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# AN INCREASE IN ACTIVITY OF SEROTONERGIC RETZIUS NEURONES MAY NOT BE NECESSARY FOR THE CONSUMMATORY PHASE OF FEEDING IN THE LEECH *HIRUDO MEDICINALIS*

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## Summary

**During the consummatory phase of feeding, in which blood is ingested, medicinal leeches display a characteristic set of behaviours: they extend their jaws, are less responsive to sensory input, produce mucus, relax the body wall and exhibit waves of peristalsis that can run the length of the body. Earlier reports suggested that this pattern of behaviour is orchestrated by serotonin released from Retzius cells in response to the appropriate sensory stimulation of the lip.**

**We have developed a semi-intact preparation in which only the nervous system in the posterior half of the leech was exposed. The front half of the leech was free to explore, bite through and feed until satiated from a blood-filled sausage casing while continuous intracellular and extracellular recordings were made from identified cells and the nerve roots of the exposed segments.**

**Prior to attachment of the animal to the feeding device, the firing frequency of the Retzius cell increased transiently during spontaneous movements or tactile stimuli to its front or posterior end. In contrast, Retzius cell activity decreased after the anterior sucker attached to the membrane of the feeding device at about the time when ingestion was initiated.**

**Increased activity of Leydig cells, which are known to modulate several circuits in the leech, was also associated with exploration. However, unlike that of Retzius cells, the activity of Leydig cells increased significantly following the onset of consumption.**

**These results suggest that increased activity of Retzius cells in midbody ganglia is not a prerequisite for the consummatory phase of feeding and raises questions regarding the role of serotonin in regulating this behaviour.**

Key words: serotonin, feeding, Retzius cell, Leydig cell, ingestion, neuromodulation, appetitive, consummatory, behaviour, leech, *Hirudo medicinalis*.

## Introduction

In many animals, feeding is a complex activity that includes an appetitive phase (involving detection, arousal, directed locomotion and prey capture) and a consummatory phase (involving ingestion; Kupfermann, 1994). In these animals, the nervous system must integrate a wide range of sensory information and generate a complex, dynamic sequence of behavioural responses made up of many components. It remains a central theme of neuroethology as to how such a sequence is generated.

The medicinal leech provides a good system in which to study the neuronal basis of behaviour. Several behavioural patterns, some of which are components of the appetitive phase of feeding, have been studied in terms of both the patterns of motor neurone activity that are generated and the neuronal networks which underlie them. These include swimming, crawling, whole-body shortening and the local withdrawal reflex (Brodfuehrer and Friesen, 1986*a*,*b*; Kristan *et al.* 1988, 1995; Brodfuehrer *et al.* 1995; Baader and Kristan, 1992; Wittenberg and Kristan, 1992; Shaw and Kristan, 1995). The consummatory phase of feeding has been the subject of far less attention, partly because of the difficulty of recording the activity of the nervous system from a feeding animal. However, the feeding behaviour of sanguivorous leeches has been described in detail.

Hungry leeches spend more time in shallow water and swim more frequently. They are more likely to swim towards a source of water waves, respond more readily to shadows and bite more frequently (Dickinson and Lent, 1984). Once attached to a host, they search for a suitable place to bite, using their front and rear suckers to crawl over the skin. On arriving at a suitable site, a leech will erect its rostral segments and bite through the skin of the host with its jaws. This marks the end

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of the appetitive phase. Strong evidence suggests that these appetitive behaviours are enhanced by serotonin. Bathing animals in, or injecting them with, serotonin increases the probability of swimming (e.g. Willard, 1981; Angstadt and Friesen, 1993) and biting (Lent and Dickinson, 1984) and causes the leech to spend less time in shallow water (Lent, 1985). Bathing isolated nerve cords in serotonin increases the likelihood of the swim motor pattern (Angstadt and Friesen, 1993). Conversely, leeches depleted of serotonin by 5,7-dihydroxytryptamine or reserpine treatment bite and swim less frequently and spend more time in deep water (Groome *et al.* 1993; O'Gara *et al.* 1991). Similar effects are seen in carnivorous leeches following serotonin depletion (Goldburt *et al.* 1994).

After biting through the skin, which starts the consummatory phase, the pharynx begins to contract rhythmically at about 2 Hz, pumping the blood into the crop. Peristaltic body waves usually accompany ingestion, but they have a lower frequency than the pharyngeal pumping (Lent *et al.* 1988). Very occasionally, dorso-ventral flexions occur which resemble swimming. The frequency of both pharyngeal pumping and body peristalsis decreases as the consummatory phase continues. During ingestion, the front of a leech will continue to feed, even during lesions to the back of the animal, showing that their response to noxious stimuli is greatly reduced. The consummatory phase of feeding can last for 30–45 min, during which time the leech can ingest up to nine times its mass in blood, a meal that can sustain it for up to a year. It has been suggested that serotonin is central to the production of this behaviour pattern (Lent, 1985; Lent *et al.* 1988).

The largest source of serotonin in the leech is the paired Retzius cells present in each of the segmental ganglia (e.g. McAdoo and Coggeshall, 1976). Retzius cells receive mechanosensory inputs *via* an unidentified population of interneurones (Szczupak and Kristan, 1995), are phasically excited during locomotion (e.g. Kristan and Nusbaum, 1983; Baader and Kristan, 1992) and have somatic receptors for serotonin (e.g. Walker and Smith, 1973; Acosta-Urquidi *et al.* 1989). They have processes both centrally and in the body wall where they decrease both the relaxation time constant of muscle contraction and the passive tension; these effects are mimicked by serotonin (e.g. Wilson *et al.* 1995; Mason and Kristan, 1982).

In other invertebrates, for example in molluscs, serotonin in conjunction with neuropeptides plays a role in modulating the arousal state induced by the presentation of food (Kupfermann and Weiss, 1982). At the cellular level, serotonin and neuropeptides modulate opposing $Ca^{2+}$ and $K^{+}$ currents that cause either potentiation or depression of the activity of the muscles involved in the mechanics of feeding and, hence, control bite strength (Brezina *et al.* 1994). In *Lymnaea stagnalis*, recordings of the activity of serotonergic cerebral giant cells using implanted wires in an intact animal show that these cells are not the command neurones for feeding, as was previously thought, but are instead modulatory, in that they enable another neurone to drive the feeding pattern (Yeoman *et al.* 1994).

Here we describe a preparation for recording the activity of the leech nervous system during feeding. Using this preparation, we confirm that the Retzius cells are activated during appetitive behaviour, but show that their activity decreases during the consummatory phase of feeding. Recordings were also made from Leydig cells, which are known to have modulatory effects on several types of behaviour in the leech. These effects include a reduction in the strength of the local bending reflex (Lockery and Kristan, 1991) and an increase in the heart rate (Arbas and Calabrese, 1990). Our data suggest that this cell, and the neuromodulator it contains, may play an important part in the regulation of consummatory behaviour.

## Materials and methods

*Hirudo medicinalis* weighing 3–3.5 g were obtained from Leeches USA (Westbury, Long Island, USA) and starved for 3–6 months at room temperature (20 °C). The dissection was performed in ice-cold leech Ringer (Muller *et al.* 1981). We used a semi-intact preparation in which the ganglia of segments M12–M18 were denervated (Fig. 1). A longitudinal cut was made through the dorsal body wall between M12 and M18 and the gut was transected and cannulated at segment M15. The gut between M13 and the cannulated opening was carefully freed from the body wall. The catheter was designed to serve three functions: to monitor the consumption of blood; to drain blood from the gut so that the length of the feeding episode, which is dependent on body distention, could be controlled (Lent, 1985); and to prevent the blood meal from leaking into the bath and affecting the chemical composition of the leech Ringer in which the preparation was bathed. The body wall was removed from M12–M18 leaving only the nerve cord connecting the rostral and caudal segments. The connective tissue stocking was removed between M14 and M16. The preparation was transferred to a dish in which the two ends of the leech could be bathed in separate chambers (Fig. 1B). The nerve cord was passed through two small holes that linked the chambers. After pinning down the exposed ganglia and the cut ends of the intact portions of the body, the holes linking the chambers were sealed with Vaseline. This ensured that the composition of the leech Ringer around the exposed ganglia remained constant for the duration of the experiment. Leeches were left to recover at room temperature for 45 min before recording.

We performed our experiments with leech Ringer in both chambers. Leech Ringer constitutes a brackish environment to the leech and probably leads to some water loss and salt gain in the intact parts of the semi-intact preparation (Wenning *et al.* 1980). Feeding seems not to be affected, however, since leeches feed until sated in leech Ringer, and the alternative, to bathe the intact parts of the preparation in pond water, would have caused damage to the tissues inside the partially open body cavity.

Once leeches had recovered from the operation, we tested

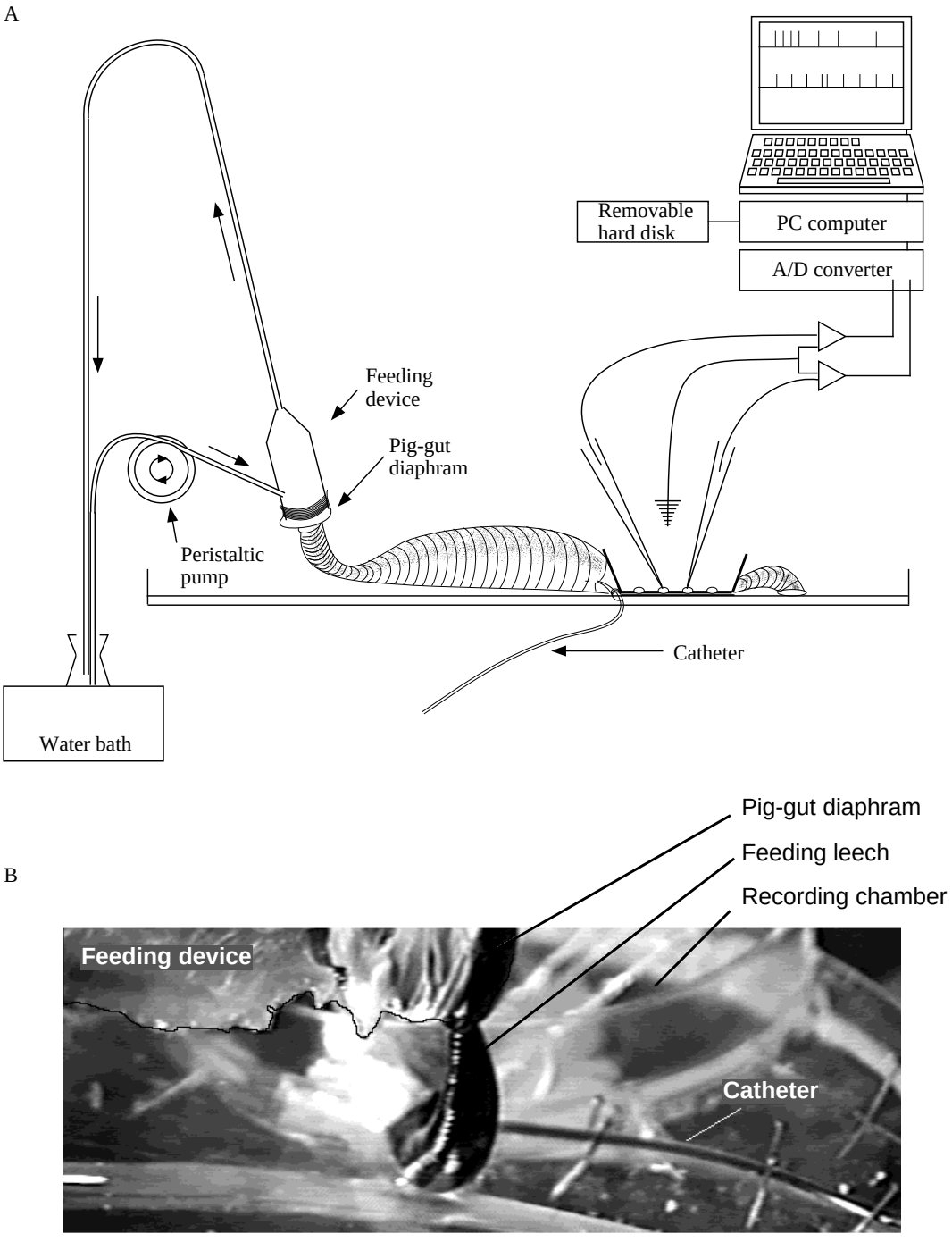


Fig. 1. The apparatus used to determine the activity of cells during feeding. (A) Schematic drawing of the apparatus. A semi-intact preparation was used in which seven of the 21 midbody ganglia (MG12–MG18) were exposed. The exposed nervous system was protected from contamination with blood leaking from the feeding device by a chamber constructed around the ganglia and sealed with Vaseline. Leeches were fed on warm blood using a device consisting of a pig-gut diaphragm (sausage casing) secured across the mouth of a modified conical tube. A peristaltic pump was used to circulate blood through the conical tube to and from a flask in a water bath. Intracellular and extracellular action potentials were recorded using conventional techniques (see Materials and methods) and stored on removable hard disks using a personal computer. (B) Photograph of a leech feeding during a recording session. The anterior of the leech (centre) can be seen suspended from the feeding device (top left). The catheter (bottom right) provides a means of draining the blood from the gut and prolonging feeding episodes. The recording chamber is visible in the background.

their response to skin stimulation and observed the behaviour of their suckers to ensure that the ventral nerve cord was intact. Our criteria were twofold. First, skin stimulation of either the intact rostral or caudal portions of the body should elicit behavioural responses at both ends and, second, leeches should coordinate the use of their two suckers appropriately. At the end of each experiment, we repeated these tests and also induced swimming, either by repetitive stimulation to the tail or by cutting off the head, and looked for coordinated swimming movements in the two ends to ensure that the nerve cord was undamaged.

Heparinised bovine blood was obtained from Animal Technology (Tyler, TX, USA), diluted to 50 % using a solution of 150 mmol $l^{-1}$ NaCl and 1 mmol $l^{-1}$ arginine and warmed to 45 °C. A peristaltic pump was used to deliver the blood mixture to a feeding device which consisted of polyethylene tubing, a 15 ml centrifuge tube and a pig-gut membrane. The tubing was arranged so that the pressure of the blood at the membrane could be adjusted (Fig. 1A).

Intracellular recordings of Retzius and Leydig cells in M14 and M15 were made at room temperature using sharp microelectrodes containing 4 mol $l^{-1}$ potassium acetate and

connected to Axoclamp-2B amplifiers (Axon Instruments, Foster City, CA, USA). A continuous record of each experiment was stored on 270 Mb removable hard disks (DataStore, Boulder, CO, USA) using a 486 PC computer, Axotape software and a Digidata A/D converter running at 2 kHz (Axon Instruments, Foster City, CA, USA). Results are presented as means ± standard error of the mean and were tested for significance using repeated-measure analysis of variance (ANOVA) which calculates the *F*-statistic. Specific comparisons between the different phases of feeding were made using the Dunnett multiple-comparison test (which calculates the *q*-statistic). The Retzius data in the different phases differed significantly in standard deviation, so the data were log-transformed. In order to use repeated-measure statistics, one out of the 10 animals from which recordings were made was dropped from the data set because the appetitive phase was interrupted by a brief period of exploration (see Fig. 2B).

## Results

### *Behaviour and description of the firing properties of Retzius and Leydig cells*

Feeding in the leech is a multi-component behaviour encompassing arousal and detection of food, directed locomotion and attachment, exploration of the host for a place to bite, biting and consumption. Each of these components may vary in duration and vigour and, using a semi-intact preparation, the whole process may take up to an hour. Correlating the activity of different neurones over the entire duration of this behaviour is therefore difficult. For the purpose of analysis, we divided each experiment into four phases: (1) a pre-appetitive phase, prior to the introduction of the feeding device; (2) an appetitive phase, in which the front of the animal reached towards and/or explored the feeding device; (3) a consummatory phase, in which biting and pharyngeal pumping was followed by the ingestion of blood; and (4) a post-consummatory phase, which began as soon as the leech had released the feeding device.

#### *Pre-appetitive phase*

During the pre-appetitive phase, leeches were largely stationary, with their front and rear suckers attached to the bottom of the dish. While they were stationary, their Retzius and Leydig cells were spontaneously active. The rate of firing was variable. Skin stimulation of either the intact rostral or caudal portions of the body elicited behavioural responses at both ends and caused a transient increase in the activity of both cell types. The periods when the animal was inactive were interrupted occasionally by spontaneous exploratory bouts, in which the front sucker detached from and explored the front of the dish (e.g. Fig. 2B). We quantified the firing frequencies of the Retzius and Leydig cells in a 2 min window immediately preceding the introduction of the feeding device. The Retzius cells fired at 0.28±0.11 Hz (*N*=9) and the Leydig cells at 0.86±0.12 Hz (*N*=9).

#### *Appetitive phase*

When the feeding device was lowered into the chamber, there was no apparent instantaneous increase in the activity of either cell type (e.g. Figs 2B, 3). After a few seconds delay, one leech began exploration spontaneously and found and fed from the feeding device without assistance (Fig. 2Aiii). In all other cases, it was necessary to initiate exploration of the device, one or more times, by detaching the front sucker with a head poke (e.g. Fig. 2Ai,ii; asterisks). The activity of both cell types appeared to increase when the front half of the animal was most active. Leeches typically found a place to attach within 5 min of exploration, and attachment was usually followed by biting and the consumption of blood. In one case, however, the leech attached transiently without pharyngeal pumping or the consumption of blood. This event was correlated with increased Retzius cell activity (Fig. 2Aiii; arrows). The average firing frequencies of the Retzius and Leydig cells in a 2 min window immediately preceding blood consumption were 0.62±0.14 Hz (*N*=9) and 1.36±0.23 Hz (*N*=9). This increase in activity was significant for both cell types (Retzius: $q$=3.16, $P$<0.05; Leydig: $q$=3.05, $P$<0.05).

#### *Consummatory phase*

The first indication that a leech would ingest blood once attached to the feeding device was the erection of pharyngeal muscles. However, to ensure that consumption had indeed occurred, we used the pumping of pharyngeal muscles to define the onset of consumption. Ingestion was then confirmed by swelling of the body and the presence of blood in the catheter. Over the 2 min following the onset of consumption, the Retzius cell had a firing frequency of 0.19±0.05 Hz (*N*=9). This was significantly less than the firing frequency during the last stages of the appetitive phase ($q$=2.67; $P$<0.05). On average, there was a highly significant increase in the firing frequency of the Leydig cell over the inital 2 min of the consummatory phase (2.06±0.14 Hz; $q$=4.24, $P$<0.01; *N*=9). Interestingly, in seven out of the nine animals, there was a rapid increase in activity after a latency of 30–60 s (e.g. Fig. 4Ai–iii). In only one of the nine animals, and in the animal that was excluded from the analysis because ingestion was interrupted by a period of exploration, did the activity of the Leydig cell fall (Fig. 4B).

The average length of the consummatory phase in these experiments (with the catheter closed) was 22±7.6 min (*N*=9). Over the course of the consummatory phase, the average firing rate of both cell types was little changed in that the firing frequencies of the Retzius and Leydig cells 2 min prior to end of the consummatory phase were 0.17±0.04 Hz (*N*=9) and 1.74±0.46 Hz (*N*=9). The activity of the Retzius cell remained significantly less than that during the pre-consummatory phase ($q$=3.16; $P$<0.05).

#### *Post-consummatory phase*

Once leeches had dropped off the feeding device, the activity of the Retzius cells almost doubled, returning to the level during the pre-appetitive phase: the average firing

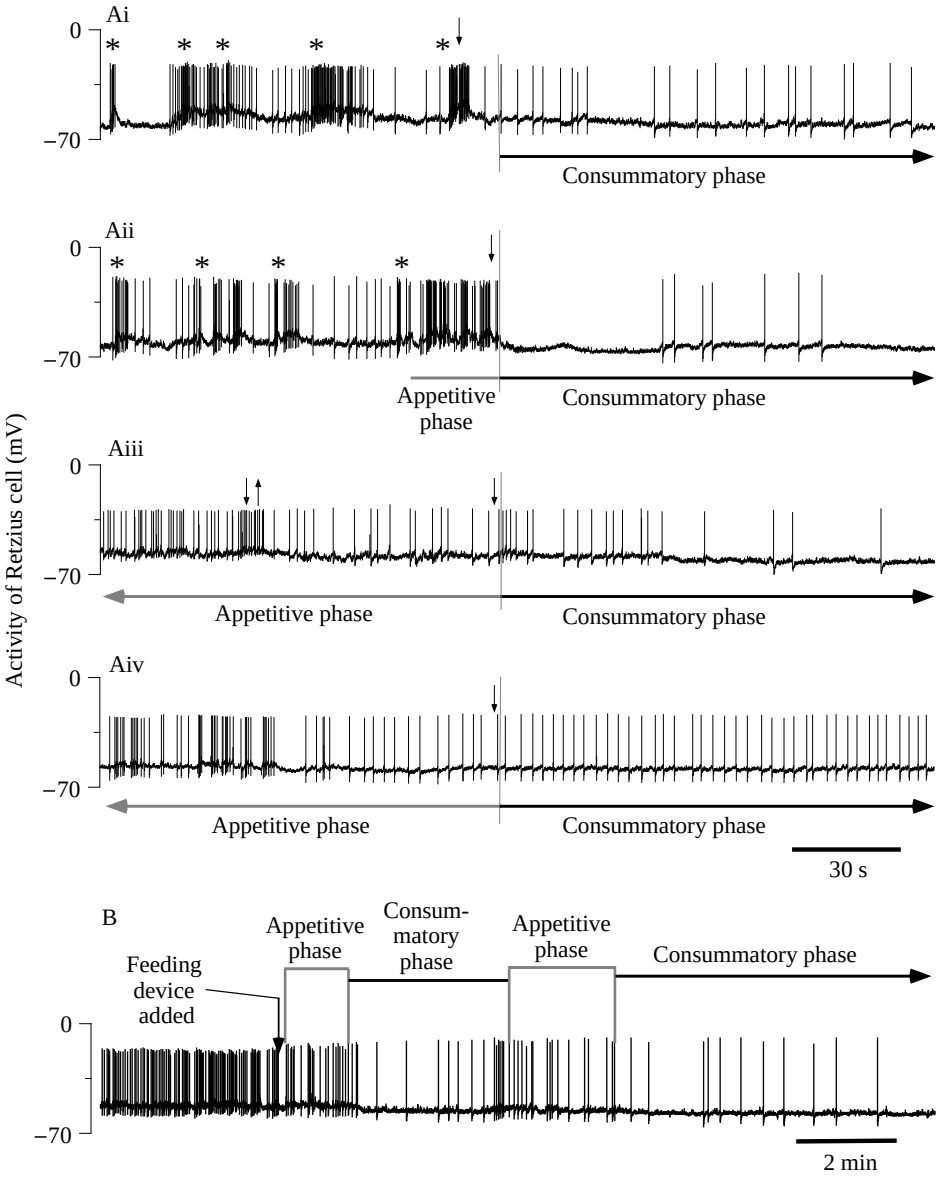


Fig. 2. Activity of the Retzius cell during the transition from the appetitive to the consummatory phase of feeding. (A) Each trace (from four different animals) shows a 4 min excerpt centred about the onset of the consummatory phase (defined by the erection of the pharyngeal muscles). In Ai,ii, the front suckers of the leeches were attached to the bottom of the dish and remained so even after the feeding device had been presented. These two animals required several head pokes (asterisks) before they detached and became sufficiently aroused to find the feeding device. Once they did, they attached rapidly (arrows) and began to ingest soon thereafter. In Aiii,iv, the leeches spent many minutes exploring the feeding device and attaching (arrows) for short periods. (B) Activity of a Retzius cell in a different animal during an aborted feeding bout. This animal began to feed within 2 min of the presentation of the feeding device. Shortly after the onset of the first consummatory phase, the firing frequency of the Retzius cell fell sharply. However, a burst of Retzius cell activity occurred 3 min later, and the front sucker detached from the feeding device. The animal began exploring the feeding device again, found a new place to attach, and resumed the consummatory phase. The animal took approximately another 20 min to feed to satiation.

frequency over the 2 min period following the consummatory phase was 0.31±0.08 Hz ($N$=9). Over the same period, the average activity of the Leydig cell fell slightly to 1.63±0.49 Hz ($N$=9).

Fig. 5 summarizes the results obtained for the firing frequency of Retzius and Leydig cells during the four phases of feeding analysed in this paper. Both cell types were affected significantly by feeding [Retzius, $F(4,8)=3.51$; $P<0.02$; Leydig, $F(2,8)=26.8$; $P< 0.001$].

## Discussion

The results described in this study (summarized in Fig. 5) suggest that the appetitive phase in the feeding behaviour of *Hirudo medicinalis* correlates with an increase in the firing frequency of both the serotonergic Retzius cell and the Leydig cell. Our data demonstrate that, during ingestion of a warm blood meal (i.e. the consummatory phase of the behaviour), the activity of the Retzius cell decreases significantly in comparison with that prevailing during the appetitive phase. In contrast, the firing frequency of the Leydig cell is elevated significantly during and possibly beyond the ingestion phase.

The results were obtained by recording from cells in posterior, midbody ganglia (M14 and M15) of a semi-intact preparation. We assume that the activity of the cells in these ganglia reflects the activity of their anterior homologues. There are several factors that justify this assumption. (1) Tactile, thermal and chemical stimulation of the head produces an increase in the activity of the Retzius cells down the length of the body (Szczupak and Kristan, 1995; Groome *et al.* 1995; also see Fig. 2). In addition, the Leydig cells form an electrically coupled network running the length of the cord (Keyzer *et al.* 1982). (2) Swimming-associated activity of the Retzius cells occurs in both anterior and posterior ganglia of isolated nerve cords and in the posterior ganglia of semi-intact preparations (Willard, 1981; Brodfuehrer and Friesen, 1986*b*). (3) Body

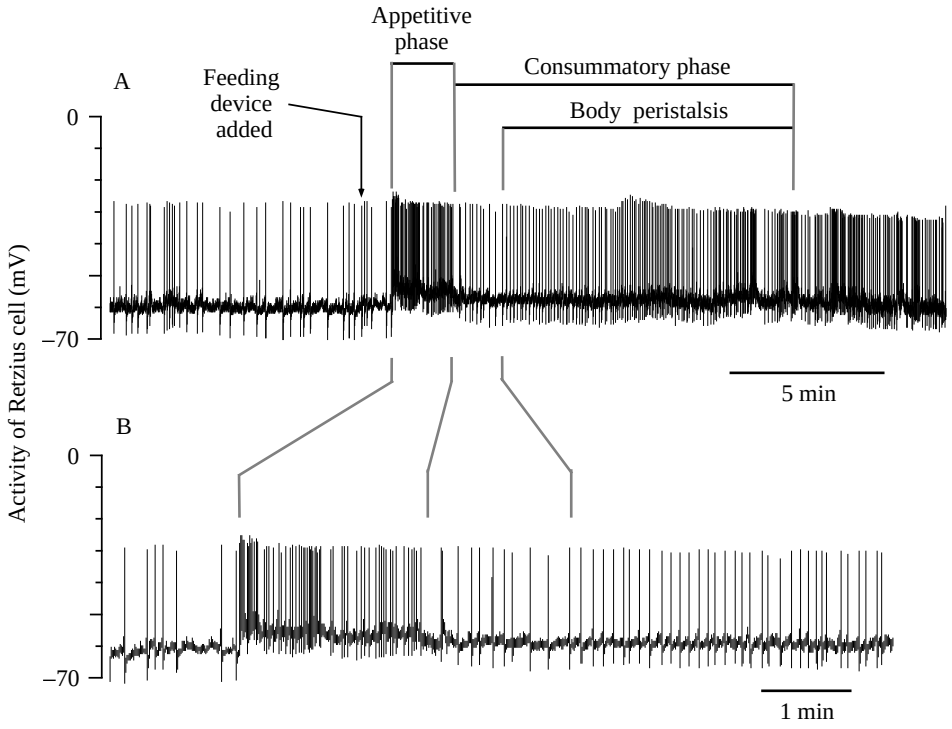


Fig. 3. The activity of the Retzius cell may be related to motor activity. (A) During the period prior to the appetitive phase, this animal remained attached to the bottom of the dish. The appetitive phase was triggered not by the proximity of the feeding device, but rather by a head poke which detached the front sucker from the substratum and led to a period of exploration (labelled appetitive phase), associated with a large increase in the activity of the Retzius cell. As in Fig. 2, the onset of the consummatory phase was marked by a dramatic reduction in Retzius cell firing frequency. The Retzius cell activity was increased during the consummatory phase in comparison with that prevailing at rest but was still lower than that recorded during the appetitive phase. This increase correlated with the onset of vigorous body peristalsis. When the animal detached from the feeding device, it attached to the bottom of the dish and the firing frequency increased further. (B) Same trace as in A, but with an expanded time scale.

peristalsis of posterior segments in intact feeding animals suggests feeding-related activity in posterior ganglia (Lent *et al.* 1988; R. J. A. Wilson and A. L. Kleinhaus, unpublished observations). (4) Nerve roots of exposed, deinnervated posterior ganglia exhibit a motor pattern during the consummatory phase of feeding which appears to be the neuronal correlate of body peristalsis (R. J. A. Wilson and A. L. Kleinhaus, unpublished observations).

Previous reports on the feeding behaviour of *Hirudo medicinalis* suggested that the activity of the serotonergic neurones, and of the Retzius cells in particular, was 'sufficient' and 'necessary' to assign a major functional role to these cells in the control of all aspects of feeding, including both the appetitive and the consummatory phases (Lent and Dickinson, 1984). The results leading to this conclusion were derived both from behavioural observations of intact animals and from electrophysiological recordings of Retzius cells in various reduced preparations.

There have been many results which suggest that the Retzius cell is important in the appetitive phase of feeding, but these were largely from indirect tests. For example, serotonin reduces the response time to a source of vibration (or possible host) towards which a leech will swim, it increases the duration of the swim and enhances the frequency at which leeches bite a Parafilm membrane warmed to 35 °C (Lent and Dickinson, 1984). The concentration of serotonin, as determined by high-pressure liquid chromatography, is higher in hungry leeches than in those that have fed recently (Lent *et al.* 1991). In addition, if the serotonin of hungry animals is depleted pharmacologically, then these animals do not bite. Retzius cell are active during crawling (J. Eisenhart, in preparation) and swimming (Willard, 1981). Heating of the prostomial lip both evokes biting in semi-intact preparations and leads to an increase in activation of the Retzius cell in the suboesophageal (Lent, 1985) and midbody (Groome *et al.* 1995) ganglia, but these preparations did not allow ingestion.

The Retzius cell, or at least the serotonin that it releases, may also be important in the consummatory phase of feeding. Serotonin increases the volume of blood ingested (Lent *et al.* 1988). On isolated pieces of tissue, it can also produce pharyngeal contractions (Lent *et al.* 1988) and salivary secretion from the body wall (Lent, 1973). Furthermore, it has been reported that serotonin depletion was more extensive in animals that fed for a prolonged period (Lent *et al.* 1991). Reducing the passive tension of the body wall is a necessary component of feeding, and a number of studies have reported that either exposure to serotonin or stimulation of Retzius cells has this effect (e.g. Mason and Kristan, 1982; Leake *et al.* 1981; Wilson *et al.* 1995).

Taken together, this body of evidence made a strong case for the involvement of Retzius cells and serotonin in feeding. However, none of the electrophysiological recordings from serotonergic cells in these previous reports was from the nervous system of feeding animals. Thus, our experiments differ from the earlier work on leech feeding in an essential way. In our preparation, behavioural observations and physiological recordings were acquired simultaneously during the appetitive and throughout the consummatory phases of

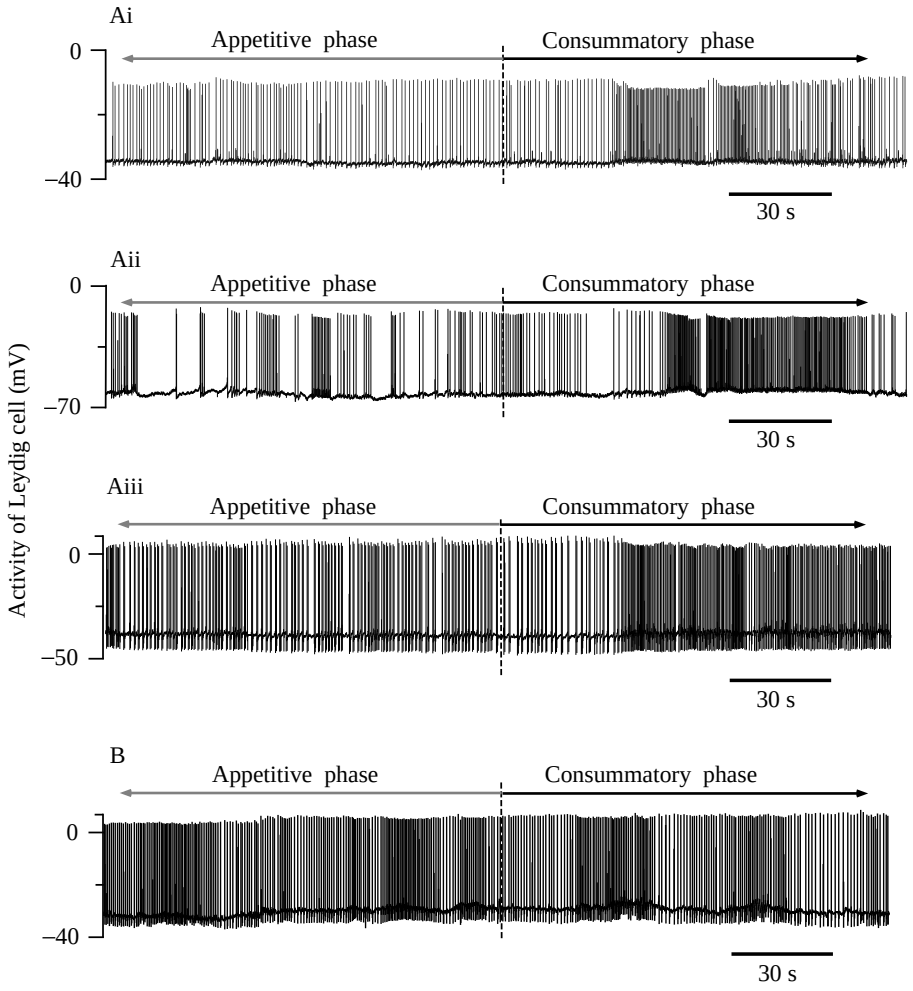


Fig. 4. The activity of the Leydig cell during feeding. Each trace (from four different animals) shows a 4 min excerpt centred about the onset of the consummatory phase (defined by the erection of the pharyngeal muscles). In seven out of nine animals, the activity of the Leydig cell increased within the first 2 min of the consummatory phase (e.g. Ai,ii,iii). (B) An example from the animal in which the activity of the Leydig cell fell.

feeding. Under these conditions, our results support the observation that the firing frequency of the Retzius cell increases during mechanosensory stimulation of the head of the animal and during movements associated with the exploration of the feeding device (i.e. during the appetitive phase). Interestingly, the Retzius cell is activated during local bending (Wittenburg *et al.* 1990; Szczupak and Kristan, 1995), whole-body shortening (Sahley, 1995) and both swimming (Willard, 1981) and crawling (J. Eisenhart, in preparation), regardless of whether locomotion is directed towards a food source. Thus, the Retzius cell and the serotonin it releases both centrally and into the body wall may serve a general modulatory or biomechanical function that is called upon whenever the animal is aroused and is likely to move or locomote.

Our findings show clearly that, as ingestion begins (within the first 2 min), the firing frequency of the Retzius cell decreases significantly, and the cell continues to fire at a relatively low rate (about 0.2 Hz) throughout the consummatory phase. In body wall preparations, Retzius cells needed to be stimulated at 0.5 Hz to have any detectable effect on basal tension (Mason and Kristan, 1982). We observed this degree of activity during the appetitive phase only, it being about three times the frequency that we observed during the initial period of ingestion (Fig. 5). Thus, the role of the Retzius cell during the consummatory phase needs to be re-evaluated.

Previously it has been shown that feeding is terminated by distention and that distention also causes a reduction in the firing frequency of the Retzius cell (Lent, 1985). However, the drop in activity of the Retzius cell that we observed immediately after the onset of ingestion (e.g. Fig. 2) occurred more rapidly than can be explained by distention alone, suggesting that other mechanisms were also involved. One possibility is that the reduction in firing frequency may be triggered by ingestion and be produced by an inhibitory pathway synapsing either directly onto the Retzius cell or onto its excitatory inputs. However, we note that in several animals, the reduction in the firing frequency of the Retzius cell occurred prior to the onset of consumption, during the later stages of the appetitive phase (cf. Fig. 2Aii and Fig. 2Aiv). Another possibility, therefore, is that serotonin released by the high firing frequency associated with exploration of the feeding device activates the autoreceptors of the Retzius cell, which in turn cause a reduction in firing frequency (e.g. Walker and Smith, 1973; Acosta-Urquidi *et al.* 1989). Alternatively, the excitatory inputs onto the Retzius cell may habituate rapidly. Further experiments are required to distinguish between these possibilities.

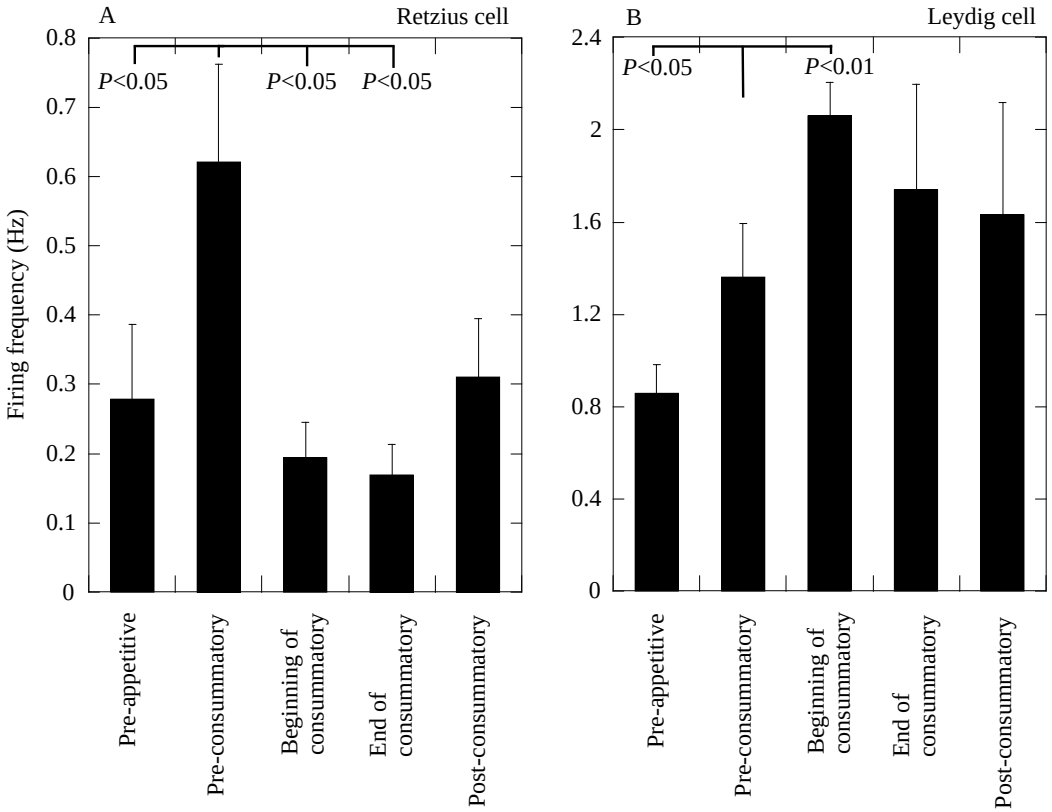


Fig. 5. Summary of activity during feeding. Average firing frequency of Retzius (A) and Leydig (B) cells over 2 min periods. Pre-appetitive period: period immediately prior to the introduction of the feeding device. Pre-consummatory and beginning of consummatory periods: periods immediately before and after the onset of ingestion, respectively. End of consummatory and post-consummatory periods: periods immediately before and after the leech released the feeding device, respectively. The pre-consummatory period was compared with other periods using the Dunnett multiple-comparison test. Values are means + S.E.M. ($N$=9).

The rapid reduction in the firing frequency of Retzius cells at the beginning of ingestion means that serotonin is not released at high levels during the consummatory phase. For serotonin released during the appetitive phase to have an influence on the different aspects of the consummatory phase (reviewed above), therefore, either it must remain in the circulation at the necessary concentration for long periods or its effects must be long-lasting. Following the stimulation of a Retzius cell, the passive tension of the piece of body wall it innervates usually returns to baseline levels within 10–15 min (Mason and Kristan, 1982). This is within the same order of magnitude as the consummatory phase of feeding, which usually lasts about 30 min. Very little is known about either the concentration or sites of serotonin release during activity or about its longevity (Leake, 1986). Clearly, this will be an important area for future research. However, it is interesting to note that the effects on pharyngeal pumping and body peristalsis of pre-incubating the leech in a solution of serotonin were significant only at the beginning of the consummatory phase (Lent *et al.* 1988).

During the appetitive phase the leech is highly responsive to sensory input and is motile, whereas during the consummatory phase the leech is far less responsive (cutting a feeding leech in half does not terminate ingestion; G. Wittenberg, unpublished observation) and is relatively quiescent. Serotonin reduces the response time to vibrational stimuli and increases both the probability of spontaneous swimming and the swim duration (Willard, 1981; Angstadt and Friesen, 1993). These effects of serotonin release are incompatible with the consummatory phase. Perhaps these central effects of serotonin on the appetitive phase are short-lived, whereas other effects are longer lasting. This would not be the first case in which the influence of serotonin depends on the target tissue (see Zhang and Harris-Warrick, 1994) or has different latencies depending on the site of release (Nusbaum and Kristan, 1986). Alternatively, the effects of serotonin that are important in the appetitive phase may be inhibited by a competing modulator or pathway. In the current set of experiments, we have shown that the activity of the Leydig cell increases during feeding. Although there is some controversy regarding the modulator it contains (Gilchrist *et al.* 1995), the Leydig cell has been shown to increase the heart rate of the leech (Arbas and Calabrese, 1990) and dramatically to reduce the strength of the local bending reflex (Lockery and Kristan, 1991). Whereas the effects on the heart rate of transient Leydig stimulation lasted less than a minute, those on the local bending reflex persisted for up to 30 min. Thus, this cell may have potent long-lasting effects that are important in feeding. The preparation described in this paper will help elucidate this and other issues related to the neuronal basis of feeding in the leech.

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