Acta Biologica Hungarica 55 (1–4), pp. 211–220 (2004)

REGULATION OF AFFERENT TRANSMISSION IN THE FEEDING CIRCUITRY OF *APLYSIA**

ELIZABETH C. CROPPER,** C. G. EVANS, J. JING, A. KLEIN, A. PROEKT, A. ROMERO and S. C. ROSEN

Department of Physiology and Biophysics, Mt. Sinai School of Medicine, One Gustave Levy Place, New York, NY 10029, USA

(Received: August 31, 2003; accepted: December 1, 2003)

Although feeding in *Aplysia* is mediated by a central pattern generator (CPG), the activity of this CPG is modified by afferent input. To determine how afferent activity produces the widespread changes in motor programs that are necessary if behavior is to be modified, we have studied two classes of feeding sensory neurons. We have shown that afferent-induced changes in activity are widespread because sensory neurons make a number of synaptic connections. For example, sensory neurons make monosynaptic excitatory connections with feeding motor neurons. Sensori-motor transmission is, however, regulated so that changes in the periphery do not disrupt ongoing activity. This results from the fact that sensory neurons are also electrically coupled to feeding interneurons. During motor programs sensory neurons are, therefore, rhythmically depolarized via central input. These changes in membrane potential profoundly affect sensori-motor transmission. For example, changes in membrane potential alter spike propagation in sensory neurons so that spikes are only actively transmitted to particular output regions when it is behaviorally appropriate. To summarize, afferent activity alters motor output because sensory neurons make direct contact with motor neurons. Sensori-motor transmission is, however, centrally regulated so that changes in the periphery alter motor programs in a phase-dependent manner.

Keywords: Invertebrate – central pattern generator – sensori-motor integration – mollusc – feeding

EFFECT OF AFFERENT ACTIVITY ON FEEDING CIRCUITRY

Although consummatory feeding in *Aplysia* is mediated by a central pattern generator (CPG), the activity of this CPG is clearly modified by afferent input under physiological conditions. For example, consummatory responses are adjusted when animals that have been making exploratory bites successfully grasp food and ingest it [9]. Additionally, animals can modify consummatory responses to adjust for particular characteristics of the ingested food [6]. For example, bite strength can be increased if increased resistance is encountered as food is ingested.

* Presented at the 10th ISIN Symposium on Invertebrate Neurobiology, July 5–9, 2003, Tihany, Hungary.

** Corresponding author; e-mail: elizabeth.cropper@mssm.edu

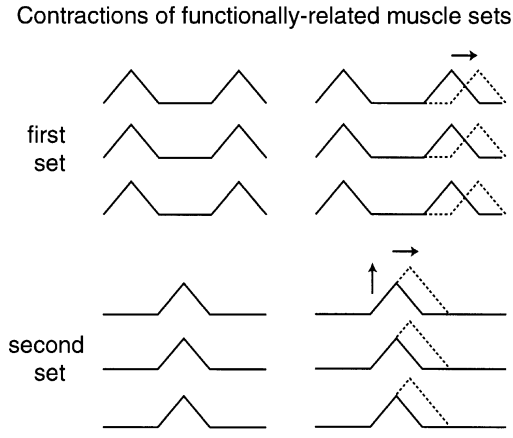

Fig. 1. When feeding movements are altered, the activity of multiple neuromuscular units is adjusted. In part this is a result of the fact that feeding movements are generally mediated via more than one set of functionally related muscles (as is indicated on the left). Additionally, when there is a change in the timing of one movement, compensatory adjustments are made in the antagonistic movement. On the right, this idea is indicated via a prolongation and enhancement of the activity in one set of muscles (dashed lines in the bottom three traces). Activity in the antagonistic muscles is therefore delayed (dashed lines in the top three traces)

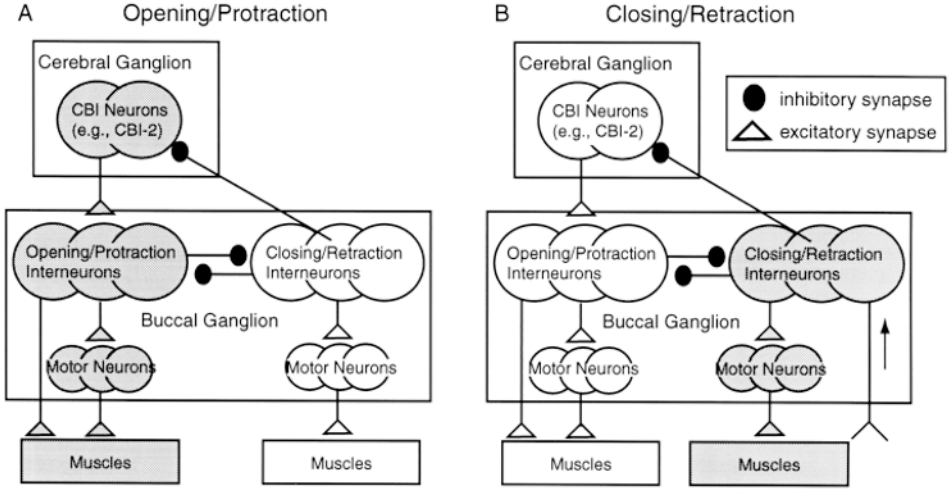

Fig. 2. Ingestive motor programs in *Aplysia* consist of at least two phases. (A) In the first phase, command-like neurons such as CBI-2 are activated, as is the circuitry that mediates radula opening and protraction (gray). (B) In the second phase, CBI-2 and the radula opening/protraction circuitry is inhibited and the circuitry that mediates radula closing and retraction is activated (gray)

When afferent-induced changes in motor programs occur, the activity of multiple neuromuscular units is adjusted (Fig. 1). In part this results from the fact that movements are mediated via more than one muscle. A change in the strength of a movement can, therefore, involve parallel changes in the activity of a number of functionally related neuromuscular systems. Additionally, consummatory motor programs consist of at least two phases; a radula protraction phase followed by a radula retraction phase (Fig. 2) [16]. Changes in the duration of one phase are, therefore, accompanied by compensatory changes in temporal characteristics of the antagonistic phase (Fig. 1). One question our research has addressed is, How do changes in afferent activity produce the widespread changes in the activity of the feeding circuitry that are necessary for changes in feeding behavior?

To address this issue we have concentrated on one change in consummatory responses that occurs when *Aplysia* that have been making exploratory bites encounter food and successfully ingest it. When this occurs a bite is converted to a bite-swallow, and radula movements are altered [3, 9]. Specifically, the radula retraction phase of behavior is prolonged and enhanced so that food will be pulled into the buccal cavity and deposited in the esophagus (Fig. 3). Since the retraction phase of behavior is prolonged, the subsequent radula protraction is delayed.

We have identified two classes of sensory neurons that are activated when bites are converted to bite-swallows, i.e., radula mechanoafferents and retraction proprioceptors [4, 10, 14, 15]. Both types of sensory neurons will presumably be activated during the radula retraction phase of ingestive motor programs [1, 4, 15]. Radula mechanoafferents will be activated as the radula closes on food [15]. These cells presumably trigger bite to bite-swallow conversions. Retraction proprioceptors will be

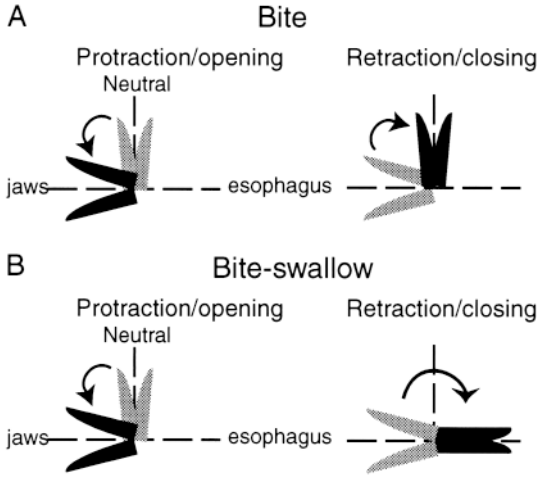

Fig. 3. Radula movements during a bite and a bite-swallow. (A) When *Aplysia* bite the radula is initially protracted and opened (left). The radula then closes and retracts so that it returns to a neutral position (right). (B) When a bite is converted to a bite-swallow the radula closing and retraction phase of behavior is enhanced and prolonged so that food will be deposited into the esophagus (right)

activated by the increase in resistance to backward rotation that occurs as food is pulled into the buccal cavity [4]. Activity in proprioceptors is likely to be at least in part responsible for the fact that closing/retraction is not simply enhanced in an all or none way when food is ingested. Instead stimulus properties are taken into account.

To determine how afferents produce widespread effects on feeding motor programs, synaptic connections of sensory neurons have been characterized [4, 13–15]. These experiments have primarily concentrated on B21 (the largest, most well-characterized radula mechanoafferent) [15], and B51 [13] (the largest, most well-characterized retraction proprioceptor) [4]. B21 and B51 are both centrally located [13, 15], and directly make a number of chemical and electrical synaptic connections in the buccal ganglion (Fig. 4) [4, 13–15]. In fact, B51 was originally described as a premotor neuron [13]. In general B21 and B51 make excitatory fast chemical or electrical connections with circuitry that mediates radula closing and retraction (Fig. 4). Increases in the activity of B21 and B51 will, therefore, directly enhance closing and retraction movements. Additionally, both cells are electrically coupled to interneurons that make fast inhibitory connections with neurons that mediate radula opening and protraction (Fig. 4). If activity in B21 and B51 prolongs the radula closing and

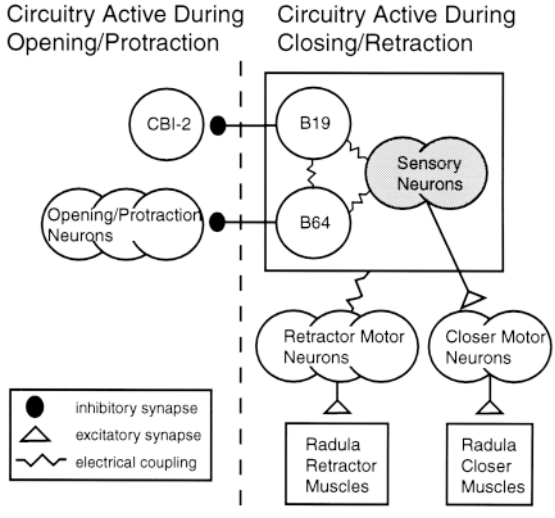

Fig. 4. Schematic summary of characterized synaptic connections of radula mechanoafferents and retraction proprioceptors (data from [4, 7, 13–15]). Mechanoafferents and proprioceptors are electrically coupled to each other so for simplicity are represented together as sensory neurons (gray). Connections with motor neurons are shown outside the box. Note that sensory neurons are electrically coupled to motor neurons that produce radula retraction, and that sensory neurons make excitatory chemical connections with motor neurons that produce radula closing. Sensory neurons are also electrically coupled to retraction interneurons (e.g. B64 and B19) (inside the box). These interneurons make inhibitory connections with the circuitry active during the radula opening/protraction phase of ingestive motor programs. Increased activity in sensory neurons can, therefore, enhance and prolong activity in the radula closing/retraction circuitry and delay the subsequent activation of the radula opening/protraction circuitry

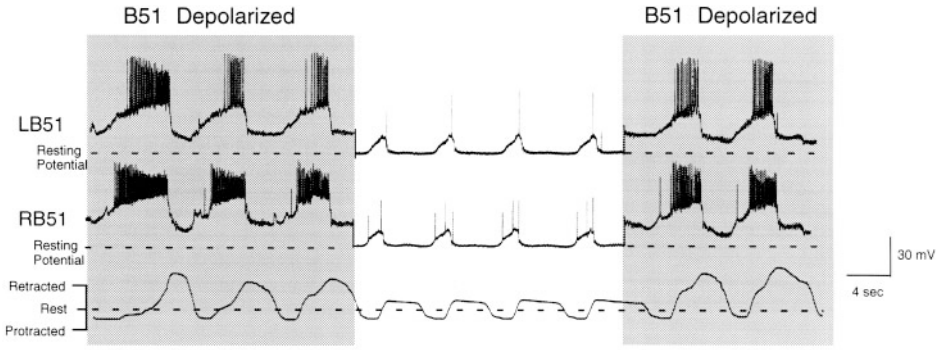

Fig. 5. Current injection into the retraction proprioceptor B51 produces significant changes in radula movements during an ongoing motor program. This motor program was induced by carbachol application to the cerebral ganglion [17]. The top two traces are intracellular recordings from the left and right B51, respectively. Dotted lines indicate the resting potentials of cells. The bottom trace is the output of a transducer that monitored radula protraction and retraction [4]. The transducer was set so that radula retraction produced an upward deflection, and radula protraction induced a downward deflection. Note that retraction movements were enhanced when B51 was depolarized so that it spiked during the retraction phase of behavior (gray sections of the recordings) (after [4])

retraction phase of motor programs it could, therefore, simultaneously inhibit the circuitry that mediates radula opening and protraction and thereby delay its activation.

To determine the effectiveness of changes in afferent activity on the feeding circuitry we have performed experiments in which we have triggered fictive feeding motor programs and then injected current into single sensory neurons to determine whether rhythmic activity is significantly altered [4, 8]. These experiments have been conducted both in the isolated nervous system [8], and in semi-intact preparations in which radula movements were monitored [4]. In general, we found that if we activated sensory neurons during the closing/retraction phase of motor programs, radula retraction movements were enhanced (e.g. Fig. 5), and the firing frequency of radula closer motor neurons was increased [8]. We have shown, therefore, that activation of sensory neurons can produce widespread changes in fictive feeding motor programs. This is a result of the fact that sensory neurons are centrally located and directly and indirectly make contact with a number of feeding neurons.

A second issue we have addressed concerns the integration of peripherally generated and centrally generated activity during a motor program. Thus, both types of sensory neurons that we have studied are striking in that they receive synaptic input during motor programs that can alter the transmission of afferent information to follower cells. We have most extensively studied this phenomenon in experiments with the radula mechanoafferent B21 and one of B21's followers, B8 [1, 5, 15]. Our initial analyses have also primarily concentrated on the regulation of afferent transmission during fictive feeding motor programs that are ingestive in nature. Below we separately discuss B21 mechanoafferent transmission during the two antagonistic phases of ingestive motor programs.

Afferent transmission during the radula opening and protraction phase of ingestive motor programs

Studies of the regulation of afferent transmission were initially triggered by the finding that the mechanoafferent B21 is activated at a time when it would not be expected that the follower neurons, the B8 cells, would receive excitatory input. Specifically, we found that B21 is peripherally activated during the radula protraction phase of fictive ingestive programs, when the tissue B21 innervates, the subradula tissue, contracts [1, 2]. Excitatory input to the B8 neurons would not be expected during radula protraction because it would tend to be counterproductive (Fig. 6). The B8 neurons are radula closer motor neurons [11, 12] so increased activity in these cells would tend to make the radula close as it protracts. This is counterproductive since it will tend to push food out of the buccal cavity (instead of pull it in). To summarize, B21 is peripherally activated during the radula protraction phase of ingestive motor programs [1]. If afferent transmission were not regulated this would tend to disrupt ingestive activity.

To determine how sensori-motor transmission might be regulated, we first studied afferent transmission under resting conditions. Interestingly, we found that B21 mechanoafferent input was not transmitted to B8. Specifically, we showed that B21-induced PSPs were not recorded in B8 if B21 was peripherally activated when it is at its resting membrane potential (Fig. 7A1) [1, 5, 15]. Afferent transmission did not occur because spikes were not actively propagated to the B21 process that is the primary point of contact with B8 (the lateral process) (Fig. 7A2) [1, 5]. In contrast, if B21 was centrally depolarized and then peripherally activated, spikes were actively propagated to the lateral process (Fig. 7B2), and PSPs were observed in B8 (Fig. 7B1). Thus, we have shown that peripherally triggered activity in B21 will not be transmitted to B8 unless B21 is also central depolarized.

When rhythmic activity is triggered in the isolated nervous system, B21 is not centrally depolarized during the radula protraction phase of the motor program [1, 5, 14]. This suggests that afferent input will not be transmitted to B8. Consistent with this

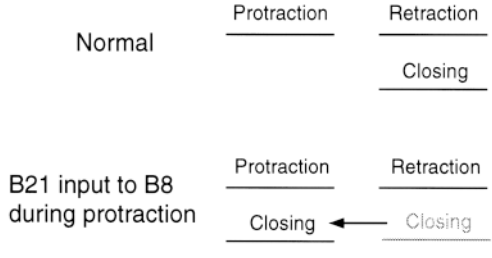

Fig. 6. During ingestive motor programs the radula closes as it retracts, which will pull food into the buccal cavity (Fig. 3B). If B21 provides excitatory input to the radula closer motor neuron B8 during the protraction phase of motor programs the radula will tend to close as it moves forward. This will be counterproductive since it will push food out of the buccal cavity

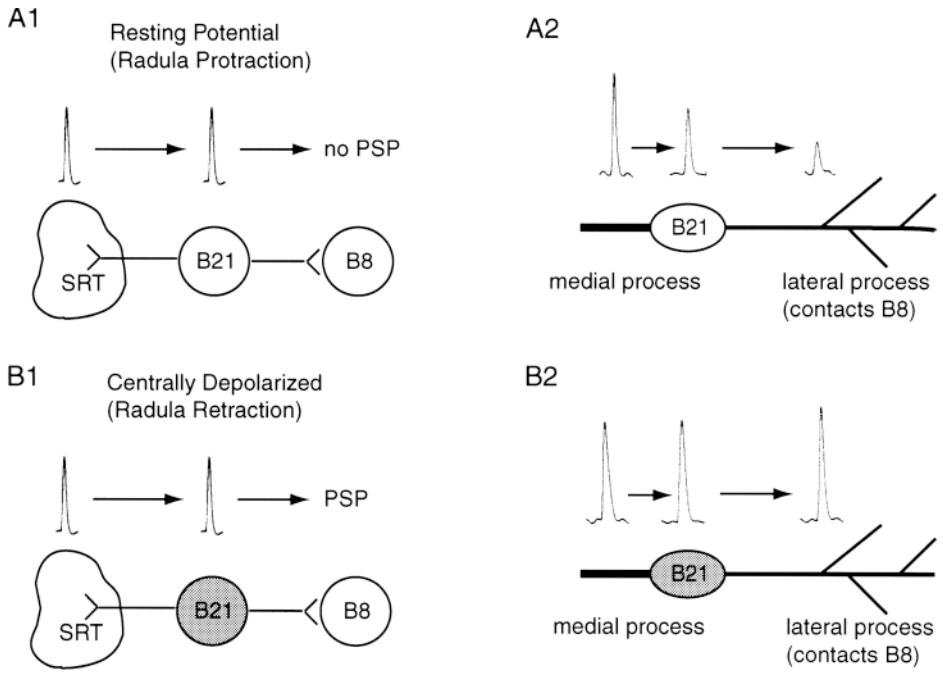

Fig. 7. B21 mechanoafferent input is only transmitted to the follower neuron B8 when B21 is centrally depolarized. (A) When B21 is peripherally activated at its resting potential, afferent transmission does not occur, i.e. postsynaptic potentials (PSPs) are not recorded in B8 (A1). Afferent transmission does not occur because spikes are not actively propagated to B21's lateral process (the primary point of contact with B8) (A2). (B) When B21 is peripherally activated while it is centrally depolarized, PSPs are recorded in B8 (B1). Afferent transmission occurs because the propagation failure is relieved and spikes are actively generated in the lateral process (B2)

idea, we have shown that when B21 is peripherally activated during the protraction of fictive ingestive motor programs spikes are not actively propagated to the lateral process (the part of B21 that contacts B8) [5]. Thus our data strongly suggest that B21 mechanoafferent input is not transmitted to B8 during the radula opening and protraction phase of ingestive motor programs.

An obvious question however, is Why is B21 activated during radula opening and protraction if mechanoafferent input is not transmitted to B8? At least in part the answer to this appears to lie in the fact that the lateral process is not the sole output region of B21 [1]. For example, B21 is electrically coupled to the buccal neuron B64 (Fig. 4) [15], and at least in part this contact appears to be made via either the soma or medial process of B21 (Fig. 8A) [1]. When spikes are not actively propagated to the lateral process, spike amplitude is decreased in medial parts of B21, but spike attenuation is much less, e.g., spikes in the soma are on average about 35 mV while on average they are about 11 mV in the lateral process (Fig. 7A2) [5]. It is possible, therefore, that although mechanoafferent information is not transmitted to B8 during

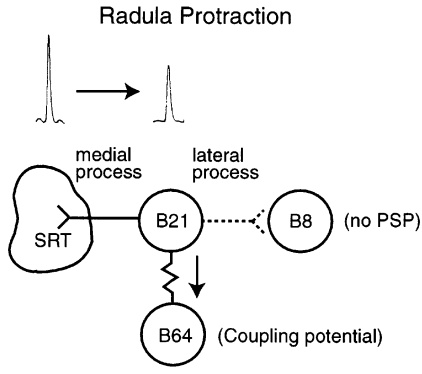

Fig. 8. Transmission of B21 mechanoafferent activity during the radula protraction phase of motor programs. During the protraction phase of ingestive motor programs B21 is not centrally depolarized. Mechanoafferent input is, therefore, not transmitted to one follower neuron, the radula closer motor neuron B8 (indicated by the dashed line). This is a result of the fact that spikes are not actively propagated to the part of B21 that contacts B8, i.e. the lateral process. During protraction, coupling potentials are, however, transmitted to a second follower, B64. The B21 contact with B64 is at least partially made via medial parts of B21 (i.e. the soma and/or medial process)

the radula protraction phase of ingestive motor programs, it is transmitted to B64 (Fig. 8A).

B64 is an interneuron that is electrically coupled to a number of retraction motor neurons (Fig. 4) [7]. Excitatory input to this cell during protraction will, therefore, tend to phase advance retraction. To assess the potential significance of B21 input to B64 during the protraction phase of motor programs we conducted experiments in which we monitored temporal characteristics of motor programs with and without activation of B21 with brief current pulses (this type of stimulation generally does not trigger active spiking in the lateral process) [1]. We found that B21 stimulation reduced protraction phase duration in a frequency dependent manner [1]. This suggests that although mechanoafferent input is not transmitted to B8 during radula protraction it can be transmitted to B64 (if B21 fires at a sufficient frequency). Radula mechanoafferent input to B64 during protraction may serve to coordinate the biomechanical state of the radula with the activity of the feeding CPG. More specifically, B21 will be most strongly activated when the radula opens quickly [1]. Presumably the radula will also protract quickly, and phase advancement of retraction will in general accelerate feeding behavior.

Afferent transmission during the radula closing and retraction phase of motor programs

Although mechanoafferent input is not transmitted to B8 when B21 is at its resting membrane potential, afferent transmission does occur when B21 is centrally depo-

larized (Fig. 7B) [1, 5, 15]. During motor programs, B21 is centrally depolarized during the retraction phase of rhythmic activity, presumably as a result of electrical coupling with retraction interneurons (Fig. 4) [15]. The depolarizations that are observed during motor programs are in fact sufficient to affect spike propagation, i.e. although spikes are attenuated in the lateral process during protraction they are full size during retraction [5]. Thus, during ingestive activity B21 is rhythmically depolarized during the radula retraction phase of the motor program. This tends to phasically gate-in mechanoafferent input to B8.

Mechanoafferent input to B8 during retraction is likely to be important when food is ingested because under these conditions consummatory responses are adjusted. Bites are converted to bite swallows and the radula closing and retraction phase of behavior is enhanced and prolonged so that food will be deposited in the esophagus (Fig. 3). During the retraction phase of ingestive behavior food contact will therefore excite the radula mechanoafferent B21. Mechanoafferent input will be transmitted to the radula closer motor B8 so that food will be more tightly grasped as it is pulled into the buccal cavity.

Concluding remarks

To summarize, we have studied two classes of sensory neurons activated during feeding in *Aplysia*. We have addressed issues such as, How do changes in afferent activity produce widespread changes in the activity of the feeding circuitry? We have found that sensory neurons are centrally located and directly and indirectly make contact with a number of feeding neurons. Afferent neurons do not, however, produce changes in motor activity that are completely determined by the state of the periphery. Instead peripherally generated and centrally generated activity is integrated so that motor output is modified in a conditional (i.e. phase-dependent) manner. At least in part this occurs via the control of active spike propagation in sensory neurons. A consequence of this arrangement is that although afferent input can produce behaviorally appropriate adjustments in ongoing activity, it cannot completely reconfigure ongoing motor programs.

ACKNOWLEDGEMENTS

We thank Klaudiusz Weiss for comments on an earlier version of this manuscript and Ayala Rosen for her invaluable assistance with the figures. This work was supported by a K02 Award (MH01267), and the following PHS Grant (MH51393, MH35564).

REFERENCES

1. Borovikov, D., Evans, C. G., Jing, J., Rosen, S. C., Cropper, E. C. (2000) A proprioceptive role for an exteroceptive mechanoafferent neuron in *Aplysia. J. Neurosci. 20,* 1990–2002.
2. Cropper, E. C., Evans, C. G., Rosen, S. C. (1996) Multiple mechanisms for peripheral activation of the peptide-containing radula mechanoafferent neurons B21 and B22 of *Aplysia. J. Neurophysiol. 76,* 1344–1351.
3. Cropper, E. C., Kupfermann, I., Weiss, K. R. (1990) Differential firing patterns of the peptide-containing cholinergic motor neurons B15 and B16 during feeding behavior in *Aplysia. Brain Res. 522,* 176–179.
4. Evans, C. G., Cropper, E. C. (1998) Proprioceptive input to feeding motor programs in *Aplysia. J. Neurosci. 18,* 8016–8031.
5. Evans, C. G., Jing, J., Rosen, S. C., Cropper, E. C. (2003) Regulation of spike initiation and propagation in an Aplysia sensory neuron: Gating-in via central depolarization. *J. Neurosci. 23,* 2920–2931.
6. Hurwitz, I., Susswein, A. J. (1992) Adaptation of feeding sequences in *Aplysia oculifera* to changes in the load and width of food. *J. Exp. Biol. 166,* 215–235.
7. Hurwitz, I., Susswein, A. J. (1996) B64, a newly identified central pattern generator element producing a phase switch from protraction to retraction in buccal motor programs of *Aplysia californica. J. Neurophysiol. 75,* 1327–1344.
8. Klein, A. N., Eisenman, J. S., Weiss, K. R., Cropper, E. C. (2000) Changes in ingestive motor programs induced by stimulation of a single sensory neuron in *Aplysia. Abst. Soc. Neurosci. 26,* 700.
9. Kupfermann, I. (1974) Feeding behavior in Aplysia: a simple system for the study of motivation. *Behav. Biol. 10,* 1–26.
10. Miller, M. W., Rosen, S. C., Schissel, S. L., Cropper, E. C., Kupfermann, I., Weiss, K. R. (1994) A population of SCP-containing neurons in the buccal ganglion of *Aplysia* are radula mechanoafferents and receive excitation of central origin. *J. Neurosci. 14,* 7008–7023.
11. Morton, D. W., Chiel, H. J. (1993) In vivo buccal nerve activity that distinguishes ingestion from rejection can be used to predict behavioral transitions in *Aplysia. J. Comp. Physiol. 172,* 17–32.
12. Morton, D. W., Chiel, H. J. (1993) The timing of activity in motor neurons that produce radula movements distinguishes ingestion from rejection in *Aplysia. J. Comp. Physiol. 173,* 519–536.
13. Plummer, M. R., Kirk, M. D. (1990) Premotor neurons B51 and B52 in the buccal ganglia of *Aplysia californica:* synaptic connections, effects on ongoing motor rhythms, and peptide modulation. *J. Neurophysiol. 63,* 539–558.
14. Rosen, S. C., Miller, M. W., Cropper, E. C., Kupfermann, I. (2000) Outputs of radula mechanoafferent neurons in *Aplysia* are modulated by motor neurons, interneurons, and sensory neurons. *J. Neurophysiol. 83,* 1621–1636.
15. Rosen, S. C., Miller, M. W., Evans, C. G., Cropper, E. C., Kupfermann, I. (2000) Diverse synaptic connections between peptidergic radula mechanoafferent neurons and neurons in the feeding system of *Aplysia. J. Neurophysiol. 83,* 1605–1620.
16. Rosen, S. C., Teyke, T., Miller, M. W., Weiss, K. R., Kupfermann, I. (1991) Identification and characterization of cerebral-to-buccal interneurons implicated in the control of motor programs associated with feeding in *Aplysia. J. Neurosci. 11,* 3630–3655.
17. Susswein, A. J., Rosen, S. C., Gapon, S., Kupfermann, I. (1996) Characterization of buccal motor programs elicited by a cholinergic agonist applied to the cerebral ganglion of *Aplysia californica. J. Comp. Physiol. [A] 179,* 509–524.