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**The timing of activity in motor neurons that produce radula
movements distinguishes ingestion from rejection in *Aplysia***

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Case Western Reserve University (Health Sciences), 1993

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The timing of activity in motor neurons that produce
radula movements distinguishes ingestion from
rejection in *Aplysia*

by

Douglas Wilson Morton

Submitted in partial fulfillment of the requirements for the
degree of Doctor of Philosophy

Thesis advisor: Hillel J. Chiel, Ph.D.

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May 1993

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GRADUATE STUDIES

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The timing of activity in motor neurons that produce radula movements distinguishes ingestion from rejection in *Aplysia*

Abstract

by

Douglas Wilson Morton

The marine mollusc *Aplysia californica* carries out biting, swallowing, and rejection responses using different movements of its radula, a structure that is used to grasp and move food. Video records during feeding show that the timing of radula closure during the radula protraction-retraction cycle constitutes a major difference between ingestion (biting or swallowing) and rejection. During ingestion, the radula is closed as it retracts. During rejection, the radula is closed as it protracts.

Using *in vivo* cuff electrodes, one can observe two different patterns of activity on nerves that are likely to mediate these radula movements. Patterns I and II are associated with ingestion and rejection, respectively, and are distinguished by the timing of radula nerve (RN) activity relative to the onset of buccal nerve 2 (BN2) activity. During these patterns, BN2 activity is temporally related to protraction and retraction movements, while RN activity is temporally related to radula closure.

Three buccal ganglion motor neurons have been characterized that are likely to contribute to these patterns. Neurons B8a and B8b project through the RN and act to close the radula. Neuron B10 projects through BN2 and acts to retract the radula. The timing of activity in these neurons can be used to distinguish the ingestion-like and rejection-like motor patterns produced by a reduced preparation. B8a, B8b and B10 are active together during the ingestion-like pattern. Activity in B8a and B8b ends prior to the onset of activity in B10 during the rejection-like pattern. During both feeding-like patterns, the activity and peripheral actions of B8a, B8b, and B10 are consistent with the neural activity and radula movements observed *in vivo* during ingestion and rejection.

These results suggest that the timing of activity in B8a and B8b relative to activity in B10 can be used to distinguish ingestion from rejection, and that this difference may contribute to the different radula movements underlying these responses. Studying the neural circuitry that mediates activity in B8a, B8b, and B10 could provide insights into how animals produce multiple behavioral responses, as well as how they decide which response to produce.

When I consider Your heavens, the work of Your fingers,
The moon and stars, which You have set in place,
What is man that You are mindful of him,
The son of man that You care for him?
You made him a little lower than God
And crowned him with glory and honor.
You made him ruler over the works of your hands;
You put everything under his feet:
All flocks and herds, and the beasts of the field,
The birds of the air, and the fish of the sea,
All that swim the paths of the seas.
O Lord, our Lord,
How majestic is Your name in all the earth!

Psalm 8:3-9

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Chapter 1

Introduction

Animals produce different behavioral responses by generating different patterns of motor neuron activity. In this dissertation, the various responses making up the consummatory feeding behavior of the marine mollusc *Aplysia californica* were studied. The goal was to identify the motor patterns associated with each feeding response and then determine the *in vivo* activity of motor neurons that contribute to these motor patterns. This dissertation specifically focused on the differences between each of *Aplysia's* feeding responses in order to develop distinguishing criteria based on the *in vivo* activity of identified motor neurons.

The remainder of this chapter introduces the specific goal and experimental approach. First, the consummatory feeding behavior of *Aplysia* will be described, and its advantages as a system for studying the neural basis of behavior will be discussed. Then, several principles underlying *Aplysia's* feeding behavior will be established by reviewing the molluscan feeding literature. Next, three of the neuronal strategies used to produce multiple behavioral responses will be introduced in order to provide insight into the strategy used by *Aplysia*. Finally,

these concepts will be combined to introduce the specific goal as well as to justify the experimental approach that was employed to address this goal.

Consummatory feeding behavior of *Aplysia californica*

The consummatory feeding behavior of *Aplysia californica* will be discussed on three different levels: behavioral, anatomical, and neurobiological. At the behavioral level, the *Aplysia* feeding system has distinct advantages because the various feeding responses are stereotyped, easily recognizable, and easily elicited. At the anatomical level, *Aplysia's* feeding apparatus is somewhat complex, and the mechanisms mediating feeding movements are not well understood. The neurobiological level is where *Aplysia's* most powerful advantage is found: many of the cells that mediate this behavior are identifiable. This raises the possibility that the neuronal circuit underlying feeding can be elucidated.

Behavioral description

Aplysia's consummatory feeding behavior is used to ingest a variety of different seaweeds (Kupfermann and Carew 1974). This behavior is made up of several types of responses, including biting, swallowing, and rejection (Kupfermann 1974a). A *biting* response represents an attempt to capture a piece of food. *Swallowing* responses are used to move a piece of captured food into the animal's mouth. *Rejection* responses are used to remove inedible objects from the animal's mouth.

The animal can switch between these different responses in a rapid and coordinated manner in response to sensory cues (Kupfermann 1974a; Chiel et al. 1986; Cropper et al. 1990a,b). Touching a piece of seaweed to the animal's lips elicits biting responses. Once a piece of food has been captured, the animal switches to swallowing responses. As long as food is present in the animal's mouth, swallowing responses continue. If an animal is tricked into swallowing an inedible object, after several swallows, the animal switches to rejection responses.

Anatomy of Aplysia's feeding apparatus

Aplysia's feeding apparatus, named the buccal mass, contains no bones. Instead of using muscles to flex skeletal joints, *Aplysia's* feeding movements are generated by shape changes in soft tissue. The muscles that mediate these shape changes have complex morphologies, are multiply innervated, and respond to a variety of neuromodulatory substances, making a complete understanding of their function very difficult. However, some general principles can be derived by reviewing the anatomy and physiology of *Aplysia's* buccal mass. Since this investigation will focus on the neural control of a structure within the buccal mass named the radula (see below for anatomy), this review will focus on how the buccal mass produces radula movements.

Aplysia's buccal mass is a complex structure made up of muscle and cartilage (Figure 1-1). Within the buccal mass is a cavity, named the buccal cavity, that serves as the animal's mouth. The buccal cavity contains a cartilaginous structure named the radula, that is used to move food into or out of the buccal cavity. The anterior end of the buccal mass contains the jaws, which

are also used to grasp food. The opening to the esophagus can be found at the posterior end of the buccal cavity.

The radula is used to grasp and move food (Kupfermann 1974a), and thus plays a central role in each of *Aplysia's* feeding responses. This structure is a tooth-covered sheet of cartilage that overlies the horseshoe-shaped I_4/I_6 muscle (Figures 1-1 and 1-2). The radula can move forward and backward within the buccal cavity (protraction and retraction, respectively, Figure 1-2a). In addition, the two halves of the radula can be moved forcefully together (radula closure, right side of Figure 1-2b), as well as moving apart (radula opening, left side of Figure 1-2b). In chapter 2 of this dissertation, it will be shown that *Aplysia* carries out biting, swallowing, and rejection responses by changing the timing of radula closure relative to protraction and retraction movements.

Eight muscles have been described in *Aplysia's* buccal mass (Scott et al. 1991). Since the precise role of these muscles in producing radula movements is poorly understood, the reader should consider the following description to be an anatomical orientation rather than a detailed explanation of buccal mass biomechanics.

The muscles of the buccal mass are identified by their morphology (Figure 1-1), and in several cases are associated with characteristic peripheral actions (Figure 1-3). The I_1 muscle is a thin sheet overlying the anterior buccal mass (left image, Figure 1-1a). Since the role of I_1 is unclear, and since this muscle is tightly adherent to the underlying I_3 muscle, I_1 and I_3 are usually treated as a single functional group (Rose and Benjamin 1979; Nagahama and Takata 1988; Scott et al. 1991). The I_3 muscle forms a thick sphincter around the jaws and

anterior buccal mass (top right image, Figure 1-1a; right image Figure 1-1b). In *Lymnaea*, a species with a similar buccal mass architecture, the muscles analogous to I_1/I_3 have been shown to contribute to jaw closure as well as to moving the radula backward in the buccal cavity (Rose and Benjamin 1979). The I_2 muscle consists of a thin sheet that wraps around the back of the buccal mass and inserts into the I_1/I_3 muscle (left images, Figures 1-1a and 1-1c). This muscle acts to pull the jaws open and push the radula forward (Scott et al. 1991; Rose and Benjamin 1979; Howells 1942). The I_4/I_6 muscle (also known as the odontophore muscle) is a thick, "U"-shaped muscle that is located immediately under the radula (right image, Figure 1-1b). This muscle has been divided into three parts, as follows (Scott et al. 1991): the two sides of the "U" correspond to the left and right I_4 muscles, while the curved, base of the "U" corresponds to the I_6 muscle. In chapter 3 of this dissertation, evidence will be presented suggesting that the I_4/I_6 muscle contributes to radula closure. The I_5 muscle (also called the accessory radula closer, or ARC muscle) wraps around the I_4/I_6 muscle and inserts on the back of the radula (lower right image, Figure 1-1a). This allows I_5 to pull the radula closed by rolling it over the surface of I_4/I_6 (Howells 1942; Cohen et al. 1978). The I_7 muscle is a small muscle that inserts on the radula sac (lower right image, Figure 1-1a). This muscle may be involved in opening the radula by pulling the radula stalk upward and forward (Scott et al. 1991). The I_8 muscle wraps around the back of the radula sac (right image, Figure 1-1c). This muscle may also be involved in opening the radula by pushing the radula sac upward (Scott et al. 1991).

Buccal mass muscles are multiply innervated, non-spiking, and subject to the effects of a variety of neuromodulatory substances (Cohen et al. 1978; Weiss et al. 1978; Cropper et al. 1987a,b, 1990a,b; Lloyd 1988; Church et al. 1991; Church and Lloyd 1991). For example, the I_5 muscle is innervated by 2 motor neurons, B15 and B16. Both B15 and B16 release acetylcholine and produce excitatory junction currents (EJCs) throughout all portions of the I_5 muscle; however, the EJCs produced by these motor neurons are quite different. This difference has been linked to the different neuropeptides released by each motor neuron (Cropper et al. 1987a,b). B15 releases the small cardioactive peptides (SCP_A and SCP_B) and produces strongly facilitating EJCs. In contrast, B16 releases myomodulin (Mm) and produces weakly facilitating EJCs. A number of groups (Cropper et al. 1987a,b, 1990a,b; Church et al. 1991; Church and Lloyd 1991) have shown that many buccal ganglion motor neurons release one or more neuropeptides. In addition, Church et al. (1991) have shown that EJC size and the time course of EJC facilitation are useful criteria for identifying buccal ganglion motor neurons.

The buccal mass is innervated by the buccal ganglion (Cohen et al. 1978; Church et al. 1991; Church and Lloyd 1991). This ganglion is bilaterally symmetric, with approximately 1000 neurons in each hemi-ganglion. Five bilaterally symmetric nerves carry axons from the buccal ganglion to the buccal mass. Four of these nerves (buccal nerve 1 (BN1), buccal nerve 2 (BN2), buccal nerve 3 (BN3), and the esophageal nerve (EN)) travel along the surface of the buccal mass before entering the muscle tissue (left image, Figure 1-1a). The fifth nerve, named the radula nerve (RN), enters the buccal mass immediately after

leaving the buccal ganglion (top right image, Figure 1-1a; right image, Figure 1-1c). BN1 innervates the thin layer of muscle and epithelium that makes up the dorsal wall of the buccal mass (Nagahama and Takata 1987; Scott et al. 1991). BN2 primarily innervates the I_1 and I_3 muscles (Nagahama and Takata 1988; Scott et al. 1991). Later in this dissertation, it will be shown that fibers in BN2 contribute to radula retraction and jaw closure. BN3 primarily innervates the I_3 and I_5 muscles (Cohen et al. 1978; Scott et al. 1991), which are believed to contribute to jaw closure, radula retraction, and radula closure (Howells 1942; Cohen et al. 1978; Scott et al. 1991). The EN bifurcates, sending one branch down the esophagus and the other to the dorsal wall of the buccal mass (Howells 1942; Scott et al. 1991). The RN has a more complex morphology. Many small nerve branches leave the common portion of the RN and innervate the I_2 muscle, contributing to protraction movements (Morton and Chiel, unpublished observations). The common portion of the RN bifurcates, forming two main divisions, the left and right RNs. These two nerves innervate the I_4 and I_6 muscles (Howells 1942; Scott et al. 1991). In chapters 2 and 3 of this dissertation, it will be shown that fibers in the left and right RNs contribute to radula closure.

Neural circuitry mediating Aplysia's consummatory feeding responses

The circuitry that mediates consummatory feeding behavior is likely to be located in the buccal and cerebral ganglia (Kupfermann 1974a,b). In this section, an important property of this circuitry, *identifiability*, will be introduced. Then the properties of identified buccal ganglion motor neurons will be reviewed.

Gardner (1971) presented the first evidence that neurons in *Aplysia*'s feeding circuitry are identifiable. An identifiable cell can be uniquely identified based on some combination of experimentally determined properties, such as: biochemical properties, electrical properties, morphology, or synaptic connections. To a good approximation, a given identifiable neuron can be treated as the same neuron in different animals. This allows the use of multiple experiments involving multiple animals to test a hypothesis involving a specific identified neuron. In other species where neurons are identifiable, it has been possible to elucidate the neural circuitry underlying specific behaviors. Examples include: the feeding pattern generators of *Helisoma* and *Lymnaea* (Kater 1974; Kaneko et al. 1978; Rose and Benjamin 1981a,b; Elliot and Benjamin 1985a,b), the central pattern generator mediating *Tritonia*'s escape swim (Getting and Dekin 1985), the pyloric network of the crustacean stomatogastric system (Selverston and Moulins 1987), the central pattern generator mediating flight in the locust (Robertson and Pearson 1982, 1983, 1985; Ramirez and Pearson 1988), the different forms of the crayfish escape swim (Krasne and Wine 1984), and the escape system of the cockroach (Ritzmann 1993).

In *Aplysia*, a number of practical criteria have been widely used for identifying neurons. The soma of each neuron tends to have a characteristic size and location (Gardner 1971). In addition, each neuron's synaptic connections (both electrical and chemical) are reliable identifying criteria (Gardner 1971; Susswein and Byrne 1988; Church et al. 1991; Church and Lloyd 1991). Finally, each motor neuron projects to the periphery via a characteristic buccal nerve (Church et al. 1991; Church and Lloyd 1991), has a characteristic pattern of

muscle innervation (Church et al. 1991), and produces either an excitatory or inhibitory junction current with a characteristic size and time course of facilitation (Cohen 1978; Cropper et al. 1987a,b; Church et al. 1991).

Motor neurons in the buccal ganglion have been extensively studied because they have large somata and release a variety of neuropeptides (Church et al. 1991; Church and Lloyd 1991) in addition to releasing a classical neurotransmitter (Gardner 1971). However, little is known about the physiological role of these neurons in producing feeding movements. Initial studies done by Cohen et al. (1978) have shown that two buccal ganglion motor neurons, B15 and B16, innervate the I_5 muscle and may play a role in closing the radula. Studies by Church et al. (1991) and Church and Lloyd (1991) have shown that the buccal mass is extensively innervated by motor neurons from the buccal ganglion. Lloyd (1988) has shown that buccal ganglion motor neurons transport large amounts of several different neuropeptides to the buccal mass musculature. A summary of the known properties of buccal ganglion motor neurons is shown in Figure 1-4.

Feeding behavior of other marine molluscs

Aplysia's buccal mass is similar to that of a number of other marine molluscs. The similarities and differences between *Aplysia's* feeding behavior and the feeding behaviors of these other species provides a useful way to illustrate some underlying principles of molluscan feeding. In this section, these underlying principles will be introduced by reviewing the molluscan feeding literature.

Feeding responses are associated with radula protraction-retraction movements

In all of the molluscan feeding systems included in this review, each feeding response is associated with a protraction and retraction movement of the radula. Three of these species, *Lymnaea*, *Helisoma*, and *Limax*, feed using a stereotyped, rhythmic behavioral response called rasping. The rasping response has three phases of radula movement: protraction, rasp, and retraction, in which the radula moves forward, rolls, and moves backward, respectively (Rose and Benjamin 1979; Kater 1974; Gelperin et al. 1978). The other three species, *Tritonia*, *Pleurobranchaea*, and *Aplysia*, use several different feeding responses to capture and consume food. These responses include biting (bite-strike in *Tritonia* and *Pleurobranchaea*), swallowing, and rejection. In these three species, the radula movements during each response have two phases: protraction and retraction (Willows 1977; Audesirk and Audesirk 1979; Croll and Davis 1981; McClellan 1982a,b; Kupfermann 1974a).

Radula movements can distinguish different feeding responses

In the species where consummatory feeding behavior is made up of multiple responses (*Tritonia*, *Pleurobranchaea*, and *Aplysia*), radula movements can be used to distinguish ingestion from rejection. The radula in these three systems is different from the radula in the three rasping systems in that it can open and close, as well as protracting and retracting. In *Tritonia*, ingestion responses (bite-strike or swallowing) are associated with the radula being closed during retraction, while rejection responses are associated with the radula remaining open during the entire response (Audesirk and Audesirk 1979). A similar picture is seen in

Pleurobranchaea (McClellan 1982a,b). *Aplysia*'s radula movements have been studied in detail only during biting responses, where the radula is closed during retraction (Kupfermann 1974a). The similarity between these three species suggests that *Aplysia*'s radula may also move differently during rejection responses.

Feeding neural circuitry is likely to reside in the buccal and cerebral ganglia

The neural circuitry mediating consummatory feeding behavior is likely to be contained in the buccal and cerebral ganglia, with command elements in the cerebral ganglion controlling pattern generation circuitry in the buccal ganglion. In *Lymnaea*, *Helisoma*, and *Limax*, the feeding pattern generator elements are found in the buccal ganglion (Rose and Benjamin 1981a,b; Elliot and Benjamin 1985a,b; Kater 1974; Kaneko et al. 1978; Delaney and Gelperin 1990a-c), while command-like cells have been found in the cerebral ganglion (Benjamin et al. 1981; Granzow and Kater 1977; Delaney and Gelperin 1990a-c). A similar picture has emerged in *Pleurobranchaea*, with the command-like paracerebral neurons located in the cerebral ganglion controlling the pattern generation circuitry in the buccal ganglion (Croll and Davis 1981; Gillette et al. 1982; Croll et al. 1985a-c). *Aplysia*'s feeding circuitry appears to have a similar architecture with command-like elements in the cerebral ganglion (Kupfermann 1974b; Rosen et al. 1991) and pattern generator elements in the buccal ganglion (Susswein and Byrne 1988; Plummer and Kirk 1990).

The pattern generator circuitry tends to be pre-synaptic to motor neurons

Pattern generator elements in these feeding systems tend to be presynaptic to the motor neurons that mediate buccal mass movements. *Lymnaea's* neuronal pattern generator is made up of 4 classes of neurons: SO, N1, N2, and N3 (Rose and Benjamin 1981a,b; Elliot and Benjamin 1985a,b). The SO neuron (slow oscillator) is a command-like neuron that drives the three phase feeding pattern when intracellularly stimulated. The N1, N2, and N3 neurons are active during protraction, rasp, and retraction, respectively, and synapse onto protractor, rasp, and retractor motor neurons (Benjamin et al. 1979; Rose and Benjamin 1979; Rose and Benjamin 1981a,b). In *Helisoma*, the cyberchron network that makes up this species' feeding pattern generator directly synapses onto motor neurons which innervate the buccal mass (Kater 1974). There is evidence that this may be partially true in *Aplysia*. Many putative elements of *Aplysia's* feeding circuit are interneurons. However, recent evidence raises the possibility that putative pattern generator elements B4/5 and B31/32 (Susswein and Byrne 1988) may be motor neurons as well as pattern generator elements (Church and Lloyd 1991; Hurwitz et al. 1992).

Pattern generator elements tend to occur in populations

The pattern generator elements in these systems tend to occur in populations (i.e. there is a high degree of parallelism). In several cases, extensive positive feedback within a population is used to produce a regenerative burst that drives the pattern generator. For example, *Helisoma's* neuronal pattern generator consists of a network of electrically coupled neurons named cyberchrons (Kaneko

et al. 1978). The degree of coupling can be controlled, allowing the amount of positive feedback to be varied, and providing a mechanism to control the duration of regenerative bursting. In *Lymnaea*, extensive positive feedback within the N1 population produces a strong burst of activity during the protraction phase of the feeding pattern (Rose and Benjamin 1981b). Strong inhibition from the N2 population is required to terminate bursting in the N1 population. In *Aplysia*, there is evidence that the pattern generator is made up of populations of neurons and that positive feedback within some of these populations produces regenerative bursting. Putative pattern generator elements B31 and B32 are indistinguishable, and are tightly electrically coupled (Susswein and Byrne 1988). The regenerative bursting ability of these cells is abolished if they are removed from the ganglion and isolated in cell culture (Chiel and You 1991), suggesting that it may be caused by positive feedback from neural circuitry within the buccal ganglion.

Strategies used to produce multiple behavioral responses

The goal of this dissertation was to identify the motor patterns underlying *Aplysia's* various feeding responses, and then to develop criteria that distinguish these responses based on the activity of identified motor neurons. Nervous systems employ a variety of different strategies to generate multiple behavioral responses. Often, it is advantageous to adopt a particular experimental approach based on the animal's behavioral strategy. This leads to two questions: What is the likely strategy underlying the consummatory feeding behavior of *Aplysia*? What is the best experimental approach for studying the neural basis of this behavior?

The various strategies used for generating multiple behavioral responses can be grouped into 3 categories: separate pattern generators, shared elements, and population vectors. The separate pattern generator strategy uses totally separate pattern generators to produce different behavioral responses. The shared element strategy uses pattern generators that share a common group of neurons to produce different behavioral responses. The population vector strategy uses a large population of neurons to shape the animal's behavioral response over a continuous range of possible responses.

In this section, a number of systems will be reviewed to illustrate some of the behavioral consequences associated with these strategies. This review will be used to indicate the likely strategy underlying *Aplysia's* consummatory feeding behavior, and to introduce the experimental approach used in this dissertation.

Separate pattern generators

Several systems have been studied in which different behavioral responses are mediated by separate, or nearly separate pattern generators. A trend suggested by these systems is that multiple behavioral responses are more likely to be produced by separate pattern generators if the responses involve totally different portions of the animal's periphery, or if one or more of the responses are optimized to be extremely fast.

In the locust *Locusta migratoria*, separate pattern generators mediate flying (Robertson and Pearson 1982, 1983, 1985; Ramirez and Pearson 1988) and walking (Burrows 1980, 1989; Ramirez and Pearson 1988; Kien 1990a,b). These two pattern generators are located in separate regions of the animal's nervous

system and apparently have only weak interactions (Ramirez and Pearson 1988). The anatomical separation between these two behaviors is nearly complete, with very few bifunctional muscles participating in both flight and walking. Ramirez and Pearson have shown that motor neurons innervating bifunctional muscles receive synaptic input from both pattern generators. In fact, in a reduced preparation, they observed that in bifunctional muscles, both the walking and flight motor patterns can be present at the same time, providing further evidence that the pattern generators are separate.

In the crustacean stomatogastric system, different pattern generators are associated with the control of different stomach regions (i.e. esophageal rhythm, gastric mill rhythm, pyloric rhythm, and cardiac sac rhythm; Selverston and Moulins 1987). These 4 rhythms are produced by circuitry located in separate parts of the stomatogastric system and can be present simultaneously, suggesting that they are produced by separate pattern generator circuits (Selverston and Moulins 1987). An interaction between the pyloric circuit and the cardiac sac circuit that involves shared control of the same stomach structure will be discussed in the following section.

In the pteropod mollusc *Clione limacina* an ongoing swimming response and a fast swim wing withdrawal response are mediated by separate neural circuits. *Clione* continuously swims to provide positive buoyancy. The animal's swim wings must be extended in order to do this. Touching one of the animal's wings, however, causes a rapid wing retraction and an immediate interruption of the swimming motor pattern. Huang and Satterlie (1990) have shown that two separate neural circuits are involved in generating and coordinating the

movements associated with these two behavioral responses: one circuit produces the swimming motor pattern, the other one produces the rapid wing retraction response. The wing retraction circuit consists of a number of mechanoafferent neurons that mediate activity in two wing retraction interneurons. These interneurons cause rapid wing retraction by directly activating wing retraction motor neurons, and stop the swim pattern via strong inhibitory synaptic connections to elements of the swimming pattern generator.

The crayfish can generate two types of tail flips (Krasne and Wine 1984): a short latency tail flip that is mediated by the giant interneuron system (giant mediated tail flip) and a longer latency tail flip that is mediated by the non-giant interneuron system (non-giant mediated tail flip). Giant mediated tail flips are stereotyped, and occur within 13-20 ms after a threatening stimulus is presented. Non-giant mediated tail flips are more variable, and occur after a longer latency (≈ 50 -500 ms). In addition, non-giant mediated tail flips can occur in bouts called "swimming". A given stimulus may elicit a giant mediated response, a non-giant response, or a giant response followed by a non-giant response. Current evidence suggests that the giant system may recruit portions of the non-giant system during giant mediated tail flips; however, these two systems appear to be largely separate. Each tail segment is innervated by two classes of motor neurons, motor giants (MoGs) and fast flexors (FFs). Each MoG innervates almost all of the flexor musculature in its hemi-segment. The FFs occur in populations, with different FFs innervating different portions of the flexor musculature. The giant interneuron system produces fast, stereotyped tail flips via powerful direct connections to the MoGs, as well as powerful indirect connections to the FFs.

The non-giant system uses a population of non-giant interneurons (many of which have not been identified) to activate only the FFs. The synaptic connections from the non-giant interneurons to the FFs are much weaker than those of the giant system, and the synaptic strength varies depending on the specific non-giant interneuron and the specific segment. This allows the non-giant tail flips to be shaped by differentially activating the non-giant interneurons. Krasne and Wine (1984) have proposed that the giant system is specialized to produce rapid, stereotyped responses to threatening stimuli, while the non-giant system is specialized to mediate a finely controlled swim response.

Shared elements

Several systems have been studied in which different behavioral responses are produced by pattern generators that share the same group of neurons. One way to share elements among several pattern generators is to have a core pattern generator circuit that is modified to produce different responses by adding or removing specific groups of neurons. Another way to share elements is to form different pattern generator circuits by changing the effective synaptic connectivity within the shared group of neurons. A trend suggested by these systems is that multiple behavioral responses are likely to be produced by pattern generators that share a group of neurons when the different responses use the same portions of the animal's periphery.

The marine mollusc *Tritonia diomedea* uses a shared group of neurons to produce either a defensive withdrawal response or an escape swim response (Gettings and Dekin 1985). During defensive withdrawals, the dorsal and ventral

flexor motor neurons are co-activated, causing the animal to pull back, away from the offending stimulus. During escape swims, however, these same motor neurons are active in a repetitive, alternating pattern. This pattern produces alternating dorsal and ventral body flexions that propel the animal several feet through the water. Getting and Dekin (1985) have shown that the animal determines which response is carried out by controlling the connectivity within the population of dorsal swim interneurons (DSIs). The DSIs are pattern generator elements during both behavioral responses. During the defensive withdrawal response, connectivity within the DSI population is mutually inhibitory. During escape swims, however, connectivity within this population is mutually excitatory, producing the regenerative bursting activity needed to generate the escape swim. The mechanism underlying this change in connectivity has not been worked out. However, it is associated with activity in the C2 neuron, which participates in the pattern generator only during escape swims (Getting and Dekin 1985).

The crustacean stomatogastric system, as mentioned above, contains several separate pattern generator circuits that control different stomach structures. An interesting example of a shared element has been found where two of these pattern generators (pyloric and cardiac sac) share control over the same stomach structure. The pyloric rhythm is an ongoing rhythm that functions to churn food in the stomach and filter it through the antrum into the small intestine. Current evidence suggests that when a large piece of food becomes stuck in the antrum, it causes the antral wall to stretch, triggering the cardiac sac rhythm (Hooper and Moulins 1989). The cardiac sac rhythm causes a large wave of contraction to

propel stomach contents back toward the esophagus, clearing the antrum of the stuck piece of food. A muscle named the cardiac sac dilator 2 is used during both responses (Selverston and Moulins 1987) and is innervated by the ventricular dilator motor neuron (VD). Hooper and Moulins (1989) have shown that VD is normally active with the pyloric circuit. When the cardiac sac circuit is active, however, VD ignores synaptic input from the pyloric circuit and participates in the cardiac sac response. This jump from the pyloric circuit to the cardiac sac circuit is associated with the rapid inactivation of VD's ability to produce plateau potentials (Hooper and Moulins 1989).

The medicinal leech uses the same anatomical structures (the longitudinal muscles) to carry out a local bending reflex, a shortening reflex, and a swimming response. At least one interneuron, cell 115, is shared by the different circuits that mediate these three responses. Lockery and Kristan (1990a,b) have shown that cell 115 is part of a population of cells that computes the direction of the bending reflex. Wittenberg and Kristan (1992a,b) have shown that cell 115 plays an important role in intersegmental coordination during the shortening reflex. Friesen (1989) has shown that cell 115 is a member of the swimming pattern generator circuit.

The shore crab, *Carcinus maenas*, produces two different gill ventilating rhythms, named forward and reverse ventilation. These two rhythms are carried out using the same peripheral structures, the scaphognathites. DiCaprio (1985) has identified two distinct motor patterns that correspond to these two ventilatory rhythms. In addition, DiCaprio (1989) has described several non-spiking interneurons that are shared by the pattern generators for these two motor patterns.

The mechanisms used to generate these two motor patterns have not been worked out. However, DiCaprio (1990) has identified a command-like neuron that can cause the pattern generator to switch from the forward rhythm to the reverse rhythm.

The feeding circuit of *Pleurobranchaea* has not been worked out in detail. However, existing evidence suggests that the feeding pattern generator contains a large number of shared neurons which undergo very similar patterns of activity during bite-strike, swallowing, and rejection responses. EMG recordings performed in intact, freely moving animals, as well as in reduced preparations show that activity in many buccal mass muscles is very similar during these three types of responses (Croll and Davis 1981; McClellan 1982b). This suggests that the same pattern generator circuit, with slight modifications, may produce all 3 types of responses (Croll and Davis 1981). This view is supported by the results of Croll et al. (1985a-c) and Mpitsos and Cohan (1986) who have described a number of neurons that participate in both ingestion-like and rejection-like motor patterns in a reduced preparation of *Pleurobranchaea*.

Population vectors

Several systems have been studied in which different behavioral responses are mediated by a population vector. In this strategy, each member of the population contributes a small amount to shaping the resulting behavioral response. As activity within the population changes, these small contributions change to produce an altered response. A trend suggested by these results is that

behaviors which are variable over a continuous range of possible responses may be mediated by a population vector.

A population of cells in the motor cortex of the rhesus monkey has activity that is related to the direction of arm reaching movements. Individual neurons within this population appear to have a broadly tuned directional response in which the neuron maximally responds to arm movements in its preferred direction while responding at lesser rates to arm movements in other directions (Schwartz et al. 1988). Within this population, it was found that the preferred directions are uniformly distributed throughout 3-D space (Schwartz et al. 1988). Georgopoulos et al. (1988) have proposed that the direction of arm reaching is computed by this population, with each neuron contributing a small amount to the resulting arm movement. Using a 3-D population vector calculated from the responses of these directionally tuned neurons, they found that this population of cells could accurately predict the direction and trajectory of arm movements before the movements began. This group's hypothesis is also supported by anatomical evidence since these neurons project to the spinal cord segments that mediate arm movements (Phillips and Porter 1977).

A cockroach escapes by turning away from its predator and then running (Camhi 1984; Ritzmann 1993). The angle of the turn can be varied to assure that the run is directed away from the predator, no matter where the predator is located. Current evidence suggests that the turn angle is determined by activity within the population of type A thoracic interneurons (TI_As; Westin et al. 1988; Ritzmann and Pollack 1988). In a hypothesis proposed by Ritzmann and Pollack (1988), the turn angle computation is distributed over the TI_A population, with

each TI_A contributing a small amount to the leg movements that produce the turn. As the position of the predator is varied, the changing directional information causes the activity of individual TI_A s to vary as well. This changed TI_A activity causes the leg movements to vary, ultimately varying the turn angle. This hypothesis is supported by evidence showing that a subset of TI_A s receives directional information from the cercal/ventral giant interneuron system, which is known to play a role in determining the position of the predator (Westin et al. 1977). This hypothesis is also supported by evidence showing that the TI_A s can produce activity in leg motor neurons via direct synaptic connections as well as via polysynaptic pathways (Ritzmann 1993).

The medicinal leech carries out a local bending reflex in which the animal flexes its body to withdraw from the site of a skin touch (Lockery and Kristan 1990a,b). The direction of the withdrawal can be varied to assure that it will always be directed away from the touch. Current evidence (Lockery and Kristan 1990a,b) suggests that the bending direction is mediated by a population of local bending interneurons (LBIs). In a hypothesis proposed by Lockery and Kristan (1990b), the withdrawal direction computation is distributed over the LBI population, with each LBI contributing a small amount to the bending direction. As the position of the skin touch is changed, the changing directional information causes the activity of individual LBIs to change as well. The changed LBI activity causes the bending movement to vary, ultimately changing the direction of bending. This hypothesis is supported by evidence showing that the LBIs receive input from the P-cells (directionally sensitive sensory neurons that

respond to skin pressure) and synapse directly onto the motor neurons that are known to mediate the bending reflex (Lockery and Kristan 1990b).

Experimental approaches for each neuronal strategy

The above examples suggest that different experimental approaches may be better suited for investigating behaviors mediated by different neuronal strategies. In the case where separate or nearly separate pattern generators are used to produce multiple responses, it may be advantageous to study separately each type of response and its underlying neural circuitry. In contrast, for cases where a shared group of neurons is used to produce different responses, it may be advantageous to study simultaneously all of the responses, focusing on the commonalities and differences between each type of response. In systems using a population vector, it may be advantageous to study simultaneously the full range of responses, working to identify the group of neurons whose activity represents the population vector.

Likely strategy underlying Aplysia's feeding responses

Two lines of evidence suggest that a shared group of neurons may be used to generate *Aplysia's* consummatory feeding responses. First, each of *Aplysia's* feeding responses use the same peripheral structure (i.e. the buccal mass). As mentioned above, when different responses use the same periphery, they tend to be generated by pattern generators that share neurons. Second, the feeding pattern generator of *Pleurobranchaea* is likely to contain many elements that undergo similar activity patterns during each type of feeding response. The similarities

between *Pleurobranchaea* and *Aplysia* provide further support for the view that many shared elements are used to generate *Aplysia*'s different feeding responses.

An experimental approach for Aplysia

The arguments presented above suggest that it could be advantageous to focus on the commonalities and differences between *Aplysia*'s consummatory feeding responses. Focusing on the commonalities will help to identify portions of the neural circuit that provide a common contribution to biting, swallowing, and rejection responses. Focusing on differences will help to identify features in the underlying motor patterns that can distinguish these responses. These distinguishing features can then be used to identify portions of the feeding circuitry that determine which response is generated.

It may be advantageous to approach the *Aplysia* feeding system as something other than a pattern generation problem. Many of the behavioral systems that have been studied in detail produce rhythmic behaviors (such as swimming, walking, or rasping). In contrast, the consummatory feeding behavior of *Aplysia* is non-rhythmic. Each biting, swallowing, or rejection response is a separate and distinct motor act. The non-rhythmicity of this behavior has an important ramification: most of the experimentally tractable tests for determining if a neuron is a pattern generator element require that the motor patterns be rhythmic (Gettings and Dekin 1985; Elliot and Benjamin 1985a,b; Selverston and Moulins 1987). It may be possible to borrow some of the techniques currently used in non-linear electrical circuit analysis (Chua and Lin 1975) to develop a

circuit element test for non-rhythmic behaviors. However, that is beyond the scope of this dissertation.

Problem to be addressed

As stated at the outset of this chapter, the goal of this dissertation was to develop criteria that distinguish biting, swallowing, and rejection responses based on the activity of identified motor neurons. Based on the following observations, I reasoned that studying the neural mechanisms underlying radula movements could provide these criteria. First, the radula plays a central role in biting, swallowing, and rejection responses. Second, data from two species with similar consummatory feeding behaviors (*Tritonia* and *Pleurobranchaea*) show that radula movements are different during different feeding responses, and suggest that the same may be true for *Aplysia*. Third, a review of systems that produce multiple behavioral responses suggests that the neural circuits producing biting, swallowing, and rejection in *Aplysia* may share a common group of neurons. These observations suggest that focusing on the similarities and differences in radula movements during these responses will facilitate the association of each response with its underlying motor pattern. It also suggests that features of these motor patterns may serve as reliable criteria for distinguishing biting, swallowing, and rejection responses.

This goal was addressed by asking three questions: Can radula movements be used to distinguish biting, swallowing, and rejection responses? Can the patterns of neural activity present on nerves that are likely to mediate radula movements be used to distinguish biting, swallowing, and rejection responses?

Can the activity of specific motor neurons be identified within these patterns of activity?

These questions were answered by performing experiments on intact, freely moving animals, as well as on reduced preparations. In chapter 2, recordings of radula movements and *in vivo* neural activity were used to develop criteria for distinguishing biting, swallowing, and rejection. A temporal relationship between radula movements and *in vivo* neural activity was also established. In chapter 3, a reduced preparation that produces ingestion-like and rejection-like motor patterns was developed. Using this preparation, the activity and peripheral actions of motor neurons that participate in the ingestion-like and rejection-like patterns were studied. In chapter 4, the significance of these results were discussed, and possible future lines of investigation were proposed.

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Fig. 1-1a-c. Anatomy of *Aplysia's* buccal mass. In each drawing, the extrinsic muscles and salivary glands have been removed for clarity. In some views, the buccal and cerebral ganglia have been removed as well. (a) Lateral view of the buccal mass. The upper left drawing shows the outside surface of the buccal mass with buccal and cerebral ganglia attached. The upper right drawing shows the buccal mass after the two ganglia, the I_1 muscle, and the I_2 muscle have been removed. The lower right drawing shows a buccal mass that has been bisected along the dorsal midline, revealing the buccal cavity and radula. (b) Dorsal view of the buccal mass. The left drawing shows the outside surface of the buccal mass. The drawing on the right shows a buccal mass in which the dorsal wall has been removed, revealing the buccal cavity and radula. (c) Caudal view of the buccal mass. The left drawing shows the outside surface of the buccal mass with the buccal ganglion attached. The right drawing shows the buccal mass with the buccal ganglion attached and the I_1 and I_2 muscles dissected away. Abbreviations: BN - buccal nerve, CBC - cerebral buccal connective, RN - radula nerve, EN - esophageal nerve.

Fig. 1-2a, b. Radula Movements. (a) Radula protraction and retraction. The radula is protracted on the left, at rest in the center, and retracted on the right. (b) Radula opening and closing. The radula is open on the left and closed on the right.

A

B

Fig. 1-3. Properties of muscles in *Aplysia's* buccal mass. This table was compiled from data presented by Howells 1942, Cohen et al. 1978, Scott et al. 1991, Lloyd 1991, Church et al. 1991, Church and Lloyd 1991, and this dissertation. Symbols: * - data presented in this dissertation, ‡ - many additional motor neurons innervate these muscles; however, the motor neurons listed in this table will be studied in greater detail in chapter 3 of this dissertation.

Muscle	Action	Innervation	Relevant motor neurons ‡
I ₁	?	BN2, BN3	
I ₂	Protract radula Open jaws	RN*	
I ₃	Close jaws Retract radula*	BN2, BN3	B10
I ₄	Close radula*	RN	B8a, B8b
I ₅	Close radula	BN3	
I ₆	Close radula*	RN	B8a, B8b
I ₇	Open radula	BN3	
I ₈	Open radula	?	

Fig. 1-4. Properties of identified motor neurons in *Aplysia's* buccal ganglion. This table was compiled from data presented by Cohen et al. 1978, Church et al. 1991, Church and Lloyd 1991, and this dissertation. Symbols: * - data presented in this dissertation, ‡ - in addition to the neuropeptide(s) listed in this table, each of these motor neurons releases a classical neurotransmitter. Abbreviations: *i* - ipsilateral, *c* - contralateral, BN - buccal nerve, RN - radula nerve.

Motor Neuron	Peripheral Actions	Muscles innervated	Projection of major axons	Peptide content [‡]
B3		<i>i</i> I3	<i>i</i> BN2	Fa
B4		<i>i</i> I7	<i>i</i> BN3	Fa
B5		<i>i</i> I7	<i>i</i> BN3	Fa
B6		<i>i,c</i> I3, I6	<i>i,c</i> BN2, BN3	SCPs
B7		<i>i</i> I6	<i>i</i> BN3	Fa, Mm, SCPs
B8a	Close Radula*	<i>i,c</i> I4, I6	<i>i,c</i> RN	Mm
B8b	Close Radula*	<i>i,c</i> I4, I6	<i>i,c</i> RN	Mm
B9		<i>i,c</i> I3, I6	<i>i,c</i> BN2, BN3	SCPs
B10	Close Jaws* Retract Radula*	<i>i,c</i> I3	<i>i,c</i> BN2	SCPs
B11		<i>i</i> I6	<i>i</i> BN3	Mm, SCPs
B15	Close Radula	<i>i</i> I5	<i>i</i> BN3	Bn, SCPs
B16	Close Radula	<i>i</i> I5	<i>i</i> BN3	Bn, Mm
B38		<i>i</i> I3	<i>i</i> BN2	SCPs
B39		<i>i,c</i> I3	<i>i</i> BN2	Fa
B43		<i>i</i> I1	<i>i</i> BN2	SCPs
B44		<i>i,c</i> I6	<i>i,c</i> BN3	Mm
B45		<i>i</i> I1	<i>i</i> BN3	Mm

Fa = FMRFamide
 Mm = myomodulin
 Bn = buccalin
 SCPs = small cardioactive peptides

Chapter 2

In vivo motor patterns associated with consummatory feeding behavior

Summary

We have studied the neural basis of consummatory feeding behavior in *Aplysia* using intact, freely moving animals. Video records show that the timing of radula closure during the radula protraction-retraction cycle constitutes a major difference between ingestion (biting or swallowing) and rejection. During ingestion, the radula is closed as it retracts. During rejection, the radula is closed as it protracts. We observed two patterns of activity in nerves that are likely to mediate these radula movements. Patterns I and II are associated with ingestion and rejection, respectively, and are distinguished by the timing of radula nerve activity with respect to the onset of buccal nerve 2 activity. The association of ingestion with pattern I is maintained when the animal feeds on a polyethylene tube, the same food substrate used to elicit rejection responses. Under these conditions, pattern I is associated with either swallowing or no net tube movement. Most transitions from swallowing to rejection were preceded by one or more occurrences of pattern I in which there was no net tube movement,

suggesting that these transitions can be predicted. Our data suggest that these two patterns can be used to distinguish ingestion from rejection.

Introduction

Many animals can rapidly and smoothly change among several different behavioral responses as environmental conditions and motivational state change. For example, during consummatory feeding in the marine mollusc *Pleurobranchaea*, the animal carries out a bite-strike response when it senses food nearby, an ingestion response once it has captured a piece of food, and a rejection response if a piece of captured food is found to be inedible (Croll and Davis 1981; McClellan 1982a). Similarly, the crustacean stomatogastric nervous system, in response to specific sensory inputs and modulating substances, generates a variety of distinct responses involving the gastric mill and stomach musculature (Selverston and Moulins 1987; Heinzel 1988a,b; Heinzel and Selverston 1988; Hooper and Moulins 1989; Weimann et al. 1991).

In order to understand the neural basis of this phenomenon, one must analyze each different behavioral response, as well as analyzing the transitions from one type of response to another. Previous studies in *Pleurobranchaea* (McClellan 1982a,b; Croll et al. 1985a-c) have shown that these analyses can be greatly facilitated by developing rigorous criteria which distinguish each type of response. These studies also suggest that if the criteria are to be applied to a reduced preparation in which most or all of the periphery has been removed, then they should be based solely on neural or neuromuscular activity.

The consummatory feeding behavior of the marine mollusc *Aplysia californica* provides several advantages for studying the neural basis of a behavior that is made up of multiple responses. The different feeding responses are easily elicited and readily distinguished from each other (Kupfermann 1974). In addition, the animal can rapidly change from one type of response to another (Kupfermann 1974; Cropper et al. 1990). Finally, this behavior is likely to be amenable to a detailed cellular analysis since many important components have been identified, including synaptic followers of identified neurons B4 and B5 (Gardner 1971), motor neurons (Cohen et al. 1978; Church and Lloyd 1991; Church et al. 1991), putative pattern generator elements (Susswein and Byrne 1988; Plummer and Kirk 1990), and command-like neurons (Rosen et al. 1991). *Aplysia's* consummatory feeding responses include biting, swallowing, and rejection (Kupfermann 1974), which are carried out using the same peripheral structure, the radula. The radula is used to move food into the buccal cavity during biting and swallowing responses, and out of the buccal cavity during rejection responses.

Since the radula plays a central role in each of these feeding responses, we sought to develop a criterion to distinguish biting, swallowing, and rejection using activity recorded from nerves which are likely to mediate radula movements. We then sought to use this criterion to study transitions from one type of feeding response to another. In order to achieve these goals, we combined videotaping of the animal's behavior with recordings of *in vivo* buccal nerve activity. A preliminary report of these results has appeared (Morton and Chiel 1991). A

slightly different version of this chapter has been published (Morton and Chiel 1993).

Materials and Methods

Aplysia californica weighing 200-350 g (Marinus, Long Beach, CA) were maintained in an aerated tank containing artificial sea water (Instant Ocean, Aquarium Systems, Mentor, OH) kept at 16°C. Animals were kept for up to 2 weeks before being used in experiments and were fed dried seaweed (laver, Sun Wing Hong Food, Ltd., Hong Kong) on a regular basis during this time. One or two days prior to the experiment, feeding was stopped in order to increase the animal's responsiveness to food.

Definition of behavioral terms

The consummatory phase of feeding consists of at least three responses: biting, swallowing, and rejection (Kupfermann 1974). These responses are described in terms of simple behavioral correlates as follows: A *bite* is a protraction and retraction of the radula which fails to capture a piece of food. The radula is open as it protracts, closes near the peak of protraction, and remains closed as it retracts. During this study, we only included bites in which the jaws were maximally opened and the radula maximally protracted (defined to be class 4 bites by Susswein et al. 1976). Sub-maximal bites (classes 1, 2, and 3) were excluded due to the difficulty in observing radula movements in the video record. We found that neural activity during sub-maximal bites was similar to that seen during class 4 bites. Bites were elicited by touching a 5 x 5 mm piece of dried

seaweed to the animal's lips. A *swallow* is an episodic inward movement of a thin strip of seaweed. Swallows were elicited by feeding the animal a strip of dried seaweed that was 2.5-5 mm wide and several centimeters long. A *rejection* is an episodic outward movement of a polyethylene tube. Rejection was elicited by first inducing biting behavior using dried seaweed, and then allowing the animal to grasp a polyethylene tube (1.27 mm OD, 150 mm long, marked at 3 mm intervals; Intramedic, Clay-Adams, Parsippany, NJ) in place of the seaweed. After several swallows, the animal usually withdrew its head slightly and then started to reject the tube.

In vivo nerve recordings

Extracellular electrodes were made of twisted pairs of insulated stainless steel wire (2.54 μ m diameter, California Fine Wire, Grover City, CA). Gold contacts were soldered to one end of each wire. The insulation was scraped from 2 mm of wire at the other ends, and the bare wires fashioned into small hooks. The electrode wires were then coated with silicon glue on all parts except for the gold contacts and the bare wire hooks, and allowed to dry for at least 24 hours. When implanted, one hook was attached to the nerve using a drop of super glue, while the other hook floated free in the hemocoel, serving as a reference.

Animals were anesthetized with an injection of 50% (v/w) of isotonic (333 mM) MgCl_2 . A 1.5 cm incision was made from a point just lateral to the left eye spot to a point just lateral and posterior to the left rhinophore. Next, a small incision was made in the left side of the I_2 muscle, exposing the large branches of the RN. Extracellular electrodes were then applied to the left branch of the RN

and the left BN2. Each electrode was held in place by a drop of super glue (Duro Quick Gel, part no. SGG-1, Loctite Corp., Cleveland, OH). Once the electrodes were in place, the skin incision was sutured closed (6-0 silk, Ethicon) with the electrodes exiting through the posterior end of the incision. Animals were allowed to recover overnight in an aerated, individual tank maintained at 16°C.

Electrode signals were amplified using an AM systems model 1700 AC amplifier (AM Systems, Everett, WA), filtered by a 100 Hz highpass filter and a 1 kHz lowpass filter, and then further amplified by a custom built variable gain amplifier. Neural signals were sampled at 2 kHz per channel and recorded on a hard disc using a PC based data acquisition system (Axotape, Axon Instruments, Foster City, CA). The animal's behavior was simultaneously recorded on video tape using a camera with a close-up lens (General Electric camcorder model 9-9610). A custom built frame counter driven by a 15 Hz clock was visible in each video frame. The frame counter clock signal was recorded with the electrode signals, allowing us to identify a behavioral event in the video record, record the frame counter value associated with that event, and then locate the event in the neural record by counting the appropriate number of frame counter clock cycles. Using this technique, events could be located in the neural record with an accuracy of ± 33 msec. Since behavioral events in this species occur on the order of seconds, this degree of precision is more than adequate. Custom software designed to read and manipulate Axotape data files was used to count the frame counter clock cycles and tag the appropriate point in the neural record.

Analysis of neural patterns

Software window discriminators were used to detect units of different sizes in the recordings of BN2 and RN activity (Plumb 1965; Rose 1971, 1972). Three different windows were used to detect the activity of the three largest units observed in BN2 recordings, while a single window was used to detect the largest units in RN recordings. Two representative patterns of neural activity are shown in Figure 2-1a. Figure 2-1b shows the window discriminator output for these patterns. Window discriminator voltage thresholds were selected based on neural activity observed during swallowing and then used to process neural activity associated with biting, swallowing and rejection. These voltage thresholds were selected individually for each animal.

Activity detected by each window discriminator was grouped into bursts using a frequency discriminator (Figure 2-1c) followed by a thresholder (Figure 2-1d). BN2 frequency thresholds were 3 Hz, 1.5 Hz, and 3 Hz for the largest, second largest, and third largest units, respectively. The RN frequency threshold was set to 2 Hz. These thresholds were selected by calculating the average frequency of each window discriminator's output for several representative biting patterns and then setting each threshold to one half of this value. Frequency thresholds were the same for all animals. In general, increasing the thresholds caused the resulting bursts to be fragmented. Decreasing the thresholds had little effect until levels near 1 Hz were reached. At this point, bursts in adjacent patterns began to fuse together. An additional criterion was imposed on the second largest BN2 unit. During many swallowing responses, activity in this unit was very intense at the start and end of the burst, while the middle of the burst

consisted of a gradual decrease in intensity followed by a gradual increase. The intensity in the mid-burst region was variable, and during some responses it fell below threshold, causing the algorithm to inappropriately divide the burst. Two people working together (DWM and HJC) manually grouped bursts with this frequency profile into a single burst. Using these criteria, approximately 90% of the burst assignments seemed unequivocal. The remaining assignments were more difficult due to some bursts having unusually low intensity, and occasionally, due to adjacent bursts blending into each other.

A quantitative analysis of these patterns was carried out using the start and end times of activity in the 3 groups of BN2 units and the large RN units (i.e. the positive and negative transitions in Figure 2-1d), as well as on the times of observed behavioral events.

Nerve lesions

Bilateral RN lesions were performed on three animals. Sham lesions, in which the RNs were exposed but not cut, were performed on two animals. We used incisions identical to those used for *in vivo* nerve recordings. In lesioned animals, the right and left RNs were cut near the point where they entered the odontophore muscle (for anatomy, see Figure 1-1c, right image). Great care was required to avoid lesioning the small nerve branches which leave the common portion of the RN and enter the I₂ muscle.

Biting responses were videotaped before and after the lesions or sham lesions were performed. Two observers, who did not know which animals were lesioned or sham-lesioned, independently viewed the video tapes of all lesioned

and sham lesioned animals. These observers were able to correctly identify the lesioned animals as well as describing the resulting deficits.

Isolated buccal mass preparation

An isolated buccal mass preparation was used to study the buccal mass movements produced by nerve shock. In this preparation, the buccal ganglion was removed by cutting the buccal nerves as close to the ganglion as possible. The buccal artery was cannulated and perfused with *Aplysia* saline (450 mM NaCl, 10 mM KCl, 22 mM MgCl₂, 33 mM MgSO₄, 10 mM CaCl₂, 10mM Glucose, 10 mM MOPS, pH 7.4-7.5) at a rate of 1 ml/min for at least 60 minutes before starting the experiment. Suction electrodes were applied to the nerve to be shocked (BN2 or the RN).

Line drawings of radula movements

Radula movements were recorded using a video camera with a close-up lens (General Electric camcorder model 9-9610). Tracings of the resulting video images were made using a 19 inch video monitor.

Results

Radula movements associated with consummatory feeding behavior

What movements does the radula undergo during biting, swallowing, and rejection? To answer this question, we videotaped radula movements during 17 bites, 40 swallows, and 10 rejections. Each type of response was observed in at least 3 different animals.

During biting (Figure 2-2a), the radula is open as it protracts, starts to close just before the peak of protraction, and is closed as it retracts. The jaws are maximally opened and the radula is often protracted beyond the jaws. This result is consistent with the observations of Kupfermann (1974).

During swallowing (Figure 2-2b), the radula is open as it protracts, closes on the seaweed at approximately the peak of protraction, and remains closed as it retracts, pulling the seaweed into the animal's mouth. The jaws are usually kept nearly closed, making it difficult to observe the radula. However, the animal will occasionally swallow with its jaws open as shown here. During swallows in which the jaws were nearly closed, we consistently observed the radula protracting with little or no seaweed movement and then retracting as the seaweed moved into the animal's mouth (data not shown). Radula opening and closing is difficult to observe with the jaws nearly closed. However, pulling on the seaweed as it moved inward revealed that it was firmly held by the radula, suggesting that the radula was closed during retraction.

When swallowing a polyethylene tube, radula movements were similar to those observed during seaweed swallows (Figure 2-2c). Tube swallowing is elicited by first causing the animal to bite using dried seaweed and then allowing the tube to be grasped in the radula. The radula is open during protraction, closes on the tube at approximately the peak of protraction, and remains closed during retraction, pulling the tube into the buccal cavity. Thus, radula movements appear to be similar when swallowing seaweed or a polyethylene tube, despite the fact that these materials have very different physical properties.

Identifying the radula movements associated with rejection was more difficult since rejection responses can only be elicited as part of a progression of different feeding responses (i.e. the animal must first swallow a polyethylene tube before rejecting it). This progression makes it difficult to identify individual responses near the transition point. It also raises the possibility that behavioral responses near the transition may not be pure responses, or may be different, previously undescribed responses. We therefore focused on chains of consecutive rejections, excluding the first rejection in each chain. This limited our observations to rejections that were not at swallowing-to-rejection transitions. We will refer to this subset of rejections as *non-transitional rejections*.

During non-transitional rejections (Figure 2-2d), the radula is closed as it protracts, opens at approximately the peak of protraction, and remains open as it retracts. The tube moves out of the animal's mouth during radula protraction. During some non-transitional rejections, we could not see the radula opening during retraction (3 out of 10 rejections). During most non-transitional rejections, the lips cover the mouth, making it difficult to record radula movements. However, the animal will occasionally carry out a non-transitional rejection with the lips pulled back, as shown in Figure 2-2d.

These data show that the timing of radula closure relative to protraction and retraction can be used as a criterion to distinguish ingestion from rejection. During each behavioral response, the radula undergoes a protraction-retraction cycle. When the animal bites or swallows, the radula is closed during the retraction portion of the cycle. When the animal rejects, the radula is closed during the protraction portion of the cycle.

Roles of buccal nerve 2 and the radula nerve in mediating radula movements

For the remainder of this study, we will be analyzing neural activity recorded from BN2 and the RN (B5 and B1, respectively, in the notation of Kandel 1979). What roles do these nerves play in mediating the radula movements shown in Figure 2-2?

BN2 may be involved in jaw closure and radula retraction. This nerve contains fibers which innervate the muscles and other tissues around the jaws (Scott et al. 1991; Nagahama and Takata 1988; Church and Lloyd 1991; Church et al. 1991). Bilateral lesions of BN2 result in a significant reduction in jaw closing forces (Chiel et al., data not shown). In an isolated buccal mass preparation, electrical stimulation of BN2 (10 Hz, 10 ms pulses) produces jaw closure (Figure 2-3a). Since the jaws close during radula retraction (Figure 2-2), we also stimulated BN2 with the radula held in a protracted position. Under these conditions, the jaw closure produced by BN2 stimulation produces retraction-like movements (Figure 2-3b).

The RN may be involved in radula closure. This nerve carries fibers that are known to innervate the muscles underlying the radula (Scott et al. 1991; Lloyd 1988; Church and Lloyd 1991). Bilateral lesions of the RNs profoundly delay the onset of radula closure, as shown in Figures 2-4a-c. Comparing the pre-lesion sequence (Figure 2-4a) to the post-lesion sequence (Figure 2-4b) shows that during the post-lesion bite, the radula opens much wider as it protracts and closes much later in the protraction-retraction cycle. In addition, a ridge around the base of the radula that is seen in the pre-lesion bite is much less prominent in the post-lesion bite (compare the last three frames of Figures 2-4a and 2-4b). The data in

Figure 2-4c show that the delay in radula closure is significant ($p < 0.002$, t-test). Electrical stimulation of the RNs (10 Hz, 10 ms pulses) in an isolated buccal mass preparation causes radula closure (Figure 2-4d).

In vivo neural activity associated with biting, swallowing, and rejection

Using 5 intact, freely moving animals, extracellular recordings of BN2 and RN activity were performed during biting, swallowing, and rejection responses. Behavioral events were recorded on video tape and then associated with the neural activity using the custom software described in the methods section. In this analysis, we directly associated radula movements with neural activity during biting responses. However, during swallowing and rejection responses, we could only associate food movement with neural activity because the radula is generally not visible during these responses. Please note that *large unit BN2 activity* refers to the collective activity of the largest 3 BN2 units (arrows 1, 2, and 3, Figure 2-1a).

Biting is associated with activity similar to that shown in Figure 2-5a. The onset of large unit BN2 activity is always preceded by a short period of large unit inactivity (bar, Figure 2-5a). In addition, the onset of large unit RN activity and the onset of large unit BN2 activity occur at approximately the same time. The biting sequence shown in Figure 2-2a and the pattern shown in Figure 2-5a are from the same bite. The numbered arrows in Figure 2-5a indicate when the numbered frames of Figure 2-2a were observed during the pattern of neural activity. The radula started to close just after arrow 1. Since this occurs shortly after the onset of large unit RN activity, it is consistent with the results of Figure

2-4 which suggest that fibers in the RN may contribute to radula closure. The radula is fully protracted approximately at arrow 3, showing that retraction starts shortly after the onset of large unit BN2 activity. This is consistent with the results of Figure 2-3b, which suggest that fibers in BN2 may contribute to radula retraction.

Seaweed swallowing is associated with activity similar to that shown in Figure 2-5b. The onset of large unit BN2 activity is always preceded by a short period of large unit inactivity (arrow, Figure 2-5b). In addition, the onset of large unit RN activity and the onset of large unit BN2 activity occur at approximately the same time. Finally, the behavioral marker indicates that the seaweed started moving into the animal's mouth shortly after the onset of large unit BN2 activity, suggesting that radula retraction may be occurring at this time.

Non-transitional rejections are associated with large unit RN activity ending prior to the onset of large unit BN2 activity (Figure 2-5c). The behavioral marker indicates that the tube moved out of the animal's mouth prior to the start of large unit BN2 activity, suggesting that protraction was occurring at this time. In 24% of non-transitional rejections (6 of 25), we observed additional large unit RN activity occurring later in the pattern (Figure 2-5d). This activity, which we called the second RN burst, will be considered in greater detail later in this paper.

During non-transitional rejections, a small, intensely active unit was reliably present in the 3-4 seconds prior to the onset of large unit BN2 activity (Figures 2-5c and 2-5d). This small unit was variably present with variable intensity during biting and swallowing responses. We found it difficult to make specific

statements concerning the activity of this unit due to the presence of many small BN2 units with nearly the same amplitude as this unit.

Three lines of evidence support the hypothesis that the large units observed in these recordings correspond to the activity of motor neurons. First, experiments in which two electrodes were placed on BN2 show that the large units in this nerve are moving toward the periphery ($N = 2$, data not shown). This suggests that these large units may be motor neurons. Second, electrical stimulation of the esophageal nerve in an isolated buccal ganglion elicits patterns of neural activity resembling those seen *in vivo* (Morton et al. 1991). This suggests that these patterns correspond to motor outputs rather than sensory inputs. Finally, nickel backfills of the RN and BN2 fill a number of large cells in the ventral cell cluster of the buccal ganglion (Morton et al. 1991; Scott et al. 1991). Since many of these large ventral cluster cells are motor neurons (Cohen et al. 1978; Fiore and Meunier 1979; Church and Lloyd 1991; Church et al. 1991; Morton and Chiel, unpublished observations), and since neurons with large somata tend to have large axons, this evidence suggests that these large units are motor neurons.

Two lines of evidence suggest that some of the motor neurons contributing to these patterns are active during all three responses. First, the amplitudes of large unit activity, especially on BN2, remained the same during biting, swallowing, and rejection, suggesting that they are produced by the same group of neurons. Second, studies using a reduced preparation (Morton and Chiel 1991) have shown that several buccal ganglion motor neurons which appear as large

BN2 or RN units contribute to both ingestion-like and rejection-like patterns of neural activity.

Analysis of activity patterns on buccal nerve 2 and the radula nerve

In order to use the patterns of Figure 2-5 to distinguish biting, swallowing, and rejection, it is necessary to develop rigorous criteria that distinguish these patterns from each other. These criteria were developed using the algorithm shown in Figure 2-1.

The pattern of large unit BN2 activity is similar during biting, swallowing, and rejection. This pattern is characterized by the sudden onset of intense activity in the third largest unit, followed by activity in the largest and second largest units (Figures 2-1a and 2-1b). It is reliably preceded by a period of large unit inactivity (bars above the top trace in Figure 2-1a). The pattern of large unit BN2 activity had a variable feature as well: the largest unit tended to be absent or weakly present during biting responses (absent in 62% of bites, present as a single spike in 13% of bites, and present as a discernible burst in 25% of bites). This tendency, however, could not be used to reliably distinguish biting from the other feeding responses.

The pattern of large unit RN activity is also similar during biting, swallowing, and rejection. This pattern consists of intense activity in several units of approximately the same size (Figures 2-1a and 1b). There are generally long periods of inactivity separating these patterns, making them easy to recognize.

We compared the timing of large unit RN activity relative to the onset of large unit BN2 activity using a variable named the *fractional overlap* (Figure 2-

1e). Our data show that high fractional overlaps are associated with ingestion responses, while low fractional overlaps are associated with rejection responses. For 40 bites and 86 seaweed swallows, the fractional overlaps were found to be 0.9 ± 0.2 and 0.9 ± 0.1 (mean $\pm$ SD), respectively. In contrast, the fractional overlaps for 27 non-transitional rejections were 0.1 ± 0.2 . Histograms of fractional overlaps associated with seaweed swallows and non-transitional rejections are shown in Figures 2-6a and 2-6b.

Based on these results, we define two patterns of *in vivo* neural activity, named pattern I and pattern II. Pattern I is associated with ingestion (biting or swallowing), and is characterized by large unit RN activity occurring together with large unit BN2 activity (fractional overlaps of 0.8-1.0). Pattern II is associated with rejection, and is characterized by large unit RN activity preceding large unit BN2 activity (fractional overlap of 0.0).

Predictive value of patterns I and II

How strong is the association between neural activity and the observed behavioral response? We have just shown that the behavioral response can be used to predict which pattern of neural activity is present. Is the converse statement true? That is, can the pattern of neural activity be used to determine which behavioral response is being carried out? This question was addressed by taking advantage of the fact that animals will both swallow and reject polyethylene tubes.

Our results suggest that the association of pattern I with ingestion is maintained when feeding on a polyethylene tube. To avoid difficulties near

swallowing-to-rejection transitions, we considered chains of swallowing responses, excluding the last swallow before the onset of rejection. This subset of tube swallows will be referred to as *non-transitional swallows*. Non-transitional swallows were associated with high fractional overlaps (Figure 2-6c). Thus, the association of pattern I with ingestion is maintained, even when the animal feeds on the same food substrate used to elicit rejection.

Pattern I can also be associated with no net tube movement. A histogram of fractional overlaps for all patterns which were associated with no net tube movement is shown in Figure 2-6d. This distribution has a large peak near fractional overlaps of 0.8, which is within the range of pattern I (i.e. fractional overlaps of 0.8-1.0).

Our data suggest that pattern I tends to be associated with inward tube movement early in the tube feeding sequence, and tends to be associated with no net tube movement later in the sequence. Figure 2-7a contains a histogram of the direction of net tube movement for all neural activity with fractional overlaps of 0.8-1.0 (pattern I) that were observed after the tube was grasped by the radula. Under these conditions, 42% of the occurrences of pattern I were associated with inward tube movements, 55% were associated with no net tube movement, and 3% were associated with outward tube movements. A histogram of tube movements for the first 3 occurrences of pattern I after the initial bite-swallow (Figure 2-7b) shows that in this subset of responses, 60% of the occurrences of pattern I are associated with inward tube movements, while 37% are associated with no net tube movement. In fact, 75% of the inward tube movements associated with pattern I were observed during the first three occurrences of this

pattern. We will further explore the temporal relationship of pattern I with inward tube movements and with no net tube movements below.

Our data show that pattern II is mainly associated with outward tube movement. The histogram of Figure 2-7c shows that 83% of the occurrences of pattern II were associated with a net outward tube movement, while 17% were associated with no net tube movement.

The data in Figures 2-7a and 2-7c suggest that pattern I and pattern II can be used to predict inward and outward tube movements, respectively. This statement is based on two observations: First, the distribution of tube movements associated with pattern I is significantly different from the distribution associated with pattern II ($p < 0.002$, Mann-Whitney U-test, Harnett 1975). Second, the distribution associated with pattern I mainly consists of inward tube movements and no net tube movements, while the distribution associated with pattern II mainly consists of outward tube movements.

We observed a significant number of patterns with intermediate fractional overlaps (i.e. fractional overlaps of 0.1-0.7) during feeding on polyethylene tubes. These patterns will be referred to as *intermediate patterns*. A histogram of the direction of net tube movement for all intermediate patterns (Figure 2-7d) shows that these patterns are associated with inward, outward, and no net tube movements, making their behavioral significance unclear.

Associating radula movements with neural activity

Is there a temporal relationship between radula movements (Figure 2-2) and neural activity (Figure 2-5) during biting, swallowing, and rejection? It is

relatively easy to establish this association for biting responses because radula movements can be directly observed. However, the radula is usually not visible during swallowing and rejection responses. For these two responses, we chose to establish an indirect association by taking advantage of the relationship between radula movements and food movements that was established in Figure 2-2.

The timing analysis shown in Figure 2-8 was used to establish the relationship between radula movements and neural activity. For the biting and swallowing portions of this analysis, we used the 40 bites and 86 seaweed swallows which have been previously discussed. For the rejection portion of this analysis, we used the 38 occurrences of pattern II which were associated with outward tube movements (Figure 2-7c). We will refer to these responses as *pattern II rejections*. By choosing this subset of rejections, we excluded the intermediate patterns (i.e. fractional overlaps of 0.1-0.7) that were associated with some outward tube movements, as well as excluding the occurrences of pattern II that were associated with no net tube movement. We reasoned that the remaining responses (i.e. pattern II rejections) would most clearly show the relationship between radula movements and neural activity during rejection.

Our analysis suggests that the onset of large unit BN2 activity occurs near the end of protraction and the start of retraction. During biting, the mean time at which the base of the radula started rolling up and back occurred on or shortly after the onset of large unit BN2 activity in all 5 animals (Figure 8a). Out of 40 bites, this event occurred after the onset of large unit BN2 activity in 38 cases. Figure 2-2a shows that during biting, protraction immediately precedes retraction, suggesting that the onset of large unit BN2 activity occurs near the end of

protraction and the start of retraction during biting responses. During swallowing, the mean time at which the seaweed started moving inward occurred shortly after the onset of large unit BN2 activity for all 5 animals (Figure 8b). In all 86 seaweed swallows, the seaweed started moving in after the onset of large unit BN2 activity. Figure 2-2b shows that during swallowing, the seaweed moves inward during the retraction portion of the protraction-retraction cycle, suggesting that the onset of large unit BN2 activity occurs near the end of protraction and the start of retraction during swallowing responses. During rejection, the mean time at which the tube stopped moving outward occurred shortly before the onset of large unit BN2 activity in all 5 animals (Figure 2-8c). In 31 of 38 cases, the tube stopped moving out prior to the onset of large unit BN2 activity. Figure 2-2d shows that during rejection, the tube moves outward during the protraction portion of the protraction-retraction cycle, suggesting that the onset of large unit BN2 activity occurs near the end of protraction and the start of retraction during rejection responses.

This analysis also suggests that large unit RN activity occurs at times of expected radula closure. Comparing the timing of RN activity (Figure 2-5) to the timing of radula closure (Figure 2-2), one can see that during ingestion responses, the radula is closed as it retracts, and large unit RN activity occurs during retraction. In addition, during rejection responses, the radula is closed as it protracts, and large unit RN activity occurs during protraction.

Our data support the hypothesis that some of the large RN units correspond to motor neurons that act to close the radula. During 18 bites in which the start of

radula closure could be seen in the video record, the onset of large unit radula nerve activity occurred between 0.5 and 2 s before the start of radula closure.

Neural activity associated with transitions from ingestion to rejection

A prominent feature of the neural activity present during swallowing-to-rejection transitions is a transition from pattern I to pattern II. An example of this is shown in Figure 2-9, which is a record of neural activity observed as the animal performed a bite, followed by a bite-swallow, and then a series of swallows and rejections on a polyethylene tube. We observed 11 transitions from swallowing to rejection in the 5 animals in which we performed BN2 and RN recordings. These 11 transitions were associated with 13 transitions from pattern I to pattern II, as follows: Nine were associated with a single transition from pattern I to pattern II. The remaining two transitions from swallowing to rejection were associated with double transitions from pattern I to pattern II (i.e. I to II to I to II). During both of these double transitions, the tube stopped moving inward during the initial group of pattern Is and started moving outward during the second group of pattern IIs. All of the intervening patterns were associated with no net tube movement.

This result suggests that swallowing-to-rejection transitions are always associated with a transition from pattern I to pattern II. Is the converse statement true? That is, are transitions from pattern I to pattern II always associated with swallowing-to-rejection transitions? We observed 16 transitions from pattern I to pattern II. 13 were associated with swallowing-to-rejection transitions as described above. The remaining 3 I-to-II transitions were not associated with swallowing-to-rejection transitions because there was no associated change in the

direction of tube movement. In two of these cases, the animals had started rejecting and then briefly changed back to pattern I with no net tube movement before returning to rejection. In the third case, the animal was swallowing and then briefly changed to pattern II with no net tube movement before returning to swallowing.

Our data suggest that it may be possible to predict behavioral transitions. Most of the transitions from pattern I to pattern II are immediately preceded by one or more occurrences of pattern I in which there is no net tube movement. An example of this is shown in Figure 2-9. We found that 14 of 16 transitions from pattern I to pattern II, and 9 of 11 transitions from swallowing to rejection were preceded by 1 or more occurrences of pattern I with no net tube movement. Overall, 81% (29 of 36) of the occurrences of pattern I that were associated with no net tube movement occurred immediately before transitions from pattern I to pattern II. Thus, our results show that when pattern I is associated with no net tube movement, the animal is likely to switch from swallowing to rejection.

Rejection-to-swallowing transitions

We observed several rejection-to-swallowing transitions. After initially swallowing the tube and starting to reject it, some animals stopped rejecting and began swallowing again before carrying out a series of rejections which finally removed the tube from the animal's buccal cavity. These rejection-to-swallowing transitions were observed a total of three different times in two different animals, and occurred in the absence of any externally applied seaweed stimulus. In all

three cases, these rejection-to-swallowing transitions were associated with a transition from pattern II to pattern I.

Neural transitions are associated with changes in pattern II

When pattern II occurs at transitions to or from other pattern types, it tends to have a second RN burst. An example of this is shown in Figure 2-9, where the first occurrence of pattern II has a second RN burst, while later occurrences do not. Of the 46 occurrences of pattern II observed in this study, 20 had second RN bursts. Eighty five percent of these patterns (17 of 20) occurred at transitions to or from other pattern types. We found that 50% (10 of 20) of the occurrences of pattern II with a second RN burst occurred at transitions from pattern I to pattern II or from pattern II to pattern I, while 35% (7 of 20) of the occurrences of pattern II with a second RN burst occurred at transitions to or from intermediate patterns.

Intermediate patterns

Our data did not suggest a clear role for intermediate patterns (i.e. patterns with fractional overlaps of 0.1 to 0.7). An example of an intermediate pattern is shown in Figure 2-9. We found that 32% (15 of 47) of the intermediate patterns occurred during the initial swallowing phase of feeding on the polyethylene tube. An additional 28% (13 of 47) of the intermediate patterns occurred during the rejection phase of feeding on the polyethylene tube. Another 28% (13 of 47) of the intermediate patterns were associated with transitions from pattern I to pattern II. In fact, 7 of the 16 transitions from pattern I to pattern II had 1 or more intermediate patterns interposed between the last occurrence of pattern I and the

first occurrence of pattern II. The remaining 9 transitions were clean transitions, in which pattern I was immediately followed by pattern II, as shown in Figure 2-9.

Discussion

Patterns I and II can be used to distinguish ingestion from rejection

Three lines of evidence suggest that patterns I and II can be used to distinguish ingestion from rejection. First, the data in Figure 2-5 show that pattern I is present when the animal bites or swallows, while pattern II is present when the animal rejects. This suggests that the animal's behavior can be used to predict which neural pattern is being generated. Second, the data in Figure 2-7 show that the converse statement is also likely to be true, that is, the pattern of neural activity can be used to predict the animal's behavior, even when the physical properties of the food are the same for both responses. Third, the data in Figure 2-8 show that components of these patterns are temporally related to the different radula movements observed during ingestion and rejection. Thus, this activity may represent the activity of motor neurons which mediate these different radula movements.

In a number of behavioral systems, neural correlates recorded *in vivo* or in semi-intact preparations have provided a good foundation for studying the neural basis of behavior (Kater 1974; Gelperin et al. 1978; Rose and Benjamin 1979; Willows 1980; Croll and Davis 1981; McClellan 1982a). There are two reasons why the neural correlates described in this paper are likely to provide a good foundation for studying the neural basis of ingestion and rejection in *Aplysia*.

First, the neural recordings were done in freely moving, intact animals with the feeding behaviors elicited by physiological sensory stimulation. Thus, we believe that these recordings reflect the native activity on these nerves, and can serve as a standard for defining ingestion and rejection in reduced preparations. Second, the neural activity in these recordings consistently distinguishes ingestion from rejection in spite of the variable duration and magnitude of individual feeding responses. This consistency suggests that the difference between patterns I and II (i.e. the timing of large unit RN activity) may be relatively insensitive to proprioceptive feedback, and therefore may be replicated in a preparation with most or all of the periphery removed.

Behavioral responses associated with pattern I

Our data strongly support the hypothesis that pattern I is associated with ingestion. During seaweed swallowing, virtually every occurrence of pattern I is associated with inward seaweed movement. When the animal feeds on a polyethylene tube, however, pattern I is often associated with no net tube movement. The histogram in Figure 2-7a shows that most of the occurrences of pattern I that were associated with no net tube movement occurred after several swallows had moved the tube well into the buccal cavity. This suggests that when pattern I is associated with no net tube movement, the tube, which is very stiff and smooth, may be caught on a fold of epithelial tissue or may simply slip out of the radula, resulting in an ineffective swallow. Since transitions from pattern I to pattern II are usually preceded by one or more occurrences of pattern I in which the tube did not move inward, this interpretation suggests that ineffective

swallows may produce sensory cues which prompt the animal to start rejecting. This also leads to the hypothesis that mechanosensory inputs from the buccal cavity may be involved in eliciting rejection responses. In fact, this has been observed in a similar species, *Tritonia* (Audesirk and Audesirk 1979).

Another possible interpretation is that sensory cues from effective tube swallows elicit a different behavioral response that is associated with pattern I, but not associated with inward tube movements. There are at least three ways in which a feeding response could be associated with no net tube movement: (1) radula protraction and retraction could decrease; (2) the jaws could close tightly on the tube, holding it in place; or (3) radula closing forces could decrease, allowing the tube to slip out of the radula more easily. As we videotaped radula movements during rejection, we recorded several cases where the animal made what appeared to be a swallowing movement; however, the tube did not move. In each of these cases, the magnitude of radula protraction and retraction appeared to be normal (data not shown). It is also unlikely that the jaws were holding the tube in place since we did not observe an increase in activity on BN2, which is known to innervate jaw closing muscles (Scott et al. 1991; Nagahama and Takata 1988; Church and Lloyd 1991; Church et al. 1991; Morton and Chiel, unpublished observations). Finally, we feel that it is unlikely that radula closing forces are decreased since we observed no decrease in the intensity of large unit activity on the RN, which is believed to contribute to radula closure based on lesion studies (Figure 2-4b), nerve shock studies (Figure 2-4d), and the temporal association of large unit RN activity with radula closure (Figure 2-8). Based on this reasoning, we feel that it is unlikely that the animal is performing a different behavioral

response with neural activity resembling pattern I; however, we cannot discount this possibility.

Are we observing the neural correlates of a decision?

At some point while feeding on a polyethylene tube, the animal makes a decision to stop swallowing and start rejecting. As this decision is being carried out, does the neural activity simply jump from the ingestion pattern to the rejection pattern? Does the ingestion pattern gradually change into the rejection pattern over several cycles? Are there totally different patterns that are interposed between the last ingestion pattern and the first rejection pattern? Two observations can be made which may address these questions.

When pattern II occurs at transitions to or from other pattern types, it tends to have a second RN burst. It is not clear if pattern II with a second RN burst is a totally different type of pattern, or if it indicates that the system is gradually leaving the ingestion state and gradually entering the rejection state. The fact that these patterns occur mainly at transitions, combined with the fact that they resemble a weak pattern I superimposed onto a pattern II, suggests that they may represent a gradual change from one behavioral state to another. However, we cannot discount the possibility that pattern II with a second RN burst is a totally different type of pattern.

Intermediate patterns were observed during a significant number (7 of 16) of the transitions from pattern I to pattern II. It is not clear if intermediate patterns are a totally different type of pattern, or if they represent a gradual change from one behavioral state to another. The fact that intermediate patterns were observed

in all parts of the tube feeding sequence (i.e. during the initial swallowing portion of the sequence, during the final rejection portion of the sequence, and at swallowing-to-rejection transitions) suggests that intermediate patterns may correspond to a totally different type of pattern. However, we cannot rule out the possibility that these patterns correspond to a mixed ingestion-rejection state that is induced by inappropriate sensory inputs due to the polyethylene tube. Another possibility is that intermediate patterns could represent the effects of modulation by sensory inputs associated with feeding on the polyethylene tube, since these patterns are rarely observed during seaweed swallowing.

Possible behavioral roles for intermediate patterns

Intermediate patterns may be used to reposition partially swallowed food. If the large RN units are motor neurons which act to close the radula, then patterns with intermediate fractional overlaps could be associated with the radula being closed during both protraction and retraction. In fact, during the experiments in which radula movements were studied in detail, 3 of the 10 non-transitional rejection responses that were recorded showed no sign of the radula opening during retraction. If intermediate patterns are associated with the radula being closed during both protraction and retraction, then these patterns could correspond to a repositioning response in which the radula is closed during protraction and retraction, moving the tube outward and then inward in an attempt to reposition it.

Intermediate patterns may correspond to the "cut" response described by Hurwitz and Susswein (1992). Cuts are a feeding response in which the animal cuts or nicks the food as it is swallowed. If the large RN units are motor neurons

which act to close the radula, then this would imply that the radula is closed during the end of protraction and start of retraction. Given the combination of linear and rolling movements that make up protraction and retraction movements (see Figures 2-2a and 2-2b), one could hypothesize that closing the radula earlier in the protraction-retraction cycle could tear or cut the food, as follows: Since the radula rolls forward as it protracts (Figure 2-2b), closing the radula earlier would grasp the seaweed strip and push it against the ventral portion of the jaws as the radula rolls toward full protraction. Pushing the seaweed strip against the jaws in this way would cause it to bend, putting a great deal of stress on the edge of the strip next to the jaws, while removing most of the stress from the other edge of the strip. During the initial portion of retraction, the radula moves linearly backward, pulling the seaweed strip with it (compare frames 3 and 4 of Figure 2-2b). This greatly increases stress on the strip, especially the portion of the strip adjacent to the ventral jaws, and could result in a cut or tear resembling that reported by Hurwitz and Susswein.

Other correlates of feeding responses

Weiss et al. (1986) have developed a reduced preparation which produces ingestion-like and rejection-like responses. By monitoring pressure in the buccal artery, this group presented evidence suggesting that radula protraction and retraction movements were made up of both active and passive components. Since our data are based on visual observations and nerve recordings using intact, freely moving animals, it is difficult to determine when radula movements are active or passive. However, it is clear that active forces must be involved in

maximally protracting the radula (as in Figure 2-2). In addition, our results suggest that retraction may have an active component, since BN2 shock can cause retraction-like movements when the radula is protracted, and since the onset of BN2 activity is temporally related to radula retraction. One way to address the question of when radula movements are active or passive is to identify some of the motor neurons appearing in these recordings and to compare their activity patterns with their peripheral actions.

Chiel et al. (1986) have recorded *in vivo* activity in the extrinsic muscles during biting, swallowing, and rejection. They found that extrinsic muscle activity was temporally related to radula protraction and retraction movements, with activity in extrinsic muscle E1 ending shortly before the end of protraction and the start of retraction during biting, swallowing, and rejection. Our observations of radula and food movements suggest that activity in E1 may end shortly before the onset of large unit BN2 activity.

Cropper et al. (1990) have recorded *in vivo* activity in the I_5 muscle (ARC muscle) during biting, swallowing and rejection. Their observations suggest that during ingestion, neurons B15 and B16 are both active, while during rejection, only B16 is active. Since the I_5 muscle is likely to contribute to radula closure, one would expect this muscle to be active as the radula retracts during ingestion and as the radula protracts during rejection. Thus, one would expect both B15 and B16 to be active after the onset of large unit BN2 activity during biting and swallowing, while expecting B16 alone to be active prior to the onset of large unit BN2 activity during rejection. In fact, Cropper et al. have shown that during biting responses, the I_5 muscle is active during retraction (supporting the

hypothesis that I_5 is active after the onset of large unit BN2 activity during biting). It would be interesting to simultaneously record *in vivo* activity from I_5 and BN2 during biting, swallowing, and rejection to test the hypothesis that I_5 is active prior to the onset of BN2 activity during rejection.

Are patterns I and II related to patterns produced by isolated buccal ganglia?

Susswein and Byrne (1988) described two patterns that can be observed in an isolated buccal ganglion. This group found that patterns 1 and 2 can occur spontaneously. In addition, pattern 2 can be elicited by nerve shock, as well as by intracellular stimulation of several buccal ganglion cells. In a separate study (Morton et al. 1991), it has been shown that esophageal nerve shock produces neural activity resembling the pattern II described in this paper. This suggests that the pattern 2 described by Susswein and Byrne may correspond to the pattern II described in this study, and thus may correspond to a rejection-like motor pattern.

Sossin et al. (1987) have described several different patterns generated by an isolated buccal ganglion in response to bath application of serotonin or different neuropeptides. It is difficult to compare the results of Sossin et al. to the results presented in this paper because we observed extracellular buccal nerve activity while they observed activity in neurons B4, B15, and B16, as well as in the I_5 muscle (ARC muscle). The present study suggests that recording from BN2 and the RN during exposure to these modulatory substances could provide insight into the patterns reported by Sossin et al.

Are extracellular units identifiable in this system?

Do the large extracellular units observed in these recordings correspond to the same cells from animal to animal? A number of groups have shown that identified buccal ganglion motor neurons have consistent peripheral projections (Cohen et al. 1978; Nagahama and Takata 1988; Morton et al. 1991; Church and Lloyd 1991; Church et al. 1991). Our extracellular recordings consistently showed characteristic patterns of activity in units having characteristic relative sizes. This consistency was maintained from pattern to pattern within a given animal, as well as from animal to animal. We cannot discount the possibility that these large units do not correspond to the same cells. However, the consistency of size and activity, combined with the identifiability of buccal ganglion motor neurons that have been studied by many different groups, suggest that the extracellular units observed in these records correspond to the same cells from animal to animal. It is important to keep in mind, however, that these two patterns can be distinguished from each other without identifying specific units.

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Fig. 2-1a-e. Definition of large unit activity using the window discriminator and frequency thresholder. (a) Raw data for BN2 and the RN. The bars above the BN2 trace mark a characteristic period of large unit inactivity that is associated with each pattern. (b) Three window discriminators were used to detect activity in the three largest BN2 units (numbered arrows in part a correspond to the numbered traces in part b). A single window discriminator was used to detect the largest RN units. Thresholds for each window discriminator were selected individually for each animal using several representative swallowing patterns. These same threshold values were then used to process the neural activity observed during all behavioral responses in that animal. (c) Output of the frequency discriminator. The instantaneous frequency was calculated by computing the reciprocal of the inter-spike interval. Closely spaced spikes caused the frequency discriminator to saturate, producing the line of dots at the top of each trace. (d) Output of the frequency thresholder. Instantaneous frequency values above the threshold value produced a positive output. Values below the threshold produced an output of zero. Thresholds were: large BN2 unit, 3 Hz; second largest BN2 unit, 1.5 Hz; third largest BN2 unit, 3 Hz; large RN units, 2 Hz. These frequency values were used for all animals during all behavioral responses. The positive and negative transitions of these signals were recorded as the start and end times of activity detected by each window discriminator during each pattern of activity. (e) Determining the fraction of the RN activity occurring after the onset of large unit BN2 activity (fractional overlap). The onset of BN2 activity reliably starts with the abrupt onset of activity in the third largest unit and always follows a period of inactivity (indicated by the bars above the top trace in

part a of this Figure). The BN2 rectangles consist of the union of activity in the 3 largest BN2 units. The *fractional overlap* is defined to be the length of the RN rectangle occurring after the start of the BN2 rectangle divided by the total length of the RN rectangle. Some patterns with low fractional overlaps had additional RN activity occurring late in the pattern, as shown here (white rectangle on right). A second radula nerve burst was defined to be a pattern of additional large unit RN activity starting at least 0.9 s after the initial RN activity had ended. Second RN bursts were not included in the fractional overlap calculation since they were not present in every case. In this Figure, the fractional overlap is 0.8 for the pattern on the left (solid black rectangles) and 0.0 for the pattern on the right (striped rectangles). Using criteria developed in the text, the pattern on the left has been classified as pattern I, while the pattern on the right has been classified as pattern II.

Fig. 2-2a-d. Line drawing sequences depicting radula movements during different consummatory feeding responses. These drawings are tracings of individual frames from a video record of each response. The number in the upper left corner of each frame in part **a** corresponds to a numbered arrow in Figure 2-5a. Corresponding frames in each sequence were taken from roughly the same points in the radula protraction-retraction cycle as follows: (1) protracting radula just after the jaws start to open, (2) mid-way to the end of radula protraction, (3) approximately the end of protraction, (4) very early retraction, (5) mid-way to jaw closing, and (6) just before jaws close. Definition of anatomical terms (refer to frame **a3**): The lips form a concave surface that ends posteriorly at the thin line marking the border of the inner lips. The inner lips form a roughly convex surface which ends at the peri-oral zone and jaws (innermost bold line). The radula (lightly stippled structure) is bordered laterally by a white, fringe-like substance (white areas inside the jaws, best seen in frame **d3**). A darkly stippled area (frames **a1** and **a2**) represents tissue located between the two halves of the radula that can be seen when the radula is open. When the radula is closed, a line indicates the separation between the two radula halves. The arrow in frame **a3** indicates the base of this separation, referred to as the base of the radula. (a) Biting. The radula is initially open and starts to close late in protraction. As the radula halves continue to roll together during retraction, a characteristic ridge develops around the base of the radula (compare frames 3 and 4). The base of the radula starts rolling backward during early retraction (compare frames 4 and 5). (b) Swallowing seaweed with the jaws open. The radula undergoes movements that are very similar to those seen during biting. The seaweed starts moving into

the animal's buccal cavity as the two halves of the radula roll together in early retraction, and continues moving into the buccal cavity throughout retraction. The arrow indicates the same point on the seaweed strip in each frame. (c) Swallowing a polyethylene tube. The radula is open during protraction, closes around the tube near the end of protraction, and pulls the tube into the buccal cavity during retraction. (d) Non-transitional rejection. The radula is tightly closed initially, and starts to open late in protraction, reflected by the disappearance of the ridge around the base of the radula (3rd frame). In addition, the white areas around the lateral edges of the radula can be seen moving laterally as the radula opens. As retraction progresses, the radula opens more widely. The markings on the tube show that the tube moves out of the buccal cavity as the radula protracts.

Fig. 2-3a, b. Possible role of BN2 in mediating jaw closure and radula retraction. (a) Electrical stimulation of BN2 in an isolated buccal mass preparation (10 Hz, 10 ms pulses), produces jaw closure (N=3). Jaw closing forces were measured using a pair of force transducing forceps that were placed between the jaws. (b) When the radula is held in a protracted position, the jaw closing movements produced by BN2 stimulation (10 Hz, 10 ms pulses) produce radula retraction (N = 3). The radula was held in a protracted position by pinning the jaws to the bottom of a sylgard-lined petri dish, attaching a silk suture to the posterior portion of the radula, leading this suture out between the jaws, and pulling the radula forward by pulling on the suture. Forces were transduced using a load cell attached to the other end of the suture.

Fig. 2-4a-d. Possible role of the RN in mediating radula closure. The biting sequences in parts **a** and **b** are from the same animal. Corresponding frames in these sequences were taken from roughly the same points in the radula protraction-retraction cycle as follows: (1) protracting radula just after the jaws start to open, (2) mid-way to the end of radula protraction, (3) approximately the end of protraction, (4) very early retraction, (5) mid-way to jaw closing, (6) just before jaws close. **(a)** Pre-lesion biting response. **(b)** Post-lesion biting response from the same animal. There are at least four differences between the pre-lesion and post-lesion bites: First, the radula is opened much wider during the post-lesion bite. Second, the duration of the post-lesion bite is much longer than that of the pre-lesion bite (time from initial radula appearance to final jaw closure was 2.8 s for the pre-lesion bite and 4.7 s for the post-lesion bite). Third, the timing of radula closure is different, occurring during late protraction in the pre-lesion bite, and during early retraction in the post-lesion bite. Finally, the characteristic ridge around the base of the radula that is seen in the pre-lesion bite is much less prominent in the post-lesion bite. **c)** Quantitative comparison of the timing of radula closure before and after bilateral lesions of the RN. Three animals were used with 9 pre-lesion and at least 8 post-lesion bites from each animal for a total of 27 pre-lesion and 26 post-lesion bites. The average time between the initial appearance of the radula during protraction and the final disappearance of the radula as the jaws close over it during retraction was 2.4 ± 0.3 s (mean $\pm$ SD) for pre-lesion bites and 4.7 ± 0.5 s for post-lesion bites. The data have been normalized so that these times appear equal. We found that, on average, the base of the radula starts rolling up and back at roughly 85% of the way through both

the pre-lesion and post-lesion bite sequences (top graph). Since the base starts rolling backward early in retraction, this result supports the observation that the end of protraction and the start of retraction occurs at roughly the same point during the pre-lesion and post-lesion sequences. In contrast, radula closure occurs 52% of the way through pre-lesion bite sequences, and 78% of the way through post-lesion bite sequences (bottom graph). These results support the observation that radula closure is delayed with respect to protraction and retraction in post-lesion animals. (d) Electrical stimulation of the RN (10 Hz, 10 ms pulses) produces radula closure in an isolated buccal mass preparation (N=3). Closing forces were measured using a pair of force transducing forceps that were placed between the two halves of the radula.

Fig. 2-5a-d. Examples of neural activity recorded from BN2 and the RN during biting, swallowing, and rejection. These examples are all from the same animal. We observed similar patterns in 5 animals for a total of 40 bites, 86 seaweed swallows, and 27 non-transitional rejections. (a) Biting. The onset of large unit BN2 activity follows a characteristic period of large unit inactivity (bar on the left), and occurs at roughly the same time as the onset of large unit RN activity. The upward arrow on the right indicates when the base of the radula started to roll up and back into the buccal cavity. The bite sequence in Figure 2-2a and the neural activity shown here are from the same bite. Each numbered arrow has a corresponding numbered frame in Figure 2-2a, and indicates when that frame was observed during the neural pattern. (b) Swallowing. The onset of large unit BN2 activity follows a short period of inactivity (arrow), and occurs at roughly the same time as the onset of large unit RN activity. The behavioral marker (bar above records) indicates that the seaweed moved into the buccal cavity after the onset of large unit BN2 activity. (c) Rejection. Large unit activity on the RN ends prior to the onset of large unit BN2 activity. The behavioral marker (bar above records) indicates that the tube stopped moving out of the buccal cavity prior to the onset of large unit BN2 activity. (d) Rejection with a second burst of activity on the RN. In this case, one RN burst ends prior to the start of large unit BN2 activity, while the second, less intense burst occurs after the onset of large unit BN2 activity.

Fig. 2-6a-d. Histograms showing that motor patterns with high fractional overlaps are associated with seaweed swallowing and tube swallowing, while motor patterns with low fractional overlaps are associated with rejection. Low fractional overlaps indicate that large unit RN activity precedes the onset of large unit BN2 activity. High fractional overlaps indicate that large unit RN activity occurs mainly after the onset of large unit BN2 activity. See Figure 2-1e for the definition of fractional overlap. (a) Histogram of fractional overlaps for all motor patterns associated with seaweed swallows. Consistent with Figure 2-5b, this histogram shows that most motor patterns associated with seaweed swallowing have fractional overlaps of 0.8-1.0 (i.e. large unit RN activity occurs mainly after the onset of large unit BN2 activity). Based on this data, motor patterns with fractional overlaps of 0.8-1.0 were classified as pattern I. (b) Histogram of fractional overlaps for all motor patterns associated with rejections that were part of a chain of consecutive rejections. The first rejection of each chain was excluded to assure that only rejections located away from swallowing-to-rejection transitions were used (i.e. non-transitional rejections). Consistent with the data in Figures 2-5c and 2-5d, this histogram shows that most motor patterns associated with non-transitional rejections have fractional overlaps of 0.0 (i.e. large unit RN activity ends prior to the onset of large unit BN2 activity). Based on this data, motor patterns with fractional overlaps of 0.0 were classified as pattern II. (c) Histogram of fractional overlaps for all motor patterns associated with tube swallows that were part of a chain of consecutive tube swallows. The last swallow in each chain was not included to assure that only tube swallows located away from swallowing-to-rejection transitions were used (i.e. non-transitional

tube swallows). This histogram shows that most motor patterns associated with non-transitional tube swallows have fractional overlaps of 0.8-1.0. Thus, seaweed swallowing and tube swallowing appear to be associated with similar motor patterns, despite the physical differences between seaweed strips and polyethylene tubes. (d) Histogram of fractional overlaps for all motor patterns associated with no net tube movement. This histogram has peaks at fractional overlaps of 0.0 and 0.8, suggesting that motor patterns classified as patterns I or II may be associated with no net tube movement as well as being associated with inward or outward movements, respectively. Inset, part A: motor patterns were classified as pattern I, pattern II, or as an intermediate pattern based on the fractional overlap values listed in this table.

Fig. 2-7a-d. Histograms of net tube movement sorted by neural pattern type showing that pattern I is mainly associated with inward tube movements or no net tube movement, while pattern II is mainly associated with outward tube movements. (a) Direction of net tube movements associated with all patterns having fractional overlaps of 0.8-1.0 (pattern I) that occurred after the tube was initially grasped by the radula. (b) Direction of net tube movements for the first three occurrences of pattern I after the tube was initially grasped by the radula. This data is a subset of the data shown in the first part of this Figure. (c) Direction of net tube movements associated with all patterns having fractional overlaps of 0.0 (pattern II). (d) Net tube movements associated with all patterns having fractional overlaps of 0.1-0.7 (intermediate patterns).

Fig. 2-8a-c. Averaged times of behavioral events relative to the onset of large unit BN2 activity. At least 5 bites, 10 seaweed swallows, and 4 pattern II rejections from each of 5 animals were used. The vertical dashed line represents the onset of large unit BN2 activity. The markers in part **a** represent single points and are shown with double sided error bars. The leading and trailing edges of the black rectangles in parts **b** and **c** represent the mean start and stop times (respectively) of food movement, and are shown with single sided error bars for clarity. Error bars correspond to one standard deviation. (a) Averaged time when the base of the radula started rolling backward during biting for 5 animals. A total of 40 bites were observed. (b) Averaged start and stop times for inward seaweed movements during swallowing for the same 5 animals shown in part **a**. A total of 86 seaweed swallows were observed. (c) Averaged start and stop times for outward tube movements during rejection for the same 5 animals shown in parts **a** and **b**. A total of 38 pattern II rejections were observed.

Fig. 2-9. Neural activity associated with biting, swallowing, and then rejecting a polyethylene tube. The bite was elicited by touching dried seaweed to the lips. The following response, termed a bite/swallow (B/S), is distinguished from a bite by the fact that it successfully captures a piece of food (in this case, the polyethylene tube). The bite/swallow was followed by four swallows and an occurrence of pattern I with no net tube movement. The animal then rejected (first pattern II). Following the rejection, an intermediate pattern occurred (fractional overlap of 0.6) that was associated with no net tube movement. The animal then carried out two more rejections. Abbreviations: INT, intermediate pattern; B/S, bite/swallow response.

Chapter 3

Motor neuron activity during feeding-like motor patterns

Summary

We have studied the neural circuitry mediating ingestion and rejection in *Aplysia* using a reduced preparation that produces ingestion-like and rejection-like motor patterns in response to physiological stimuli. Using this preparation, we have characterized three buccal ganglion motor neurons that produce specific movements of the radula and buccal mass. B8a and B8b act to close the radula. B10 acts to close the jaws and retract the radula. The patterns of activity in these neurons can be used to distinguish the ingestion-like and rejection-like motor patterns. B8a, B8b and B10 are active together during the ingestion-like pattern. Activity in B8a and B8b ends prior to the onset of activity in B10 during the rejection-like pattern. Our data suggest that these neurons may undergo similar patterns of activity *in vivo*. During both feeding-like patterns, the activity and peripheral actions of B8a, B8b, and B10 are consistent with radula movements observed during ingestion and rejection. In addition, the extracellular activity produced by these neurons is consistent with neural activity observed *in vivo*.

observed in these motor neurons contribute to the different radula movements which underlie ingestion and rejection.

Introduction

Nervous systems produce various behavioral responses by generating different patterns of motor neuron activity (motor patterns). For example, the marine mollusc *Tritonia diomedea* activates its dorsal and ventral flexor motor neurons simultaneously to perform a defensive withdrawal response, while activating these same motor neurons in a rhythmic, alternating pattern to carry out an escape swim (Getting and Dekin 1985). Similarly, the three different scratch reflexes of the spinal turtle are carried out by changing the timing of activity in knee flexing and knee extending motor neurons relative to activity in hip protracting and retracting motor neurons (Robertson and Stein 1988). Studying the neural circuitry involved in generating different motor patterns could provide insight into the mechanisms of circuit reorganization, decision making, and movement coordination that underlie these complex behaviors.

An important step in studying a neural circuit that generates multiple motor patterns is to identify motor neurons that contribute to each pattern. One strategy for accomplishing this step is to develop a reduced preparation that produces the desired motor patterns and is amenable to intracellular recording (Getting and Dekin 1985, McClellan 1982a,b; Croll et al. 1985a-c; Selverston and Moulins 1987; Robertson and Stein 1988). It is usually necessary to remove most or all of the animal's periphery to do this, which raises the possibility that the resulting patterns may not have behavioral relevance. Behavioral relevance can be assessed

in a number of ways: First, one can use *in vivo* neural activity to develop rigorous criteria to distinguish the responses being studied and then determine if the patterns produced by the reduced preparation meet these criteria. Second, one can identify motor neurons, determine their peripheral actions and activity patterns in the reduced preparation, and then verify that these observations are consistent with the animal's movements. Finally, under some circumstances, one can determine the *in vivo* activity of specific neurons and then verify that these patterns are replicated in the reduced preparation.

The consummatory feeding behavior of the marine mollusc *Aplysia californica* is a useful system for studying the mechanisms used to produce multiple motor patterns. This behavior is made up of several different responses, including biting, swallowing, and rejection (Kupfermann 1974), that are carried out using different movements of the animal's radula. Using *in vivo* recordings from peripheral nerves that are likely to mediate radula movements (buccal nerve 2 (BN2) and the radula nerve (RN)), two different motor patterns have been associated with these feeding responses (see chapter 2). *In vivo* patterns I and II are associated with ingestion (biting or swallowing) and rejection, respectively, and can be distinguished by the timing of RN activity relative to the onset of BN2 activity.

In this study we identified motor neurons that are likely to contribute to *in vivo* patterns I and II. To accomplish this goal, we developed a reduced preparation that produces ingestion-like and rejection-like patterns of neural activity. We then identified and characterized motor neurons that project through either BN2 or the RN. Finally, we determined the activity of these motor neurons

during the *in vitro* motor patterns. A preliminary report of these results has appeared (Morton and Chiel 1991, 1992).

Materials and Methods

Aplysia californica weighing 150-350 g (Marinus, Long Beach, CA) were kept in aerated aquaria containing artificial sea water (Instant Ocean, Aquarium Systems, Mentor, OH) maintained at 16°C. These animals were fed on a regular basis and were kept under these conditions for up to two weeks before being used in an experiment. Animals were anesthetized with a 75% (v/w) injection of isotonic MgCl_2 (333 mM). The dissection was carried out in a mixture of 50% artificial sea water and 50% isotonic MgCl_2 . Desheathing was done in a mixture of 50% *Aplysia* saline (for the composition of *Aplysia* saline, see below) and 50% isotonic MgCl_2 .

Extracellular and Intracellular Recording Techniques

Extracellular recordings of neural activity were done using standard techniques. Briefly, electrodes were made by gently heating polyethylene tubing (1.27 mm OD, Clay-Adams) in a Bunsen burner flame until soft, and then drawing the tube to a fine taper. This taper was then trimmed to an appropriate size. The nerve was suctioned into the tapered end of this tube, and connected to the input of an AC coupled differential amplifier (model 1700, AM Systems, Everett, WA) using a silver-silver chloride electrode wire. Extracellular signals were filtered with a 1 kHz lowpass filter and a 100 Hz high pass filter. These

frequency settings were selected to reproduce, as closely as possible, the conditions used in chapter 2 for *in vivo* recordings of buccal nerve activity.

Intracellular electrodes were made from single barreled glass microelectrodes (AM systems, part no. 6150) pulled on a Flaming-Brown pipet puller (Model P-80, Sutter Instruments Corp., Novato, CA). Electrodes were filled with 4M potassium acetate. Lucifer yellow fills were done using electrodes containing 4% lucifer yellow (lucifer yellow was obtained from Sigma Chemical Co., St. Louis, MO) in 1M lithium acetate. Neurons were filled over a 60-90 minute period using a continuous hyperpolarizing current of 1-5nA. Intracellular electrode signals were amplified using an DC amplifier (model 1600, AM systems, Everett, WA) and filtered with a 1 kHz lowpass filter. All intracellular recordings were made from the soma.

Intracellular and extracellular signals were further amplified using a custom built variable gain amplifier and then sampled at 2 kHz or 10 kHz per channel. Digitized data were recorded on a hard disc using a PC based data acquisition system (Axotape, Axon instruments, Grover City, CA).

Recording muscle activity in the reduced preparation

Recordings from buccal mass muscles were performed using the electrode design of Cropper et al. (1990b; for muscle anatomy, see Scott et al. 1991). Briefly, electrodes were made from insulated stainless steel wire (California Fine Wire, Grover City, CA, 2.54 μ m diameter). Two mm of insulation was stripped from one end of this wire, and a gold contact was soldered to the other end. The bare wire end of the electrode was inserted into the muscle from which activity

was to be recorded, and a small drop of super glue (Duro, Quick Gel, part number SGG-1, Loctite Corp., Cleveland, OH) was applied to the muscle to hold the wire in place. Recordings from the I_1/I_3 muscle (jaw sphincter muscle) were made from the middle portion of the muscle (mm region, in the notation of Nagahama and Takata 1988). The I_1/I_3 designation refers to the fact that these electrodes passed through the sheet-like I_1 muscle in order to penetrate the I_3 muscle, and thus transduced signals from both muscles. The I_4 muscle (odontophore muscle) was exposed by either cutting small holes in the radula, or by carefully dissecting away the entire radula. Signals were recorded from the lateral surface of I_4 immediately beneath the radula.

Muscle recordings were amplified using an AM systems model 1700 AC amplifier, filtered with a 500 Hz lowpass and a 10 Hz highpass filter. These signals were further amplified by a variable gain amplifier before being digitized at 2 kHz and recorded on a hard disc using the PC based data acquisition system.

Measurement of forces produced by the buccal mass

Jaw closing forces were measured using a pair of custom built force transducing forceps. Radula retraction forces were measured using a load cell (model FT03, Grass Instruments Corp., Quincy, MA). The load cell and forceps outputs were amplified using an instrumentation amplifier (model 3170, Daytronic Corp., Miamisburg, OH) configured for a 5 Hz bandwidth. These signals were further amplified using a custom built variable gain amplifier before being sampled at 2 kHz and recorded on a computer disc using the PC based data acquisition system.

Reduced preparation producing feeding-like patterns of neural activity

The reduced preparation consists of the buccal ganglion, the buccal mass, the cerebral ganglion, and the animal's lips (Figure 3-1). The cerebral-buccal connectives were left intact bilaterally. The lips were attached to the cerebral ganglion via the upper and lower labial nerves and the anterior tentacular nerves. In some early experiments, the lip innervation was cut on the right side.

We chose this preparation as a minimal system in which the animal's own sensory inputs could be used to elicit patterns. A similar preparation has been used to elicit feeding-like patterns in a reduced preparation of *Limax maximus* (Gelperin et al. 1978). This preparation is also similar to one used by Rosen et al. (1991) to study the properties of command-like neurons that have been implicated in controlling the consummatory feeding behavior of *Aplysia*.

Gross dissection was done as follows. The lips and anterior tentacles were cut away from the body along a line just anterior to the rhinophores and just posterior to the anterior border of the foot. The buccal mass was separated from the lips and anterior tentacles by cutting circumferentially along the posterior border of the jaw cartilage, and was left attached to the buccal ganglion via the left and right RNs and the left BN2 (these nerves were further dissected after motor neuron identification, as described below). The lips were isolated from the buccal and cerebral ganglia in a separate chamber sealed with a Vaseline/mineral oil mixture. The branch of the buccal artery feeding the lips, and the branch of the buccal artery feeding the buccal mass were separately cannulated.

During motor neuron identification, polysynaptic pathways were suppressed by introducing a high divalent cations saline (3xCa^{2+} , 3xMg^{2+}) into the cannulas

and the chamber containing the ganglia. Perfusion with high divalent cations saline was allowed to continue for at least 60 minutes with a cannula flow of 1 ml/min before attempting to identify motor neurons. A suction electrode was applied to the right BN2 shortly after perfusion was started.

Once the desired neurons had been identified, the intracellular electrodes were removed. Then the loose connective tissue surrounding the RN and the small nerve branches leaving the common portion of the RN were dissected away. The left BN2, which was still intact, was cut at this time. The right RN was then cut and a suction electrode applied to it.

To prepare for the behavioral portion of the experiment, *Aplysia* saline was introduced into the cannulas and gradually washed into the ganglion and lip chambers over a 60 minute period. Cannula flow was 2 ml/min. Once the saline wash was completed, the buccal mass cannula was gently removed. This greatly reduced the chances of sensitizing the preparation, presumably by reducing potentially painful mechanical forces on the buccal mass. The preparation was then allowed to sit undisturbed for at least 30 minutes before re-impaling the identified neurons with intracellular electrodes.

Different stimulation protocols were used to elicit the different feeding-like patterns. *In vitro* pattern I was elicited by applying seaweed extract to the lips. This extract was prepared by soaking 5g of dried seaweed (Laver, Hun Sing Wong Foods Ltd., Hong Kong) in 40 ml of *Aplysia* saline for 60 minutes and then using a press to obtain the extract. *In vitro* pattern II was elicited by pushing a glass rod between the halves of the radula with a force of 45-90 mN (5-10 g).

Preparations that did not produce both *in vitro* patterns were excluded from this study. As our proficiency and experience with this preparation increased, we found that the fraction of preparations producing both *in vitro* patterns approached 80-90%. The preparations producing only one pattern usually produced *in vitro* pattern II (rejection-like pattern). Often, these preparations showed signs of sensitization, with the lips contracting in a withdrawal-like movement in response to any stimulus.

We have found two steps which greatly increase the percentage of preparations that produce both feeding-like patterns: First, we only used animals displaying spontaneous biting and head waving movements. Second, starting 1-2 days prior to the experiment, feeding was suspended and the animal was transferred to a warm tank (20°C). The effectiveness of this latter step was originally reported by Weiss et al. (1986).

Salines

The following salines were used in this investigation. In each case, pH was adjusted to 7.4-7.5. One liter of *Aplysia* saline: 450 mM NaCl, 10 mM KCl, 22 mM MgCl₂, 33 mM MgSO₄, 10 mM CaCl₂, 10 mM glucose, and 10 mM MOPS. One liter of high divalent cations saline (3xCa²⁺, 3xMg²⁺): 270mM NaCl, 6mM KCl, 120 mM MgCl₂, 33mM MgSO₄, 30mM CaCl₂, 10 mM glucose, and 10 mM MOPS. MOPS was purchased from Sigma Chemical Co. (St. Louis, MO). Salts were purchased from Fisher Scientific (Springfield, NJ). Glucose was purchased from GIBCO (Grand Island, NY).

Results

The purpose of this investigation was to identify motor neurons that are likely to contribute to the motor patterns associated with ingestion and rejection responses. We approached this problem in three steps. First, we developed a reduced preparation that produces ingestion-like and rejection-like patterns. Second, we identified and characterized motor neurons that project through BN2 or the RN (B5 and B1, respectively, in the notation of Kandel 1979), the nerves used to define motor patterns *in vivo*. Finally, we determined the activity patterns of these motor neurons during the ingestion-like and rejection-like patterns produced by the reduced preparation.

Terms used to describe neural activity

The following terms were used in chapter 2 to describe the *in vivo* motor patterns associated with consummatory feeding in *Aplysia*. *Large unit BN2 activity* refers to activity in the largest three groups of BN2 units (arrows 1, 2, and 3, Figure 3-2a). *The onset of large unit BN2 activity* is a characteristic feature in every pattern, and is indicated by a starred arrow (Figures 3-2a and 3-2b). *Large unit RN activity* refers to activity in the largest group of RN units (arrow 4, Figure 3-2a). *The fractional overlap* is a measure of the timing of large unit RN activity relative to large unit BN2 activity. This quantity is formally defined in Figure 3-2.

Reduced preparation producing feeding-like patterns

Application of physiological stimuli to the reduced preparation produces feeding-like responses. A food stimulus (application of seaweed extract to the lips and peri-oral zone) elicits multiple repetitions of a motor pattern that resembles the *in vivo* neural activity associated with ingestion responses. This pattern is characterized by large unit RN activity occurring mainly after the onset of large unit BN2 activity (Figures 3-2a and 3-2c). We have named this motor pattern *in vitro* pattern I. The food stimulus typically elicits 5-10 cycles of *in vitro* pattern I.

Gentle mechanical stimulation of the buccal mass (lightly pushing a glass rod between the two halves of the radula with a force of 45-90 mN) elicits multiple repetitions of a motor program that resembles the *in vivo* neural activity associated with rejection. This pattern is characterized by large unit RN activity ending prior to the onset of large unit BN2 activity (Figures 3-2b and 3-2d). We have named this motor program *in vitro* pattern II. Before the onset of *in vitro* pattern II activity, the preparation typically produces 1-2 motor patterns that resemble *intermediate patterns*, another type of *in vivo* pattern whose behavioral significance is unclear (see chapter 2, page 58). These initial intermediate patterns were excluded from this study. *In vitro* pattern II activity typically stopped within 30-60 seconds after the mechanical stimulation ended.

The reduced preparation consistently produced *in vitro* pattern I in response to the food stimulus and *in vitro* pattern II in response to the mechanical stimulus. Repeated application of the food stimulus, with 5-10 minutes between each stimulus, elicited repeated bouts of *in vitro* pattern I. Similarly, repeated

application of the mechanical stimulus, with 5-10 minutes between each stimulus, elicited repeated bouts of *in vitro* pattern II. In addition, alternating between the food and mechanical stimuli, with 5-10 minutes between each stimulus, elicited alternating bouts of *in vitro* pattern I and *in vitro* pattern II, respectively.

Comparison of in vitro and in vivo patterns

Our data show that during both *in vitro* patterns, the structure of BN2 activity resembles that seen *in vivo*. Both *in vivo* and *in vitro*, BN2 activity starts with the sudden onset of intense activity in the third largest group of units (arrow 3, Figures 2-1a, 3-2a, and 3-2b) followed by activity in the largest and second largest groups of units (arrows 1 and 2, Figures 2-1a, 3-2a, and 3-2b).

Our data also show that during both *in vitro* patterns, the structure of RN activity resembles that seen *in vivo*. Both *in vivo* and *in vitro*, RN activity is characterized by activity in several large units of about the same size separated by long periods of inactivity in these units (Figure 2-1a, 3-2a, and 3-2b).

In chapter 2, it was shown that the fractional overlap, a quantitative measure of the timing of large unit RN activity, could be used to distinguish ingestion from rejection. The fractional overlap is defined to be the fraction of RN activity occurring after the onset of large unit BN2 activity (see Figure 3-2 legend). As an illustration, the pattern in Figure 3-2a has a fractional overlap of 0.9, while the pattern in Figure 3-2b has a fractional overlap of 0.0. It was shown in the previous chapter that the *in vivo* neural activity observed during biting and swallowing had fractional overlaps of 0.9 ± 0.2 and 0.9 ± 0.1 , respectively (mean

$\pm$ SD, 40 bites, 86 seaweed swallows) while the *in vivo* neural activity observed during rejection had a fractional overlap of 0.1 ± 0.2 ($N = 27$).

The motor patterns produced by the reduced preparation have fractional overlaps similar to those observed *in vivo*. We based our calculations on the patterns produced by 5 reduced preparations in response to food and mechanical stimulation. For food stimulation, the fractional overlaps were calculated for all neural patterns observed during the stimulation period. For mechanical stimulation, the fractional overlaps were calculated for all patterns occurring after the initial intermediate patterns and within 60 s after the end of the stimulation period. We found that patterns elicited by seaweed extract stimulation had fractional overlaps of 0.8 ± 0.1 (mean $\pm$ SD, $N = 38$). Patterns elicited by mechanical stimulation had fractional overlaps of 0.04 ± 0.2 ($N = 37$).

These results suggest that in response to a food stimulus or an inappropriate mechanical stimulus, the reduced preparation generates the appropriate ingestion-like or rejection-like motor patterns.

Motor neurons that project through the radula nerve

We found that two large buccal ganglion neurons, named B8a and B8b (Gardner 1971; Church and Lloyd 1991), send axons through the RN and act to close the radula. Figure 3-3a is a *camera lucida* drawing of a lucifer yellow fill of one of these neurons showing that its axon enters the common portion of the RN, bifurcates, and then sends a branch down the left and right RNs. Stimulating these neurons produces bilateral radula closure ($N = 25$, Figure 3-3b). Figure 3-3c shows that these neurons appear as large units in both the right and left RNs.

Recordings of activity in the I_4 muscle in a preparation consisting of the buccal mass and the buccal ganglion with the left and right RNs intact revealed large EJC's appearing bilaterally that persisted in high-divalent cations saline (Figure 3-3d). These EJC's were either weakly facilitating or non-facilitating (Figure 3-3e). We could find no evidence of electrical coupling between these two neurons ($N = 4$) or between these neurons and their homologs in the contralateral hemi-ganglion ($N = 3$).

In this study, we identified neurons B8a and B8b based on their soma location, their ability to produce bilateral radula closure when stimulated, and their appearance as large units in extracellular recordings of the RN. In 6 preparations, the morphology of either B8a, B8b, or both was verified using a lucifer yellow fill.

Based on our observations, we could only distinguish B8a from B8b using soma location. Since this is considered to be a relatively weak criterion, we adopted the convention of Church and Lloyd (1991) in which a letter following the neuron's number designator denotes the inability to uniquely identify that neuron as an individual. Our designations for B8a and B8b are consistent with the notation of Church and Lloyd (1991), with the soma of B8b located lateral to that of B8a.

A motor neuron that projects through buccal nerve 2

We found that a large buccal ganglion neuron named B10 (Gardner 1971; Church et al. 1991; Church and Lloyd 1991) sends its axon through BN2 and acts to close the jaws and retract the radula. Figure 3-4a shows that this neuron has a

bipolar morphology, sending axons to the ipsilateral and contralateral BN2. In a preparation in which BN2 was left intact bilaterally, stimulating B10 produced a sphincter-like constriction of the anterior buccal cavity and closing-like movements of the jaws ($N = 4$; top two traces, Figure 3-4b). In chapter 2, it was shown that fibers projecting through BN2 can cause retraction-like movements when the radula is held in a protracted position. We therefore performed experiments in which this neuron was stimulated while the radula was held in a protracted position with a silk suture attached to the base of the radula. Under these conditions, we found that the sphincter-like anterior buccal cavity contraction produced by B10 stimulation could produce retraction-like movements of the radula ($N = 3$; bottom two traces, Figure 3-4b). Figure 3-4c shows that this neuron appeared bilaterally as a large unit in extracellular recordings of BN2. This neuron produced EJC's in the center of the left and right I_1/I_3 muscles (mm region, in the notation of Nagahama and Takata 1988) that persisted in high-divalent cations saline (Figure 3-4d). These EJC's showed evidence of facilitation (Figure 3-4e).

In this study, we identified B10 based on soma location, its appearance as a large unit in both the left and the right BN2, and its ability to produce small, facilitating EJC's in the I_1/I_3 muscle. After most experiments involving B10, its morphology was verified using a lucifer yellow fill.

Activity of B8a and B8b during in vitro patterns I and II

The *in vitro* activity of B8a and B8b is consistent with observations of *in vivo* neural activity during ingestion and rejection. During *in vitro* pattern I, B8a

and B8b activity occurs mainly after the onset of large unit BN2 activity (Figure 3-5a). During *in vitro* pattern II, activity in these neurons ends prior to the onset of large unit BN2 activity (Figure 3-5b). During both *in vitro* patterns, B8a and B8b contribute to the large unit activity seen on the RN (Figure 3-5c). Simultaneous action potentials in B8a and B8b produce a larger, compound action potential in the RN record (Figure 3-5d). The contribution of B8a and B8b to large unit RN activity, combined with the similarity of *in vitro* and *in vivo* RN activity, suggests that a portion of the large unit RN activity observed *in vivo* may represent the activity of B8a and B8b.

The *in vitro* activity and peripheral actions of B8a and B8b (putative radula closers) are consistent with the radula movements observed during ingestion and rejection. Using the onset of large unit BN2 activity as an approximate marker for the end of protraction and the start of retraction (see chapter 2, page 59), our results suggest that B8a and B8b are active during the retraction portion of *in vitro* pattern I (ingestion-like pattern) and the protraction portion of *in vitro* pattern II (rejection-like pattern). This is consistent with *in vivo* observations that the radula is closed as it retracts during ingestion, while being closed as it protracts during rejection (Figure 2-2).

Activity of B10 during in vitro patterns I and II

The *in vitro* activity of B10 (putative jaw closer and radula retractor) is consistent with *in vivo* neural activity observed during ingestion and rejection. During *in vitro* patterns I and II, B10 becomes active with the onset of large unit BN2 activity (Figures 3-6a and 3-6b). This neuron contributes to activity in the

third largest group of BN2 units (Figure 3-6c). The similarity of *in vivo* and *in vitro* activity in this group of BN2 units suggests that the *in vitro* activity of B10 is consistent with *in vivo* observations.

The *in vitro* activity and peripheral actions of B10 are consistent with the radula movements observed during ingestion and rejection. B10 becomes active with the onset of large unit BN2 activity. Since the onset of large unit BN2 activity is an approximate marker for the start of retraction, our data suggest that B10 is active when the radula is near its maximally protracted position and is starting to retract. Thus, this neuron, which can produce retraction-like movements when the radula is protracted (Figure 3-4b, bottom traces), could be functioning as a retractor by squeezing the radula-odontophore backwards using a sphincter-like contraction of the I_1/I_3 muscle. B10 may also contribute to jaw closure as the radula retracts, which is also consistent with the jaw movements observed during ingestion and rejection (Figure 2-2).

Motor neuron activity distinguishes in vitro patterns I and II

The relative timing of activity in B8a, B8b, and B10 can be used to distinguish *in vitro* pattern I from *in vitro* pattern II. Our data suggest that during *in vitro* pattern I, all three neurons are active together (Figures 3-5a and 3-6a). However, our data suggest that during *in vitro* pattern II, activity in B8a and B8b ends prior to the onset of activity in B10 (Figures 3-5b and 3-6b).

How important are B8a and B8b to radula closure?

The results presented above suggest that B8a and B8b may contribute to the control of radula closure during ingestion and rejection. How significant is this contribution? We addressed this question in two steps. We first estimated the relative contribution of each buccal nerve to radula closure by stimulating each left and right nerve pair while measuring the resulting radula closing forces. Then, in a different set of preparations, we estimated the portion of the RN mediated closing force that is attributable to B8a/b by comparing the closing force produced by intracellular stimulation of these cells to the closing force produced by stimulating the RNs.

Nerve stimulation experiments suggest that fibers in the RN and BN3 contribute significantly to radula closure. RN stimulation and BN3 stimulation produced the two strongest closing forces in all three preparations tested (Figure 3-7a). In addition, each doubling of the stimulation rate on these two nerves produced an approximate doubling of the closing force. Doubling the stimulation frequency for BN1, BN2, or the EN, however, did not produce a consistent increase. Examining several representative *in vivo* patterns, we found that depending on the type of response, the frequency of BN2 units ranged between 2 and 15 Hz (depending on the size of the unit as determined in Figure 2-1), while the frequency of large unit RN activity ranged between 3 and 12 Hz. These frequencies tended to be lower during biting responses, and higher during swallowing and rejection responses.

Our data also suggest that the B8a/b neurons contribute significantly to the closing force mediated by the RNs. In 3 preparations, we first determined the

radula closing force produced by B8a/b stimulation. These neurons were fired at a frequency between 5 and 10 Hz. We then cut the RNs, attached extracellular electrodes, and stimulated the RNs at approximately the same rate at which the B8 neurons fired. Using this protocol, we found that stimulation of either B8a or B8b produced approximately 10% of the force produced by subsequent stimulation of the RNs (left and right bars, Figure 3-7b). In addition, stimulation of both B8a and B8b produced approximately 20% of the force produced by subsequent RN stimulation (center bar, Figure 3-7b).

Activity in B4/5 during in vitro patterns I and II

B4 and B5 are easily identified buccal ganglion neurons that have been extensively studied in this system due to their widespread synaptic connections (Gardner 1971; Church and Lloyd 1991) and their activity during motor patterns (Sossin et al. 1987; Susswein and Byrne 1988; Plummer and Kirk 1990; Rosen et al. 1991). Since many of these groups have used B4/5 activity as a timing reference during buccal ganglion motor patterns, we elected to record the activity of these neurons during *in vitro* patterns I and II. We identified B4 and B5 by soma size, soma location, and the ability of these neurons to produce fast IPSPs in other large buccal ganglion neurons (Gardner 1971). Our data show that the onset of activity in these neurons occurs just before the onset of large unit BN2 activity during both *in vitro* patterns (Figure 3-8).

Discussion

Are in vitro patterns I and II behaviorally relevant?

A major concern in developing a reduced preparation is whether or not the patterns produced by the preparation are behaviorally relevant. There are several lines of evidence suggesting that the preparation described in this paper is producing ingestion-like and rejection-like patterns. First, during *in vitro* patterns I and II, the extracellular activity observed in the RN and BN2 is qualitatively similar to the activity observed *in vivo* during ingestion and rejection. Second, the timing of RN activity relative to the onset of BN2 activity during *in vitro* patterns I and II is quantitatively similar to that seen *in vivo*. Third, the stimuli used to elicit these *in vitro* patterns are similar to the stimuli used to elicit ingestion and rejection *in vivo*.

An important difference between the *in vivo* and *in vitro* patterns is the way in which *in vitro* pattern II is elicited. *In vivo* pattern II (i.e. rejection) is elicited by leading the animal through a progression of different behavioral responses that results in tricking the animal into swallowing an inedible object (a polyethylene tube). In contrast, *in vitro* pattern II is elicited by mechanically stimulating the buccal mass in the absence of chemostimulation. One could argue that *in vivo*, rejection is elicited in response to mechanical stimulation of structures located within the buccal cavity as the animal attempts to swallow the polyethylene tube. If this is the case, then the protocol used to elicit *in vitro* pattern II could be consistent with the protocol used to elicit rejection *in vivo*. However, we cannot discount the possibility that the stimuli used to trick the animal into swallowing the tube may play a role in shaping the subsequent rejection responses.

It is important to keep in mind that *in vivo*, pattern I is associated with more than one type of behavioral response. *Aplysia's* consummatory feeding behavior is made up of biting, swallowing, and rejection responses. The two ingestion responses (biting and swallowing) are associated with *in vivo* pattern I, while rejection responses are associated with *in vivo* pattern II. Because *in vivo* pattern I cannot distinguish biting from swallowing, it is not clear whether *in vitro* pattern I represents a biting response, a swallowing response, or some combination of the two. Since the stimulus used to elicit *in vitro* pattern I elicits biting responses in intact, freely moving animals, one would expect *in vitro* pattern I to represent a biting-like response. However, we cannot discount the possibility that it represents a swallowing response or some combination of biting and swallowing.

Do these neurons undergo similar patterns of activity in vivo?

Two lines of evidence suggest that the activity of B8a, B8b, and B10 during *in vitro* patterns I and II is similar to the activity that these neurons undergo during ingestion and rejection. First, the peripheral actions and *in vitro* activity of these neurons are consistent with the radula movements observed during ingestion and rejection. During the ingestion-like pattern, B8a and B8b (radula closers) and B10 (radula retractor) are active together, consistent with the observation that during ingestion, the radula is opened as it protracts and closed as it retracts. During the rejection-like pattern, B8a and B8b activity ends prior to the onset of B10 activity, consistent with the observation that during rejection, the radula is closed as it protracts and open as it retracts. Second, the extracellular activity produced by B8a, B8b, and B10 is consistent with the extracellular activity

observed *in vivo*. B8a and B8b appear as large RN units (Figure 3-5). Our fractional overlap calculations show that the timing of activity in these units during *in vitro* patterns I and II closely resembles the timing of large unit RN activity during ingestion and rejection. B10 contributes to the early portion of BN2 activity as a characteristically sized unit (Figure 3-6). The timing of activity in this unit during *in vitro* patterns I and II resembles that of similarly sized BN2 units during ingestion and rejection (Compare Figures 2-5 and 3-6).

Active and passive movements during ingestion and rejection

Weiss et al. (1986) have developed a reduced *Aplysia* preparation that produces ingestion-like and rejection-like responses. By monitoring pressure in the buccal artery, this group has presented evidence suggesting that radula protraction and retraction movements are made up of both active and passive components. Since most of the buccal mass innervation is cut in our preparation, it is difficult to directly compare our results to those of Weiss et al. However, our results suggest that there is an active component to the early portion of radula retraction and that identified neuron B10 is likely to contribute to this component. B10 acts to close the jaws and retract the radula (Figure 3-4b). In addition, this neuron becomes active with the onset of large unit BN2 activity during both *in vitro* patterns (Figure 3-6). Since the onset of large unit BN2 activity is an approximate marker for the start of retraction (chapter 2, page 59), this suggests that B10 is active during the early portion of radula retraction *in vivo*, and is thus likely to contribute to retraction movements.

Do the ingestion and rejection pattern generators share a common set of neurons?

There are many similarities between *in vitro* pattern I (ingestion-like pattern) and *in vitro* pattern II (rejection-like pattern), raising the possibility that there may be a significant amount of common circuitry shared by the ingestion and rejection pattern generators. Both *in vivo* and *in vitro*, recordings of BN2 activity suggest that many motor neurons undergo similar patterns of activity during these two responses. We have confirmed this observation for one BN2 motor neuron, B10 (Figure 3-6). In addition, the timing of activity in identified neurons B4 and B5 is similar during these two responses. B4 and B5 have widespread central synaptic connections (Gardner 1971; Rosen et al. 1982; Susswein and Byrne 1988; Plummer and Kirk 1990; Church and Lloyd 1991; Rosen et al. 1991), may play a role in shaping motor output during patterned activity (Nagahama and Takata 1989, 1990), and may have a role as command-like elements controlling the feeding circuitry (Susswein and Byrne 1988).

Similarities between ingestion and rejection have been reported in another marine mollusc, *Pleurobranchaea californica*. Using EMG recordings from intact, freely moving animals, Croll and Davis (1981) found that the activity in most buccal muscles was similar during ingestion and rejection. They hypothesized that the neural circuitry producing these responses consisted of a single pattern generator that underwent minor modifications to produce ingestion or rejection. Croll et al. (1985a-c) went on to show that a number of command-like elements participated in both ingestion-like and rejection-like patterns,

providing more direct evidence that the circuits which generate ingestion and rejection share a common group of neurons.

Our data show that there are differences between the neural circuits mediating ingestion and rejection. This can be seen in the sub-threshold activity of B8a and B8b. During *in vitro* pattern I, B8a and B8b activity is associated with a broad, slow depolarization (Figures 3-5a and 3-6a). During *in vitro* pattern II, however, B8a and B8b activity is associated with a series of large EPSPs. This clear difference in sub-threshold activity suggests that different presynaptic elements are mediating activity in B8a and B8b during these two motor patterns.

These observations suggest that it may be advantageous to approach this system as a single pattern generator that produces more than one behavioral response, rather than as a set of separate pattern generators, with each one producing a different response. In other systems where this strategy has been applied, it has led to the identification of shared neurons and mechanisms of neuronal reorganization. Examples include the lobster stomatogastric system (Hooper and Moulins 1989) and *Tritonia* escape (Gettings and Dekin 1985).

Timing of activity in B8a, B8b, and B10: neural correlate of a decision

Since *Aplysia's* consummatory feeding behavior is made up of several different responses, the animal must decide which response to carry out. This decision is shaped not only by sensory information (Kupfermann 1974; Chiel et al. 1986; Cropper et al. 1990a; previous chapter), but also by learning (Susswein et al. 1986) and by motivational state (Kupfermann 1974; Kupfermann and Carew 1974).

In the previous chapter, it was shown that the timing of radula closure during the radula protraction-retraction cycle comprises a major functional difference between ingestion and rejection responses. In this chapter, we have shown that the timing of activity in B8a/b relative to activity in B10 is likely to contribute to that difference, and thus represents a neural correlate of the animal's decision to either ingest or reject. Studying the neural circuitry which mediates activity in B8a, B8b, and B10 could provide insight into the mechanisms of decision making and neural reorganization that underlie the generation of *Aplysia's* consummatory feeding behavior.

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Fig. 3-1. Diagram of the reduced preparation used to produce feeding-like patterns of neural activity. This preparation consists of the buccal and cerebral ganglia, as well as the lips and the buccal mass. The buccal and cerebral ganglia are connected bilaterally via the cerebral-buccal connectives. The lips are connected to the cerebral ganglion bilaterally via the upper labial nerves, the lower labial nerves, and the anterior tentacular nerves. The buccal mass is connected to the buccal ganglion via the left RN. Extracellular recordings of neural activity in the right RN and the right BN2 were performed by applying suction electrodes to these nerves. The lips were isolated in a separate chamber. Chains of ingestion-like motor patterns (*in vitro* pattern I) were elicited by a food stimulus (applying seaweed extract to the lips). Chains of rejection-like motor patterns (*in vitro* pattern II) were elicited by a gentle mechanical stimulus (gently pressing a glass rod between the two halves of the radula) in the absence of any chemostimulation. Abbreviations: CG - cerebral ganglion, BG - buccal ganglion, BN - buccal nerve, EN - esophageal nerve, RN - radula nerve, CBC - cerebral-buccal connective, ATN - anterior tentacular nerve, ULN - upper labial nerve, LLN - lower labial nerve.

Fig. 3-2a-d. Feeding-like patterns produced by the reduced preparation. Arrows 1, 2, and 3 indicate the largest, second largest, and third largest BN2 units, respectively. These 3 groups of units are collectively referred to as large unit BN2 activity. Arrow 4 indicates the units referred to as large unit RN activity. The starred arrows indicate a characteristic feature of every pattern referred to as the onset of large unit BN2 activity. The shaded rectangles under the patterns indicate the extent of large unit BN2 and RN activity. The *fractional overlap* is a measure of the timing of these rectangles. It is defined to be the length of the RN rectangle occurring after the leading edge of the BN2 rectangle, divided by the length of the RN rectangle. The fractional overlap can range from 0.0 to 1.0. (a) *In vitro* pattern I is generated in response to the application of a food stimulus to the lips, and is characterized by large unit RN activity occurring mainly after the onset of large unit BN2 activity (starred arrow). The fractional overlap for this pattern is 0.9. (b) *In vitro* pattern II is generated in response to gentle mechanical stimulation of the buccal mass. Large unit RN activity ends prior to the onset of large unit BN2 activity (starred arrow). The fractional overlap for this pattern is 0.0. The patterns in part a and b of this Figure are from the same preparation. (c) A chain of *in vitro* pattern I responses that were elicited by a food stimulus. (d) A chain of *in vitro* pattern II responses that were elicited by a mechanical stimulation. The responses in parts c and d of this Figure are from the same preparation.

Fig. 3-3a-e. Properties of B8a and B8b. The different parts of this Figure contain data from different preparations. In each case B8a or B8b were identified using the criteria listed in the text. The a/b designation denotes the fact that we could only distinguish B8a from B8b using soma location, which is considered to be a relatively weak criterion (Church and Lloyd 1991). The soma of B8b is located lateral to that of B8a. (a) A camera lucida drawing of a lucifer yellow fill of B8a. The axon of this neuron bifurcates, sending branches down the left and right RN. B8a and B8b are found on the rostral side of the buccal ganglion. (b) Stimulation of the left or right B8b with the left and right RNs intact produces bilateral radula closure. Simultaneous stimulation increases the closing force. (c) Intracellular stimulation of B8a shows that it appears as a large unit in extracellular recordings of the left and right RNs (left traces). In the same preparation, shocking the RN (20Hz, 10ms pulses, 1 s duration) elicits a motor pattern in which B8a contributes to large unit RN activity (right traces). (d) Action potentials and excitatory junction currents of B8b (EJCs) in *Aplysia* saline (left traces) and high divalent cations saline (right traces). These data are from the same preparation. Action potentials were elicited by injecting depolarizing current. EJCs were recorded from the lateral surface of the I_4 muscle, immediately beneath the surface of the radula. (e) A series of action potentials in *Aplysia* saline showing weak facilitation of the resulting EJCs. Abbreviations: BN - buccal nerve, RN - radula nerve, CBC - cerebral-buccal connective, EN - esophageal nerve.

A RS

B

C

D

E

Fig. 3-4a-e. Properties of B10. The different parts of this Figure contain data from different preparations. In each case, B10 was identified using the criteria listed in the text. (a) *Camera lucida* drawing of a lucifer yellow fill showing that this neuron projects into the ipsilateral and contralateral BN2. B10 is found on the caudal side of the buccal ganglion. (b) Stimulation of B10 in a buccal mass-buccal ganglion preparation with BN2 intact bilaterally produces jaw closure and a sphincter-like contraction of the anterior buccal mass (top two traces). Jaw closure forces were measured using a pair of force transducing forceps. If the radula is held in a protracted position, then the anterior buccal mass contractions produced by B10 stimulation produce retraction-like movements of the radula (bottom two traces). Retraction forces were measured using a load cell. (c) Injecting depolarizing current shows that B10 appears as a large unit in extracellular recordings of the ipsilateral and contralateral BN2 (left traces). In the same preparation, shocking the RN (20 Hz, 10 ms pulses, 1 s duration) produces a motor pattern in which this neuron contributes to activity in the third largest group of BN2 units (right traces). (d) Action potentials and the associated I_1/I_3 EJC's in *Aplysia* saline (left traces) and high divalent cations saline (right traces). These data are from the same preparation. Action potentials were elicited by injecting depolarizing current. These EJC's were recorded from the center of the I_1/I_3 muscle (mm region, in the notation of Nagahama and Takata 1988). (e) A series of action potentials in *Aplysia* saline showing facilitation of the resulting EJC's. Abbreviations: BN - buccal nerve, RN - radula nerve, CBC - cerebral-buccal connective, EN - esophageal nerve.

Fig. 3-5a-d. Activity of B8a and B8b (putative radula closers) during *in vitro* patterns I and II. All of the data in this Figure are from the same preparation. Similar patterns of activity were observed in 22 preparations (5 with both B8a and B8b, 17 with either B8a or B8b). The starred arrows indicate the onset of large unit BN2 activity. (a) *In vitro* pattern I (ingestion-like pattern). B8a and B8b are active during the pattern of large unit RN activity. (b) *In vitro* pattern II (rejection-like pattern). B8a and B8b are again active during the pattern of large unit RN activity. (c) Injecting depolarizing current into these neurons produces 1:1 action potentials in the RN record. These RN units are large and clearly show that B8a and B8b contribute to the large unit RN activity seen during both *in vitro* patterns. (d) Simultaneous action potentials in B8a and B8b produce a larger, compound action potential in the RN. Simultaneous action potentials were elicited by injecting trains of depolarizing current pulses into both B8a and B8b.

Fig. 3-6a-c. Activity of B10 (putative radula retractor) and B8a (putative radula closer) during *in vitro* patterns I and II. All of the data in this Figure are from the same preparation. Similar patterns of activity in B10 were observed in 4 preparations. The starred arrows indicate the onset of large unit BN2 activity. (a) *In vitro* pattern I (ingestion-like pattern). B10 and B8a are active together. (b) *In vitro* pattern II (rejection-like pattern). B10 becomes active with the onset of large unit BN2 activity. B8a activity ends prior to the onset of large unit BN2 activity. (c) Injecting depolarizing current into B10 shows that it appears as a large BN2 unit and contributes to the intense activity observed just after the onset of large unit BN2 activity. Injecting depolarizing current into B8a shows that it contributes to the large unit RN activity seen during both patterns.

Fig. 3-7a, b. B8a and B8b contribute significantly to radula closing forces. (a) Electrical stimulation of each left and right buccal nerve pair (10 ms pulses; 5, 10, and 20 Hz) shows that powerful radula closing movements are produced by BN3 or RN stimulation. Each nerve pair was stimulated 3 times at each different frequency. Radula closing forces were transduced using the force transducing forceps. The three panels show results from three different preparations. (b) The actions of B8a/b contribute significantly to the radula closing force produced by RN stimulation. Each column corresponds to a different preparation, and shows the closing force produced by B8a/b stimulation as a percentage of the closing force produced by bilateral RN stimulation at approximately the same frequency. The left column compares B8a closing force to that of RN shock. In the center column, the closing forces produced by 3 different simultaneous stimulations of B8a and B8b are compared to forces produced by RN shock. The right column compares the closing forces produced by B8b and RN shock.

A

B

Fig. 3-8a, b. Activity of B4 and B5 during *in vitro* patterns I and II. All of the data in this Figure are from the same preparation. Similar patterns of activity were observed in B4, B5, or both in 7 preparations. The starred arrows indicate the onset of large unit BN2 activity. (a) *In vitro* pattern I (ingestion-like pattern). Both B4 and B5 become active just before the onset of large unit BN2 activity. (b) *In vitro* pattern II (rejection-like pattern). The timing of activity in B4 and B5 is similar to that seen during *in vitro* pattern I.

Chapter 4

Summary and future directions

Major results and their significance

Ingestion and rejection have been associated with their underlying motor patterns

A major result of this dissertation is that ingestion and rejection responses have been associated with specific patterns of motor neuron activity. This association was made at three different levels. At the behavioral level, the radula movements underlying ingestion responses are different from those underlying rejection responses. Thus, the muscles (and motor neurons) controlling the radula undergo different patterns of activity during ingestion and rejection. At the level of *in vivo* neural activity, recordings from nerves that are likely to mediate these different radula movements revealed two patterns of activity, one associated with ingestion, and one associated with rejection. This activity was temporally related to the radula movements underlying ingestion and rejection, suggesting that it may represent motor neurons which mediate these movements. At the level of a nearly isolated nervous system (i.e. the reduced preparation), ingestion-like and rejection-like motor patterns were observed in response to stimuli that elicit ingestion or rejection *in vivo*.

This result allows a portion of a behavioral question (i.e. what is the neural basis of feeding behavior?) to be re-cast as a pattern generation question (i.e. how are the ingestion and rejection motor patterns generated?). Relatively few systems have been studied in which a behavior made up of several mutually exclusive responses is produced by neural circuitry that is amenable to a detailed cellular analysis. Thus, the *Aplysia* feeding system represents an opportunity to study the mechanisms of neural reorganization and decision-making that underly this class of complex behavior.

The likely in vivo activity of several motor neurons has been determined

A second major result of this dissertation is that the likely *in vivo* activities of three motor neurons, B8a, B8b, and B10, have been determined during ingestion and rejection. B8a and B8b appear as large units in extracellular recordings of the RN. These 2 cells act to close the radula. B10 appears as one of the third largest group of units in extracellular recordings of BN2. This cell acts to close the jaws and retract the radula. Using the reduced preparation, it was shown that the extracellular activity associated with these cells is consistent with the extracellular activity occurring *in vivo* during ingestion and rejection. In addition, the peripheral actions and activity of these motor neurons during ingestion-like and rejection-like motor patterns are consistent with the radula movements observed during ingestion and rejection.

Establishing the *in vivo* activity of motor neurons is a crucial step in relating the pattern generating circuit to the observed behavior. Knowing the *in vivo* activity of a motor neuron can help focus a search for the premotor neurons (and

pattern generator elements) which mediate that activity. In addition, the likely *in vivo* activity and peripheral projections of these motor neurons can be used to understand the biomechanics of *Aplysia's* buccal mass, as was done for the buccal mass of the pond snail, *Lymnaea stagnalis* (Rose and Benjamin 1979; Benjamin et al. 1979).

A criterion that distinguishes ingestion from rejection has been identified

A third major result of this dissertation is that the timing of activity in B8a/b relative to activity in B10 has been shown to be a reliable criterion for distinguishing ingestion from rejection. This criterion is unique because it has been linked to the actual radula movements that distinguish ingestion from rejection. It is also unique because it can be used to determine which feeding response is most likely to be occurring in the absence of any additional behavioral information.

This distinguishing criterion represents an important tool for understanding how *Aplysia's* nervous system generates multiple behavioral responses. For example, in the stomatogastric system, focusing on the similarities and differences between different motor patterns has revealed several sites of neural reorganization, such as the VD neuron participating in both the pyloric and cardiac sac circuits (Hooper and Moulins 1989), the fusion of two pattern generators to form a third, different pattern generator (Dickenson et al. 1990), and the participation of neurons in both the pyloric and gastric mill circuits (Weimann et al. 1991). Since *Aplysia's* feeding pattern generator is likely to be presynaptic

to the motor neurons, studying the premotor neurons to B8a/b and B10 is likely to reveal sites of reorganization that underly these different motor patterns.

Radula movements distinguish ingestion from rejection

A fourth major result of this dissertation is that radula movements were shown to distinguish ingestion from rejection. During ingestion (biting or swallowing), the radula is opened as it protracts and closed as it retracts. During rejection, the radula is closed as it protracts and opened as it retracts. Radula movements during biting have been previously characterized by Kupfermann (1974). However, the results of this dissertation constitute the first characterization of radula movements during swallowing and rejection responses. These results also represent the first report that the radula is used for two different purposes (i.e. moving food either into or out of the buccal cavity) by changing the timing of radula closure relative to protraction and retraction movements. Since the neuronal circuitry controlling radula movements is likely to be amenable to a detailed cellular analysis, *Aplysia* may be an advantageous system for studying the neural mechanisms underlying the multifunctional use of a peripheral structure.

A non-rhythmic behavior has been characterized

A fifth major result of this dissertation is that a non-rhythmic behavior has been characterized. *Aplysia's* feeding behavior is made up of biting, swallowing, and rejection responses. Each of these responses is a separate and distinct motor act that can occur individually as well as in repetitive chains which approximate

rhythmic behavior. In addition, the animal can rapidly switch among these different responses as sensory cues change (Figure 2-9). These behavioral switches are particularly frequent when the animal feeds on complexly structured foods. For example, when eating the fronded seaweed, *Laurencia*, the animal breaks off and ingests small pieces of food by repeatedly switching between several different feeding responses (Chiel et al. 1990).

Most studies of the neural basis of behavior have focused on rhythmic behaviors because relatively simple phase resetting experiments can be used to identify pattern generator elements. However, the results of these studies may not be representative of the majority of behaviors, which are non-rhythmic. *Aplysia's* consummatory feeding behavior has a number of advantages for studying the neural basis of a non-rhythmic behavior: the neurons are identifiable (making the nervous system amenable to a detailed cellular analysis), the different feeding responses are stereotyped, and these responses can be distinguished by the timing of activity in specific motor neurons. Thus, using intracellular techniques, it may be possible to identify some of the mechanisms involved in decision making, peripheral coordination, and pattern generation that underly this non-rhythmic behavior. If tests to identify pattern generator elements in non-rhythmic systems can be developed, then it may also be possible to determine the neural circuit underlying this behavior.

A group of shared elements is likely to mediate biting, swallowing, and rejection responses

A sixth major result of this dissertation is that the pattern generators which mediate biting, swallowing, and rejection responses were shown to be likely to share a common group of neurons. This statement is supported by several lines of evidence: First, the literature survey done in Chapter 1 suggests that when different behavioral responses share the same anatomical structures, the responses are likely to be mediated by a shared group of neurons. Second, both *in vivo* and in the reduced preparation, many motor neurons undergo similar patterns of activity during biting, swallowing, and rejection responses, suggesting that portions of the pattern generator circuitry may do the same. Third, neurons B4 and B5, which may be command-like neurons (Susswein and Byrne 1988), undergo similar patterns of activity during ingestion-like and rejection-like patterns in the reduced preparation. Finally, the ingestion and rejection patterns were never present at the same time, either *in vivo* or in the reduced preparation. The simultaneous presence of two different motor patterns that are not synchronized with each other is considered to be strong evidence for the existence of separate pattern generators and has been observed in systems such as locust (Ramirez and Pearson 1988) and the stomatogastric system (Selverston and Moulins 1987).

Understanding this result is crucial to successfully exploiting the advantages of the *Aplysia* feeding system. The likelihood that a shared group of neurons mediates biting, swallowing, and rejection suggests that future investigations should study each of these responses simultaneously, focusing on the similarities and differences between them. This approach is more likely to identify the shared and unshared portions of the biting, swallowing, and rejection pattern generators,

and will help to focus future investigations dealing with multifunctionality, decision-making, and the generation of multiple motor patterns.

Aplysia is one of several similar feeding systems

The results of this dissertation open up an opportunity to compare *Aplysia's* feeding circuitry to that of other similar molluscan species, such as *Tritonia* and *Pleurobranchaea*. Comparing the nervous systems of species with similar, but non-identical peripheries has provided useful insights into the relationship between neuronal architecture and peripheral structure in the crustacean stomatogastric system (Katz and Tazaki 1992).

It may also be advantageous to compare the molluscan feeding systems to the stomatogastric system. The molluscan pattern generator circuitry is likely to be made up of premotor neurons, while the stomatogastric pattern generator circuitry includes the motor neurons (Selverston and Moulins 1987). This extra layer of neurons separating the molluscan pattern generator circuitry from the periphery affords an extra degree of freedom which could have profound effects on the architecture of the pattern generator. Comparing the molluscan pattern generator circuitry to that of the stomatogastric system could provide insights into the effects of this additional degree of freedom.

Limitations of these results

This investigation deliberately focused on the similarities and differences between biting, swallowing, and rejection responses instead of trying to characterize the entire feeding system. Using this strategy, it was shown that the

timing of radula closure relative to protraction and retraction movements distinguishes ingestion from rejection, and that motor neurons B8a and B8b are likely to have a role in controlling the timing of radula closure. It is important, however, to keep in mind that many additional motor neurons are involved in generating feeding movements. It is also important to keep in mind that the biomechanics of the buccal mass are not well understood.

How important is it to completely characterize the activity of all buccal ganglion motor neurons during ingestion and rejection? This may not be necessary to answer questions involving the activity of B8a, B8b, and B10. Such questions include: What neural mechanisms underly the generation of more than one motor pattern? How does the animal decide which motor pattern to generate? In contrast, questions involving buccal mass biomechanics and the function of various motor pools can only be addressed if additional buccal ganglion motor neurons are characterized. Some of these questions include: Are there any additional motor neurons that contribute to the change in timing of radula closure? What additional motor pools are involved in producing radula protraction and retraction movements? What significance does the pattern of large unit BN2 activity have? Are radula movements produced by co-contraction of antagonist muscle pairs? Are additional motor pools recruited as the force produced by the buccal mass increases? What are the roles of the various modulatory neuropeptides released by buccal ganglion motor neurons? Can biting responses be distinguished from swallowing responses?

Is it necessary to have a complete understanding of buccal mass biomechanics? The experimental strategy used in this dissertation was designed

to allow the neural circuitry to be studied without understanding buccal mass biomechanics. By combining cuff electrode recordings of *in vivo* neural activity with intracellular recordings of motor neuron activity in a reduced preparation, the likely *in vivo* activity of specific motor neurons was determined. The fact that the reduced preparation produces feeding-like patterns even though it is almost completely deafferented suggests that there is a strong centrally generated component to these motor patterns. This, in turn, suggests that knowing the *in vivo* activity of motor neurons during specific behavioral responses will allow the neural circuitry to be studied in the context of generating those patterns. It is important to keep in mind, however, that an understanding of the buccal mass biomechanics may be necessary to understand how the system responds to perturbations, such as changes in the size, texture, or toughness of the food being ingested.

Within these limitations, the results of this dissertation provide a foundation for studying *Aplysia's* feeding pattern generator. Two different *in vivo* motor patterns have been identified, one associated with ingestion, the other associated with rejection. A preparation has been developed that produces ingestion-like and rejection-like motor patterns and is amenable to intracellular recording techniques. The activity of several motor neurons has been identified during these ingestion-like and rejection-like patterns. This activity can be used to distinguish these two motor patterns.

Future Directions

This investigation associated biting, swallowing, and rejection responses with their underlying motor patterns, and then identified some of the motor neurons that contribute to these motor patterns. A logical next step would be to attempt to identify premotor neurons that mediate activity in these motor neurons. This section introduces two possible lines of investigation involving premotor neurons that mediate activity in the motor neurons identified in this study.

A group of shared elements may control activity in B4, B5, and B10

Studying the premotor neurons mediating activity in neurons B4, B5, and B10 could reveal the portions of *Aplysia's* nervous system that are shared between the ingestion and rejection pattern generators. The results of this investigation suggest that B4, B5, and B10 are likely to undergo similar patterns of activity during ingestion and rejection (Figures 3-6 and 3-8). This suggests that the premotor neurons mediating activity in these neurons may also undergo similar patterns of activity during ingestion and rejection, and that these premotor neurons may therefore be shared between the ingestion and rejection pattern generators. Thus, studying these premotor neurons could provide insight into how networks dynamically reorganize to produce different patterns of neural activity.

Timing of activity in B8a/b represents the neural correlate of a decision

Studying the premotor neurons which mediate activity in B8a and B8b could provide insights into how *Aplysia* decides which feeding response to generate, as well as how that decision is made in the context of the animal's current

environment, past experiences, and motivational state. The results of this investigation suggest that the timing of activity in B8a and B8b relative to activity in B10 represents a neural correlate of the animal's decision to ingest or reject (Figures 3-5 and 3-6). Several groups have shown that a variety of factors contribute to this decision, such as: chemosensory and mechanosensory cues (Kupfermann 1974), satiation (Susswein et al. 1976), learning (Susswein et al. 1986; Schwarz and Susswein 1986), and egg-laying (Arch and Smock 1977; Pinsker and Dudek 1977).

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