THE FUNCTIONAL MORPHOLOGY AND MOTOR INNERVATION OF THE BUCCAL MASS OF TRITONIA HOMBERGI

BY A. G. M. BULLOCH* AND D. A. DORSETT

Marine Science Laboratories, Menai Bridge

(Received 25 May, 1978)

SUMMARY

The anatomy of the buccal mass and the function of nine principal muscle groups involved in the feeding movements, are described for the mollusc Tritonia hombergi. Anatomical and physiological studies on some 40 neurones along the posterior border of the buccal ganglia indicates that many are primary motoneurones to the muscles of the buccal mass. The feeding cycle may be divided into three phases of muscle activity termed Protraction, Retraction and Flattening, which are correlated with the patterned bursting observed in P, R and F motoneurone groups within the motoneurone population. A fourth group of motoneurones are thought to innervate muscles to the outer lips which are active during protraction. The patterned output of impulses in the buccal nerves during feeding cycles confirms that the motor control of the muscle groups may be explained in terms of the sequential activation of the P, R and F cells.

INTRODUCTION

The neural control of feeding in gastropod molluscs has been the subject of a number of recent studies. The advantages of this system are several; the buccal muscles are anatomically distinct and directly innervated by central motoneurones (Heyer, Kater & Karlsson, 1973), sequences of feeding movements can be obtained from preparations of the isolated buccal mass, and the motor output associated with feeding can be generated by the isolated buccal ganglia (Rose, 1968, 1971; Kater & Rowell, 1973; Kater, 1974; Davis, Siegler & Mpitsos, 1973; Siegler, Mpitsos & Davis, 1974; Woollacott, 1974). More recently these investigations have been extended to two species of the genus Tritonia (Longley, 1976; Willows, 1977; Bulloch, 1977). The present paper describes the feeding behaviour and specifies the function of approximately 40 buccal motoneurones in Tritonia hombergi. A second paper deals with the neural mechanisms underlying the patterned motor output (Bulloch & Dorsett, 1979).

* Present address: Department of Zoology, University of Iowa, Iowa City, Iowa 52242, U.S.A.
MATERIALS AND METHODS

*Tritonia hombergi* were obtained by dredging around the coast of Anglesey and the Isle of Man. They were kept for periods of up to 2 months under circulation in the laboratory and fed periodically on supplies of fresh *Alcyonium digitatum*. The anatomy and histology of the buccal mass was studied by dissection of fresh and Bouin-fixed material, and by conventional wax-sectioning techniques. For the latter, animals of approximately 2–4 cm were used. They were first relaxed in 4% MgCl₂, fixed for 24 h, washed and dehydrated in Cellosolve, and embedded in Ester wax. Serial sections were cut at 8 μm and stained with Azan. Observations on the feeding behaviour were made on fully grown animals 12–15 cm in length, which had been starved for 5–10 days. Individuals were placed in large glass dishes with sea water containing several pieces of *Alcyonium*. The bite made by the jaws could usually be observed through the body wall, and timed observations were made of the feeding sequence.

Two types of preparation were used for the electrophysiological studies. The semi-intact preparation consisted of the anterior third of the animal, in which all neural connexions to the head and oral veil were left intact. In the isolated buccal mass preparation, all nerves to the cerebro-pleuro-pedal ganglia were cut, except for the cerebro-buccal connective. In both cases the buccal ganglia were stabilized on a wax-covered platform passed down the oesophagus. The experiments were conducted in perspex tanks supplied with continuously flowing sea water, in which the preparations remained in good condition for up to 36 h.

Conventional electrophysiological recording techniques were used. Microelectrodes were filled with either 3 M-KCl, 2 M-KAc or 3% CoCl₂ and normally had a resistance of 10–30 MΩ. For electrical stimulation of nerve trunks, 2 ms square pulses were delivered at rates of 5–20 s⁻¹ through flexible polythylene suction electrodes filled with sea water. Similar electrodes were used to record E.J.P.s from the surface of muscle fibres. For the anatomical investigations, similar electrodes filled with 1 M-CoCl₂ were used to backfill central and peripheral nerve trunks (Pitman, Tweedle & Cohen, 1972). These preparations were developed in 1% NH₄S solution, dehydrated in alcohol containing a trace of ammonium hydroxide, and cleared in methyl salicylate. These preparations were photographed or drawn within 48 h.

**Anatomy of the buccal mass**

The gross features of the anatomy were obtained by dissection, but sections proved useful in revealing the orientation of sub-surface muscle fibres and non-muscular structures. The four major features of the buccal mass are:

1. A pair of strong, chitinous jaws extending the length of the buccal mass, hinged anteriorly and having serrated cutting edges (Fig. 1).

2. A radula supported by two sets of muscles and divided into two anteriorly facing lateral lobes. The median, inward facing edges of these lobes being separated by a fleshy membrane posteriorly. The radula teeth are numerous, arranged in parallel rows, and of uniform size except for a mid-ventral spur, where they are considerably enlarged. The radula is used for grasping and manipulation rather than rasping the food.
Fig. 1. Stereodiagrammatic representation of the buccal mass of *T. hombergi* viewed from the left side. The diagram includes sectional views taken anterior and posterior to the oesophagus to show internal structure. The external musculature has been removed from the upper half of the mid-section to show the radula in the resting position. M 1–6, Intrinsic muscles; M 7, 8, extrinsic muscles; IL, OL, inner and outer lips; J, jaws; OC, oral canal; Oe, oesophagus; R, radula; Me, fleshy membrane.
The inner lips are a pair of fleshy pads which partially cover the anterior face of each radula lobe.

An outer buccal lip which encircles the buccal mass aperture, rather like a sphincter muscle.

The buccal mass occupies the anterior one third of the body cavity and is joined to the mouth on its ventral side by an infolded oral canal. The outer layers of the jaws and radula teeth, and the faces of the inner lips that appose the radula give staining reactions that indicate the presence of chitin.

The musculature of the buccal mass

The principal muscle systems within the buccal mass may be considered as nine groups, here designated as M 1–9 (Fig. 1). Where possible the action of each muscle has been checked by direct electrical stimulation of the fibres in quiescent isolated preparations.

The intrinsic muscles

M 1. Jaw opener. This muscle consists of a strip of fibres running transversely over the jaw hinge and inserted on to the lateral edges of the jaws anteriorly. Stimulation of this muscle reliably caused wide separation of the jaws, without affecting the radula or inner lips. With very wide separation of the jaws, the inner lips were often displaced laterally, due to passive stretching of M 4 whose fibres also attach to the jaws.

M 2a and b. Anterior and posterior jaw closers. Muscle 2a represents a pair of muscle blocks underlying the anterior third of the jaws. Anteriorly they are joined by a narrow ligament running transversely under the hinge. M 2b is a similar pair of larger muscle blocks at the posterior end of the jaws. Together they encircle the opening of the buccal mass with a powerful ring of muscle. In the resting state the jaws are slightly open, but stimulation of either of these paired muscles resulted in jaw closure.

M 3. Superficial dorsal muscles. These thin bands of muscle are located on the anterior dorsal surface of the buccal mass, extending from the upper edges of the jaws to the anterior dorsal boundary of M 5. Stimulation of one or two of these muscles resulted in a movement of the radula towards the jaws. The concerted activity of the whole group protracts the radula into the cavity between the open jaws.

M 4. Inner lip retractor. M 4 are a small pair of muscles inserted on to the dorsal edges of each inner lip. The majority of fibres run from the inner lips to the outer edges of the posterior half of each jaw. A small band of fibres connects the lips, running transversely just behind the hinge. Stimulation of M 4 by inserting an electrode between the M 3 bands, resulted in the inner lips being withdrawn laterally and posteriorly. During this movement the inner lips drew the radula lobes apart, exposing the fleshy lobe. When viewed from above, inner lip retraction produces a characteristic narrowing of the buccal mass anterior to the oesophagus. The lips and the associated muscles were previously described as jaw closers (Thompson, 1962).

M 5. Lateral radula retractor. This name belies the complexity of this largest buccal muscle, which consists of several layers of interdigitating fibres. To simplify its structure, it may be considered to consist of three components. The most ventral of these (Layer 1) is the thickest, composed of many transverse laminae typical of radial muscle systems such as the tongue. In this it has a structure closely resembling the
Motor innervation of buccal mass in Tritonia

Fig. 2. Sagittal section through the buccal mass, viewed from the left side, to illustrate the relative positions of the radula (R), odontophore (O) and inner lips (IL) during a buccal cycle. (1) Radula and inner lips at rest (1a, dorsal view). (2) At end of Phase 1, odontophore protruded with ventral spur of radula between jaws. Inner lips retracted and radula flattened (2a, dorsal view). (3) End of Phase 2, radula and odontophore retracted, inner lips relaxed. (4) End of Phase 3, inner lips retracted and radula flattened. Note the 'narrowing' and 'doming' denoting inner lip retraction and radula retraction respectively.

The posterior ventral fibres of Layer 1 insert on the outer edge of the jaws, whereas the dorsal and anterior fibres support the underside of the radula. Layer 2 overlies Layer 1, the fibres being inserted on the lateral borders of the radula, passing ventrally and medially to interdigitate with those of Layer 1. Layer 3 fibres form a thin superficial sheet above Layer 2 at the radula membrane, and are inserted on to the outer edges of the jaws.

It was only possible to stimulate the superficial fibres of M 5 directly (Layer 3), although deeper layers may have also been affected to some extent. Such stimulation caused the radula to retract posteriorly and dorsally. On anatomical grounds, Layers 2 and 3 appear to be capable of separating the two lobes and flattening the radula, but this response was not obtained.

Viewed from the dorsal aspect, M 5 stimulation resulted in a distinct 'doming' of the dorsal buccal mass posterior to the oesophagus (Fig. 2).

M 6. Medial radula retractors. These are a pair of substantial muscles inserted under the medial groove of the radula and fanning out posteriorly to the outer edges of the jaws, where they attach posterior to those of M 5 (Layer 3). Stimulation of either M 6 muscle caused the ipsilateral radula lobe to be retracted and folded in towards the midline. The ipsilateral side of the buccal mass 'domed' in a similar manner as described above.

M 7–9. Extrinsic buccal muscles. Three groups of extrinsic muscles have been identified and designated as M 7, 8 and 9.

M 7 is a series of muscle bands originating on the jaw perimeter and inserting
ventrally on to the outer buccal lip. The bands of M 8 are narrow and overlie those of M 7, inserting ventrally on to the wall of the oral canal. This group of muscles includes several which extend to the oral canal from origins close to the oesophagus. The individual fibres of M 8 interdigitate with those of muscles M 9, which pass from the outer buccal lip to the oral opening, and together comprise much of the oral canal musculature.

The function of these extrinsic muscles have not been investigated. On anatomical grounds M 7 appears capable of retracting the outer lip, whereas M 8 and 9 are likely to be involved in extrusion of the buccal mass into the oral canal.

**Feeding behaviour**

*Tritonia hombergi* is invariably associated with the soft coral *Alcyonium digitatum* upon which it feeds exclusively (Alder & Hancock, 1845; Thompson, 1962, 1976; Thompson & Brown, 1976; Yonge & Thompson, 1976). The feeding mechanism has been described by Thompson who noted that pieces of food were detached by the jaws and passed to the oesophagus by movements of the radula. Thompson also considered a piece of the coral could be detached with a single bite but our observations show the animals remain firmly attached to the colony for several minutes before separation of the piece is achieved. When a starved animal first encounters the food the following sequence of events was recorded. The head and oral veil was raised and moved from side to side. Almost immediately the oral canal was everted and the buccal mass partially extruded so that the jaws and retracted outer lip could be seen. The everted oral canal forms two lobes laterally, suggesting eversion results from a local increase in haemocoelic pressure. ‘Exploratory’ movements lasted from several seconds to a few minutes, suggesting chemical or tactile investigation of the food. A violent spasm occurs as the jaws open and close in an attempt to grasp the colony. Several attempts were made before such efforts were successful, but once achieved the colony was gradually cut away by periodic ‘bites’ with the jaws. The animal then moved away and one or two further cycles occurred as the food was transferred from the lumen of the buccal mass to the oesophagus. On several occasions the radula was visible during the transfer process and moved in phase with the jaw movements, retraction coinciding with jaw closure. The entire sequence of buccal cycles lasts between 3 and 6 min, the interval between bites varying between 10 and 30 s. These observations were based on ten animals, and none fed again within the experimental period. The maximum number of pieces of *Alcyonium* found in the oesophagus in over 120 dissections never exceeded three.

**Feeding movements in the isolated preparation**

The isolated buccal mass preparation commonly undergoes periods of vigorous cyclic activity which can be divided into three phases of movement.

**Phase 1. Protraction.** In this phase the jaws open widely, while the outer and inner buccal lips are withdrawn to expose the jaws. (Fig. 2). Concurrently the radula swings forward and ventrally (protracted) and is flattened, separating the lobes and exposing the fleshy membrane. At the end of this phase the ventral spur lies in the space between the jaws, but does not protrude to any distance. The combined radula and lip movements create a space in the anterior of the buccal cavity. Phase 1 movements are attributed to activation of M 1, 3 and 4.
Phase 2. Retraction. The radula lobes fold together and the whole organ rotates posteriorly towards the oesophageal opening. This is accompanied by jaw closing and 'doming' of the posterior buccal mass. The movements are thought to result from activation of M₁a, b, 5 and 6.

Phase 3. Flattening. With the radula in the retracted position, the inner lips retract a second time and the radula is again flattened. The anterior buccal mass undergoes the characteristic narrowing signifying M₄ activation. One or more of the M₅ layers may contribute to the flattening observed here and in Phase 1.

Although details of the buccal mass activity cannot be seen in the feeding animal, the inter-bite intervals corresponds closely to the range of cycle periods observed in the isolated buccal mass. In view of the regular and co-ordinated pattern of this activity, it seems reasonable to suppose it represents the normal feeding sequence that occurs in intact animals.

The cycle duration varies with the preparation, but generally the period observed during continuous activity is 10–15 s, agreeing well with the inter-bite intervals observed in the intact feeding animals.

The identification of buccal motoneurones

The symmetrical buccal ganglia lie posterior to the oesophagus on the dorsal side of the buccal mass (Figs. 1, 3). The positions of 22 somata in each ganglion can be recognized from preparation to preparation. The two giant neurones 4 and 5 (Fig. 3) serve as markers relative to which the position of other cells can usually be referred. For the smaller neurones the grid reference system is useful. From each ganglion three pairs of nerves supply the buccal mass (Figs. 3, 4), the oral canal also receiving innervation by cerebral nerve 4. Other connexions are implied by stimulating peripheral stumps of buccal nerves (Bulloch, 1977). Stimulation of the gastro-oesophageal nerve BN 4 proved to be an effective way of generating buccal mass activity, a short train of stimulus pulses regularly causing one or more complete cycles
of buccal mass movement in previously quiescent preparations. Buccal cycles evoked in this way were indistinguishable from those of spontaneous cycles.

Several criteria were used to identify buccal motoneurones.

1. The demonstration of a characteristic burst pattern and integrative synaptic inputs recorded from the soma, which correspond to one or more phases of buccal movement observed during spontaneous or induced cyclic activity (Fig. 5).

2. Intracellular stimulation of the neurone consistently produced a movement of buccal muscles or associated structures which can be recognized as part of the feeding sequence.

3. Demonstration of direct innervation of a muscle by recording a 1:1 EJP that followed the soma spike with constant latency and at high frequency.

4. Demonstrating an axon of the cell in one or more peripheral nerve trunks by electrophysiological or visual (cobalt iontophoresis) methods.

The above criteria were applied to the 22 identifiable neurons along the posterior border of each buccal ganglia, and the results are summarized below.

Criteria 1 and 2. The initial experiments indicated that neurones satisfying these criteria could be designated as P (Protractor), and R (Retractor) or F (Flattener) cells, which were active during Phase 1, 2 or 3 of the buccal cycle respectively, and could cause some or all of the movements associated with a particular phase (Figs. 3, 5). The F cells are often active during Phase 1 in addition to Phase 3, being consistent with the
Motor innervation of buccal mass in Tritonia

Fig. 5. Two paired recordings from different preparations showing the phase relationships between P, R, F and M cells. Both M and P cells fire in Phase 1, the R cell fires in Phase 2, when F and M cells are inhibited. The F cells burst in Phase 3. Unlike the F cells, M cells often show e.p.s.p.'s during the large inhibitory wave. Calibration: 50 mV, 5 s.

radula flattening movements during both these phases. There is a fourth category of motoneurone which can retract the outer buccal lip and has been termed the M (mouth opener) cell.

The P cells show a burst of spikes during Phase 1, this being preceded by a slowly accelerating train of e.p.s.p.s peculiar to these neurones. Occasionally P cells fire during other phases, but are most active in Phase 1. Driven activity in P cells (commonly located in positions 2, 12, 13) by intracellular depolarization, results in jaw opening and radula protraction. The R cells burst during a depolarizing wave experienced in Phase 2, and are inhibited during Phase 1 and often in Phase 3. Stimulation of R cells causes radula retraction and/or jaw closure, and it is possible to divide R cells into sub-groups according to their pattern of muscle innervation (Bulloch 1977). The two giant neurones 4 and 5 are consistently identified as R cells, the others commonly occupying positions 1, 3, 6–9, and 21.

In contrast to the P and R cells, most F cells burst twice per cycle, although the phase 1 activity is short and often lacking altogether. The two bursts are separated by a large non-symmetrical hyperpolarization that occurs during Phase 2 and is termed the Long Inhibitory Wave (LIW) (Fig. 5). Stimulation of an F cell usually causes radula flattening and inner lip retraction. Externally, the movements are associated with a narrowing of the anterior buccal mass and some swelling of M 5. The most common locations of F cells are positions 10, 11, and 14–17. Occasionally other neurones were found exhibiting an F-type burst pattern, which did not cause these
Table 1. Innervation pattern and movements caused by activation of buccal motoneurones

<table>
<thead>
<tr>
<th>Cell</th>
<th>Muscles innervated</th>
<th>Movements caused</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-R</td>
<td>—</td>
<td>RR</td>
</tr>
<tr>
<td>2-P</td>
<td>1, 3</td>
<td>JO, RP</td>
</tr>
<tr>
<td>3-R</td>
<td>6</td>
<td>RR</td>
</tr>
<tr>
<td>4-R</td>
<td>5, 6</td>
<td>RR</td>
</tr>
<tr>
<td>5-R</td>
<td>2b, 5, 6 (i and c)</td>
<td>JC, RR</td>
</tr>
<tr>
<td>6-R</td>
<td>—</td>
<td>JC</td>
</tr>
<tr>
<td>7-R</td>
<td>5, 6</td>
<td>RR</td>
</tr>
<tr>
<td>8-R</td>
<td>5, 6</td>
<td>RR</td>
</tr>
<tr>
<td>9-R</td>
<td>5, 6</td>
<td>JC, RR</td>
</tr>
<tr>
<td>10-F</td>
<td>4</td>
<td>RF</td>
</tr>
<tr>
<td>11-F</td>
<td>4</td>
<td>RF</td>
</tr>
<tr>
<td>12-P</td>
<td>—</td>
<td>JO</td>
</tr>
<tr>
<td>13-P</td>
<td>—</td>
<td>RP</td>
</tr>
<tr>
<td>14-F</td>
<td>—</td>
<td>RF</td>
</tr>
<tr>
<td>15-F</td>
<td>—</td>
<td>RF</td>
</tr>
<tr>
<td>16-F</td>
<td>—</td>
<td>RF</td>
</tr>
<tr>
<td>17-F</td>
<td>—</td>
<td>RF</td>
</tr>
<tr>
<td>18-M</td>
<td>—</td>
<td>MO</td>
</tr>
<tr>
<td>19-M</td>
<td>—</td>
<td>MO</td>
</tr>
<tr>
<td>21-R</td>
<td>2a</td>
<td>JC</td>
</tr>
</tbody>
</table>

JO, JC, Jaws open/closed; RR, RF, radula retracted/flattened; MO, mouth open; i, ipsilateral; c, contralateral.

movements when stimulated. It is possible that these cells are not primary motoneurones.

Information on the M cells is limited at present, but neurones in positions 18, 19 receive Phase 2 inhibition similar to the F cells, but emerge from it firing continuously or with an impulse train that accelerates into Phase 1. Driving an M cell results in a slight retraction of the outer lips exposing the jaws.

**Criterion 3.** Simultaneous recordings from the neurone soma and the muscle provide the most positive means of motoneurone identification (Fig. 6, Table 1). Muscle E.J.P.s typically followed some spikes at rates up to 20 s⁻¹, and facilitation was apparent at rates as low as 1 s⁻¹. Anti-facilitation or fatigue was often seen at stimulation rates in excess of 10 s⁻¹.

A P cell was found to innervate muscles M 1 and M 3, which explains its ability to cause jaw opening and protraction movements during Phase 1 (Fig. 6, Table 1). A number of F cells were found to innervate M 4 and produce retraction of the inner lips, but it was not possible to demonstrate their innervation of M 5. Recordings were only made from the surface fibres of M 5 (Layer 3), and the F cells may innervate deeper components of this muscle in Layers 1 and 2.

The R cells innervate radula retractor muscles, jaw closer muscles and some innervate both (Table 1). These findings are confirmed by visual observation of movements generated by R cell stimulation, but during the feeding cycle all members of the R cell population appear to be activated contemporaneously. The R cells may be organized into functional sub-groups, based on the muscles they innervate. Preliminary results suggest that neurones in the same sub-group may be electrically coupled.
**Motor innervation of buccal mass in Tritonia**

Recordings from the surface of the muscle fibres show E.J.P.s of different sizes occurring spontaneously, suggesting that the fibres are innervated polyneuronally. The latency of the E.J.P.s relative to the soma spike vary between 70 and 140 ms (Fig. 6).

**Criterion 4.** Experiments confirming the existence of peripheral axons from all the identified neurones (except 6 and 20) are summarized in Table 2. The inclusion of centrally located sensory neurones is made unlikely by data included under criteria 1–3.

With the exception of cell 5, all the buccal motoneurones had ipsilateral axons, some appearing in different nerve trunks in different preparations (e.g. cells 10 and 11). This may be due to mis-identification, or some natural variability in the neurone population.

Each buccal nerve was backfilled using the cobalt chloride diffusion technique on several preparations. These preparations showed considerable variability in detail of cells filled, and identification was subjective and based on position relative to the giant neurones 4 and 5. Most preparations stained numbers of small and unidentified neurones, especially through the cerebro-buccal connective (BN 5). The only identified neurones stained through this trunk are cells 4 and 5 which are also notable.
Table 2. Axon distribution of buccal motoneurones shown by various techniques

<table>
<thead>
<tr>
<th>Cell</th>
<th>Nerve recording</th>
<th>Cobalt backfill</th>
<th>Intracellular cobalt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-R</td>
<td>2</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>2-P</td>
<td>3</td>
<td>2, 3</td>
<td>—</td>
</tr>
<tr>
<td>3-R</td>
<td>3</td>
<td>3</td>
<td>2 (c)</td>
</tr>
<tr>
<td>4-R</td>
<td>1, 2, 3, 5</td>
<td>1, 2, 3, 5</td>
<td>1, 2</td>
</tr>
<tr>
<td>5-R</td>
<td>1, 2, 3, 5 (i and c)</td>
<td>1, 2, 3, 5 (i); 3 (c)</td>
<td>1, 2, 3 (i and c)</td>
</tr>
<tr>
<td>6-R</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7-R</td>
<td>—</td>
<td>2, 3</td>
<td>—</td>
</tr>
<tr>
<td>8-R</td>
<td>2</td>
<td>1, 2</td>
<td>—</td>
</tr>
<tr>
<td>9-R</td>
<td>2 or 3</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>10-F</td>
<td>2 or 3</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>11-F</td>
<td>2 or 3</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>12-P</td>
<td>3, 4</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>13-P</td>
<td>3, 4</td>
<td>1, 3</td>
<td>—</td>
</tr>
<tr>
<td>14-F</td>
<td>—</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>15-F</td>
<td>2 or 3</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>16-F</td>
<td>3</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>17-F</td>
<td>2 or 3</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>18-M</td>
<td>1</td>
<td>1, 3</td>
<td>—</td>
</tr>
<tr>
<td>19-M</td>
<td>—</td>
<td>1, 2</td>
<td>—</td>
</tr>
<tr>
<td>21-R</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>G</td>
<td>4</td>
<td>4</td>
<td>—</td>
</tr>
</tbody>
</table>

i, Ipsilateral; c, contralateral.

Fig. 7. Outline diagrams of buccal ganglia showing results of cobalt backfilling of nerve trunks. A, BN 1 of L and R side filled together. Identifiable neurones numbered, dotted outlines indicate cells 4 and 5. i, Sub-surface cells. B, RBN 2, dotted outlines denote position of 4 and 5. C, RBN 3, many small cells stained in addition to those identified, including some on contralateral side. D, RBN 5, cerebrobuccal connective. Ganglia viewed from ventral aspect. Apart from ipsilateral giant cells, four clusters of smaller neurones on ventral surface take up the stain.
Motor innervation of buccal mass in Tritonia

For the extensive branching of the axon. More detail on cell morphology was obtained by intracellular iontophoresis of cobalt, which showed neurones 3, 4 and 5 to be bipolar and gave detail of the dendritic fields.

Motor patterns in the buccal nerves

Rhythmic motor output patterns in molluscan buccal nerves were first described by Rose (1968, 1971) and Davis et al. (1973). The three phases of the buccal cycle in T. hombergi are reflected by patterned bursting of the impulse traffic in the buccal nerves 1–3 (Fig. 8). This activity is entirely motor, no afferent pattern being detected in the peripheral stumps of the nerves. BN 3 contains axons from the P, R and F cells (Table 2) and the three phases can readily be distinguished in this nerve. The P and F cells account for Phase 1 activity, the R cells for Phase 2, while the F cells alone are the probable source of the Phase 3 units. Bursts corresponding to the three phases are also observed in BN 2, which contains axons from both F and R cells. Phase 2 activity in this nerve is often sub-divided (Fig. 8C) which may provide evidence for
sub-grouping the R cells. As yet, this observation is not confirmed by the intracellular recordings. Three features are apparent in recordings from BN 1, a vigorous Phase 1 burst, some Phase 2 activity, and several units firing more or less uniformly except during Phase 2. The R cells (e.g. 4 and 5) could account for the Phase 2 units, and the other activity may come from the M cells. Thus there is no major feature of the impulse patterns from the buccal nerve trunks that cannot be assigned to one of the three phases of motor activity described above.

Complex burst patterns have also been recorded from BN 5 (the cerebro-buccal connective), in which it is also possible to recognize three phases. Transection of the nerve shows this activity is mostly ascending to the cerebral ganglia, but its function is uncertain (Davis et al. 1973). With both cerebro-buccal connectives cut, correctly phased bursts are still observed in the buccal motoneurones, and cyclic activity of the buccal mass continues.

**DISCUSSION**

**Functional anatomy and feeding behaviour.** The present study has expanded earlier descriptions of the buccal mass of *Tritonia hombergi* (Alder & Hancock, 1845; Thompson, 1962) and has demonstrated the function of various muscle systems. The anatomy closely resembles that given for the Pacific species, *T. diomedea* (Willows, 1977), but there appear to be differences in the feeding behaviour of the two species.

The feeding behaviour of *T. hombergi* differs considerably from that of pulmonate molluscs such as *Helisoma* (Kater, 1974), where the radula is used for rasping. In *Tritonia* the jaws are the principal means by which a piece of Alcyonium is cut off, and contrary to earlier reports (Thompson, 1962), several bites are necessary before this is achieved. Detachment with a single bite is probably only feasible if the colony is fully expanded. The feeding mechanism shows some differences from the Pacific species, *T. diomedea* (Willows, 1977). This animal shows two phases of consummatory behaviour: there is an initial bite-strike in which the radula protrudes from the buccal mass and grasps its prey (the sea whip, *Virgularia*) before pulling it inside, there then follows a sequence of comparatively slow buccal cycles during which the prey is swallowed. During the latter period the radula remains within the buccal cavity, the stalk of the sea whip being broken off by the radula movements rather than the jaws. *T. hombergi* does not show a distinct bite-strike, the initial grasping of the prey by the jaws being followed by a repeated sequence of identical jaw-radula cycles until the food is detached and transferred to the oesophagus. Radula retraction may assist detachment by raising the posterior edges of the cut, allowing the jaws access to fresh tissue. An important function of the radula is thought to be the transport of detached food to the oesophagus. The Phase 1 movements create a large space for the food in the lumen, and separate the two lobes of the radula. During Phase 2 the radula lobes fold back, grasping the food and transporting it dorsally towards the oesophagus. As the radula flattens in Phase 3, it releases the food and enables it to enter the oesophagus, possibly assisted from beneath by the swelling odontophore muscle. The difference in the behaviour of the two species may be explained as a result of the diet. *Virgularia* withdraws rapidly into the substrate when disturbed, making a rapid attack essential. *Alcyonium* cannot escape and is consumed at leisure.

**Motor control of the buccal mass.** An important feature distinguishing the feeding
Motor innervation of buccal mass in Tritonia

mechanism of Tritonia from other gastropods such as Helisoma and Pleurobranchaea (Kater, 1974; Siegler, Mpitssos & Davis, 1974) is the division of the buccal cycle into three phases rather than two. The motor output to the intrinsic buccal musculature is not derived from two alternating sets of motoneurones, but from three groups here designated as the P, R and F cells. The cellular mechanisms underlying this generation of the motor output will be the subject of another paper (Bulloch & Dorsett, 1979).

In semi-intact preparations of this type it is impractical to satisfy rigid criteria for identifying motoneurones with each experiment, but on separate occasions the identified cells 1–19, 21 have satisfied at least three of the required criteria.

Representatives of the P, R and F groups have all been shown to innervate specific muscles, and on this basis these cells have been designated motoneurones. This probably also applies to the M cells, but their muscle innervation has yet to be demonstrated.

The patterned discharge of the motoneurones shows a precise relationship to the three phases of activity observed in the buccal mass and is consistent with the motor action and innervation of the neurones themselves. The extrinsic muscles M 7 are thought to be controlled by the M cells, but the neurones innervating muscles M 8 and 9 are as yet unidentified. As the oral canal is innervated by the cerebral nerve CN 4, and stimulation of this nerve results in a movement of the buccal mass towards the oral opening (Bulloch, 1977), M 9 may be supplied by cerebral neurones. A similar situation exists in T. diomedea, where stimulation of the distal ends of cerebral nerves can elicit jaw closure. This does not happen in T. hombergi, but stimulation of BN 2 and 3 achieves this result. Buccal motoneurones may supply the jaw closer muscle through the cerebro-buccal connective and cerebral nerves. The two R cells 4 and 5 have axons in the connective but it is not known if they emerge in cerebral nerve trunks.

The patterned activity in buccal nerves 1–3 provides confirmation that the cycle of buccal movements is controlled by the P, R and F cells. Additionally, the traffic in BN 1 could be accounted for by the M cells. The behaviour appears to result from a programme of neural interactions generated centrally within the buccal ganglia. There appears to be little flexibility in its operation. For example, jaw movements are not observed in the absence of movements of the radula during spontaneous or stimulated cycles of activity. The level of cellular impulse activity may vary in excitability or intensity, but the three components of the cycle always occur together.

The authors gratefully acknowledge the assistance of Anastasia Bulloch in preparing the figures. The project was supported by grant B/RG/65141 from the S.R.C. to D.A.D.

The authors wish to record their grateful thanks to Mr J. Rodford for the production of Fig. 1.

REFERENCES


