

THE ROLE OF THE MELIBE BUCCAL GANGLIA IN FEEDING BEHAVIOR

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A series of anatomical, physiological and behavioral experiments were conducted to determine the role of the *Melibe* buccal ganglia in feeding. The small, paired buccal ganglia are located on the surface of the esophagus, communicate with each other via a long buccal-buccal connective, and with the brain via bilaterally symmetrical cerebral-buccal connectives. They also innervate the anterior and posterior regions of the esophagus, and the paired salivary glands. Stimulation of the cerebral-buccal connectives causes slow rhythmic contractions of the esophagus, and stimulation of either the anterior or posterior buccal nerves results in single contractions of the esophagus. Removal of both buccal ganglia does not impair the ability of animals to capture food, but it has a significant impact on the transfer of captured prey through the esophagus. These data, taken together, indicate that the *Melibe* buccal ganglia do not influence the capture of food, but rather control movements of the esophagus which are necessary to transport food from the mouth to the stomach.

KEY WORDS: *Melibe leonina*, buccal ganglia, feeding behavior, swallowing, lesions, gastropod feeding.

INTRODUCTION

Gastropod buccal ganglia innervate extensive areas of the buccal mass, pharynx, salivary glands, esophagus, and stomach and serve important functional roles in the control and modulation of feeding behavior. Due to their suitability for investigations of the neural basis of behavior buccal ganglia have been studied extensively in a number of different species (*Aplysia*, Kupfermann, 1974; Cohen *et al.*, 1978; Lloyd *et al.*, 1988; *Helisoma*, Kater, 1974; *Limax*, Gelperin *et al.*, 1978; *Lymnaea*, Benjamin and Rose, 1979; Elliot and Benjamin, 1985a, b; *Pleurobranchaea*, Gillette and Gillette, 1983; *Tritonia*, Willows, 1980; Lloyd and Willows, 1988; Willows *et al.*, 1988; *Planorbis*, Arshavsky *et al.*, 1988). Although the feeding behaviors, and the types of prey consumed, differ widely between gastropods, certain organizational and functional characteristics of buccal ganglia appear to have been conserved during the course of evolution (Benjamin, 1983). In general,

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buccal ganglia contain sensory neurons, motor neurons that innervate the feeding organs, a network of interneurons which generate the biting, chewing and swallowing rhythms, and regulatory neurons that modulate the output of the network.

Recordings from buccal nerves in semi-intact preparations of several species indicate that the buccal ganglia play a major role in the acquisition and processing of food. They generate the various rhythmic feeding patterns and control the intrinsic muscles of the radula and buccal mass, as well as the salivary glands, pharynx, and esophagus (Benjamin, 1983). Additional evidence indicating that buccal ganglia play a critical role in the consummatory aspects of feeding behavior comes from studies in *Aplysia*. When Kupfermann (1974), cut both the cerebral-buccal connectives between the brain and the buccal ganglia, thereby preventing activation of the buccal motor programs by descending interneurons and sensory neurons, he observed that food would still elicit appetitive aspects of feeding, such as head waving, but animals were unable to eat the food.

Swallowing and movements of the buccal mass that transport food through the esophagus to the stomach, also appear to be under the control of neurons in the buccal ganglia. Willows (1980) has identified buccal ganglion neurons which are involved in swallowing behavior in *Tritonia diomedea* and characterized their rhythmic activity. Both ingestion and egestion motor programs have been recorded from isolated and semi-isolated *Pleurobranchaea* buccal ganglia (Croll and Davis, 1981). Finally, Lloyd *et al.* (1988) have demonstrated that identified peptidergic neurons in the buccal ganglia of *Aplysia* control gut motility. Thus, it appears as if gastropod buccal ganglia contain neural circuits which control chewing, swallowing, egestion, and the transport of food through the esophagus to the stomach, as well as aspects of food acquisition.

The nudibranch *Melibe leonina* has a unique feeding behavior for a gastropod, which is related to the fact that it lacks both a radula and buccal mass (Agersborg, 1921; Gosliner, 1987). It uses movements of its large oral veil to capture food and bring it in contact with the mouth (Agersborg, 1921; Hurst, 1968; Ajeska and Nybakken, 1976; Watson and Trimarchi, this issue). In addition, *M. leonina* lacks structures for chewing food and therefore prey are engulfed whole and transported, without chewing, to the stomach. While the process of capturing food is rather complex, the gut movements controlled by the buccal ganglia are relatively simple.

Despite the fact that *Melibe* lack a buccal mass, they have retained a pair of small buccal ganglia. Their general anatomy and pattern of innervation suggest that they are involved in the control of gut motility. In the present study we tested this hypothesis by examining how removal of the buccal ganglia affected feeding and the movement of food through the alimentary canal. In addition, we mapped the buccal ganglia and demonstrated that stimulation of the buccal nerves cause contractions of the esophagus. These data suggest that the *Melibe* buccal ganglia are not necessary for the capture of prey, but rather are responsible for controlling the transfer of food from the mouth to the stomach.

METHODS

Animals

All animals were collected, using SCUBA, from eel grass beds located around the San Juan Archipelago (Washington), and shipped to the University of New Hampshire Coastal Marine Laboratory. Animals were maintained in seawater tables which were continuously perfused with filtered seawater from Portsmouth Harbor (6–12°C). Every 3–4 days the *Melibe* were fed a small amount of the material which was filtered from the flow-through seawater system. During the time of our feeding experiments this filtrate consisted primarily of barnacle nauplii.

Anatomy of the Buccal Ganglia

The nervous system of freshly dissected animals was observed with a dissecting scope, drawn and photographed.

Stimulation of the Buccal Ganglia Nerves

To determine the potential motor function of particular buccal nerves we stimulated them in partially dissected animals. *Melibe* were pinned in a perfusion chamber and an incision was made in the skin, extending from over the mouth to just above the junction of the esophagus and the stomach. The incision was held open with pins, so that suction electrodes could be placed on different nerves, and a thread connected to a Grass FT.03 force transducer could be attached to various locations along the musculature of the alimentary canal. A Grass S88 stimulator and isolation unit was used to supply pulses of various strengths, durations and frequencies to the suction electrode. The output of the force transducer was recorded on a Grass Model 79 polygraph.

Lesioning the Buccal Ganglia

Animals to be lesioned were placed on their side in a Sylgard-lined dish, and held in place with pins that secured the animals but did not penetrate their integument. A 0.5 cm incision was made in the integument of the neck region (Figure 1), and the exposed buccal ganglia were removed with a fine forceps. Care was taken to cause as little damage as possible to the surrounding musculature, connective tissue and non-buccal nerves. Once both buccal ganglia were removed the incision was sutured closed using fine silk thread glued to a minuten pin. The entire surgical procedure lasted approximately 15 minutes. Sham-operated animals were treated identically except the buccal ganglia were not removed. Control animals did not undergo surgery (n=9).

All animals were then placed in aquaria, where they fed on *Artemia* (concentration of 1500/L) for five days. *Artemia* are not a natural food source for *Melibe* in the Puget Sound, and they are digested very slowly; thus the quantity of *Artemia* present in the

digestive tracts of experimental animals can be used as an indicator of post-surgery feeding efficacy.

After feeding for 5 days the animals were weighed, and dissected for analysis of gut contents. The number of *Artemia* in each of the four gut regions (esophagus, stomach, gastric sac, intestine) were counted. Other observations recorded were the color of the stomach diverticuli, the size, color and viability of the salivary glands, locomotor activity, and posture of each animal. After the 5 day recovery and feeding period 11 of the lesioned animals and 9 of the sham-operated animals were either dead, or dying; leaving 10 healthy lesioned and 14 healthy sham-operated animals for data analysis. Data were tabulated and non-parametric statistical analysis, Mann-Whitney U, was conducted on the resulting data.

RESULTS

Anatomy of the Buccal Ganglia

Melibe buccal ganglia are located lateral to the esophagus, just posterior to the mouth (Figure 1, Figure 2). In comparison to the buccal ganglia of most gastropods they are quite small (200 μm in diameter), with fewer neurons (approximately 30–40/ganglion, Falk *et al.*, 1990). They are separated by an unusually long buccal-buccal connective, which positions them on opposite sides of the esophagus. There are 2 major nerves projecting from each buccal ganglion, in addition to the buccal-buccal connective. The posterior root runs along the surface of the esophagus, branching extensively and apparently innervating this tissue at various points along its length (Figure 1). At the base of the stomach diverticuli the posterior root connects to the gastric ganglion (Hurst, 1968).

The buccal anterior root bifurcates close to the ganglion (Figure 2). One branch becomes the cerebral-buccal connective and the other projects anteriorly. This anterior root innervates the salivary gland, anterior esophagus and the most posterior region of the mouth (Figure 1). Another very small root exists the buccal-buccal connective near its midpoint and innervates the esophagus immediately under the buccal ganglia.

Removal of Buccal Ganglia

Both lesioned and sham-operated animals followed a similar time course of recovery from surgery. Approximately twelve hours post-surgery the animals were behaving normally; carrying out activities such as swimming, mating and laying eggs. Of more interest, all animals appeared to feed normally (Figure 3), extending and contracting their oral veil in the stereotypical manner described in the the previous paper (Watson and Trimarchi, this issue).

After three to four days the surgical wounds were fully healed and the sutures had been sloughed off (Figure 4). Approximately 50% of the lesioned and sham-operated animals began to die at this time, and most of these subjects were dead after 5 days. The cause of death in these surgically treated animals was not clear, but

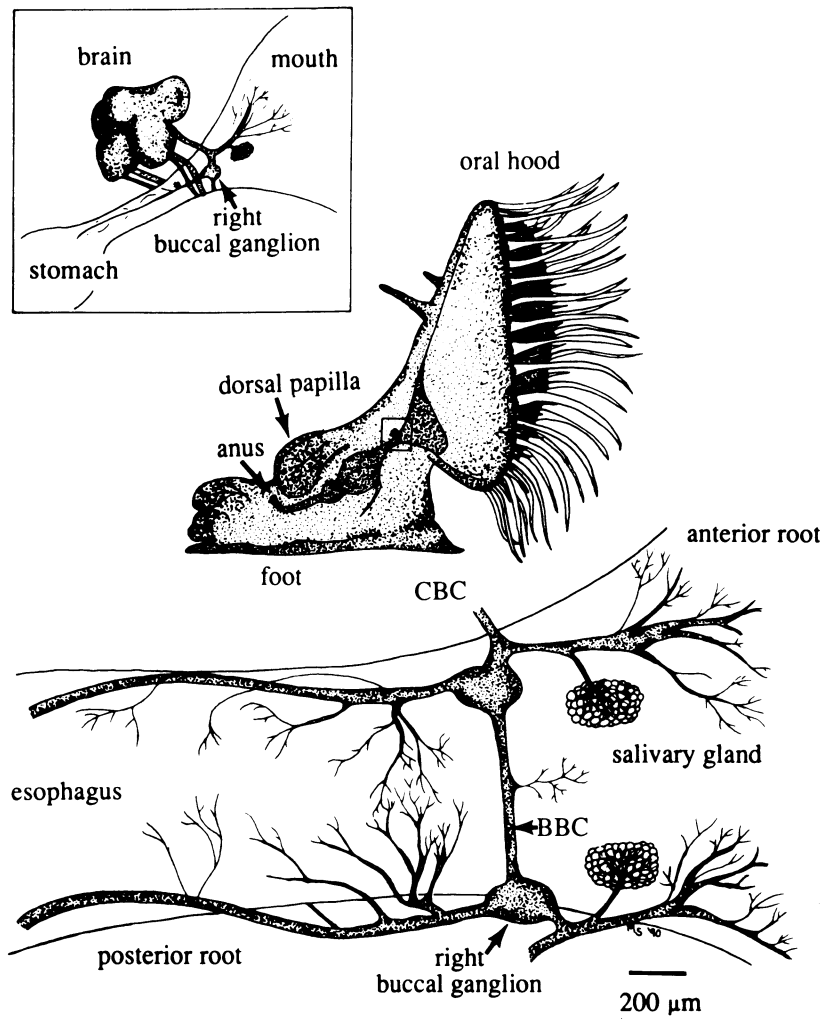


Figure 1 The anatomy of the *Melibe* buccal ganglion. An overview of the location of the internal digestive organs is illustrated in the center drawing. The boxed area is shown magnified in the upper left of the figure, to demonstrate the orientation of the brain, one buccal ganglion and one salivary gland. A magnified ventral view of the paired buccal ganglia is presented at the bottom of the figure. Note the extensive branching of the anterior and posterior buccal nerves, as well as the innervation of the salivary glands. Calibration bars are not presented for the top two figures because of the variability between animals of different sizes. The calibration bar in the bottom figure is typical for a large (5 inch long) *Melibe*.

