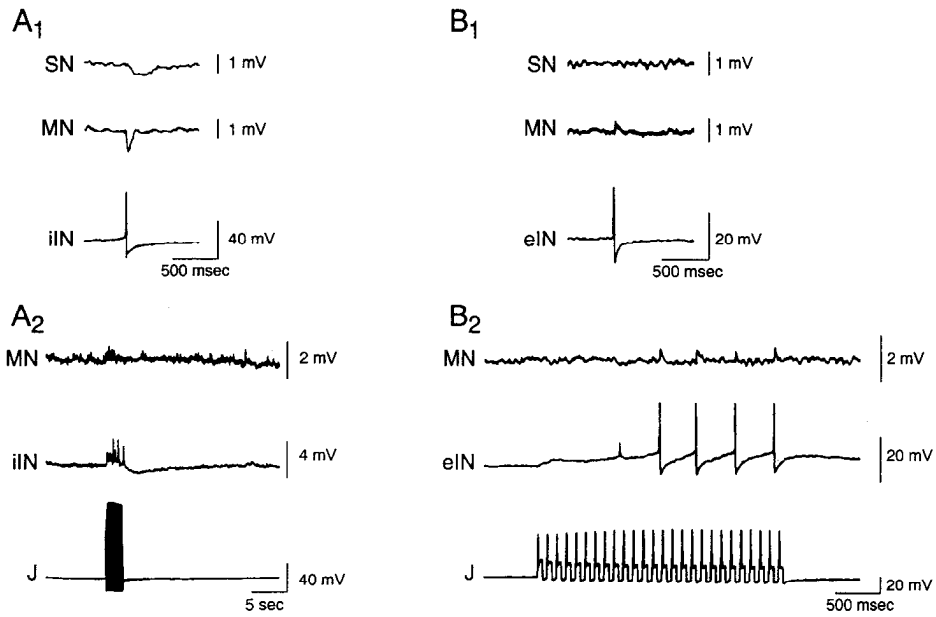

Figure 9. Input to pleural interneurons from the J cells. *A,* Mixed input to an inhibitory interneuron (*iIN*) from a J cell (*J*). A_1, A single spike in the inhibitory interneuron produced an IPSP in both a sensory neuron (*SN*) and motor neuron (*MN*). A_2, A burst of spikes in a J cell produced fast excitatory synaptic input followed by a slow IPSP in the inhibitory interneuron. *B,* Spikes elicited in an excitatory interneuron (*eIN*) by a J cell. B_1, A single spike in the excitatory interneuron produced a fast EPSP in a motor neuron. It did not produce a response in the sensory neuron. B_2, A burst of spikes in a J cell produced excitatory input that was sufficient to elicit spikes in the excitatory interneuron, which each produced a fast EPSP in the motor neuron. Spikes in the interneuron in *B* were clipped by the pen recorder.

sensory neurons by intracellular stimulation of a single neuron. The observation of J cell–triggered slow depolarizations in association with an increase in the excitability of the sensory neurons is consistent with the idea that the principal action of at least some of the J cells may be reduction of a K^+ current such as the S-current (Klein et al., 1982, 1986; Baxter and Byrne, 1990a,b). However, some of the membrane responses observed in the sensory neurons, such as the IPSPs, the multicomponent PSPs, and the fast PSPs, were not consistent with a simple reduction of the S-current. Hence, activation of the J cells apparently leads to changes in more than one conductance in the sensory neurons. This is further supported by the observation of PSPs that were not associated with any apparent change in input conductance in the sensory neurons, which could occur if activation of a J cell led to simultaneous modulation of more than one current in opposite directions or if the modulation of a single current by activation of a J cell caused a change in the membrane potential of the sensory neuron that then caused other, voltage-sensitive currents to be modulated.

It is interesting that the J cells can produce responses in the tail sensory neurons that involve the differential expression of two forms of modulation that have been observed in the sensory neurons, namely, changes in excitability and changes in spike duration. This dissociation parallels that which has been reported during development (Marcus and Carew, 1990), and which has been revealed using various modulatory transmitters (Abrams et al., 1984; Raymond et al., 1989; D. A. Baxter and J. H. Byrne, unpublished observations), the 5-HT antagonist cyproheptadine (Mercer et al., 1991), and agonists and antagonists of two kinase systems, cAMP-dependent protein kinase (PKA) and protein kinase C (Baxter and Byrne, 1990a,b; Braha, 1990; Sugita et al., 1992a,b). The above results provide some insights into the possible mechanisms of J cell action, but detailed pharmacological and voltage-clamp analyses will be necessary to understand more fully the cellular events underlying the various PSPs observed and the effect of the J cells on excitability.

It is also interesting that the stimulation of some J cells could produce conventional (fast) PSPs in the pleural sensory neurons. This is the first report of this type of synaptic connection to mechanosensory neurons mediating a withdrawal reflex in *Aplysia,* although fast PSPs have been reported in the proprioceptor and mechanoafferent neuron C2 in *Aplysia* (Weiss et al., 1986) and in other invertebrate sensory neurons (see, e.g., Tabata and Alkon, 1982; Kirk, 1985). It is possible that fast PSPs have not been reported previously in the pleural sensory neurons because most studies of these neurons have employed preparations in which the cerebral ganglion was removed. Even with the cerebral ganglion present, the J cells are not spontaneously active, so one would not expect to observe any effects mediated by these cells unless spikes were specifically elicited in them.

It was not determined which, if any, of the effects of the J cells were direct and which were mediated by polysynaptic connections. The usual method of distinguishing direct connections as those that persist in a solution containing high divalent cations was not a good criterion in the present case because of the likelihood that the long distance between the J cells in the cerebral ganglion and their targets in the pleural and pedal ganglia would result in a conduction block (Waxman, 1971). The answer to this question therefore must await detailed anatomical studies. Some of the PSPs produced by the J cells did follow spikes in the J cells at a short and constant latency and may therefore have been monosynaptic. Other PSPs did not follow spikes in the J cells in a one-to-one manner, suggesting that at least some of the effects of the J cells were mediated by interneurons. The functional connections of the J cells to the pleural interneurons described in the present study could mediate the IPSPs in the sensory neurons and most of the effects of the J cells on the motor neurons. No interneurons have been described in *Aplysia* that produce fast or slow EPSPs or an increase in excitability in the sensory neurons. These effects may have been produced directly by the J cells, or they may have been mediated by as yet unidentified interneurons.

The J cells have parallel excitatory and inhibitory effects at multiple loci in the tail-withdrawal circuit

In general, the modulatory neurons that have been described for the withdrawal reflexes have distributed effects on the cir-

cuits for the reflexes. For instance, L29 projects to sensory neurons, motor neurons, and interneurons of the siphon-gill withdrawal circuit (Hawkins et al., 1981a; Frost et al., 1988; Fischer and Carew, 1993), as well as at least one interneuron of the tail-siphon withdrawal circuit, LPl17 (Cleary and Byrne, 1986, 1993), and LPl17 projects to both sensory neurons and motor neurons of the tail-siphon withdrawal circuit as well as motor neurons and interneurons of the siphon-gill withdrawal circuit (Cleary and Byrne, 1985, 1986, 1993). Likewise, the J cells have distributed effects on the tail-siphon withdrawal circuit. In addition to their effects on the sensory neurons, the J neurons have effects on the motor neurons and several classes of interneurons in the pleural ganglion. The observation of functional connections from the J cells to LPl17 indicates that the J cells can, via these connections, also affect the siphon-gill withdrawal reflex. Connections may also exist between the J cells and additional interneurons, such as RPl4 and RPl5 in the right pleural ganglion, that were not included in the present experiments, which were conducted on left pleural-pedal ganglia.

In contrast to L29 and CB1, which can both be considered excitatory, and L16, LPl16, RPl4, and RPl5, which are all inhibitory to the withdrawal reflex circuits, the J cells have both excitatory and inhibitory effects at each cellular locus in the circuit. Even the effects of individual J cells can have apparently mixed or opposing effects. For example, a single J cell can produce depolarization of one sensory neuron, and simultaneously produce hyperpolarization of another (Fig. 6). The observation of different effects in different target neurons in response to a single J cell may reflect differences in the receptors present on the target neurons or differences in the strengths of connections of the J cell to different target neurons. Alternatively, a single J cell may be acting on the different targets through different interneurons.

Implications of the excitatory and inhibitory connections of the J cells for behavioral plasticity

The synaptic effects of the J cells indicate that they, and, by extension, head stimuli, can contribute to both the facilitation and the inhibition of the tail-withdrawal reflex circuit. The overall effect in any particular instance may depend on many factors. In a single preparation as many as 15 J cells with effects on the tail-withdrawal circuitry were observed. Each J cell projected to a subset of the potential target neurons in the tail-withdrawal circuit and could have different effects on different targets. Furthermore, excitatory and inhibitory synapses were observed between some of the interneurons to which the J cells projected (data not shown). Hence, the effect of a head stimulus on the tail-withdrawal reflex might depend on how many or which subset of J cells are activated, and how strongly they are activated (which components of a multicomponent connection are activated or which target interneurons receive strong enough input to elicit spikes). Furthermore, the functional effects of the multiple inputs from J cells to individual neurons in the tail-siphon withdrawal circuit may depend on the location of those inputs on the target neuron. Our data do not reveal whether the effects we recorded in the soma result from direct inputs to the soma or whether these effects were propagated from other regions of the neuron, such as the spike initiation zone. The location of inputs could affect not just the functional consequences of each individual input, but also the integration of multiple inputs.

Plasticity in the J cells is also likely to be an important factor in their modulation of the reflex. Rosen et al. (1979) reported low-frequency homosynaptic depression in the connections of the J cells to the B cells in the cerebral ganglion. We also observed depression in the responses of neurons of the tail-withdrawal circuit to repeated stimulation of the J cells. Therefore, the effect of activating the circuit containing the J cells could depend on the level of depression in both the J cells and their interneuron targets. Furthermore, the idea that neurons such as the J cells, with multiple effects on the tail-withdrawal circuit, are themselves subject to heterosynaptic as well as homosynaptic plasticity is an intriguing possibility. It has been shown that the neurons in the J cluster are modulated by several transmitters (Rosen et al., 1989), and so it seems likely that there exists modulatory circuitry that heterosynaptically regulates the ability of the J cells to activate and modulate the tail-siphon withdrawal circuit.

Thus, not only can the modulatory input to the tail-withdrawal circuit from any particular J neuron have more than one component, but both the absolute and the relative strength of those components can change with time and perhaps with the recent activity of the network (i.e., the modulatory synapses of the J cells are themselves plastic). These results indicate that activation of the same circuit by a head stimulus could have different, even opposite, effects on the circuitry for the defensive withdrawal behaviors under different conditions. This is especially interesting in light of behavioral studies (Mackey et al., 1987; Marcus et al., 1988) indicating that a stimulus can produce either facilitation or inhibition of a withdrawal reflex, depending on the time after the stimulus, on parameters of the stimulus such as intensity and duration, and on the behavioral state of the animal (e.g., whether the reflex had been habituated). In addition, several studies suggest that all behaviors may be influenced by the ongoing behavioral pattern of the animal (Cleary and Byrne, 1985, 1986, 1993; Teyke et al., 1990, 1991; Botzer et al., 1991; Ziv et al., 1991). Hence, the modulatory effects of a stimulus on the withdrawal reflexes may also depend on which behaviors were occurring during the application of the modulatory stimulus and activation of the modulatory circuitry.

Multiple sensory and modulatory functions of the J cells

The J cells seem to serve both sensory and modulatory roles in the neural circuits of *Aplysia*. Some of the functional connections of the J cells appear to serve the traditional sensory role of transmitting information to neurons of the response circuitry via fast synaptic potentials. For instance, the J cells produced fast EPSPs in the tail motor neurons and have been shown previously to produce fast PSPs in several motor neurons involved in feeding (Rosen et al., 1979, 1982). Other effects of the J cells, such as the slow EPSPs and the effects on excitability in the pleural sensory neurons, indicate that they also activate or are part of a modulatory circuit for the tail-siphon withdrawal circuit. Slow PSPs from J cells to their targets in the cerebral ganglion have not been reported. This could be due to differences in protocol between this and previous studies, or it could be that the J cells have different roles in these different circuits.

It will be interesting to determine in future anatomical studies whether the J cells mediate both sensory and modulatory functions directly, or whether certain functions require intervening interneurons. Several populations of sensory neurons have been described in *Aplysia* that produce both transient synaptic potentials and prolonged modulatory effects in their follower neurons. The histaminergic sensory neuron C2 conveys mechanosensory information from the mouth of the animal to motor

neurons involved in feeding via fast synaptic potentials, and it produces slow, modulatory EPSPs in other neurons, including the metacerebral cells (Chiel et al., 1990). Radula mechanoafferents B21 and B22 contain the modulatory peptide SCPb (small cardiac peptide b) and produce slow EPSPs in motor neuron B15 that can modulate its excitability (Miller et al., 1992; Rosen et al., 1992). Furthermore, neurons in all of the identified clusters of mechanoafferents, including the J cluster, contain mRNA for a peptide, sensorin-A, that seems to function as an inhibitory transmitter on a subpopulation of follower cells of the mechanoafferent neurons (Brunet et al., 1991). This peptide may mediate some of the inhibitory effects of the J cells.

Modulatory function has also been ascribed to some motor neurons in *Aplysia*. Cholinergic motor neurons B15 and B16, for instance, contain a number of modulatory peptides (Cropper et al., 1987a,b, 1988; Lloyd et al., 1987). In other model systems, there are also instances in which neuromodulation is produced by neurons with a sensory or other functional role. In the stomatogastric systems of crustaceans, for example, the gastropyloric receptor cells are primary mechanoreceptors that produce rapid cholinergic synaptic potentials in neurons of motor circuits and that also release 5-HT and exert prolonged neuromodulatory actions on the motor circuits (Katz and Harris-Warrick, 1989, 1990; Katz et al., 1989). In *Tritonia*, the serotonergic neuron C2 contributes to the modulation of the swim CPG of which it is an integral component (Frost and Getting, 1989).

A general principle is emerging that neuromodulation is not necessarily a function that is distinct from or extrinsic to other functional circuits of the nervous system. Rather, neuromodulatory processes appear to be part of the normal function of many circuits, and neuromodulatory function can be embedded in neurons that play other essential roles in those circuits. Thus, the identification and characterization of additional neurons with modulatory functions will be important for understanding, not just the special role of neuromodulation in learning and memory, but also functions of neuromodulation such as the ongoing integration and coordination of behavior.

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