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ON THE TERMINATION OF INGESTIVE BEHAVIOUR BY THE MEDICINAL LEECH

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SUMMARY

Hungry leeches, *Hirudo medicinalis*, ingest blood meals averaging 890 % of their mass in 29 min. Ingestion is terminated as a result of distension of the body: experimentally distending leeches as they feed causes an immediate cessation of ingestion and inhibits any subsequent biting behaviour; if distension is circumvented by various experimental procedures, leech ingestive periods are prolonged significantly. Ingestion is not terminated as a result of fatigue, chemical cues or mass change. Distension also underlies satiation, for removing blood from the crops of recently fed leeches qualitatively alters their satiated behaviour to biting. Biting is not a defensive reaction to injury.

In rostral ganglia, impulses of the serotonergic Retzius (RZ) and LL neurones evoke the physiological components of ingestion. Localized warming of the prostomial lip induces impulses in these large effector neurones. Distending the body wall tonically hyperpolarizes the RZ and LL cells. This inhibitory response to distension is conducted from the mid-body to the anterior neurones *via* the ventral nerve cord. Distensive inhibition antagonizes the synaptic excitation evoked in RZ and LL neurones by thermal stimulation. Thus, a stimulus which evokes feeding synaptically excites 5-HT neurones and a stimulus which terminates ingestion inhibits them. The integration of these inputs controls the expression of leech feeding behaviour and these connections match precisely a model proposed to regulate the ingestive behaviour of blowflies.

INTRODUCTION

Hungry leeches respond to chemical, mechanical and thermal cues in initiating ingestion (Mann, 1962). Nevertheless, thermal stimulation alone will evoke biting by the gnathobdellid leech, *Hirudo medicinalis* L. Surface temperatures of 1°C above ambient are sufficient, but the highest frequencies of biting occur at 35–40°C (Dickinson & Lent, 1984), which approximates the body temperature range of their mammalian prey (Prosser, 1973). Biting is also evoked by chemicals; however, sodium and arginine are required for ingestion (Elliot, 1986). *Hirudo* are distended

Key words: leech, behaviour, Retzius cells, serotonin, 5-hydroxytryptamine, ingestion, satiation, distension.

by blood meals averaging 890% their mass, and the ensuing satiation lasts approximately 12 months (Dickinson & Lent, 1984). Satiated leeches are characterized by an absence of biting and lift their heads rapidly and repeatedly away from warm surfaces.

To feed, a leech attaches its anterior sucker and moves three semicircular jaws back and forth, cutting the host's skin. As blood flows into the buccal cavity, it is pumped into the crop by pharyngeal peristalsis. Leech saliva contains anticoagulant and is secreted from many cell bodies (Marshall & Lent, 1984) onto the cutting edges of the jaws. A localized warming of the prostomial lip stimulates salivation, biting and peristalsis.

Two colossal Retzius cell bodies (RZ, 50–100 μm in diameter) reside in each of 32 ganglia of the leech segmental nervous system, including four ganglionic homologues composing the suboesophageal ganglion (SubEG; Fig. 1; Wilson & Lent, 1972). RZ cells synthesize and release serotonin (5-hydroxytryptamine, 5-HT; Rude, Coggeshall & VanOrden, 1969; Henderson, 1983) and project axons into the periphery *via* lateral roots. Impulses of anterior RZ cells cause salivation (Lent, Dickinson & Marshall, 1983) and high-frequency bursts evoke twitches of the jaws. Two large, lateral neurones, the LL cells, are exclusive to the first segment of the SubEG (Fig. 1) and also contain serotonin. LL cells project axons peripherally *via* the cerebrobuccal roots (Lent, 1985*b*) and their activity produces pharyngeal peristalsis

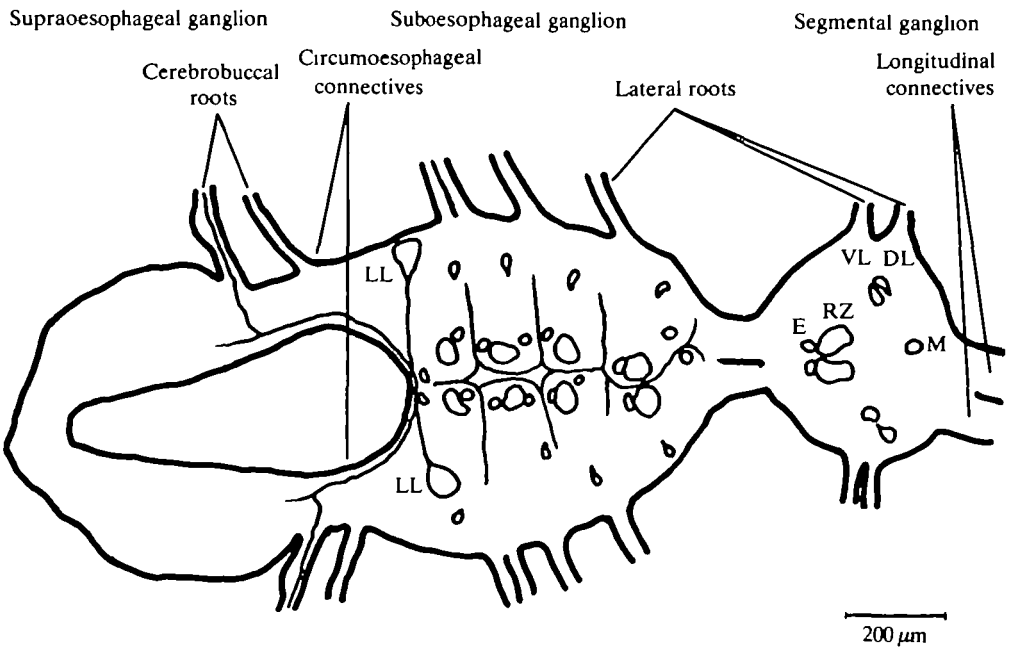

Fig. 1. Anterior ganglia of the medicinal leech *Hirudo medicinalis* L. Ganglia were reacted with rabbit serotonin antibody and stained by secondary reactions with horseradish peroxidase conjugated with goat second antibodies. The serotonergic neurone cell bodies are illustrated in all ganglia and only the axons of the LL cells are traced into roots. The interneurones in the first segmental ganglia are labelled.

(Lent & Dickinson, 1984*a*). Warming the prostomial lip excites RZ and LL cells synaptically and increases their impulse frequency (Lent & Dickinson, 1984*a*).

If ingestion were terminated neuronally, a potential route of feedback is the inhibition of the 5-HT neurones. Feedback could be exerted for minutes to terminate ingestion, or for months to inhibit biting during satiation. In either case, it would probably antagonize the excitation from lip warmth.

We report here upon behavioural experiments which establish that body wall distension underlies the termination of leech ingestive behaviour. Electrophysiological experiments demonstrate that distension tonically hyperpolarizes serotonergic effector neurones. Distensive inhibition of these neurones is integrated with their excitation from warming the lip. This integration appears to regulate the expression of leech feeding behaviour.

MATERIALS AND METHODS

Hirudo were obtained from European suppliers 7 months prior to any experiments reported here. They were maintained in glass, gallon jars filled partially with aerated pond water (Muller, Nicholls & Stent, 1981), on a 12 h: 12 h light: dark cycle at 15 °C. Leeches weighed between 1·5 and 2·5 g unless otherwise noted. Feeding status was assessed by measuring the frequency with which leeches bit a 35 °C surface (Dickinson & Lent, 1984). If one bit, it was defined operationally as hungry; most leeches were satiated upon receipt. As months passed, increasing numbers of leeches became hungry.

Behaviour

Biting frequency was assessed by placing leeches upon a 35 °C aluminium plate covered with a Parafilm sheet (American CanCo, Greenwich, CT). The temperature of the plate was maintained with a thermostatically controlled water bath. Individual leeches were placed on the film beneath inverted 50 mm plastic Petri dishes, weighted with 50 ml bottles filled with ice. Weighting precluded escapes and instances where leeches bit upon sides of the dish rather than the film. After a 10 min trial, the dish outline was impressed in the film, the animal removed, and the number of triradiate bite marks was counted under a dissecting microscope. Biting behaviour is thigmotactic and approximately 95 % of the marks were at the right angle made by the dish with the Parafilm.

To study behavioural effects of distension upon ingestion, we transected four leeches at the level of segmental ganglion 13, where the crop joins the intestine. Flexible, 2·5 mm o.d., polyethylene tubing was inserted into the crop opening, and secured by tying surgical silk tightly around the body wall (Fig. 2A). After 1 h, the leeches were allowed to feed through Parafilm stretched over a test tube containing 'artificial blood' warmed to 37–39 °C. The free end of the tubing from the crop was connected by a valve to a 20 ml syringe or fed back into the test tube of blood. Artificial blood was made of 50 vols% discarded erythrocytes suspended in Liebowitz L-15 culture medium. Leeches were submerged in pond water during ingestion. All

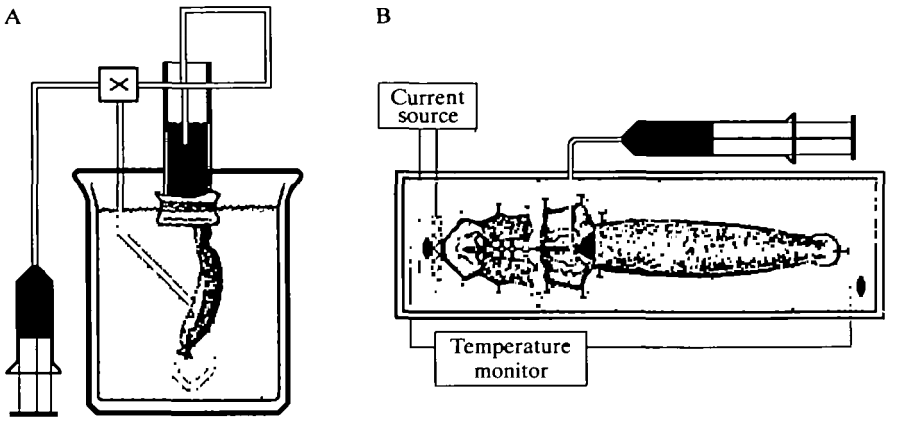

Fig. 2. (A) Diagram of a cannulated leech feeding through Parafilm on warm 'artificial blood' in a test tube. The volume of the body is controlled by the syringe when it is connected to the crop in one valve position. When the valve is in the alternative position, the ingested blood is returned continuously to the test tube. (B) The semi-intact, electrophysiological preparation. The head of the leech is dissected and positioned over the Sylgard section of the recording chamber. The posterior body is secured to the wax section of the chamber and communicates with the head by the ventral nerve cord only. The body is distended reversibly by Ringer from the syringe connected to the crop by polyethylene tubing. The lip is warmed by the power output from d.c. current passing through an insulated resistor. The thermistors, on the heat source and in the bath, are used to monitor lip temperature differentially.

descriptions of the behaviour of cannulated leeches reported here are based upon at least six observations of six trials. Observations were made on at least three different animals.

Experiments in which distension was circumvented and ingestion prolonged were terminated after 2 h without recording individual durations. Distension was avoided by cutting through the body wall or returning ingested blood back into the feeding tube. The time for control leeches to ingest to satiation is $28{\cdot}9 \pm 1{\cdot}9$ min ($N = 27$) (Lent, Fliegner & Freedman, 1986). Two hours, then, is a four-fold prolongation of behaviour and is 15 times above the 99 % confidence interval of mean feeding time.

Statistical data are presented as the arithmetic mean x, ± 1 S.E.M. (N, number of observations) unless otherwise noted. Animal size is given by the median mass x $\pm$ range (g). Biting of distended and non-distended leeches was compared with a 2×2 contingency test (X^2, 1 df, Sokal & Rohlf, 1981).

Electrophysiology

We used glass micropipettes filled with 4 mol l^{-1} potassium acetate (resistance, 30–50 MΩ) for intracellular recording and electrical potentials were amplified using standard methods (e.g. Muller *et al.* 1981). The leech physiological saline (Ringer) consisted of ($\times 10^{-3}$ mol l^{-1}): NaCl, 115·0; KCl, 4·0; $CaCl_2$, 8·0; Tris-maleate, 10·0 (pH 7·4).

We used two types of preparations for electrophysiological experiments. For distension experiments, leeches were dissected in a rectangular Lucite chamber in

which the first one-third was lined with transparent Sylgard (Dow Chemical, Midland, MI) and the rear two-thirds lined with a mixture of paraffin and Tackiwax (Central Scientific, Chicago, IL). This chamber enabled a darkfield illumination of ganglia in the head through the Sylgard in order to visualize and impale neurone cell bodies, while providing a wax substratum for pinning out the posterior segments of the body securely (Fig. 2B). The body was separated from the head between segmental ganglia 3 and 4, leaving the ventral nerve cord as the sole neuronal and mechanical interconnection. The head was pinned and dissected ventral side up, providing direct access to LL and RZ somata. The posterior body portion was pinned loosely, dorsal side up. A 2·5 mm polyethylene tube was inserted into the open end of the crop near the fourth segmental ganglion and secured within the body by ligation with surgical silk. A 20 ml syringe was attached to the free end of the tubing and used hydraulically to distend or relax the crop and overlying body wall structures. In experiments not requiring distension, the head was dissected similarly and the body was discarded.

Thermal stimulation

The dorsal edge of the prostomial lip was warmed locally by heat from a 0·25 W, 2·2 kΩ resistor driven with d.c. current from a regulated power supply. The resistor was insulated with heat-shrink tubing, mounted on a manipulator and positioned under visual control (Fig. 2B). Lip temperature was monitored differentially by two thermistors (time constant, 300 ms): one situated upon the stimulating resistor and the other in the Ringer of the recording chamber. Thus, the temperature recorded is a maximum at the prostomium, which could not be secured tightly to the resistor and remained capable of limited movements.

Figures

Electrophysiological traces were obtained from a Brush 220 ink-writing oscillograph or by photographing the oscilloscope face. These traces, and original drawings, were digitized, labelled on a Macintosh Computer and reproduced with a LaserWriter printer (Apple Inc). The ganglia and neurones composing Fig. 1 were traced by means of a *camera lucida* mounted on a compound microscope.

RESULTS

Behaviour

Hungry *Hirudo* attach the anterior sucker and bite before initiating ingestion. During ingestion, pharyngeal peristaltic movements of the head are clearly visible. As ingestion is terminated, pharyngeal movements cease and, after 2–3 s, the sucker is detached. Control leeches ingest satiating meals averaging 890 % of their initial mass in 28·9 ± 1·9 min ($N = 27$) (Lent *et al.* 1986). Sensory or physiological mechanisms which could terminate ingestion include the following. (1) Leeches sense the distension of the body wall which results from voluminous blood meals and

this provides a cue for termination. (2) During ingestion, the muscles of the jaws and pharynx fatigue, resulting in termination. (3) Leeches fall from their prey because the anterior sucker is incapable of supporting the accumulated mass of their meals. (4) The crop contents change as blood is ingested and chemicals within the ingestate provide the cue for termination. We manipulated the volume and contents of the crop in behavioural experiments designed to differentiate between these mechanisms.

Distension

Cannulated leeches (Fig. 2A) attached to and bit the warm Parafilm on feeding tubes. Ingestive behaviour appeared normal despite the tube emerging from the crop. When the body of these feeding leeches was distended by introducing 4–8 ml of Ringer, ingestion stopped immediately. The cessation displayed a normal behavioural sequence, even if the leech had been feeding for only 10 min.

If distension were maintained after they had detached, the cannulated leeches would neither attach to nor bite upon the feeding tube. However, they attached and bit immediately upon removing the distension. This initiation of ingestion occurred within 1 h of terminating the preceding ingestive bout. Without a reduction in crop volume, leeches usually initiate an additional bout of ingestion only after a non-biting period lasting many months (Dickinson & Lent, 1984).

If a bite fails to draw blood, the leech will usually re-explore the surface and bite again. To examine the effects of distension on the frequency of biting, we injected Ringer directly into the crops of leeches ($1{\cdot}2 \pm 0{\cdot}1$ g) with a syringe (26 gauge needle). Before injection, the six animals bit a 35°C surface at $0{\cdot}22$ bites min^{-1} (Fig. 3). The injection of 5 ml of Ringer reduced the frequency to $0{\cdot}07\ min^{-1}$ and none of the leeches bit after they had been fully distended with 8 ml. Experimental distension abolished biting by leeches which had not eaten for at least 7 months. Further, these distended leeches acted as if satiated by repeatedly lifting their heads from the test surface.

To ascertain whether the decrease in biting was in response to distension or to repeated injections, Ringer was removed with a syringe (20 gauge). After a 50% volume reduction, biting returned at a frequency of $0{\cdot}12\ min^{-1}$. The frequency increased to $0{\cdot}17\ min^{-1}$ when all but 0·5 ml of Ringer was removed by manual expression through a small incision (0·5 cm). Hence, biting frequency decreases with distension in an approximately linear manner (approx. $0{\cdot}025\ min^{-1}\ ml^{-1}$) and is not apparently affected by injections or cuts.

We conducted a similar experiment on 15 hungry leeches which all bit the test surface. They were injected with enough Ringer to distend them fully. In each instance, biting stopped and they acted as if satiated. Subsequently, most of the Ringer was removed and they again bit the test surface. These two experiments comprise 49 observations of biting by leeches with relaxed bodies and 27 observations of non-biting from the same animals when distended. Distension of the body has a statistically significant effect upon biting behaviour ($X^2 = 76$; $P \ll 0{\cdot}001$).

These results raise the possibility that distension underlies satiation. Thus, we examined biting behaviour of 50 leeches which had fed within the previous 2 months.

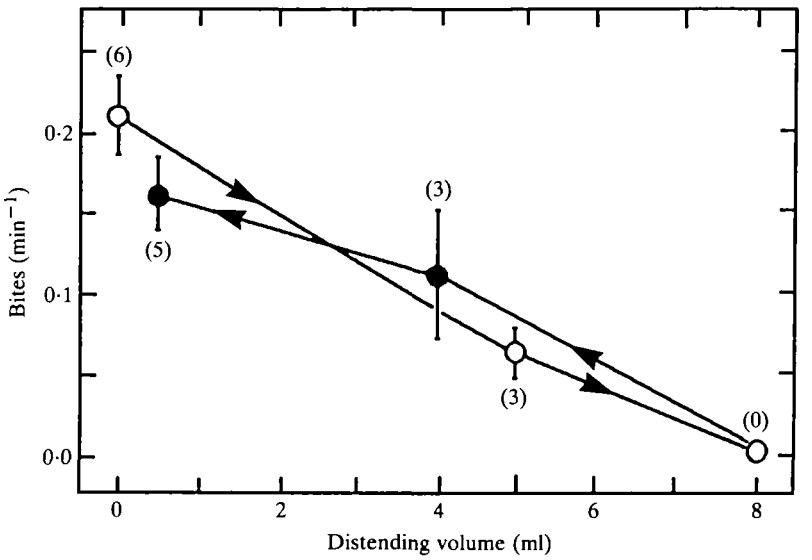

Fig. 3. Biting frequency of leeches is an inverse function of body distension in millilitres. The frequency of biting by six uniformly sized (1·2 g) leeches injected with Ringer decreases linearly with the volume of Ringer in the crop. The number of leeches which bit at each level of distension is in parentheses. Open circles, Ringer being injected; filled circles, Ringer being removed.

These leeches were obviously distended and only one bit. The 49 non-biting leeches lifted their heads repeatedly from the warm surface. Next, we cut through the body walls of 25 of them and removed the blood from the crops. After this procedure, 24 of the relaxed leeches bit the test surface. This biting was exhibited within 1 h of removing the distending blood. The one exception was the leech which bit when distended. Again, biting is altered significantly by body wall distension ($X^2 = 42{\cdot}3$; $P \ll 0{\cdot}001$).

Biting could be a defensive reaction to the injuries we inflicted on the leeches. To test this, we cut the posterior suckers of 15 recently fed leeches with a scalpel. None of these injured, distended animals bit. This finding is reinforced by data from all the experiments above. First, consider the 70 trials on undistended leeches: of these, 21 were intact, and 21 bit. Of 49 trials on injured leeches, 48 bit. The behaviour of these two groups of undistended *Hirudo* appears similar and is statistically independent of injury ($X^2 = 0{\cdot}43$; $P = 0{\cdot}52$).

Next, consider the data from the 92 trials on *distended* leeches: of 50 intact leeches, 1 bit. Of 42 injured leeches, none bit. The behaviour of these two groups appears similar and the absence of biting by distended *Hirudo* is statistically independent of injury ($X^2 = 0{\cdot}85$; $P = 0{\cdot}36$). By pooling all observations, biting was seen in 118 of 119 trials on undistended animals. Biting was absent in 123 of the 124 trials on distended leeches. Biting, then, depends upon body relaxation ($X^2 = 235$; $P \ll 0{\cdot}001$).

Ingestion without distension

The preceding experiments establish that body distension terminates ingestion and subsequently inhibits biting. Next, we tested the role of distension in terminating ingestion in a period of 29 min. To circumvent the distension of cannulated leeches (Fig. 2A) during ingestion, their crop contents were returned continuously to the tube from which they fed. Cannulated leeches ingested normally, but they ingested the recirculating blood for 120 min.

Next, distension was circumvented by cutting through the body wall and underlying crop with a scalpel in 20 otherwise intact leeches. When presented with the feeding tube, each leech bit, exhibited pharyngeal pumping and the ingested blood escaped through the incision. These leeches did not distend and they ingested for 2 h. Combining data from cut and cannulated leeches yields 26 observations of ingestive prolongation in the absence of distension. This 120 min duration was compared to the 28·9 min control duration with a one-tailed *t*-test (Sokal & Rohlf, 1981) and they differ significantly ($P \ll 0{\cdot}001$). If undisturbed, most incised leeches stop ingestion within 4 h.

To control for the cuts required in these experiments, we examined the ingestive period of two groups of small leeches ($0{\cdot}5 \pm 0{\cdot}1$ g). After their posterior suckers had been cut through, seven leeches were allowed to feed until satiated. They ingested for $23{\cdot}6 \pm 4{\cdot}0$ min ($N = 7$). We cut the body walls of another group with similar-sized incisions. These animals did not distend and they ingested for $109 \pm 15{\cdot}6$ min ($N = 7$): 4·3 times longer. The difference between these groups is highly significant ($P < 0{\cdot}001$, *t*-test, paired comparisons; Sokal & Rohlf, 1981). Thus, injury *per se* has no effect upon ingestive duration unless it also affects body distension.

Fatigue

These results demonstrate that leeches are capable of prolonging their ingestive behaviour for 2 h. Pumping by pharyngeal muscles is obvious throughout the prolonged behaviour. It is improbable, therefore, that fatigue by ingestive muscles underlies the termination of ingestion in 29 min.

Mass

Leeches were submerged in pond water for these feeding experiments. Blood and water have similar densities, hence leech mass would be unlikely to have a major role in termination. Nevertheless, to examine any potential effects, we tied 5-g weights to the posterior suckers of five 1·5-g leeches. Were mass a cue in termination, they would have ingested approximately 450% their mass in 15 min: weighted leeches ingested 910% in 31 min.

To examine mass more quantitatively, we measured the effective force of the leech anterior sucker. Leeches ($1{\cdot}2 \pm 0{\cdot}1$ g) were allowed to attach their suckers to the stainless steel pan of a top-loading balance holding 100 g. Each of 10 animals was gripped by its posterior sucker and pulled steadily upwards until it detached or the balance reached zero. If the sucker detached, the weight on the balance face was

subtracted from 100 g to calculate the maximal force of the sucker. The sucker supported 89·4 ± 4·2 g ($N = 10$). Most 1·2-g leeches ingest blood meals weighing between 9·8 and 11·6 g (±1 S.D.). Thus, the anterior sucker of a leech supports at least eight times the mass of its blood meal. Ingestive termination, then, is not effected by a passive pulling of leeches from their prey by the accrued mass of their blood meals.

Chemical cues

When we distended the crops of cannulated leeches with Ringer, rather than blood, ingestion was terminated immediately in all trials. Cannulated leeches were distended with air and their ingestion was also terminated normally. Thus, distension of the crop with diverse media terminates ingestion.

To examine any effects of blood chemicals on termination, we varied the ratio of erythrocytes to culture medium to 25 and 75 vols% and discerned no obvious effect upon feeding time. Six leeches were fed upon L-15 lacking erythrocytes and they terminated ingestion normally. However, they ingested 282 ± 35 % ($N = 6$) of their mass in 8·17 ± 0·83 min ($N = 6$): about one-third of the time and volume of controls. Thus, blood cells are not required for termination and appear to prolong ingestion.

Many of the results from the above experiments assist in evaluating the roles of blood-borne chemicals in termination. Cannulated leeches fed for 120 min while the buccal cavity and crop were bathed by recirculating blood. The 20 leeches whose feeding was prolonged by body incision had the buccal cavity and crop irrigated continuously with fresh blood: they fed for 120 min. Hence, chemicals ingested with blood do not cause termination of ingestion in 0·5 h.

Electrophysiology

We examined the effects of behaviourally relevant stimuli upon the electrophysiology of serotonergic effector neurones in preparations such as those depicted in Fig. 2B. Forcing Ringer into the crop distended the body and affected the membrane potential and impulse frequency of LL and RZ cells in the head. Fig. 4 illustrates that Retzius cells were hyperpolarized for the duration of distension. When the body was distended with a small volume, the RZ cells' impulse frequency was reduced below spontaneous levels (top); however, when fully distended, all impulse activity was abolished (bottom). The effects of distension were transmitted anteriorly by the ventral nerve cord: the only pathway between the body and head. Fig. 5 shows a similar abolition of LL cell impulses by distension. LL cells were hyperpolarized by 3–5 mV throughout the distension, which lasted for up to 5 min. Therefore, body distension inhibits RZ and LL cell impulses. This inhibition was recorded 20 times (five preparations). The hyperpolarizations did not result from movement artifacts produced by filling the crop. Such artifacts did occur on occasion and depolarized the neurones by increasing transmembrane leakage and shunting the membrane potential towards zero. Movement artifacts also increased action potential frequency.

The stimulus bars in these figures indicate the distension and begin as Ringer was injected. The crop was distended fully with 5–8 ml, until it resisted introduction of

additional fluid. Note that RZ cells fired a small burst at the onset of distension (Fig. 4). This brief excitation was observed six times (three preparations). Stimulus bars end when the Ringer was removed. As the body relaxed, RZ and LL cells repolarized to resting levels and their impulses returned to frequencies indistinguishable from those seen before distension.

LL cell inhibition arrives from distended midbody segments *via* the longitudinal connectives: a distance of at least eight segments. Because leaks and imperfect seals around the crop often precluded maintenance of constant distension, we did not use elevated Mg^{2+} levels as a test of whether LL cells were hyperpolarized by calcium-dependent chemical synapses; primarily because an absence of inhibition could not be attributed, with confidence, to the ionic substitution. Nevertheless, this inhibition is probably mediated by chemical synapses. During hyperpolarization, synaptic noise decreased, suggesting increased membrane conductance (Fig. 5). Further, neurones are not known to be hyperpolarized by electrotonic junctions for periods exceeding 1 s (Spray & Bennett, 1985; and personal communication).

Warming the lip of the leech synaptically excites anterior serotonin neurones into high-frequency impulses (Lent & Dickinson, 1984*a*). Repetitive stimulation evokes similar depolarizations and impulse numbers if the inter-stimulus interval exceeds

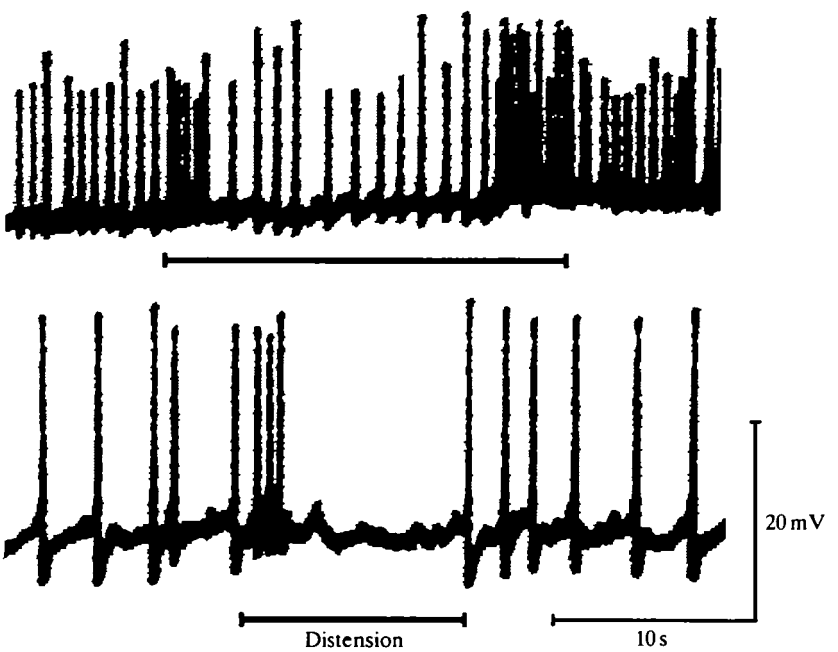

Fig. 4. Retzius cells are hyperpolarized by body wall distension. At the top, the body wall was partially distended. This RZ cell fired a brief impulse burst at the onset and offset of distension. The spontaneous frequency of 1·6 Hz decreased to 1·0 Hz during distension, during which the RZ was tonically hyperpolarized by approximately 2 mV. At the bottom, spontaneous RZ impulses of 0·5 Hz were completely abolished when the body was distended fully. The neurone was excited briefly at the onset of the stimulus and its impulses returned to precontrol spontaneous frequencies when the body was relaxed.

1 min. The thermal excitation of these neurones was found to interact with their distension-induced inhibition. In Fig. 6, for example, an LL cell was depolarized by lip warmth and fired 11 action potentials with the body relaxed. However, distending

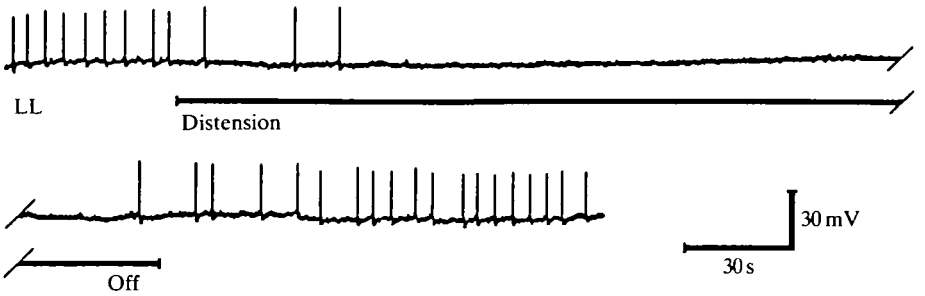

Fig. 5. Body wall distension hyperpolarizes the LL cell tonically by approximately 3 mV and inhibits the spontaneous impulse activity of 0·2 Hz. The LL cell was hyperpolarized and quiescent for the duration of the stimulus (5 min, in this example). Note that synaptic noise appears to decrease during hyperpolarization.

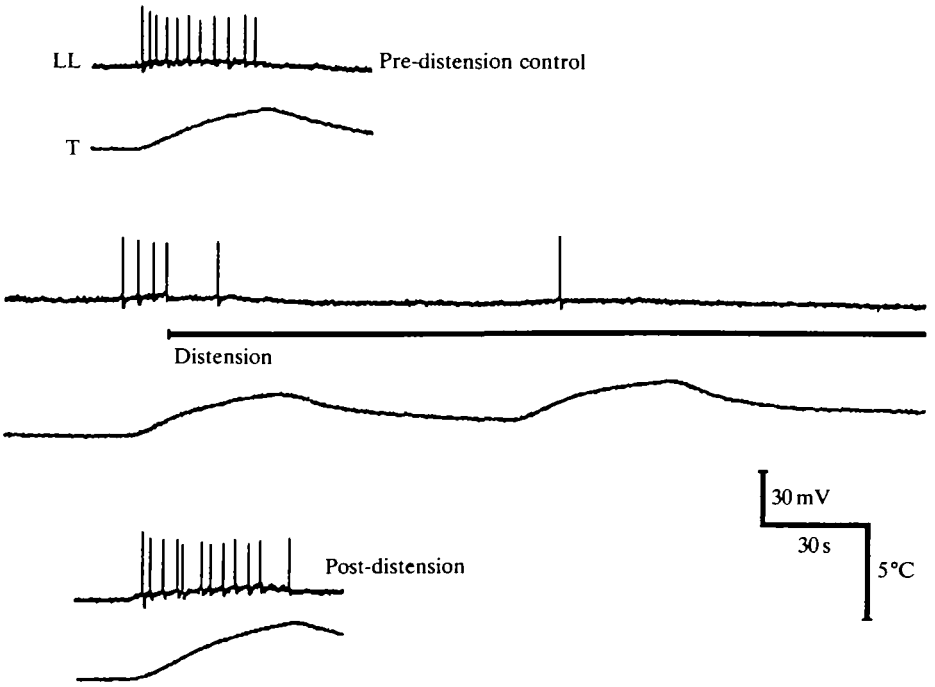

Fig. 6. LL cells integrate excitatory and inhibitory synaptic inputs. Top, the phasic, synaptic response of an LL cell to a 40-s warming of the lip by about 3°C before distension. The LL cell was not firing spontaneously, but when the lip was warmed it fired 11 action potentials during the phase of rising temperature. Middle, during a similar thermal stimulus, the body wall was distended, reducing the subsequent train to five impulses. A third warming stimulus during maintained distension resulted in a single impulse. Bottom, a post-distension thermal response of 12 action potentials illustrates that the excitation is fully expressed in the absence of distension.

the body during a thermal stimulus 2 min later interrupted the generation of a similar spike train. Distension was maintained and a third thermal stimulus (after 1·5 min) evoked one action potential. The responsiveness of the LL cell to lip warming returned to pre-distension levels after the body had relaxed. Similar integration of distensive inhibition and thermal excitation was seen in RZ cells, but is not shown here. The inhibitory influence of body distension appears to outweigh the excitatory influence of lip warmth in both of these effector neurones.

DISCUSSION

Our behavioural experiments lead to the conclusion that body distension which results from voluminous blood meals is the stimulus mediating the termination of leech ingestion. If a feeding leech is distended, by various fluids, ingestion is terminated immediately. The leech then acts as if satiated and does not bite for as long as distension is maintained. With smaller distending volumes, leeches still bite and biting frequency is proportional to the injected volume. Satiated leeches withdraw from warm surfaces, but bite immediately after the blood distending their crops is removed. Hence, we infer that distension inhibits biting during the many months of satiation. Our findings explain the anecdotal report of Indian leech traders who restore appetite to their engorged leeches by piercing them with a needle and squeezing out the ingested blood (Bhatia, 1941).

For leeches allowed to distend while feeding, ingestion is terminated in 0·5 h. If distension is circumvented, by cannulation or incision, ingestive behaviour is significantly prolonged. The Parafilm through which 10 intact animals were feeding for other experiments became occluded by clots, preventing their distension. These leeches exhibited ingestive behaviour for 90 min. Thus, leeches, whether intact, cut or cannulated, prolong ingestion in the absence of distension. These findings explain a practice of medieval phlebotomists who cut the posterior suckers from leeches to increase the amounts of blood they removed (Bossche, 1639). This practice circumvented distension and prolonged ingestion. The 2- to 4-h duration of prolonged ingestion could be produced by fatigue or by the depletion of serotonin from nerve terminals in peripheral effectors. Ingestion significantly reduces the amount of serotonin in leech ganglia (Lent, 1985*a*).

Distension appears to be involved generally in controlling ingestion. In insects, distension feeds back neurally and reduces the volume of blood ingested by the bug *Rhodnius* (Maddrell, 1963) and of sugar solutions ingested by the blowfly *Phormia* (Dethier & Gelperin, 1967). Non-nutritive bulk in the gut reduces the amount of algae required to satiate the herbivorous gastropod, *Aplysia* (Susswein & Kupfermann, 1975). Meal size in mammals is determined in part by stomach distension (Deutsch, Gonzalez & Young, 1980). Hence, distension is implicated in ingestive regulation by animals which represent four major phyla and exhibit diverse patterns of feeding.

Distension appears to produce the behaviour of satiation. In the months following a blood meal, leech body mass increases anabolically while its crop volume decreases.

Sensory neurones would necessarily continue to monitor the changing levels of distension. Endocrine factors might influence satiation to some degree since the feeding cycles of leeches from temperate climates are associated with seasonal changes (Mann, 1962). Serotonin levels of the leech nerve cord have been found to vary annually (Stenzel & Neuhoff, 1976).

The location of the distension receptors which are the presynaptic source of the RZ and LL cell inhibition is unknown. Our experiments suggest that receptors are in the mid-body and their influence is interganglionic. The gastrointestinal nervous system (Leake, Griffith & Burnstock, 1985) is not necessary for the inhibition: only the ventral cord was intact in our experiments. Ingested or injected fluids not only distend the crop and overlying muscular body wall of the leech but they also elongate the nerve cord and compress the reproductive and excretory organs. All these structures are potential loci for putative mechanoreceptors which monitor distension.

Identifying sources of neuronal feedback at the segmental level will be an important step in analysing the synaptic effects of distension upon the 5-HT neurones. Such analysis is feasible only if the distension-sensing cells are represented over several serial segments. More than one class of receptor is probable; Fig. 4 shows the brief excitation of the RZ cell as distension begins. The crop is filled by pharyngeal peristalsis and any inhibition of serotonergic neurones during this period would interfere with their release of 5-HT which excites the pharynx, the jaws and the salivary glands. An analysis of the feedback from the leech distension-sensing system onto the serotonergic effector neurones is needed.

Impulses of serotonergic effector neurones evoke the physiological components of feeding (Lent & Dickinson, 1984*a*), and neuronal serotonin is necessary for both the behavioural and the physiological components of feeding (Lent & Dickinson, 1984*b*). Thus, the criteria of sufficiency and necessity which establish the functional role of these neurones in feeding behaviour are met. Stimuli which control ingestion synaptically affect the impulse activity of 5-HT effector neurones. Lip warmth excites them, body distension inhibits them, and they integrate these disparate inputs into their impulse frequency output. These results constitute a corollary to the criteria of function and demonstrate that behaviourally relevant stimuli result in the neurones firing in the appropriate context. RZ activity was reported recently to inhibit muscular contractions of the genitals (Leake, 1986). RZ activity is likely to inhibit the movements of sexual behaviour which, if expressed, would interfere with efficient feeding.

Kristan & Nusbaum (1983) have proposed that 5-HT released by RZ cells in segmental ganglia modulates hormonally the activity of neurones of the central pattern generator for swimming. Our findings suggest that, at least in rostral ganglia, RZ cells are effector neurones which drive feeding behaviour, rather than neuroendocrine cells with long-term hormonal effects. In electrophysiological experiments, we removed the major dorsal and ventral blood vessels, and this operation precluded any delivery of neurohormones *via* the blood. The responses of salivary

glands and jaw muscles to RZ cell impulses were evident in less than 1 s. Clearly, such rapid serotonergic effects are direct and not mediated hormonally.

A model for the expression of feeding behaviour by blowflies has been proposed by Dethier & Gelperin (1967). Nerve transection experiments led them to suggest that peripheral excitation for feeding is integrated with inhibitory feedback from distension receptors to control expression of ingestive behaviour. In the leech, serotonergic effector neurones drive feeding behaviour. These cells are excited by a peripheral stimulus which initiates feeding, inhibited by a stimulus which terminates ingestion, and integrate these disparate inputs into their firing frequency. Varying the level of distension inhibits 5-HT neurones and reduces biting behaviour proportionally. This synaptic integration appears to determine the expression of leech feeding behaviour and corresponds accurately to the model proposed for blowfly ingestion. These synaptic interactions in leech constitute an example of neuronal integration which is responsible for the expression of biologically important behaviour.

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