

PHARYNGEAL MOVEMENTS DURING FEEDING SEQUENCES OF *NAVANAX INERMIS* (GASTROPODA: OPISTHOBRANCHIA) IN SUCCESSIVE STAGES OF DISSECTION

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SUMMARY

Feeding in *Navanax inermis* Cooper was filmed and analysed after various dissections. In preparations with a cut through the body wall exposing the pharynx and buccal ganglia, completely normal feeding was observed. In addition to seven motor acts previously described in intact animals, an eighth act, peristalsis, was observed. In preparations with the pharynx excised from the animal but attached to the buccal ganglia, four motor acts were observed: flaring, expansion, contraction and peristalsis. In addition to increasing information about the nature of feeding movements in *Navanax*, these data indicate that preparations suitable for neurophysiological studies are capable of producing a variety of feeding acts.

INTRODUCTION

Neurobiological studies combining behavioural and cellular observations must establish links between the two levels. Since dissected or anaesthetized preparations are often used in cellular studies, it is difficult to demonstrate that a cellular event is related to a specific behaviour. This problem is less acute when examining simple invertebrate behaviour, which can be performed even after radical dissections (Kandel, 1976). However, as more complex behavioural processes are examined, the problem of bridging levels of analysis arises in these animals as well.

This paper demonstrates a preparation that ties together behavioural and cellular levels: we observe a complex behavioural sequence in progressively more dissected preparations. In late stages of dissection the entire sequence no longer occurs, but individual acts from which the sequence is composed are still recognizable. In the future, it should be possible to investigate cellular events underlying specific behavioural components, and then reconstruct the entire sequence. A previous paper (Susswein *et al.* 1984) demonstrated seven distinct motor acts that make up feeding sequences in intact *Navanax*:

Key words: *Navanax*, feeding, motor pattern.

(1) *Protraction* of the pharynx, initiated by touch of food to the tentacles.

(2) *Lip flaring*, occurring simultaneously with protraction; the lips are an anterior extension of the pharynx.

(3) *Prey seizure: strike*, initiated by contact of food to the lips. This is a fast lunge of the pharynx towards the prey.

(4) *Prey seizure: lip closure*, occurring simultaneously with the strike. This movement consists of the lips snapping shut around the prey.

(5) *Expansion* of the pharynx, initiated by food in the anterior pharynx. This movement sucks prey into the pharynx.

(6) *Retraction* of the pharynx, which may occur as an alternative movement to prey seizure if food is withdrawn; if prey seizure occurs, retraction follows both successful and unsuccessful attempts to capture prey.

(7) *Contraction* of the pharynx, leading to expulsion of the pharyngeal contents. This can occur instead of expansion or subsequent to it, and may represent prey rejection, or clearing of the pharynx in preparation for a new attempt at prey seizure.

Recently, Leonard & Lukowiak (1984) have published an ethogram of *Navanax*. In their terminology, 1-Coil, 2-Strike, 3-Hold and 4-Relax seem to correspond with (1) protraction and lip flaring, (2) prey seizure, (3) expansion and (4) contraction, respectively.

Data below confirm the picture of feeding outlined above, and indicate that feeding acts of known function occur in preparations suitable for cellular analysis. A similar preparation has been used to determine, by optical methods, activity during feeding of many units simultaneously (London, Zecevic & Cohen, 1984; Zecevic, London & Cohen, 1985).

MATERIALS AND METHODS

Subjects were *Navanax inermis* (Pacific Biomarine, Venice, California). Techniques for maintaining *Navanax* and for filming and analysing feeding behaviour have been described (Susswein *et al.* 1984).

In some preparations pharyngeal movements were filmed in the same individual while it was intact, after each of a number of enlargements of a hole through the skin above the pharynx had been made, and after the pharynx had been completely excised. Progressive dissection allowed observation of a movement in an individual at different stages of dissection. However, by the later stages animals had been exposed to very rough handling and had performed many feeding responses, which produced habituation of feeding (Susswein & Bennett, 1979). To overcome this problem, radical dissection was sometimes performed without previous feeding.

To make a 'pharyngeal window', a fold of skin was lifted with fine forceps, and a quick cut removed the fold. Animals contracted when cut, but quickly relaxed. Feeding movements were elicited and filmed within minutes of the cut.

In experiments with a pharyngeal window, a finger was often placed over the gut to prevent herniation of the gut and pharynx through the window. If herniation

occurred, feeding movements ceased. When the gut and pharynx were pushed back into the animal, feeding movements proceeded. If the gut and pharynx could not be returned to the interior, dissection to an isolated pharynx preparation was performed.

In experiments utilizing an excised pharynx, the gut and pharynx were pinned to a wax-covered dish. The buccal ganglia innervating the pharynx remained in place throughout. In some experiments the circumoesophageal ganglia remained attached to the buccal ganglia by way of the cerebrobuccal connectives, but all potential connections from the circumoesophageal ganglia to the buccal musculature were severed. In other experiments, the cerebrobuccal connectives were cut and the circumoesophageal ganglia removed.

RESULTS

Preparations with a window through the body wall

The first stage of dissection was cutting a window through the body wall, exposing the pharyngeal musculature.

Figs 1 and 2 show feeding sequences in which the same individual sucked a large *Bulla* partially into the pharynx and then rejected it, before and after a ventral cut over the posterior pharynx. The sequences were strikingly similar: prey seizure was followed by pharyngeal expansion and contraction, leading to expulsion of the oversize prey. Time for prey seizure was almost identical (Fig. 1A–D and Fig. 2A–D), while expansion and contraction were somewhat slower following the

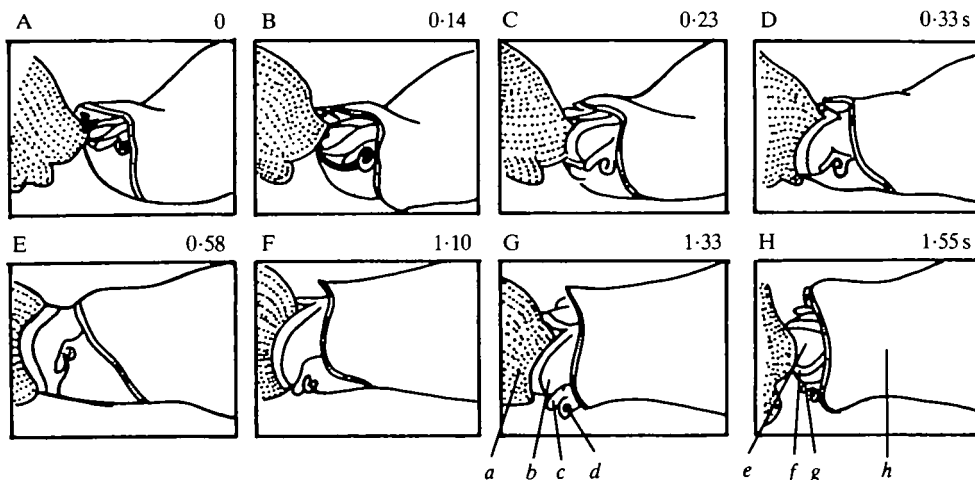


Fig. 1. Line drawing traced from film of a feeding sequence in an intact animal. The sequence consists of prey seizure, expansion and contraction of the pharynx leading to expulsion of the prey. At the start of the sequence (A), the pharynx is already protracted and the lips are flared. In this and following figures numbers in each frame represent seconds following the first frame. *a*, edge of the *Bulla* prey; *b*, pharynx; *c*, tentacle; *d*, anterior lateral fold; *e*, mouth; *f*, lips; *g*, tentacle; *h*, foot.

cut. The data indicate that strike, lip closure, expansion and contraction are minimally affected by the pharyngeal window. The first frames in Figs 1 and 2 show a pharynx that is already protracted and flared, indicating that the ability to perform these acts is unaffected.

Fig. 3 shows a preparation with a large cut exposing the entire ventral pharynx and the buccal ganglia. The cut was not preceded by smaller cuts, or by feeding

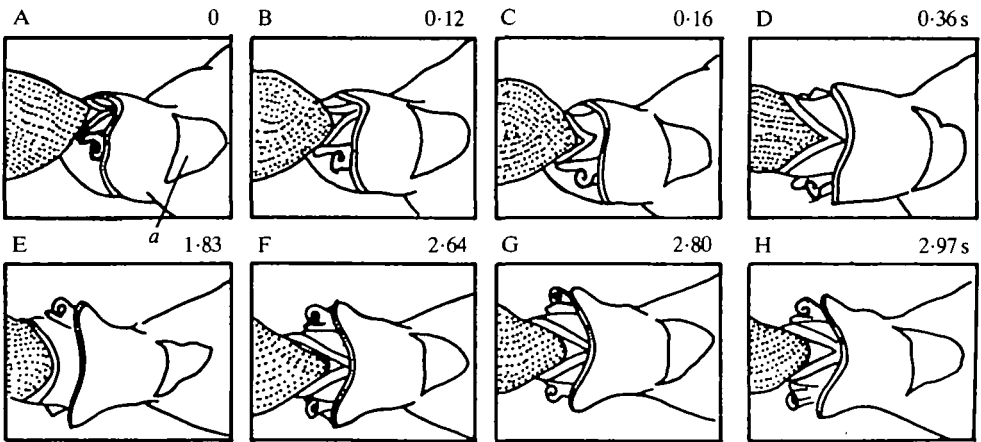


Fig. 2. Line drawing traced from film of a feeding sequence from the same individual as in Fig. 1, following a cut which exposed the posterior pharynx. Events occurring before and after the cut are similar. *a*, the pharyngeal window exposing the interior of the animal.

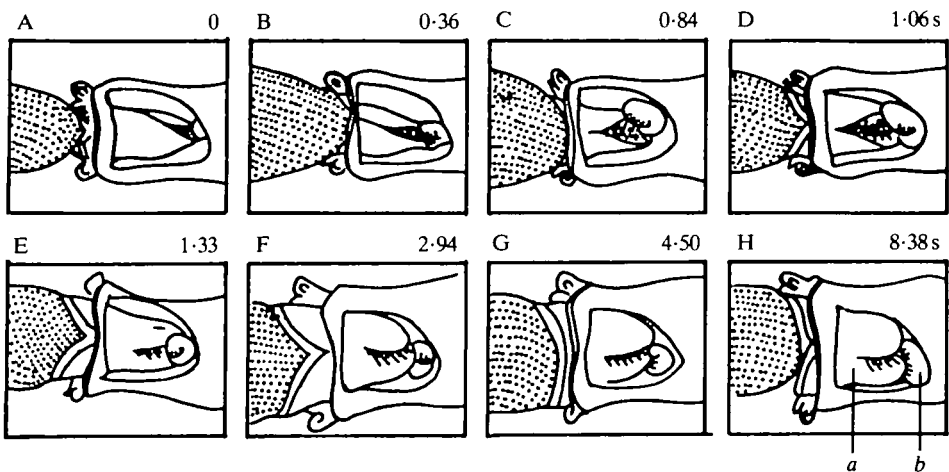


Fig. 3. Line drawing traced from film of a feeding sequence in an individual in which a large window exposed the ventral pharynx, buccal ganglia and anterior gut. Shaded areas of the pharynx represent a fold in the ventral pharynx. Much of the expansion (C-F) consists of opening this fold. As the pharynx expands, it occupies more of the area exposed by the window, pushing the anterior gut posteriorly. *a*, pharynx; *b*, gut.

movements. Fig. 3A shows the rest position of the pharynx, which is somewhat expanded. Before prey seizure the posterior pharynx constricted (Fig. 3B). In this sequence the lips are not seen; when the lips are visible, protraction and flaring occur at this time (Susswein *et al.* 1984). Prey seizure began at Fig. 3C and ended at Fig. 3E. Prey seizure is characterized by rapid movement of the pharynx, and even at a speed of 64 frames s^{-1} the pharynx changes from frame to frame (Susswein *et al.* 1984). Fig. 3C–E shows that during prey seizure the pharynx lunged outwards, and prey entered the anterior pharynx; considerable pharyngeal expansion also occurred. Prey seizure was followed by a much slower expansion of the pharynx (Fig. 3E–G), most prominent posteriorly. The pharynx remained expanded for a considerable time (Fig. 3G–H). The prey was eventually released, and left the mouth. The data indicate that normal feeding occurs even when the pharyngeal window is very large, exposing the entire ventral pharynx and buccal ganglia.

Movements subsequent to prey seizure push food from the pharynx into the gut. Fig. 4 shows a sequence in which 3 s after prey seizure (not shown) peristalsis occurred. At the start (Fig. 4A), the pharynx was relatively expanded. The anterior pharynx then contracted, followed by a posterior contraction, while the anterior region remained contracted (Fig. 4A–E). The form of the contraction is consistent with two bands of innervation anteriorly and posteriorly. With small prey the movement pushes an expanded portion of the pharynx containing prey towards the gut. Peristalsis ended with a coordinated action of the gut and pharynx, the gut enlarging to accommodate the prey. Peristalsis was a relatively slow movement, taking close to 1.5 s.

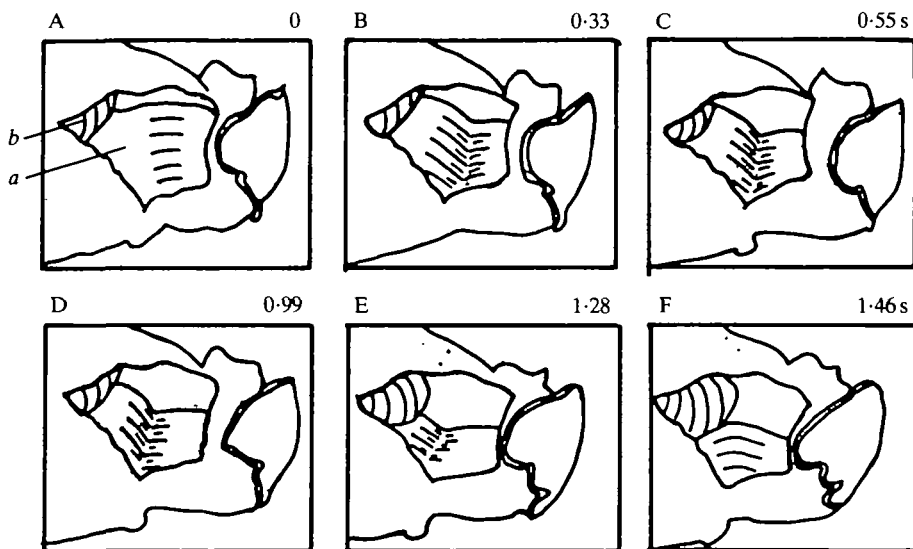


Fig. 4. Line drawing traced from film of a sequence in which the ventral pharynx was exposed during peristalsis, which consists of a contraction of the pharynx that moves posteriorly, pushing an expanded area into the gut. *a*, pharynx; *b*, gut.

The CNS-isolated pharynx preparation

The next stage of reduction examined was the excised pharynx and anterior gut attached to the buccal ganglia and, *via* the cerebrobuccal connective, to the circumoesophageal ganglia.

Expansion, contraction and peristalsis were readily observed in this preparation. Fig. 5 shows a modest expansion that took over 4.5 s. Fig. 6 shows a sequence in which expansion (not shown) was followed by contraction and prey expulsion. Contraction occurred as a constriction beginning posteriorly, pushing prey out of the pharynx. Contraction took over 3.8 s in the sequence, compared to about 3 s in intact animals (Susswein *et al.* 1984). At the end of the contraction the mouth was still open, as it is in intact animals (Susswein *et al.* 1984) and in animals with a window through the body wall (Fig. 2). Fig. 7 shows a peristaltic movement that pushed a bolus of material within an expanded region of the pharynx towards the gut. Peristalsis lasted over 6 s (Fig. 7B–F), as compared to about 1.5 s (Fig. 4) in preparations with a window. A similar result was also illustrated by Bennett (1974).

Although well-organized feeding movements occurred in this preparation, sensory control was impaired. Peristalsis was difficult to elicit and was observed only occasionally. In contrast, expansion was easier to obtain than in more intact animals: placing food directly into the pharynx, or tactile stimuli to the lips or the posterior pharynx, occasionally elicited expansion in the CNS-isolated pharynx preparation, but never elicited expansion in intact animals (Susswein *et al.* 1984), or in animals with a window through the skin.

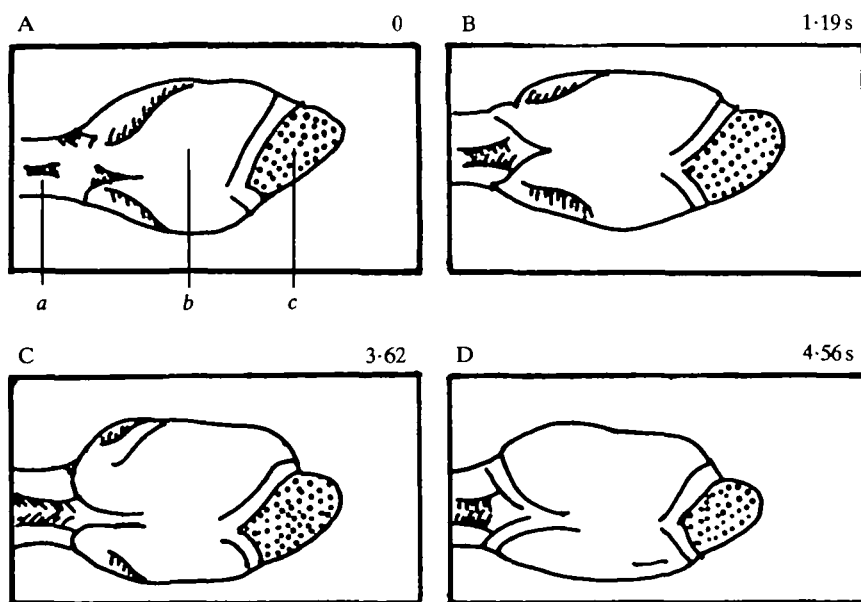


Fig. 5. Line drawing of expansion in the CNS-isolated pharynx preparation. The view is ventrally and laterally. The stimulus for expansion is a piece of *Bulla* prey placed within the pharynx. (A) The pharynx in the rest state. (B–D) The pharynx expands. *a*, gut; *b*, pharynx; *c*, food.

Protraction, strike and retraction were never observed in the CNS-isolated pharynx preparation, probably because these acts depend in part on extrapharyngeal musculature. More surprisingly, we never observed the lips to snap shut. This may mean that some muscles responsible for the movement are extrapharyngeal, or that activity of extrapharyngeal muscle receptors initiates the movement.

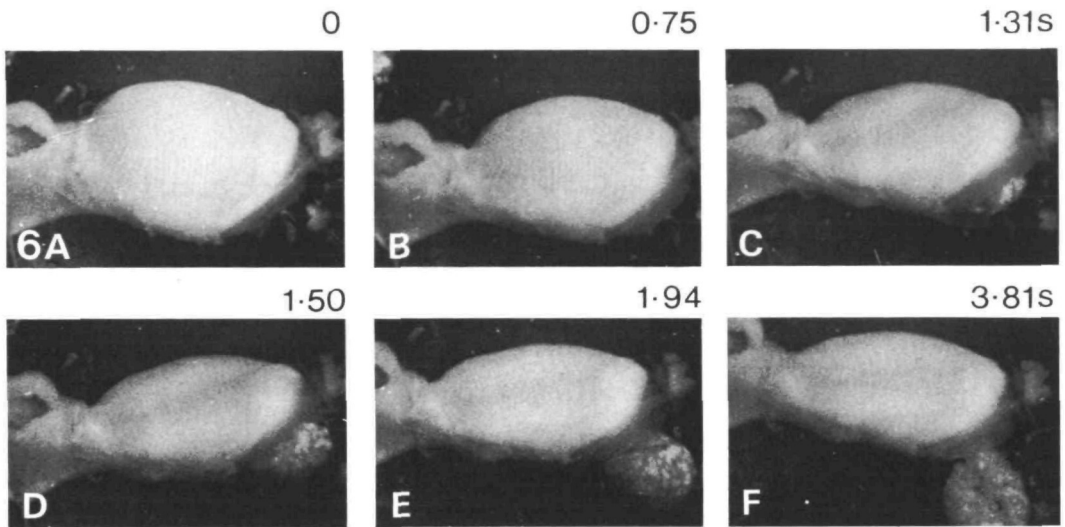


Fig. 6. Expulsion of prey, caused by contraction of the pharynx. (A) The pharynx is expanded, and a piece of *Bulla* meat is present inside the pharynx. (B-F) The piece of *Bulla* is slowly pushed out of the pharynx as it constricts. Magnification, $\times 1.1$.

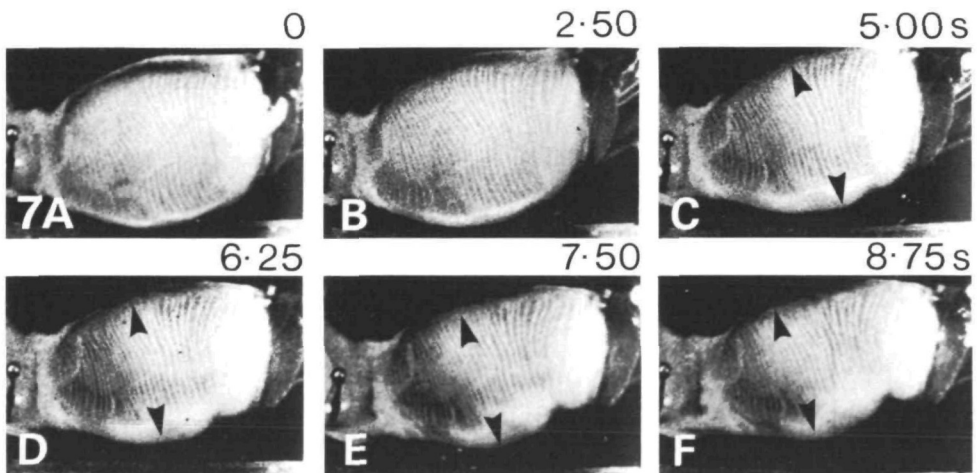


Fig. 7. Peristalsis in the CNS-isolated pharynx preparation. (A) The pharynx begins in a partially expanded state. (B-F) The pharynx constricts, anteriorly, then posteriorly, pushing a partially expanded region (arrowheads) of the pharynx posteriorly. The prey is contained within the partially expanded region (arrowheads). Magnification, $\times 1.2$.

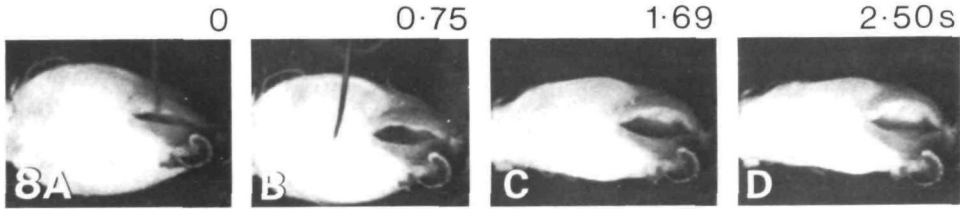


Fig. 8. Flaring of the anterior pharynx, with constriction of the posterior pharynx, in the buccal ganglia-isolated pharynx preparation. (A) The stimulus for the movement is rubbing the length of a pin on the lips. The initial state of the pharynx is partially expanded. (B–D) Constriction of the posterior pharynx, while the lips flare. Magnification, $\times 0.8$.

The buccal ganglia-isolated pharynx preparation

The next stage of reduction examined was the pharynx and anterior gut innervated by the buccal ganglia alone. In this preparation, expansion and contraction movements virtually identical with those shown in Figs 5 and 6 were observed, indicating that neural circuitry entirely within the buccal ganglia is sufficient for these acts. Peristalsis was not observed, but since it occurred only rarely in the CNS-isolated pharynx preparation as well, the absence of this movement does not mean that the movement is due to circuitry present in the circumoesophageal ganglia. Tactile stimulation of the pharyngeal walls evoked motoneurone activity suggestive of peristalsis in more radically dissected preparations with only buccal ganglia present (Spira, Spray & Bennett, 1980).

Contraction movements were easily evoked after the circumoesophageal ganglia had been removed. Fig. 8 shows a sequence in which a tactile stimulus to the lips (touch of the side of a pin) produced a constriction of the body of the pharynx and lip flaring. This was a slow movement, occurring over 2.5 s, similar to the time course of flaring in intact animals (Susswein *et al.* 1984).

DISCUSSION

The neural basis of feeding is being studied in many gastropods, as a model for understanding the control of a complex motor act (for reviews see Benjamin, 1983; Kohn, 1983). In some gastropods, feeding preparations suitable for intracellular analysis are obtained only with great difficulty (Weiss, Koch & Kupfermann, 1986). By contrast, our data indicate that in *Navanax* normal feeding movements occur when a window exposes the pharynx and buccal ganglia. This preparation is sufficiently dissected for electromyographic recordings to be made from pharyngeal and extrapharyngeal muscles, and for extracellular and intracellular recordings to be made from the buccal ganglia. Multi-unit recording by optical methods has been carried out during feeding behaviour in a similar preparation (London *et al.* 1984; Zecevic *et al.* 1985). Normal feeding does not occur in more radically reduced preparations, but flaring, expansion, contraction and peristalsis movements are readily recognized, although the movements are often slower and elicited by less-specific stimuli. Preparations with the isolated pharynx attached to the buccal ganglia

are wholly suitable for detailed physiological analysis (Spira & Bennett, 1972; Woollacott, 1974; Spira *et al.* 1980; Spray, Spira & Bennett, 1980*a,b*).

Our results conflict with those of Woollacott (1974), who found that noxious stimuli associated with dissection produced permanent withdrawal of the phalliform organs, sensory structures near the lips that initiate feeding (Murray, 1971). The difference is probably due to herniation of the gut and pharynx in Woollacott's experiments: the drawings by Woollacott (1974) indicate that substantial herniation had occurred. We found that dissected preparations fed only when the gut and pharynx were within the body cavity.

Our observations indicate that two previously identified acts, flaring and contraction, are similar: in both, the posterior pharynx constricts while the anterior pharynx is expanded. Although further studies may demonstrate that different pattern generators, motoneurons or muscles are involved, on a behavioural level the movements are distinguished only by the stimuli eliciting them, and by their context. Since expulsion of pharyngeal contents and mouth opening should precede an expansion for maximal effectiveness, the movements may in fact be the same. A common pattern generator may underlie both acts, perhaps by being driven by two kinds of sensory inputs.

It is not clear what muscles act during the strike. Activity of the protractor muscles, an increase in coelomic pressure by body wall circumferential muscles, contraction of pharyngeal circumferential muscles or 'inverse jet propulsion' through expansion may all contribute in intact animals. In dissected preparations, coelomic pressure could not contribute.

Radial muscles causing expansion are active in the resting state (Fig. 3). During feeding, these muscles are active in flaring, prey seizure and expansion. In flaring, the anterior pharynx is expanded, whereas in later movements more posterior regions also expand (Fig. 3). Previous studies (Hall, Spray & Bennett, 1983; Levitan, Tauc & Segundo, 1970; Spira & Bennett, 1972; Spira *et al.* 1980) have demonstrated electrical coupling between radial muscle motoneurons. Coupling is modulated by synaptic input, presumably allowing the motoneurons to function both independently and as a unit (Spira & Bennett, 1972; Spira *et al.* 1980). The present data confirm that both local and global expansion movements occur.

Circumferential muscles are active in peristalsis, contraction and lip closure. Also, in flaring, the posterior pharynx is constricted. In all four movements, specific areas of the pharynx are constricted, while others are expanded. Peristalsis consists of a sequential constriction of the anterior and posterior pharynx; in contraction and flaring, the posterior pharynx is constricted while the anterior pharynx is expanded. When the lips snap, a specialized group of anterior circumferential muscles may be active (Cappell *et al.* 1979; Cappell, Spray, Hall & Bennett, 1980; Zimering, Spray & Bennett, 1981), while the anterior pharynx just behind the lips remains expanded. Circumferential motoneurons are electrically coupled to one another, but coupling is functionally reversed by chemical synaptic input. Motoneurons innervating a given region inhibit motoneurons innervating more posterior regions of the pharynx (Bennett, Zimering, Spira & Spray, 1985; Spira *et al.* 1976; Zimering *et al.* 1981).

Reversal of coupling probably occurs during peristalsis and lip closure, but is unlikely to be functional in contraction and flaring.

Studies on other gastropods (for reviews see Benjamin, 1983; Kohn, 1983) have shown that feeding movements are driven by central pattern generators. Although many sensory stimuli can initiate or modulate feeding, the motor sequences that make up feeding are relatively fixed. In contrast, this and a previous paper (Susswein *et al.* 1984) indicate that feeding in *Navanax* seems to consist of a chain of discrete acts which can combine in different ways to form sequences appropriate to a specific prey stimulus.

Cellular studies on feeding in *Navanax* to date have utilized a number of reduced preparations. These studies have produced information on properties of receptor fields and synaptic interactions between primary sensory neurones (Spray *et al.* 1980*a,b*) as well as on effects on muscles of identified motoneurones (Cappell *et al.* 1979, 1980; Spira & Bennett, 1972; Spira *et al.* 1976, 1980; Woollacott, 1974; Zimering *et al.* 1981). Electrical and chemical synaptic interactions between motoneurones and between motoneurones and interneurones have also been described (Bennett *et al.* 1985; Hall *et al.* 1983; Levitan *et al.* 1970; Murray, 1971; Spira & Bennett, 1972; Spira *et al.* 1976, 1980; Woollacott, 1974). The present study explores the behavioural abilities of a number of preparations allowing considerable latitude for application of electrophysiological techniques. By using these preparations, it should be possible to determine the role of specific cells in producing a behavioural sequence. An entire feeding sequence can then be built by tying together a number of specific acts.

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