Afferent-Induced Changes in Rhythmic Motor Programs in the Feeding Circuitry of Aplysia

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Shetreat-Klein, Avniel N. and Elizabeth C. Cropper. Afferent-induced changes in rhythmic motor programs in the feeding circuitry of Aplysia. J Neurophysiol 92: 2312–2322, 2004. First published June 2, 2004; 10.1152/jn.00137.2004. A manipulation often used to determine whether a neuron plays a role in the generation of a motor program involves injecting current into the cell during rhythmic activity to determine whether activity is modified. We perform this type of manipulation to study the impact of afferent activity on feeding-like motor programs in Aplysia. We trigger biting-like programs and manipulate sensory neurons that have been implicated in producing the changes in activity that occur when food is ingested, i.e., when bites are converted to bite-swallows. Sensory neurons that are manipulated are the radula mechanoafferent B21 and the retraction proprioceptor B51. Data suggest that both cells are peripherally activated during radula closing/retraction when food is ingested. We found that phasic subthreshold depolarization of a single sensory neuron can significantly prolong radula closing/retraction, as determined by recording both from interneurons (e.g., B64), and motor neurons (e.g., B15 and B8). Additionally, afferent activity produces a delay in the onset of the subsequent radula opening/protrusion, and increases the firing frequency of motor neurons. These are the changes in activity that are seen when food is ingested. These results add to the growing data that implicate B21 and B51 in bite-to-bite swallow conversions and indicate that afferent activity is important during feeding in Aplysia.

INTERLUDE

Many motor behaviors are mediated by networks [central pattern generators (CPGs)] that can generate rhythmic output in the absence of afferent input (e.g., Delcomyn 1980). Under physiological conditions, however, CPGs often receive sensory input so that output is adjusted to compensate for changes in the periphery. In fact, in some cases, sensory neurons are considered to be an integral part of a CPG (e.g., Pearson et al. 1983). In many systems, therefore, the generation of behaviorally relevant motor programs cannot be understood unless processes that integrate peripheral and central activity are characterized. A number of studies that have provided insights into how this type of integration occurs have utilized experimentally advantageous invertebrate preparations. We are studying sensorimotor integration during feeding behavior in one such preparation, the marine mollusc Aplysia.

A number of studies have shown that rhythmic activity can be triggered in the feeding circuitry of Aplysia in preparations in which connections between the nervous system and peripheral structures are severed (Church and Lloyd 1994; Hurwitz et al. 2003; Jing and Weiss 2001, 2002; Jing et al. 1999, 2003; Morgan et al. 2000, 2002; Proekt and Weiss 2003; Rosen et al. 1991; Sanchez and Kirk 2000, 2001; Susswein et al. 1996). These results indicate that there is a feeding CPG. Under physiological conditions, however, feeding in Aplysia shows a great deal of plasticity that is likely to be induced by afferent input. For example, when Aplysia are in the general vicinity of food, they will generate ingestive responses, bites, even if they are not able to grasp food (Kupfermann 1974). Bites consist of two antagonistic sets of radula movements; radula opening and protrusion is followed by radula closing and retraction. When an animal bites, the radula opens and protracts vigorously, but it does not fully retract, and activity in radula closing/retraction motor neurons is relatively brief (Weiss et al. 1986). If bites are successful and the radula does close on food, bites are converted to bite-swallows, i.e., radula closing and retraction is enhanced and prolonged so that food will be pulled through the buccal cavity and deposited in the esophagus (Cropper et al. 1990; Kupfermann 1974; Weiss et al. 1986). Thus bite to bite-swalllow conversions are presumably triggered by afferent activity, e.g., by afferent input from the site of food contact (the radula).

Afferent input that triggers bite to swallow conversions is likely to be provided, in part, by radula mechanoaferents (RMIs) (Miller et al. 1994), such as the identified neuron B21 (Rosen et al. 2000b). RMs are relatively low threshold mechanoreceptors that have receptive fields on the biting surface of the radula (Miller et al. 1994; Rosen et al. 2000b). These neurons are activated whenever anything touches the radula; therefore they will be activated when food contact occurs and/or when the radula closes on food. Other sensory neurons that are likely to be activated when bites are converted to bite-swallows are proprioceptors that are activated when retraction movements are induced, i.e., retraction proprioceptors (RPs) (Evans and Cropper 1998). RPs are activated when the resistance to backward rotation is increased (as it can be when food is pulled into the buccal cavity) (Evans and Cropper 1998). The largest and best-characterized RP is a neuron originally described as an interneuron, B51 (Plummer and Kirk 1990).

RMIs and RPs are located in the buccal ganglion (Evans and Cropper 1998; Miller et al. 1994; Plummer and Kirk 1990; Rosen et al. 2000b). Previous studies have characterized synaptic connections of the identified neurons B21 and B51 and have shown that both cells are electrically coupled to, or make excitatory connections with, radula closing and retraction interneurons and motor neurons (Evans and Cropper 1998; Klein et al. 2000; Plummer and Kirk 1990; Rosen et al. 2000b). Both
neurons are therefore depolarized via central input during retraction (Borovikov et al. 2000; Evans et al. 2003; Rosen et al. 2000a,b). During biting-like motor programs, this depolarization is often subthreshold or only triggers low-frequency activity. When food is ingested, however, retraction activity in B21 and B51 would be increased (via peripheral activation). In this study, we show that increases in activity in B21 and B51 will change the activity of the feeding circuitry during biting-like motor programs in a manner consistent with the bite to bite-swallow conversion.

METHODS

Animals

Experiments were performed on 100- to 150-g Aplysia californica (Marinus, CA) maintained in 14–16°C holding tanks. Prior to dissection, animals were anesthetized by injection of isotonic MgCl₂ (50% of body weight). The cerebral and buccal ganglia were removed, and all nerves were severed that connect the buccal ganglion with the buccal mass and esophagus. The paired radula nerves were cut close to where they disappear into the buccal mass to leave a sufficient length for extracellular recording. Additionally the I2 nerve was initially left intact. Its innervation of the I2 muscle was subsequently severed with the aid of a dissecting microscope. Ganglia were placed in a Sylgard-coated dish and pinned (the buccal ganglion rostral side up, the cerebral ganglion ventral side up). The buccal and cerebral ganglia were pharmacologically isolated by surrounding the cerebral ganglion with a circle of silicone vacuum grease (Dow Corning, Midland, MI) and by gently placing a polyethylene ring on top of the layer of grease.

Electrophysiology

Neurons were impaled with single barreled glass microelectrodes filled with 2 M potassium acetate, beveled to an impedance of 6–12 MΩ. Two microelectrodes were connected to an AxoClamp 2B (Axon Instruments, Burlingame, CA), and two to Getting Model 5A amplifiers (Getting Instruments, Iowa City, IA). Two extracellular suction electrodes were connected to Grass Model P511 amplifiers (Grass Medical Instruments, Quincy, MA). Outputs from the amplifiers led to a PCM recording adapter model 3000A (Vatron Technology) connected to a Sony VCR for archival data storage. Output from the Vetter PCM was split so that it passed to an oscilloscope (Tektronix Model 5111), a thermal printer (AstroMed MT9500), and an ITC-16 A-D converter (Instrutech, Long Island, NY) connected to a Macintosh G4 computer (Apple Computer, Cupertino, CA). Data were acquired and analyzed using Axograph v4.0 software (Axon Instruments). When stimulator-driven current injection was needed, it was provided by either TTL pulses from a Grass S48 stimulator (for simple pulse trains) triggering current via the “step activate” port on the AxoClamp, or in the case of more complex protocols, by output programmed into the AxoGraph program, and sent via the ITC-16 interface into the “ext command” port of the AxoClamp.

Solutions

Normal artificial seawater (ASW) was composed of (in mM) 460 NaCl, 10 KCl, 11 CaCl₂, 55 MgCl₂, and 10 HEPES (pH 7.6). All salts were from Sigma (St. Louis, MO). Modified ASW (2:1 ASW) (Trudeau and Castellucci 1992), for suppressing polysynaptic activity, contained twice the normal concentration of Mg²⁺ and 1.25 times the normal concentration of CaCl₂.

Generation and modification of motor programs

Biting-like motor programs were generated by replacing the ASW bathing the cerebral ganglion with a solution of 3 mM carbachol in ASW (Susswein et al. 1996). Protraction was monitored by extracellular recordings from the I2 nerve, which contains the axons of the motor neurons that innervate a muscle that produces protraction movements, the I2 muscle (Hurwitz et al. 1996) (i.e., the I2 nerve innervates the I2 muscle). Retraction was monitored by extracellular recordings from the RN, in which high-frequency activity immediately following protraction is indicative of ingestive-like activity (Morton and Chiel 1993a). Intracellular recordings were obtained from sensory neurons and one to three additional previously characterized interneurons or motor neurons.

In experiments in which effects of increases inafferent activity were studied, sensory neurons were intracellularly depolarized with DC to achieve a firing rate of 12–16 Hz. Physiological firing patterns of sensory neurons during bite to bite-swallow conversions have not been described, but these types of increases in afferent activity are observed when sensory neurons are peripherally activated in semi-intact preparations (Borovikov et al. 2000; Evans and Cropper 1998). Depolarization was initiated at the start of high-frequency activity in B8 or the RN and was continued until just after retraction terminated to insure that the termination of retraction was not simply due to the cessation of current injection.

Presentation of data

Typically, the effect of an afferent neuron on the motor program was shown by comparing three cycles of activity: the cycle before the sensory neuron was stimulated (labeled before), the cycle in which the sensory neuron was stimulated (labeled during), and the cycle following sensory neuron stimulation (labeled after). Duration reflects the duration of depolarization from baseline during retraction. For neurons that were strongly activated during retraction, duration reflects the duration of high-frequency firing. Frequency reflects the number of spikes divided by the duration of spike burst during retraction. Significance was determined using repeated-measure ANOVA with Fischer’s PLSD posthoc analysis comparing the three cycles of activity. All values are given as mean ± SE.

RESULTS

Application of carbachol to the cerebral ganglion produces ingestive-like motor programs (Susswein et al. 1996). Figure 1, A and B, shows two examples of carbachol-generated motor programs, demonstrating the timing of activity of several buccal neurons. The ingestive motor program consists of three components. Synchronous activity in the opening/protraction neurons occurs first, seen in Fig. 1A as activity in B61 and the I2 nerve, which carries axons of motor neurons (including B61) innervating the major protractor muscle, the I2 muscle (i.e., the I2 nerve innervates the I2 muscle).

Opening/protraction is followed by closing/retraction. During closing/retraction, there is relatively high-frequency activity in motor neuron B8 (which generates radula closing; Morton and Chiel 1993b; Orekhova et al. 2001), and B64, a retraction interneuron (Hurwitz and Susswein 1996). Radula mechanosensory B21 and motor neuron B15 (implicated in radula closing and retraction; Cohen et al. 1978; Cropper et al. 1990; Orekhova et al. 2001) also receive depolarizing inputs during this time, but are generally not strongly activated. The protraction motor neuron B61, on the other hand, is hyperpolarized during closing/retraction. Also seen in Fig. 1 is activity in the RN. Large amplitude extracellular activity in this nerve is due to activity in radula closer neurons. The fact that, in the carbachol-generated motor programs, radula closer activity is phase locked to retractor activity provides good evidence that these programs are indeed ingestive-like.
A third component of activity can be seen in Fig. 1 as well. It appears as a gap between the end of closing/retraction and the beginning of the subsequent opening/protraction. There is little to no bursting activity in the I2 or RN nerves at this time, but as shown in Fig. 1B, there is high-frequency activity in neuron B52. B52 makes inhibitory connections with the closing/retraction circuitry but it does not make excitatory connections with the opening/protraction circuitry, and it does not appear to have any motor output of its own. B52 is active at this time. We refer to this as the postretraction component of the motor program.

**Stimulation of B21**

Bites are converted to bite-swallows when food contacts the radula and the radula closes on food during closing/retraction (Weiss et al. 1986). Presumably this stimulation of the radula surface will activate radula mechanoreceptors, including B21. In these experiments, B21 was therefore phasically activated during retraction. In initial experiments, we sought to determine whether increases inafferent activity could produce changes in the activity of characterized motor neurons that produce radula closing or retraction [Fig. 2, J)]. Activation of B21 did in fact produce a significant increase in the duration of activity of the radula closer motor neuron B8 (before, 3.35 ± 0.27; during, 7.11 ± 0.59; after, 3.41 ± 0.32 s; Fig. 3A) and in the radula closer/retractor B15 (before, 3.47 ± 0.50; during, 7.91 ± 1.15; after, 4.03 ± 0.70 s; Fig. 3B). Additionally, B21 stimulation produced a significant increase in the firing frequency of these two motor neurons (B8—before, 16.70 ± 1.66; during, 19.40 ± 1.72; after, 15.70 ± 1.66 Hz; B15—before, 1.27 ± 0.95; during, 4.23 ± 1.11, after, 1.66 ± 0.67 Hz; Fig. 3, A and B).

If B21 stimulation was in fact producing a widespread reconfiguration of motor programs, we would additionally expect to see changes in the activity of radula closing/retraction interneurons [Fig. 2, J)]. We therefore examined effects of B21 stimulation on a characterized (Hurwitz and Susswein 1996) retraction interneuron, B64. We observed a significant increase in the duration of B64 activity (before, 3.10 ± 4.20; during, 7.03 ± 1.44; after, 3.44 ± 0.33 s; Fig. 4). Moreover, the activity in B64 remains phase-locked to the high-frequency activity in the RN, indicating that the alteration of the motor program by B21 is a coordinated, multilevel effect.

Physiologically, it is not desirable for closing/retraction to overlap with opening/protraction. Since B21 stimulation prolongs closing/retraction, one of the following must occur. The postretraction component of the motor program and opening/
Figure 6 summarizes the effects of B21 stimulation on temporal characteristics of motor programs. B21 significantly increased radula closing/retraction duration (from 2.3 to 4.7 s). There was no significant effect on the duration of the postretraction component of the motor program. Consequently, cycle period increased (from 10.4 to 12.7 s).

B21 is electrically coupled to much of the circuitry that mediates radula retraction (Rosen et al. 2000b). This circuitry includes both retraction interneurons (e.g., B64) and retraction motor neurons (e.g., B15). Previous studies have shown that interneuron stimulation alters parametric features of motor programs (e.g., Dembrow et al. 2003; Hurwitz et al. 1997; Jing and Weiss 2001, 2002; Jing et al. 2003; Teyke et al. 1993). Since electrical coupling exists between B21 and motor neurons, it was possible that the prolongation of closing/retraction was due to the secondary depolarization in coupled cells rather than the activation of B21. To determine whether directly activating motor neurons could recreate this effect, we performed experiments in which we injected current into the accessory radula closer motor neuron B15. Current injection
duration—before, 3.13 ± 0.36; during, 6.77 ± 0.49; after, 3.04 ± 0.27 s; B8 firing frequency—before, 8.59 ± 1.63; during, 14.80 ± 1.10; after, 8.35 ± 1.40 Hz; Fig. 8A). Additionally we observed a significant increase in the duration of depolarization in radula retractor motor neuron B15 (before, 3.13 ± 0.71; during, 4.85 ± 0.67; after, 3.68 ± 0.68 s; Fig. 8B) and a significant increase in the duration of activity of retraction interneuron B64 (before, 3.01 ± 0.56 s; during, 4.77 ± 1.10; after, 3.23 ± 0.91 s; Fig. 9). Thus B51 stimulation prolonged and enhanced radula closing/retraction.

To determine whether B51 stimulation could delay radula opening/protraction (as does B21 stimulation), we monitored the duration of inhibition in the protraction interneuron B52. B51 stimulation significantly increased the duration of B52 inhibition (before, 2.47 ± 0.54; during, 4.84 ± 0.31; after, 2.34 ± 0.50 s; Fig. 10). Of note, the prolonged inhibition seen in B52 during B51 activity is of a different character than the inhibition B52 receives during closing/retraction when B51 is not active. When B51 fires, B52 appears to receive a more complex inhibition accompanied by a slow excitation (delineated by the black arrow in Fig. 10).

It has been shown that B52 manifests postinhibitory rebound excitation (Plummer and Kirk 1990). It was therefore possible that the increased duration of inhibition seen when B51 was active would lead to an increased rate of B52 firing immedi-

FIG. 6. Effect of B21 depolarization on temporal characteristics of motor programs. Data were obtained in experiments such as the one shown in Fig. 3. Length of each bar represents average total cycle period. Depolarization of B21 increased cycle period (before, 10.40 ± 1.30; during, 12.70 ± 1.50; after, 10.90 ± 1.13 s; ANOVA F(2,3) = 12.65 (P < 0.01); Fisher’s PLSD before, during P < 0.005 and during, after P < 0.01; n = 4). Each bar is subdivided to indicate the components of the motor program, i.e., protraction, retraction, and the postretraction component (interval between end of retraction and onset of next protraction). Increase in cycle period induced by B21 occurred as a result of an increase in the duration of retraction without significant change in the other 2 components of the motor program. Values for retraction are as follows: before, 2.31 ± 0.11; during, 4.67 ± 0.32; after, 2.16 ± 0.17 s [ANOVA F(2,3) = 27.60 (P < 0.001); Fisher’s PLSD before, during P < 0.001 and during, after P < 0.001; n = 4]. Depolarization of B21 did not significantly alter duration of protraction or postretraction component. Values for protraction are as follows: before, 4.38 ± 1.03; during, 4.55 ± 1.16; after, 4.20 ± 0.83 s. Values for the protraction component are as follows: before, 3.69 ± 0.53; during, 3.44 ± 0.25; after, 4.50 ± 0.73 s (n = 4).

into B15 had no significant effect (Fig. 7A vs. B). Closing/retraction duration was not prolonged, and moreover, despite the continued injection of current into B15, there was no increase in the duration of B15 activity. Rather, B15 firing ceased due to a barrage of inhibitory postsynaptic potentials (IPSPs) at the end of closing/retraction.

Stimulation of B51

A second type of sensory neuron that presumably plays a role in the bite to bite-swallow conversion is the cell B51 (Evans and Cropper 1998). In a previous study, which was conducted in a semi-intact preparation, we showed that DC current injection into B51 alters radula movements during carbachol-induced motor programs, i.e., when B51 is continuously depolarized, radula retraction movements are enhanced (Evans and Cropper 1998). Previous work did not, however, study the effect of phase specific activation of B51. Additionally, effects of B51 stimulation on the feeding circuitry were not specifically described.

We injected current into B51 neurons during closing/retraction and found a significant increase in the firing frequency and duration of activity of radula closer motor neuron B8 (B8 protraction retraction
Duration (seconds)

FIG. 7. Example of an experiment in which effects of B21 depolarization and effects of motor neuron depolarization were compared. A: current injection into the ARC motor neuron B15 had no effect on the duration of retraction. In this experiment, protraction duration was decreased. This effect on protraction duration was not consistently observed. B: in contrast, current injection into B21 did prolong retraction (n = 3). A and B are from the same preparation.
During rhythmic activity, B21 could also be activated and could mediate the observed changes in motor programs (Fig. 11). Together, these results suggest a temporal directionality in the B21 to B51 connection.

**Relationship between B21 and B51**

B21 and B51 exhibit bi-directional electrical coupling (Fig. 11). Thus it is possible that when current is injected into B51 during rhythmic activity, B21 could also be activated and could mediate the observed changes in motor programs. In a similar vein, when current is injected into B21, B51 could be activated. To determine whether either or both of these possibilities occur, we performed experiments in which we induced changes in motor programs and recorded from both cells. When current was injected into B51, B21 was not activated (Fig. 12). Activity in B21 is therefore not essential for B51-induced increases in retraction duration.

In contrast, when current was injected into B21, B51 was activated in seven of nine preparations (Fig. 13). B51 activity was increased in duration (before, 3.39 ± 0.31; during, 7.16 ± 0.84; after, 3.29 ± 0.21 s), and the B51 firing frequency was increased (before, 0.48 ± 0.36; during, 5.17 ± 1.80; after, 0.50 ± 0.24 Hz). Interestingly, however, spiking in the two neurons did not occur simultaneously. The current injection into B21 immediately triggered action potentials in B21. However, spiking in B51 was initiated with a delay, and when B51 began to spike, IPSPs were observed in B21, and spiking ceased (Fig. 13). This suggests that, when the two cells are activated, changes in motor programs are initially triggered by activity in B21 (Fig. 14A). Subsequently, however, it is B51 that primarily mediates increases in retraction duration (Fig. 14A2). Together, the experiments shown in Figs. 11–13 suggest a temporal directionality in the B21 to B51 connection.
In experiments such as the one shown in Fig. 13, it should be noted, however, that depolarizing current was injected into B21 throughout retraction (despite the fact that spiking in B21 had ceased). Because there is electrical coupling in the retraction circuitry, the increase in retraction duration could therefore have been due to current injection in B21 (and not due to B51 activity). To determine whether this was the case, we performed experiments in which we stopped injecting current into B21 when spiking was initiated in B51. Under these conditions, we continued to see a significant prolongation of the retraction phase of the motor program (Fig. 15).

As noted above, we did not always observe spiking in B51 when injecting current into B21 triggered changes in motor programs. This suggests that, although B51 is often activated when B21 induces changes in motor programs, B51 is not essential for alterations in motor programs. To further explore this issue, we triggered changes in motor programs by injecting current into B21 and hyperpolarizing both the left and right B51 neurons. We found that the duration of the retraction phase of the motor program could still be prolonged (before, 2.01 ± 0.18; during, 4.01 ± 0.83; after, 1.88 ± 0.18 s; Fig. 16). To ensure that changes in retraction duration were not simply due to the change in the B51 membrane potential, we also looked at the effect of hyperpolarizing B51 without depolarizing B21. As expected (Evans and Cropper 1998), this produced a decrease in the duration of retraction. Our data indicate therefore that B51 is not essential for B21-induced alterations in motor programs.

Although it is not essential for B21-induced alterations in motor programs, it might be expected that B51 activity would impact the magnitude of the increase in retraction duration that is observed. To determine whether this is the case, we analyzed data to determine whether increases in retraction duration induced by B21 when B51 was hyperpolarized were different from increases in retraction duration that were observed when B21 activated B51. We found that retraction duration was doubled when B21 was stimulated without B51 (i.e., increased to 4.5 ± 0.8 s), but was tripled when B21 and B51 were stimulated together (i.e., increased to 7.7 ± 1.2 s). This was a significant difference (P < 0.05). Thus, although B51 is not essential for B21-induced alterations in motor programs, increases in protraction duration are more pronounced when B51 is activated.

**FIG. 11.** B21 and B51 are electrically coupled. Depolarizing (left column) and hyperpolarizing (right column) current injected into B21 and B51. Two electrodes were placed in the cell into which current was being injected to insure accurate measurement of voltage changes while injecting current. In all panels, the top trace is the voltage response of the coupled cell, the middle trace is the voltage response of the cell into which current is being injected, and the bottom trace (marked I) is the current command. A: electrical coupling measured by current injection into B21. Coupling ratio B21:B51 was 20.7:1 ± 3.8 mV for hyperpolarizing pulses and 12.9:1 ± 1.9 mV for depolarizing pulses. Average ratio was 18.0:1. B: electrical coupling measured when current was injected into B51. Ratio B51:B21 was 15.2:1 ± 2.2 mV for hyperpolarizing pulses and 12.3:1 ± 1.6 mV for depolarizing pulses. Average ratio was 13.8:1 ± 0.4 mV (n = 3).

**FIG. 12.** Depolarization of B51 does not recruit B21. DC current injection into B51 during retraction phase (bar under top trace) prolonged the central depolarization in B21 but did not trigger spiking in B21 (before, 1.57 ± 0.84; during, 1.68 ± 0.75; after, 2.17 ± 1.23 Hz; ANOVA F(2,9) = 0.94 (P = not significant); n = 10).

**FIG. 13.** Depolarization of B21 can recruit B51. When depolarizing current was injected into B21 (bar under top trace), spiking was triggered in B51 after a delay, resulting in a longer duration and higher frequency of B51 activity. (duration: before, 3.39 ± 0.31; during, 7.16 ± 0.84; after, 3.29 ± 0.21 s; ANOVA F(2,8) = 23.6 (P < 0.0001); Fisher’s PLSD before, during P < 0.0001 and during, after P < 0.0001: n = 9; frequency: before, 0.48 ± 0.36; during, 5.17 ± 1.80; after, 0.50 ± 0.24 Hz; ANOVA F(2,8) = 7.80 (P < 0.005); Fisher’s PLSD before, during P < 0.005 and during, after P < 0.005; n = 9).
that these cells can be peripherally activated with stimuli likely present during bite to bite-swallow conversions (Borovikov et al. 2000; Evans et al. 2003; Miller et al. 1994; Rosen et al. 2000a,b). Additionally, previous studies characterized central synaptic connections of B21 and B51 and showed that both cells make direct excitatory connections with the radula closing/retraction circuitry (Klein et al. 2000; Plummer and Kirk 1990; Rosen et al. 2000a,b). One purpose of this study was to determine whether changes in afferent activity are transmitted to the feeding circuitry during biting-like motor programs and whether these changes are consistent with the bite to bite-swell conversion. Determining whether B21’s known connections would in fact be able to alter ongoing motor programs was a crucial step in understanding B21’s capabilities given that studies have shown that synaptic pathways can be heterosynaptically inhibited by other neurons of the neural network (e.g., Blitz and Nusbaum 1997; Chiel et al. 1988; DiCaprio 1999; Grillner and Wallen 1985; Nusbaum et al. 1997; Segev 1990; Storozhuk and Castellucci 1999). More specifically, Rosen et al. (2000a) have shown that activity of other neurons can significantly reduce B21’s output.

**FIG. 14.** Schematic model of B21- and B51-induced changes in retraction. B21 depolarization alters radula protraction. When this occurs, B51 is also activated in some cases (A1 and A2) but not others (B). When B51 is activated, its spiking occurs with a delay so that initially effects of the retraction circuitry are primarily mediated by B21 (A1). When B51 is activated, B21 is inhibited (indirectly), and effects on the retraction circuitry are primarily mediated by B51 (A2).

**FIG. 15.** Sustained depolarization in B21 is not needed for the prolongation of retraction. Depolarizing current was injected into B21 at the start of retraction. Current injection was eliminated as soon as B51 became active (dotted line). Nevertheless retraction was prolonged [before, 4.09 ± 0.01; during, 9.73 ± 1.50; after, 3.44 ± 0.63 s; ANOVA, F(2,2) = 9.79 (P < 0.05); Fisher’s PLSD before, during P < 0.05 and during, after P < 0.05; n = 3].

**FIG. 16.** B51 activity is not an obligate component of B21-induced alterations in motor programs. Both the right and left B51 neurons were hyperpolarized for the duration of this experiment. During 1 retraction, neuron B21 was depolarized with direct intracellular current (bar under top trace). Retraction was prolonged, despite the absence of spiking activity in either B51 neuron [before, 2.01 ± 0.18; during, 4.01 ± 0.83; after, 1.88 ± 0.18 s; ANOVA F(2,4) = 8.96 (P < 0.01); Fisher’s PLSD before, during P < 0.01 and during, after P < 0.01; n = 6].
The effects of B21 and B51 were determined using carbachol-generated motor programs. Analysis of these motor programs provided insights about the fundamental nature of the neural network underlying motor programs. For instance, we noted that neuron B52 fired at the highest frequency during a period in the motor program in which the network appeared otherwise relatively quiescent (labeled as the postretraction component in Fig. 1). A study by Evans and Cropper (1998) found that, immediately following the retraction phase, there was a short burst of activity in the radula opener motor neuron B48, but this burst did not persist for the entire duration of activity in B52. Hence, although the Aplysia ingestive motor program is generally regarded as biphasic (e.g., Church and Lloyd 1994; Evans et al. 1999; Morgan 1999), our results indicate that a triphasic description of the motor program is also possible (see Murphy 2001 for a recent review).

It is possible to reconcile the triphasic motor program with the apparently biphasic ingestive behavior. Evans et al. (1999) recorded from B52 while monitoring radula movements. Examination of the simultaneous B52 recording and radula movement record shown in Fig. 13 of Evans and Cropper (1998) actually shows a two-phase trajectory from the peak of retraction to the subsequent peak of protraction. Immediately following the peak of retraction, one sees a shallow protraction trajectory, indicating a relatively slower moving forward of the radula. After this, there is an abrupt, steep trajectory protraction, which may be the shallow protraction trajectory, indicating a relatively slower moving forward of the radula. It was a short burst of activity in the radula opener motor neuron B52 during a period in the motor program in which the network appeared otherwise relatively quiescent (labeled as the postretraction component in Fig. 1). A study by Evans and Cropper (1998) found that, immediately following the retraction phase, there was a short burst of activity in the radula opener motor neuron B48, but this burst did not persist for the entire duration of activity in B52. Hence, although the Aplysia ingestive motor program is generally regarded as biphasic (e.g., Church and Lloyd 1994; Evans et al. 1999; Morgan 1999), our results indicate that a triphasic description of the motor program is also possible (see Murphy 2001 for a recent review).

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Changes in motor programs induced by B21 and B51 together differ from those triggered by B21 alone in that the increase in the duration of radula closing/retraction is greater when both B21 and B51 are activated. In general, an increase in the duration of activity in a feeding neuromuscular system will generate an increase in the amplitude of the evoked movement (i.e., feeding muscles are generally nonspiking and contraction amplitude is determined by the total number of spikes within a given burst of activity; e.g., see Cohen et al. 1978). Recruitment of B51 therefore presumably increases retraction movements. It is therefore possible that in some cases bite-swallow conversions can be triggered via activation of B21 alone. This may occur when a small piece of floating seaweed is ingested that can be readily pulled into the buccal cavity. In contrast, when a larger piece of food is ingested or when food that is attached to a substrate is ingested, the increased resistance during retraction may ensure recruitment of B51. B51 recruitment may in turn produce necessary enhancements in retraction movements. Furthermore, B21 causes B8 to fire longer and at a higher frequency. Firing of B8 produces centripetal activity in B51 (Evans and Cropper 1998). Thus B21 firing is even more likely to produce activation of B51 in an intact behaving animal.

In the isolated nervous system, we showed that, when B51 is recruited by B21, the two sensory neurons are for the most part not co-active. Spiking is immediately triggered in B21, but it is not immediately triggered in B51. When B51 does finally spike, B21 receives inhibitory input and spiking ceases. Thus in the isolated nervous system, B21 activity predominates early in retraction, whereas B51 activity predominates later in retraction (Fig. 14). With the periphery present, afferent activation could obviously occur in a different manner, e.g., B51 could be strongly peripherally activated early in retraction. Interestingly, however, we would not expect this to the case given what is currently known about feeding movements and...
the mechanisms that peripherally activate B21 and B51. Thus radula movements and the activity of the feeding circuitry are slightly out of synch (i.e., neural activity precedes movement) (Evans and Cropper 1998). When food is ingested, it will, therefore, presumably contact the radula at peak protraction, which will occur as activity in the retraction circuitry is initiated. Thus B21 will presumably be peripherally activated at the beginning of the retraction phase of the motor program. In contrast, B51 will not be peripherally activated until retraction has occurred, at least to some extent. This will occur later during the retraction phase of the motor program. Thus central and peripheral mechanisms are likely to work together to create a sequential pattern of afferent activation during bite to bite-swell conversions. Functionally, this pattern of activity may create a situation in which B21 (and other RMs) primarily trigger bite to bite-swell conversions. In contrast, B51 may predominantly insure that the magnitude of the evoked movement is appropriate for the food ingested.

In conclusion, a goal of our current research is to utilize the experimentally advantageous features of the \textit{Aplysia} feeding system to study sensorimotor integration during a rhythmic motor program. These results (taken together with previous work) establish B21 and B51 as physiologically characterized afferents that can be used to study direct effects of afferent activity on pattern generation. With the possible exception of B4/5 (Fiore and Geppetti 1981; Jing and Weiss 2001) and B52 neurons (Evans et al. 1999; Nargeot et al. 2002), other \textit{Aplysia} neurons that can be used for these studies have not yet been identified.

A C K N O W L E D G M E N T S

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R E F E R E N C E S


Hurwitz I, Kupfermann I, and Weiss KR. Different roles of neurons B63 and B34 that are active during the protraction phase of buccal motor programs in \textit{Aplysia californica}. J Neurophysiol 78: 1305–1319, 1997.


Hurwitz I and Susswein AJ. B64, a newly identified central pattern generator element producing a phase shift from protraction to retraction in buccal motor programs of \textit{Aplysia californica}. J Neurophysiol 75: 1327–1344, 1996.


Morton DW and Chief HJ. In vivo buccal nerve activity that distinguishes ingestion from rejection can be used to predict behavioral transitions in \textit{Aplysia}. J Comp Physiol 172: 17–32, 1993a.


Murphy AD. The neuronal basis of feeding in the snail, 

H. l. s. o. m. i. a., with comparisons to selected gastropods. Preg Neurobiol 63: 383–408, 2001.


