Outputs of Radula Mechanoafferent Neurons in *Aplysia* are Modulated by Motor Neurons, Interneurons, and Sensory Neurons

STEVEN C. ROSEN, 1 MARK W. MILLER, 2 ELIZABETH C. CROPPER, 3 AND IRVING KUPFERMANN 1

1Center for Neurobiology and Behavior, New York State Psychiatric Institute and College of Physicians and Surgeons of Columbia University, New York, New York 10032; 2Institute of Neurobiology and Department of Anatomy, University of Puerto Rico Medical Science Campus, San Juan, Puerto Rico 00901; and 3Department of Physiology and Biophysics and Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, New York 10029

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**INTRODUCTION**

The transmission of sensory information from the periphery to the nervous system is modulated both at the level of primary afferents (Brooke et al. 1997; Gu and MacDermott 1997; Hill et al. 1997; Passaglia et al. 1998; Pasztor and Macmillan 1990) and at various stages of processing in the CNS (Blakemore et al. 1998; Filion et al. 1998; Geyer and Braff 1997; Gottlieb et al. 1998; Motter 1993; Steinman and Steinman 1998). Modulation of sensory information is involved both in basic sensory processing, such as enhancement of contrast, and in complex cognitive processes, such as attention. In both invertebrates and vertebrates there is evidence that inputs into the nervous system can be modulated so that the nature and intensity of afferent input is variable. Sometimes the variability is a function of other sensory inputs or of the state of motor systems that generate behavior. A form of sensory modulation was investigated in the *Aplysia* feeding system at the level of a radula mechanoafferent neuron (B21) that provides chemical synaptic input to a group of motor neurons (B8a/b, B15) that control closure and retraction movements of the radula, a food grasping structure. B21 has been shown to receive both excitatory and inhibitory synaptic inputs from a variety of neuron types. The current study investigated the morphological basis of these heterosynaptic inputs, whether the inputs could serve to modulate the chemical synaptic outputs of B21, and whether the neurons producing the heterosynaptic inputs were periodically active during feeding motor programs that might modulate B21 outputs in a phase-specific manner. Four cell types making monosynaptic connections to B21 were found capable of heterosynthetically modulating the chemical synaptic output of B21 to motor neurons B8a and B15. These included the following: 1) other sensory neurons, e.g., B22; 2) interneurons, e.g., B19; 3) motor neurons, e.g., B82; and 4) multifunction neurons that have sensory, motor, and interneuronal functions, e.g., B4/5. Each cell type was phasically active in one or more feeding motor programs driven by command-like interneurons, including an egestive motor program driven by CBI-1 and an ingestive motor program driven by CBI-2. Moreover, the phase of activity differed for each of the modulator cells. During the motor programs, shifts in B21 membrane potential were related to the activity patterns of some of the modulator cells. Inhibitory chemical synapses mediated the modulation produced by B4/5, whereas excitatory and/or electrical synapses were involved in the other instances. The data indicate that modulation is due to block of action potential invasion into synaptic release regions or to alterations of transmitter release as a function of the presynaptic membrane potential. The results indicate that just as the motor system of *Aplysia* can be modulated by intrinsic mechanisms that can enhance its efficiency, the properties of primary sensory cells can be modified by diverse inputs from mediating circuitry. Such modulation could serve to optimize sensory cells for the different roles they might play.
 Effects of Aplysia can be modulated so as to enhance its efficiency (Kupfermann et al. 1997), the properties of the primary sensory cells can be modified so as to optimize their function for the different behavioral roles they might play.

METHODS

Subjects and preparations

The subjects were Aplysia californica weighing 300–450 g. Two types of preparations were used: a buccal mass and an odontophore preparation. The buccal mass preparation was used to identify motor neurons, to determine the synaptic connections of the RM neurons specifically to motor neurons, and to characterize the behavioral significance of buccal motor programs that were initiated by chemical and/or electrophysiological means. It consisted of a partially dissected buccal mass that was removed from the body wall by a series of anterior, posterior, and lateral cuts. The anterior end of the buccal mass was freed from the body cavity by a cut through the anterior part of the jaws. The posterior end was freed by a cut through the esophagus. A series of lateral cuts through the extrinsic muscles of the buccal mass, connective tissue, and the anterior aorta completed the dissection. The portion of the anterior aorta leading to the buccal artery was cannulated and tied off so that the buccal mass could be perfused with artificial seawater (ASW) to simulate normal blood pressure and blood flow. The buccal mass preparation included the buccal and cerebral ganglia and the radula with most of the attached muscles that form the odontophore. Particular care was taken to preserve the buccal mass innervation provided by the radula nerve and buccal nerve 3.

The odontophore preparation was used to determine the receptive fields, response properties, and in some instances, the synaptic connections of RM neurons (Rosen et al. 2000). The odontophore was cut longitudinally to isolate it from the lower muscular wall of the buccal mass. The excised odontophore included the chitinous radula, its supporting membranes and muscles, and the innervation provided by the buccal and cerebral ganglia via the radula nerve and cerebral-to-buccal connective. The dissected odontophore rested on the cut surface, leaving the entire radula facing upward. The attached ganglia were pinned to a silicone elastomer (Sylgard) pedestal. A partial section of the anterior muscles was sometimes made to visualize the 14, radula closer muscles, and the 15, accessory radula closer (ARC) muscles. Contraction of each of these muscles when its motor neurons were fired was used to establish the identity of the motor neurons.

Electrophysiology

Neurons were impaled with double-barreled microelectrodes using techniques previously described (Rosen et al. 2000). A modified odontophore preparation was used (Fig. 1) in experiments aimed at examining the mechanisms of synaptic modulation of the outputs of RM neurons and in studies describing orthodromic and antidromic conduction of RM neuron action potentials. In this preparation, the radula with its support tissue was placed in a recording chamber along with the buccal and cerebral ganglia. Only the radula nerve innervation was preserved. One of the paired lateral branches of the radula nerve was cut, and the proximal cut end was drawn into a suction electrode to obtain extracellular recordings of action potentials in the nerve root traveling either toward the periphery (centrifugally, away from the center) or toward the ganglion (centripetally). Centripetal action potentials could arise from the intact radula nerve branch when the ipsilateral radula-half was mechanically stimulated. Centrifugal action potentials could result from central activation of either the left or right B21 RM neuron. Combinations of up to three neurons in the buccal ganglion were impaled with microelectrodes to electrically stimulate the cells or to make intracellular recordings of action potentials and synaptic potentials. A fourth channel was used for extracellular nerve recordings (A-M Systems, Differential Amplifier).

Mechanical and chemical stimulation

The recording chamber and the chemical and mechanical stimuli used in this study were identical to those previously described (Rosen et al. 2000).

Morphology

Neurons were filled with one of four fluorescent dyes that were delivered by iontophoretic ejection from microelectrodes. Aqueous solutions of the following dyes were prepared: 1) a 3% solution of 5(6)-carboxyfluorescein dye (Kodak), 2) a 5% solution of Lissamine-Rhodamine (Molecular Probes, Eugene, OR); 3) a 2% solution of Lucifer yellow CH (Molecular Probes, Eugene, OR); and 4) a 3% solution of Cascade Blue hydrazide; and 4) a 3% solution of Lissamine-Rhodamine (Molecular Probes, Eugene, OR). To reduce the active transport of the dye out of the cells (Steinberg et al. 1987), probenecid (10 mM

FIG. 1. Schematic diagram of the modified odontophore preparation and the experimental arrangement. The radula with its support tissue provided by the odontophore was placed in a recording chamber along with the buccal and cerebral ganglia (cerebral ganglion not shown). Radula innervation provided by the radula nerve (rad. n.) was preserved. One of the paired lateral branches of the radula nerve was cut. The proximal cut end was drawn into a suction electrode to make extracellular recordings of action potentials. Combinations of 3 neurons in the buccal ganglion were impaled with microelectrodes to electrically stimulate the cells or to make intracellular recordings of synaptic potentials and action potentials. B8a, B15, and B82 are identified motor neurons. B4 is a multifunction cholinergic cell. Numbers 1–7 designate peripheral and central portions of a B21 RM cell as follows: 1, lateral process; 2, soma; 3, medial process; 4, contralateral process; 5, radula process; 6, left radula branch; 7, right radula branch.
final concentration) was added to the ASW bathing medium, and the preparation was kept for 24–48 h at 4°C (Rosen et al. 1991). The unfixed tissues were viewed with a fluorescence microscope (Nikon Optiphot) using cubes (V-2A, B-2A, and G-1B) appropriate for simultaneous visualization of the multiple dyes, which fluoresce at different wavelengths. Confirmation of cell morphology was made with Lucifer yellow fills, followed by fixation in paraformaldehyde, and clearing in methyl salicylate.

RESULTS

Four types of input neurons evoke monosynaptic postsynaptic potentials (PSPs) in RM neuron B21 (Rosen et al. 2000). These include the following: 1) sensory neurons, e.g., B22; 2) interneurons, e.g., B19; 3) motor neurons, e.g., B82; and 4) multifunction neurons, that have sensory, motor, and interneuronal functions, e.g., B4/5 (Gardner 1971; Jahan-Parwar et al. 1983; Rosen et al. 1982). To determine whether each type of input neuron could produce heterosynaptic modulation of the putative chemical synaptic output of B21, we simultaneously monitored the synaptic outputs of B21 to motor neurons B8a (Church and Lloyd 1991; Gardner 1977; Morton and Chiel 1993) and B15 (Cohen et al. 1978). B8a innervates a part of the I4 muscle. B21 evokes a fast EPSP in B8a that shows marked synaptic facilitation, particularly if B21 is repetitively fired at relatively high frequency. B15 innervates the ARC (I5) muscle. Firing of B21 evokes fast electrotonic potentials and a slow, presumably chemical excitatory postsynaptic potential (EPSP) in B15.

In the sections that follow, we first present data on dye fills of B21 and various neurons that provide synaptic input to it. These studies were designed to help clarify the possible sites of action of the modulatory inputs. We also show the effects of coactivation of B21 and each of the various input neurons to B21 on the chemical EPSPs that B21 evokes in B8a and B15. Finally, we investigated the firing patterns of selected input (modulator) neurons, during buccal motor programs, particularly phase-specific activity produced during motor programs driven by command-like interneurons CBI-1 and CBI-2 (Rosen et al. 1991).

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FIG. 2. Drawings of a set of B21 RM neurons that have each been paired with another identified cell that has either an electrical, chemical, or dual electrical and chemical synaptic connection to it. Drawings are based on pairs of cells where one member (B21) was filled 5-(6)-carboxyfluorescein dye and the other (B8a, B15, B4, B22, B19, or B82) was filled with Cascade Blue hydrazide. A and B: morphologies of 2 motor neurons (B8a and B15, respectively) that receive the putative chemical synaptic output of B21. B15 receives dual input. C: morphology of a multifunction B4 neuron that provides inhibitory, chemical synaptic input to B21. D–F: morphologies of a sensory neuron (B22), an interneuron (B19), and a motor neuron (B82), respectively, that are coupled to B21 via nonrectifying electrical synapses. Because transmission at an electrical synapse is bidirectional, the latter cells could provide B21 with input or receive its output.
previously reported descriptions of B21 morphology (Fig. 2, top left). The appearance of the filled B21 cells was consistent with B22, B19, or B82) was filled with Cascade Blue hydrazide. (yellow-green) and the connected cell (i.e., B8a, B15, B4/5, B21). In a series of experiments (n = 18), pairs of cells located in the left buccal hemiganglion were identified by electrophysiological criteria and filled with different dyes. In each experiment, the B21 neuron was filled with 5-(6)-carboxyfluorescein (yellow-green) and the connected cell (i.e., B8a, B15, B4/5, B22, B19, or B82) was filled with Cascade Blue hydrazide. The appearance of the filled B21 cells was consistent with previously reported descriptions of B21 morphology (Fig. 2, A–F, topmost cell except in D). The cell is bipolar, having a lateral and medial process. The lateral process arises from the soma and extends to the lateral limit of the ipsilateral buccal hemiganglion. The medial process projects to the contralateral buccal hemiganglion via the buccal commissure. In the buccal commissure the medial process bifurcates and sends a major branch into the root of the radula nerve. This branch bifurcates at the point where the radula nerve root gives rise to the left and right radula nerves (the various processes and the somata are drawn and denoted by numbers in Fig. 1).

Within the limitations of light microscopy, visualization of the processes of B21 together with the motor neurons with which it makes chemical synaptic connections revealed that probable sites of contact were primarily located on the lateral process of B21 and the initial segment of the motor neuron.

**Morphology**

A light microscopic examination of the morphological features of RM neuron B21 and of the identified buccal neurons that represent its inputs and outputs was undertaken to obtain basic anatomic information that might contribute to an understanding of the mechanisms of heterosynaptic modulation of B21. In a series of experiments (n = 18), pairs of cells located in the left buccal hemiganglion were identified by electrophysiological criteria and filled with different dyes. In each experiment, the B21 neuron was filled with 5-(6)-carboxyfluorescein (yellow-green) and the connected cell (i.e., B8a, B15, B4/5, B22, B19, or B82) was filled with Cascade Blue hydrazide. The appearance of the filled B21 cells was consistent with previously reported descriptions of B21 morphology (Fig. 2, A–F, topmost cell except in D). The cell is bipolar, having a lateral and medial process. The lateral process arises from the soma and extends to the lateral limit of the ipsilateral buccal hemiganglion. The medial process projects to the contralateral buccal hemiganglion via the buccal commissure. In the buccal commissure the medial process bifurcates and sends a major branch into the root of the radula nerve. This branch bifurcates at the point where the radula nerve root gives rise to the left and right radula nerves (the various processes and the somata are drawn and denoted by numbers in Fig. 1).

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B8a is the lateral-most neuron of the ventral motor neuron cluster of the buccal ganglion. Dendritic processes of B8a arise from its initial axon segment as it courses medially through the hemiganglion toward the buccal commissure and radula nerve (Fig. 2A). The B8a dendrites are interspersed among the terminal branches of the lateral process of the B21 neuron, but are not seen in close proximity to the terminal branches of the medial process or soma of B21 (Fig. 2A). Similarly, dendrites arising from the initial segment of motor neuron B15 are interspersed with the terminal branches of the lateral process of B21, but not with the terminal branches of other portions of the cell (Fig. 2B). The soma of B15 is located in the medial portion of the buccal hemiganglion, in close proximity to the soma of B21. B15 projects an axon laterally toward the root of buccal nerve 3. The initial segment of B15 runs parallel to the B21 lateral process, and the fine processes of the two cells intermingle extensively.

In contrast to the relatively restricted spatial limitations on possible contact sites between B21 and its follower motor neurons, with which it makes chemical synaptic connections, the dye-fill experiments indicated that the neurons that provide input to B21 could contact B21 at its lateral process, its medial process, its soma, or at some combination of these regions. Neuron B4/5, which produces a chemically mediated inhibitory postsynaptic potential (IPSP) in RM neuron B21 (Rosen et al. 2000), has numerous processes in close proximity to the medial process and soma of B21 (Fig. 2C). Moreover, as the axon of B4/5 traverses the buccal hemiganglion, from the medially located B4/5 soma toward the root of buccal nerve 3, it gives rise to additional branches in the vicinity of the lateral process of B21. Most of these branches, however, pass above the lateral process of B21 and do not appear to make contact with it. Neuron B22 is a cell similar to B21, in that it is an SCP-containing radula mechanoafferent neuron. It makes an electrical synaptic connection to B21 (Rosen et al. 2000). B22 has numerous filamentous processes that appear to contact the medial and lateral processes of B21 (Fig. 2D). Moreover, the soma of B22 often abuts the soma of B21. Interneuron B19 (Fig. 2E) and motor neuron B82 (Fig. 2F) also make nonrectifying electrical synaptic connections to B21 (Rosen et al. 2000). The processes of B19 and B82 appear to contact the lateral process and soma, but not the medial process of B21.

**Effect of firing of B4/5 on signal transmission of sensory cell B21**

Previous work showed that B21 receives monosynaptic chemical inhibitory input from the multifunction neurons B4/5. Thus B4/5 activity might block signal transmission of B21 to one or more of its follower cells. The following experiments examined this hypothesis.

**Firing of B4/5 Can Block Peripheral Sensory Input to B21 and Its Follower Motor Neurons Such as B82.** To examine the possible functional significance of the inhibitory input that B4/5 evokes in B21, B21 was activated by means of controlled, tactile stimuli applied to the radula surface. In the modified odontophore preparation (see Fig. 1), a suction electrode attached to a branch of the radula nerve was used to monitor the afferent volley and ensure that the tactile stimulus evoked a relatively consistent sensory response (Fig. 3). In the absence of the firing of B4/5, the tactile stimulus evoked spikes in B21. B82, which receives excitatory electrical input from B21 and perhaps other sensory neurons (Rosen et al. 2000), depolarized and fired (Fig. 3, left and right). When neuron B4/5 was fired in the physiological range of 20 Hz or greater by injection of depolarizing intracellular current, the tactile stimulus either failed to evoke spikes in B21 or evoked fewer spikes than in the control condition (Fig. 3, middle). Under these conditions B82 showed a reduced excitatory input and often failed to spike. The results suggest that firing of B4/5 may block...
spikes originating in the mechanosensitive peripheral processes of B21. The spikes presumably are blocked at some point between their entrance into the ganglion and the cell body. Based on the morphology of B21 (Fig. 2C), it is possible that B4/5 could act to block B21 spikes via synaptic terminals at the medial process and soma of B21 (Fig. 1, regions 2 and 3), thereby preventing orthodromic spikes from reaching the soma and/or lateral process of B21 (region 1) where the connections to B82 are likely to be present (Fig. 2F).

FIG. 7. Coactivation of multifunction neuron B4/5 with RM neuron B21 produced depression of synaptic transmission between the sensory cell and its follower motor neurons, B8a and B15. Repetitive spiking was elicited in B21 by delivery of trains of intracellular current pulses (4-s trains of 20-ms pulses at a 12-Hz rate). Evoked synaptic responses were simultaneously recorded in motor neurons B8a and B15 (controls at left and right). The evoked responses in B8a consisted of facilitating EPSPs leading to action potentials (filtered by the chart recorder). The evoked responses in B15 consisted of small electrotonic potentials and a slow EPSP. When B4/5 was fired simultaneously with B21, the B21 evoked chemical EPSPs in B8a and B15 were abolished (middle). Note, however, that the electrotonic synaptic potentials in B15 persisted. After termination of B4 activity, the evoked synaptic responses in B8a and B15 were rapidly restored. Recordings were made in artificial seawater (ASW). Amplitude calibrations for each of the traces as shown.

FIRING OF B4/5 CAN BLOCK CENTRIFUGAL AS WELL AS CENTRIPETAL B21 SPIKES. Consistent with the preceding interpretation are experiments in which B21 was activated by central input and antidromic spikes were recorded in the periphery. Central input to B21 was provided by depolarizing B82, which is electrically coupled to B21. When B82 was stimulated so that it fired at physiological rates (8–16 Hz), it evoked electrotonic potentials in B21, which could trigger action potentials that propagated to the periphery. Extracellular recording from the radula nerve indicated a one-to-one correspondence between distal impulses and intracellular spikes recorded in the soma of B21 (Fig. 4, left and right). When neuron B4/5 was fired at 20 Hz, at the same time that B82 was stimulated, the B82 spikes no longer evoked action potentials that could be recorded either in the soma of B21 or at its peripheral processes in the radula nerve (Fig. 4, middle). Thus

FIG. 8. A: depolarizing, constant-current, intracellular stimulation of CBI-2, sufficient to elicit repetitive spiking (bottom left) drove an ingestive motor program that included rhythmic bursting of neurons B4 and B19. Although stimulated with constant current, CBI-2 exhibited bursting activity due to phasic inhibitory feedback from the buccal ganglion, in part mediated by the activity of B19. The accelerated firing of CBI-2 is correlated with protraction of the odontophore and jaw opening. Vertical lines indicate the cessation of CBI-2 bursting, which based on observations of movements of the buccal mass, correlates with the transition from protraction (pro.) to retraction (retr.). Both B4 and B19 are active during the radula closure phase of the biting cycle; however, the activity of B4 leads that of neuron B19 and occurs before the transition. B: during the motor program driven by a moderate level of firing of CBI-2, B21 receives periodic inhibitory and excitatory synaptic inputs that occur before and after the phase transition from protraction to retraction.
B4/5 activity appears to block spikes in B21 that are propagated either toward or away from the cell body.

The blockade of B21 action potentials was also evident when B4/5 fired spontaneously during the time that B21 was intrasomatically stimulated so that it fired a train of spikes and evoked EPSPs that were simultaneously recorded from the ipsilateral and contralateral B8a motor neurons (Fig. 5). Presumably the ipsilateral EPSPs in B8a are due to action potentials traveling from a spike initiating zone near the B21 soma along the lateral process of B21, which appears to contact B8a (Fig. 2A). The EPSPs in the contralateral B8a are presumably mediated by spikes in B21 traveling in the opposite direction along the medial axon of B21, which passes to the contralateral hemiganglion. When spontaneous bursts of B4/5 action potentials occurred simultaneously with the intracellular stimulation of B21, the IPSPs evoked in B21 blocked the induced action potentials in B21 so that spikes could not travel in either direction, and the EPSPs in both the contralateral and ipsilateral B8a were blocked (Fig. 5, right column). The evoked inhibition of B21 spiking lasted ~500 ms, after which time both the ipsilateral and contralateral EPSPs reappeared (see Fig. 5, last 3 spikes in B21).

Chemical synaptic output of B21 is dependent on the membrane potential of the cell

The inhibitory synaptic input of B4/5 to B21 not only could function to block spikes in B21, but could, in principle, modulate the synaptic output of B21 even in the absence of spike blocking. This might be a consequence of the fact that synaptic outputs typically are dependent on the tonic membrane potential of the presynaptic cell. In Aplysia, tonic depolarization of the presynaptic terminal results in a graded increase of the transmitter released by a presynaptic spike (Shapiro et al. 1980). To further explore the effects of presynaptic membrane potential on the synaptic output of B21, we altered the steady-state membrane potential of B21 over a range of 15 mV and examined the consequences on the chemical synaptic output of B21 to B8a. Spikes in B21 were evoked by means of brief depolarizing pulses. Over the range of steady-state holding potentials examined, progressive depolarizations resulted in a graded increase in the EPSP B21 evoked in B8a (Fig. 6). The size of the B21 spike appeared to be largely unchanged over the range of membrane potentials studied, suggesting that the effect was not due to blockage of the B21 spike.

Effect of the firing of B4/5 on the chemical PSPs that B21 evokes in B8a and B15

When a train of action potentials was produced in B4/5 at the same time that a train of high-frequency spikes was elicited in B21, the excitation that B21 normally evoked in its follower motor neurons B8a and B15 was reduced or completely blocked (Fig. 7), even though the soma spikes in B21 were not blocked. Spikes were not blocked because B21 was directly fired by strong, repetitive depolarizing pulses. In the absence of the firing of B4/5, the firing of B21 at 12 Hz evoked facilitating
EPSPs in B8a that produced action potentials (Fig. 7, left). Firing of B21 also produced small electrotonic potentials and a slow EPSP in neuron B15. Simultaneous firing of both B21 and B4/5 resulted in abolition of the facilitating EPSPs and spikes evoked in B8a/b and B15. The synaptic potentials were also evoked when B4 was fired at high frequency (right).

Incorporation of the firing of B4 into buccal motor programs driven by CBI-1 and CBI-2

To begin to understand the functions of the inhibitory synaptic input produced in B21 by neurons B4/5, we examined the timing of the phasic firing of B4/5 during two different feeding motor programs: namely an ingestive program driven by command-like interneuron CBI-2 and an egestive motor program driven by mechanosensory interneuron CBI-1 (Rosen et al. 1991). In previous studies that utilized a buccal mass preparation (Rosen et al. 1988), it was shown that depolarizing, constant current, intracellular stimulation of CBI-2 produced a robust buccal motor program that usually generated rhythmic biting-like movements of the buccal mass. Simultaneous video recording of the buccal mass movements and CBI-2 activity, indicated that during the initial phase of each biting cycle, accelerated firing of CBI-2 was correlated with the opening of the jaws and the protrusion of the odontophore with the radula open (Rosen et al. 1988). During the inhibitory phase of CBI-2 activity, the odontophore was retracted and the radula and jaws
Excitatory and inhibitory effects of interneuron B19 on chemical EPSPs that B21 evokes in B8a and B15

In addition to intraganglionic interneurons such as B4/5, the buccal ganglion contains interganglionic interneurons that project their axons to the cerebral ganglion via the cerebral-buccal connective. To determine whether this type of neuron firing during an ingestive motor program, we monitored the activity of B4/5 at the same time that CBI-2 was fired to drive a motor program in the odontophore preparation. Moreover, we used the termination of the CBI-2 burst as a marker of the phase transition between retraction and retraction. As suggested by previous reports (Church and Lloyd 1994; Rosen et al. 1991), we found that B4/5 exhibited vigorous rhythmic bursting when CBI-2 received inhibition. A detailed analysis, however, indicated that in five of six preparations examined, B4/5 began to fire 1 s or more before the spiking in CBI-2 was terminated (Fig. 8A). Although B4/5 fired in phase with interneuron B19, which fires during the retraction phase of the CBI-2 driven program, B4/5 typically began to fire before the onset of firing in B19. Early firing of B4/5 relative to B19 was similar to the relatively early firing of B4/5 when compared with the firing patterns of numerous retraction phase motor neurons (Church and Lloyd 1994). As indicated in Fig. 8B and elsewhere (Rosen et al. 2000), B21 receives phasic inhibitory and excitatory input when a motor program driven by moderate levels of CBI-2 stimulation is initiated and maintained. To help determine whether the inhibitory input correlated with the onset of the B4/5 burst, a slower motor program was driven by relatively weak firing of CBI-2 (Fig. 9). Under these conditions the interval of time between the onset of intense B4/5 bursting and the onset of B19 bursting increased, as did the duration of the bursting of each of these cells. The resulting motor program showed that the onset of the inhibitory input correlated with the onset of the B4/5 burst and is presumably due at least in part to the IPSPs that B21 receives from B4 and B5. Despite the fact that B4/5 produced inhibition in B21, late during a burst of firing of B4/5, B21 exhibited an abrupt return to resting potential, or a depolarization beyond resting potential. The depolarizing potentials corresponded to the time that B19 exhibited its peak of bursting activity and are consistent with the fact that B19 activity excites B21 (Rosen et al. 2000). The data suggest that during the feeding cycle, the activity of B4/5 occurs at or about the phase transition from protraction to retraction, and this should not be equated with the transition from radula opening to radula closing, which is not necessarily in exact synchrony with retraction (Rosen et al. 1998).

Unlike the relatively late burst of B4/5 activity evoked by CBI-2, CBI-1 evoked a nonrhythmic rejection program characterized by a short-latency burst of B4/5, often followed by a second burst (Church and Lloyd 1994; Rosen et al. 1991). In recordings of the synaptic potentials evoked in B21 during the motor program driven by CBI-1, an inhibitory burst of input was observed (Fig. 10, left arrow). The latency of this burst is similar to that previously shown for the burst of B4/5 (Rosen et al. 1991). The burst occurred in the absence of firing of radula closure motor neuron B8a, a pattern expected for a rejection response. There were also indications of a second inhibitory burst (Fig. 10, right arrow), which might be related to the second B4/5 burst that is often evoked by CBI-1.

Figure 13. Interneuron B19 produced heterosynaptic modulation of discrete EPSPs that RM neuron B21 evoked in motor neuron B8a: A: B21 was repetitively stimulated with intracellular current pulses of 20 ms duration so that the cell fired action potentials at a rate of 8 Hz (middle traces). During a brief period in which successively evoked EPSPs in B8a increased in amplitude (synaptic facilitation, not shown), a stable EPSP of constant amplitude was produced (top traces). When depolarizing intracellular current was injected into the soma of B19 for 4 s, the cell fired a burst of action potentials (bottom left trace). The net effect of the current injection was to increase the amplitude of the evoked EPSPs recorded in neuron B8a (top left trace, middle portion). When hyperpolarizing intracellular current was injected into the soma of B19, the net effect of the current injection was to decrease the amplitude of the evoked EPSPs recorded in neuron B8a (top right trace, middle portion). B: an expanded time base for recordings of data presented in A showed the effects of intracellular current injection into B19 on unitary EPSPs that action potentials elicited in B21 evoked in neuron B8a. Top pair of traces shows control B21 spikes and evoked EPSPs recorded in B8a before (left) and after (right) B19 was depolarized. Middle traces show the effects of B19 depolarization (B19 depol.) on the B21 spike and on the EPSP it evoked in neuron B8a. Note the increase in both the B21 spike amplitude and the B8a EPSP amplitude. Bottom pair of traces shows B21 spikes and evoked EPSPs recorded in B8a before (left) and after (right) B19 was hyperpolarized (middle, B19 hyperpol.). Note the decrease in both the B21 spike amplitude and the B8a EPSP amplitude.
Follower motor neurons were decreased in amplitude (Fig. 12, constant current pulse, the EPSPs that B21 evoked in its follower motor neurons (Fig. 12). Conversely, when B19 was hyperpolarized by a B8a and B15 (Fig. 12). EPSPs that B21 normally evoked in its follower motor neurons were decreased in amplitude (Fig. 12D). Under conditions in which B21 was fired at high frequency, the EPSPs that B21 evoked in its follower motor neurons were decreased in amplitude (Fig. 12C, compare with A and D). Under conditions in which B21 was fired at high frequency, the EPSPs that B21 evoked in B8a exhibited temporal summation, and it was not clear to what extent changes in the magnitude of the summed potential were due to changes in the size of the individual PSPs rather than to changes in the rate of decay of the PSPs. By firing B21 at a relatively low rate (10 Hz), it was possible to see discrete PSPs it evoked in B8a (Fig. 13A), and these were revealed more clearly at a fast sweep speed (Fig. 13B). Under this condition, when B19 was depolarized 20 mV above its resting potential, the amplitude of individual EPSPs could increase by 150–200% of controls (Fig. 13A, left; B, top). The increase in EPSP amplitude persisted as long as B19 was depolarized and almost immediately returned to control levels on termination of the B19 current injection. On the other hand, when B19 was hyperpolarized, the individual EPSPs that B21 evoked in B8a decreased to 40–60% of control (Fig. 13A, right; B, bottom). Again the effect was short-lived.

Incorporation of the firing of B19 into the motor program driven by CBI-2

B19 was consistently found to exhibit rhythmic bursting activity during the ingestion-like motor program driven by CBI-2 (Figs. 8A and 9). As previously noted, its activity was confined to the odontophore retraction phase (n = 20 preparations). Unlike B4/5, its bursting did not bridge the transition from protraction to retraction.

Excitatory and inhibitory effects of the firing of motor neuron B82 on PSPs that B21 evokes in B8a/b and B15

It was previously shown (Rosen et al. 2000) that B21 has a particularly strong electrical synaptic input from motor neuron B82. As was the case for B19, depolarization and firing of B82 increased the summed EPSPs that B21 evoked in follower motor neurons B8a and B15 (Fig. 14B, compared with controls, Fig. 14, A and D). Conversely, hyperpolarization of B82 decreased the summed EPSPs that B21 evoked in follower motor neurons B8a and B15 (Fig. 14C, compared with controls, Fig. 14, A and D). The effects of hyperpolarization or depolarization of B82 could be seen in individual EPSPs when B21 was repetitively fired at 8 Hz and B82 was either depolarized or hyperpolarized 20 mV from its resting membrane potential (Fig. 15). In the control condition when B82 was at rest (Fig. 15, middle pair of traces), the amplitude of the EPSPs that B21 evoked in B8a went from <1.0 mV for the first few EPSPs to an average of 8.2 mV. When B82 was depolarized by 20 mV (Fig. 15, top pair of traces), the amplitude of the EPSPs that B21 evoked in B8a went from <1.0 mV for the first few EPSPs to an average of 10.4 mV. When B82 was hyperpolar-
The amplitude of the EPSPs that B21 evoked in B8a went from 1.0 mV for the first few EPSPs to an average of 6.2 mV. The final amplitude for the summated potentials was 30 mV for the control, 45 mV for the depolarized, and 15 mV for the hyperpolarized conditions.

Incorporation of B82 into the motor programs driven by CBI-1 and CBI-2

In an attempt to understand the possible functional significance of the modulatory actions of motor neuron B82 on the outputs of sensory neuron B21, variations of the membrane potential of B82 were recorded during bite-like motor programs driven by firing CBI-2. During such motor programs, B82 received phasic depolarizing synaptic inputs that caused it to fire periodic bursts of spikes (Fig. 16). In the example shown, CBI-2 exhibited a typical pattern of bursting interrupted by strong inhibitions. The onset of the inhibition is coincident with the onset of odontophore retraction, which occurs when the radula is fully closed. Each B82 burst occurred during the same interval that B21 received its rhythmic excitatory synaptic input. B82 was also found to fire during motor programs driven by CBI-1 and during similar spontaneous programs associated with egestive movements of the buccal mass (Fig. 17). B82 received excitatory synaptic inputs during a cycle of the egestive program sufficient to cause it to fire late in the program, in conjunction with radula closer motor neurons (see spikes in the radula nerve recording). B82 spiking also occurred when B21 received excitatory input during the motor program.

Other radula mechanoafferent neurons can produce phase-dependent modulation of the synaptic output of B21

To determine whether other sensory cells could modify the output of B21, we examined the effects of other immunoreactive synaptic inputs. B82 was also found to fire during motor programs driven by CBI-1 and during similar spontaneous programs associated with egestive movements of the buccal mass (Fig. 17). B82 received excitatory synaptic inputs during a cycle of the egestive program sufficient to cause it to fire late in the program, in conjunction with radula closer motor neurons (see spikes in the radula nerve recording). B82 spiking also occurred when B21 received excitatory input during the motor program.

FIG. 15. Changes in the membrane potential of motor neuron B82 produced heterosynaptic modulation of the facilitating EPSPs that RM neuron B21 evoked in motor neuron B8a. B21 was repetitively stimulated with trains of intracellular current pulses of 20 ms duration so that the cell fired action potentials at a rate of 8 Hz (2nd, 4th, and 6th traces). When intracellular current injection depolarized neuron B82 by 20 mV (B82 depol.), the facilitating EPSPs that B21 evoked in B8a increased in amplitude (top trace) compared with the control condition when B82 was at its normal resting potential (3rd trace). Records show the net effects of synaptic facilitation and temporal summation of successively evoked unitary EPSPs. When intracellular current injection hyperpolarized neuron B82 by 20 mV (B82 hyperpol.), the facilitating EPSPs that B21 evoked in B8a decreased in amplitude (5th trace) compared with the control condition. Temporal summation produced a smaller overall depolarization of B8a.

FIG. 16. Phase-specific bursts of action potentials recorded in motor neuron B82 during the motor program driven by command-like interneuron CBI-2. Depolarizing, constant-current, intracellular stimulation of CBI-2, sufficient to elicit repetitive spiking, drove a motor program that included phasic bursting of neurons (e.g., B8a). The phasic synaptic input that caused B82 to fire rhythmic bursts of action potentials overlapped the transition from CBI-2 bursting to CBI-2 inhibition. Phase-specific synaptic input to B21 is also shown.

FIG. 17. Phase-specific burst of action potentials in motor neuron B82 during a spontaneous cycle of an egestion-like buccal motor program. Control recordings were made of radula nerve (Rad. n.) activity (extracellular) and intracellular B4 activity. In this experiment B21 was repeatedly stimulated with trains of brief (20 ms), depolarizing, intracellular current pulses sufficient to evoke full-blown spikes. Note that the current pulses failed to evoke spikes during the intervals of intense B4 bursting.
tive RM neurons, which are known to be electrically coupled to B21 (Rosen et al. 2000). We found that the effects of membrane potential changes of RM neuron B22 on the outputs of B21 were similar to that of other cells that are electrically coupled to B21 (e.g., B19 or B82). Specifically, when a RM neuron was depolarized and fired, the chemical EPSPs that B21 evoked in the follower motor neuron B8a and B15 were increased in amplitude (Fig. 18B) compared with control conditions (Fig. 18A and D). When B22 was hyperpolarized, the EPSPs that B21 evoked in its follower motor neurons were decreased in amplitude (top and below top traces). D: following control responses that were similar to A when the B22 cell was not stimulated.

**DISCUSSION**

In a previous study we found that the synaptic output of B21 exhibits a type of plasticity that is the outcome of previous activity of the synapse. This type of plasticity has been termed “homosynaptic plasticity” to distinguish it from heterosynaptic plasticity, which results from the action of one synapse on another synapse (Kandel 1976). The data in the present paper indicate that the various synaptic outputs of RM neuron B21 can be enhanced or depressed by means of extensive heterosynaptic inputs provided by remarkably diverse categories of other neurons. The heterosynaptic modulation differs in a number of respects from the well-known examples of presynaptic depression and facilitation, extensively documented for sensorin-containing mechanosensory neurons involved in defensive reflexes in *Aplysia* (Byrne and Kandel 1996). First, unlike cells mediating defensive responses, the modulation of B21 is very short lasting and appears to be related to the alterations of membrane potential that the heterosynaptic activity produces in it. Second, the modulation of B21 can occur rhythmically, and in phase with motor output to the structure (radula) that contains the B21 sensory endings. The gain of the B21 transmission can be either increased or decreased at different times, because the cell receives both excitatory as well as inhibitory synaptic inputs.
B21 is in intercommunication with at least three major classes of neurons involved in generating buccal mass movements: 1) other radula sensory neurons, 2) buccal motor neurons, and 3) pattern-generating interneurons and/or premotor cells (Fig. 20, left). In addition, B21 receives a depolarizing input from at least one modulatory neuron, the metacerebral cell (MCC) (Alexeeva et al. 1998). The extensive synaptic inputs from the various classes of neurons provide an opportunity for different types of neuronal processing to adjust the gain of the sensory signals coming from the radula (see also Fischer and Carew 1993). The inputs from motor neurons and interneurons provide a simple means for an efferent discharge (Grusser 1995) to functionally adjust sensory input. Inputs from other sensory cells can serve to ensure that sensory flow into the nervous system only occurs in response to appropriate stimuli, e.g., those contacting at least a minimal amount of the receptive field. In addition, as has been suggested for electrically coupled mechanoreceptors in the crayfish (El Manira et al. 1993), the coupling could serve to facilitate sensory transmission conveyed by the conjoint activation of functionally related afferents.

Interconnections with motor neurons provides a means of enhancing the output of B21

B21 makes diverse types of synaptic connections with motor neurons such as B8a/b, B15, and B82 (Fig. 20, right). The electrical connections to motor neurons such as B15 and B82 provide a source of depolarizing input to B21 directly related to the excitation and firing of the motor neurons. It should be noted that not all motor neurons provide excitatory input to B21, and that input from motor neurons that fire during the phase of radula closing is particularly prominent. Because B21 has effects on pattern-generating neurons (see Reciprocal interactions with higher order control elements), modulation of its outputs by motor neurons provides one means by which the activity of motor neurons can influence pattern generation in mollusks (Hurwitz et al. 1994; Staras et al. 1998).

Multifunction neurons B4/5 mediate phasic inhibition of B21

A major source of inhibition that serves to decrease the synaptic output of B21 is provided by the firing of B4/5 (Fig. 20, right), which is a paired multifunctional interneuron that produces synaptic outputs to numerous buccal motor neurons (Gardner 1971; Jahan-Parwar et al. 1983; Ono 1989; Rosen et al. 1982). The IPSPs that B4/5 and homologous neurons produce on motor neurons appear to alter the timing of the firing of the motor neurons (Nagahama and Takata 1990), but an additional role for the inhibitory outputs of B4/5 may be to suppress or gate sensory information to the feeding network by actions on RM cells. Our evidence suggests that this gating might be due to a direct effect of membrane potential of the RM cells on transmitter release. Gating may also involve spike failure, which is consistent with morphological data indicating
that the terminals of B4/5 contact B21 at its soma and along its thick medial axon (Fig. 2C), which is a likely site of spike failure. An integrative role for spike failure of the axons of afferent processes has been suggested for a variety of systems (Chiel et al. 1990; Mar and Drapeau 1996; Van Essen 1973).

B4/5 activity appears to block spikes in B21 that are propagated either toward or away from the cell body. The functional significance of centrifugally directed spikes is unknown. The findings that B21 contains neuroactive peptides and that the fine peripheral processes of the cell in the subradula tissue contain varicosities (Miller et al. 1994; Rosen et al. 2000) suggest the possibility that centrifugal spikes might evoke the release of bioactive peptides in the subradula tissue.

B4/5 appears to fire intensely just before the transition from odontophore protraction to retraction. The inhibition of B21 produced by B4/5 may help ensure that sensory stimulation of the radula during ingestive behavior (e.g., biting) does not result in RM outputs that can contribute to premature radula closure during the transition from protraction to retraction. Later, in the retraction phase of a biting cycle, heterosynaptic inhibition of the RM cells is terminated or replaced by heterosynaptic facilitation, and this may enable radula stimuli to enhance radula closing on food objects, or perhaps adaptively regulate the force of closure, according to the mechanical properties of the food. Thus the alterations of the gain of the sensory signal may function as a form of attentional device that permits the transfer of information only at moments of time that the information is functional. Movement-related inhibitory input to primary sensory mechanoafferents is a prominent feature in arthropods, in which diverse roles for this type of gating has been suggested (Burrows 1996; Krasne and Byran 1973). Similar functions are also served by gating of higher order sensory information, as in saccadic suppression in vertebrates (Lee and Malpeli 1998).

It should be noted that B4/5 may also be active during egestive (rejection) movements as well as ingestive movements, and that its firing phase relative to motor neurons is different for the different behaviors (Church and Lloyd 1994). It may be that during the interval in which the open radula moves backward toward the esophagus to grasp inedible objects, it is crucial that RM sensory signals produced by objects contacting the radula be suppressed so that the excitatory input to radula closer neurons decreases. When the radula then closes on the object and moves forward, excitation and termination of suppression of B21 could facilitate the regulation of radula closure. When the radula opens and releases the object, it would be useful to again suppress B21 function with a second burst of B4/5 activity, so that the object is not grasped and drawn back into the esophagus. Just as motor neurons and muscles can be engaged in multiple behaviors, sensory neurons also typically function in different behaviors. Thus it makes functional sense for their response properties and outputs to be optimized according to the specific needs of the behavior they are engaged in. The heterosynaptic inputs from motor neurons, interneurons, and other sensory cells, may function to provide this optimization.

FIG. 21. Schematic diagram depicting a postulated mechanism whereby the chemical synaptic transmission between B21 and its follower motor neurons is modulated by 3 states of neurons that affect the membrane potential of the cell (see text for details of the postulated mechanisms). Regions of neurons exhibiting action potentials or substantial depolarizations are indicated in black. The extent of transmitter release is indicated by the number of small black dots outside of the synaptic terminals.
Reciprocal interactions with higher order control elements

B21 has electrical synaptic connections to pattern-generating interneurons such as B19, and it has been shown that B21 is also coupled to pattern-generating neurons such as B64 and B51 (Evans and Cropper 1998; Rosen et al. 2000; C. G. Evans and E. C. Cropper, personal communication). These neurons fire during the radula closure/retraction phase of the digestive motor program, suggesting that a sensory neuron such as B21 by virtue of its central connectivity might also contribute to, and therefore be a part of, the underlying mechanisms of the feeding pattern generation (Evans and Cropper 1998; Pearson 1993). Pattern generator interneuron B19 can provide input to, as well as receive input from B21 by means of electrical connections. B19 also projects an axon to the cerebral ganglion and provides phase-specific inputs to cerebral-to-buccal interneurons, which are involved in the initiation and patterning of buccal ganglion programs (Rosen et al. 1991). It is interesting that for mammalian rhythmic feeding motor programs, there is also evidence that primary sensory neurons generate both antidromic and orthodromic spikes and may be involved in the dual roles of sensory processing as well as motor pattern generation (Lund et al. 1998).

Output states of B21

Figure 21 is a cartoon illustration of our thinking of how the outputs of B21 are regulated (darkened portions of cells in Fig. 21 indicate regions of active spikes; synaptic output is represented by dots). B21 can be thought of as operating in three overlapping states in which its synaptic output is either low, completely blocked, or enhanced. In the resting state, during which the radula is partially open (Fig. 21, rest/open), and neuronal activity is minimal, peripheral afferent spikes, at least at low frequency, invade only part way to the main branches and terminals of the cell (see Fig. 7 in Rosen et al. 2000). Thus in this condition little or no synaptic output from the cell is present. During the initial part of the radula closing phase (Fig. 21, early close), activity of cells such as B4/5 hyperpolarizes B21, resulting in a direct reduction of its synaptic outputs, as well as an indirect reduction that results from blockade of afferent spikes (see Fig. 3). Finally, during the late phase of radula closing (Fig. 21, late close), excitatory inputs from cells such as B19, result in increased invasion of the peripheral spikes as well as enhanced transmitter release (see Figs. 6 and 13).

Optimization of sensory processing

The synaptic inputs that impinge on B21 provide the cell with a means of altering its gain as a function of context that is defined by other sensory inputs as well as by signals provided by circuitry associated with motor pattern generation. In addition, the transmission of information by the RM neurons is conditioned by local contraction of the subradula tissue (Cropper et al. 1996). The modulatory affects at the RM cells are provided, in part, directly by a corollary discharge of the motor neurons and various classes of interneurons that constitute the mediating circuitry that controls the generation of movements. In the crayfish, sensory gain is decreased by central signals to protect sensory cells from reafferent activity that can produce depression of the synaptic output of the sensory cells (Grusser 1995; Krasne and Byran 1973). This is unlikely to be a func-

tion of the depression of RM gain in Aplysia because the outputs of the RM cells do not exhibit homosynaptic depression. Furthermore, because the modulation at the RM cells is rhythmic, it is unlikely to serve a function such as has been suggested to occur in the crayfish in which an extrinsic modulatory system associated with a particular behavioral state serves to ensure that incompatible classes of responses (e.g., feeding and escape) do not occur simultaneously (Krasne and Lee 1988; Krasne and Wine 1975). It has been suggested that in mammals sensory inputs are adaptively modified by the highly complex circuitry of the cerebellum, which acts as an extrinsic input to the mediating circuitry that generates the behavior (Bower 1997; Courchesne 1997; Miall et al. 1996). The current results indicate that in Aplysia, considerable modulation of sensory signals can be accomplished by the sensory, motor, and interneuronal circuitry that actually mediates behavior. Considerable data already indicate that the efficiency of motor systems in Aplysia is improved not only by the action of specialized extrinsic modulatory systems that are not involved in generating behavior, but also by peptidergic co-transmission within the intrinsic circuitry that generates responses. The outputs of the radula sensory neurons are also affected by extrinsic modulatory systems, in particular, the serotonergic metacerebral cells (Alexeeva et al. 1998). The array of inputs to the RM sensory neurons of Aplysia is an example of a parallel, distributed organization that provides an opportunity for different classes of mediating neurons to directly influence, and in turn, be influenced by sensory processes.

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