

SHORT COMMUNICATION

THE BEHAVIOURAL STATE OF SATIATION IN THE LEECH IS REGULATED BY BODY DISTENSION AND MIMICKED BY SEROTONIN DEPLETION

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The behavioural state of an organism consists of an array of constituent behaviours or responses which may be altered differentially as the behavioural state of an animal changes (Kupferman *et al.* 1991). The medicinal leech, *Hirudo medicinalis*, exhibits a long cycle in feeding behaviour (Dickinson and Lent, 1984). When they have not fed for some months, *Hirudo* exhibit an array of appetitive behaviours that are associated with the state of hunger. These leeches rest near the water surface and spontaneously initiate swimming bouts. Shadows, mechanical shocks or surface ripples alert hungry *Hirudo*, after which they swim towards a point source of water waves. Upon contacting a surface, leeches cease to swim and will explore the surface by crawling. If warmth is encountered at this time, hungry leeches will bite; biting is typically localized in or near surface folds.

During the ensuing consummatory phase of hunger, leeches ingest blood meals which increase their volume by approximately nine times and which distend their body walls. Distension acts as a stimulus that terminates feeding and produces the behavioural state of satiation (Lent and Dickinson, 1987). Recently fed leeches bite rarely, swim infrequently and appear to select greater depths in water. Hunger alternates with satiation in this behavioural cycle whose period is approximately a full year.

Neuronal serotonin controls a variety of behavioural and physiological components of leech ingestive behaviour (Lent and Dickinson, 1984). Serotonin enhances spontaneously generated bouts of swimming (Willard, 1981) as well as swimming in response to water vibration (Brodfuehrer and Friesen, 1984; Lent and Dickinson, 1984). In addition, serotonin increases the frequency of biting by hungry leeches and evokes biting by satiated ones. Ganglionic serotonin levels are decreased after feeding (Lent *et al.* 1991) and pharmacological lesions of serotonin-containing neurones decrease biting frequency in a manner reminiscent of satiation (Lent and Dickinson, 1984; O'Gara *et al.* 1991).

We sought to describe quantitatively these two feeding states of *Hirudo* by measuring a number of clearly definable behavioural responses during hunger and satiation. We quantified several behaviours (phototaxis, thigmotaxis, geotaxis, swim initiation latency and biting frequency) that are known to be or are hypothesized to be associated with the feeding status of this animal. We assessed correlation between tests of each category of

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behaviour as one measure of relative importance of each behaviour to feeding status. Then, we assessed the effect of feeding, pharmacological reduction of neuronal serotonin or artificial distension of the leech body wall on these behaviours. These tests allowed us to evaluate the relationship between serotonin levels and body wall distension in the feeding-related behavioural state.

Leeches were obtained from Leeches USA (Westbury, NY) and maintained in glass aquaria without feeding until used. To measure phototactic responses, animals were placed in 1cm of pond water in aquaria that were half covered with opaque paper, creating equal areas of light and dark. Leeches were scored positively phototactic if they rested in the light region after 5min of exploration. Thigmotaxis was evaluated by recording the position of the anterior sucker of leeches with respect to the corners of the aquaria. After 5min, leeches within 1cm of one of the four corners were ranked as positively thigmotactic. Geotaxis was measured by placing leeches in a hemispherical tank filled with 24cm of pond water. After 5min, the distance of the anterior sucker from the water surface was measured. Leeches whose anterior sucker was above the water surface were given a score of zero. To measure the latency of swim initiation, leeches were placed in aquaria containing 2cm of pond water and allowed to come to rest. On the opposite side of the tank, a stimulus of surface ripples was generated by air bubbles emerging from the vinyl tubing connected to an aquarium pump. Response latency was measured from the beginning of stimulation until a leech initiated swimming towards the source of ripples with release of its posterior sucker. Biting frequency was assessed by placing individual leeches under a Petri dish on a sheet of Parafilm resting on a 0.5cm aluminium surface in contact with water at 38°C from a recirculating bath. After 5min trials, the bites in the Parafilm were counted.

All behavioural tests were performed at room temperature ($21\pm 1^\circ\text{C}$) with individual leeches, which were housed in separate containers between assays. Each of the five tests was performed no more than once in a 24h period. Each individual was tested for each behaviour with at least 12 trials. For each behavioural assay, data are presented as the arithmetic mean value \pm one standard error of the mean for each individual. Significant relatedness between categories of behaviour was assessed with critical-value *t*-tests of correlation. To assess the effect of feeding or neurotoxin on behavioural state, leeches were assigned such that the two groups were equivalent in mean biting frequency. There was little group-related difference for other behavioural scores using this sorting criteria (see Fig. 2). Behavioural alterations produced by these treatments, or by distension, were assessed statistically with paired Student's *t*-tests.

Unfed leeches were positively phototactic in 72 of 286 trials ($25.2\pm 2.6\%$ using the mean of means from 27 leeches) and positively thigmotactic in 140 of 273 trials ($51.3\pm 3.0\%$). These leeches selected an average water depth of $17.3\pm 2.4\text{mm}$ ($N=234$ trials, 33 leeches), initiated swimming at an average latency of $20.4\pm 1.5\text{s}$ ($N=258$ trials, 33 leeches) and bit a warm surface at an average frequency of $0.36\pm 0.02\text{min}^{-1}$ ($N=194$ trials, 33 leeches). For all leeches tested ($N=33$), the behaviours of swim latency and bite frequency correlated significantly ($r=0.81$, $P\leq 0.01$), with an inverse relationship. There appeared to be a size component (Fig. 1), as suggested by analysis of this relationship in groups of leeches ranging from 0.2 to 0.6g ($N=8$, $r=0.88$, $P\leq 0.05$) and from 0.6 to 1.0g

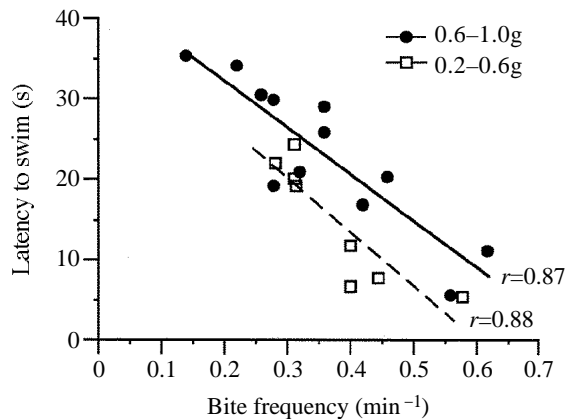


Fig. 1. Correlation of feeding-related behaviours in two size groups of unfed *Hirudo*. Leeches that more frequently bit a warm surface were also more responsive to vibrational stimuli as measured by the latency of swim initiation in response to surface ripples. Each point represents the mean behavioural score for an individual leech, with 10–12 trials each. Error bars are omitted for clarity; in both groups a significant correlation ($P \leq 0.05$) between bite frequency and latency to swim was found.

($N=12$, $r=0.87$, $P \leq 0.01$). This correlation might have behavioural significance. Leeches that respond rapidly to disturbances in the water may be more likely to ingest blood once they encounter an appropriate feeding site than leeches that are less sensitive to water vibration. There was no obvious interrelationship between any of the other behaviours assayed; all other behavioural scores correlated with $r \leq 0.40$.

Unfed leeches were initially assessed for hunger using a bite frequency of 0.1 min^{-1} as a lower limit below which they were excluded from experiments in which feeding or neurotoxin treatments were employed. Comparison of the effect of these treatments was performed on 23 leeches designated to one of these two treatments. The mean bite frequencies in these groups differed by less than $0.01 \text{ bites min}^{-1}$.

Unfed leeches ($N=11$) were fed on a 1:1 mixture of mammalian erythrocytes and L-15 tissue culture fluid (Whittaker MA Bioproducts, Walkersville, MD). Body weight increased by a factor of 5.72 ± 0.51 after feeding. Leeches were assayed for behaviour over the following 2 weeks (Fig. 2A). Three behaviours were significantly altered. Depth in the water column increased by 68.3% to $31.4 \pm 3.5 \text{ mm}$ ($P \leq 0.025$). Swim latency increased by 102.1% to $51.9 \pm 2.5 \text{ s}$ ($P \leq 0.0005$). Biting frequency decreased by 85.8% to $0.05 \pm 0.01 \text{ min}^{-1}$ ($P \leq 0.001$). Phototaxis and thigmotaxis were not significantly altered by feeding.

The neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) effectively depletes serotonin from the leech nervous system (Glover and Kramer, 1982; Lent, 1984). Twelve unfed leeches were injected with 5,7-DHT (Sigma Chemical Co., St Louis, MO) to assess the effect of serotonin depletion on behavioural state. The neurotoxin ($50 \mu\text{l g}^{-1}$ of 1 mmol l^{-1} 5,7-DHT) was injected into the ventral sinus surrounding the nervous system between segmental ganglia 3 and 4 (after Lent, 1984). Injected leeches were returned to

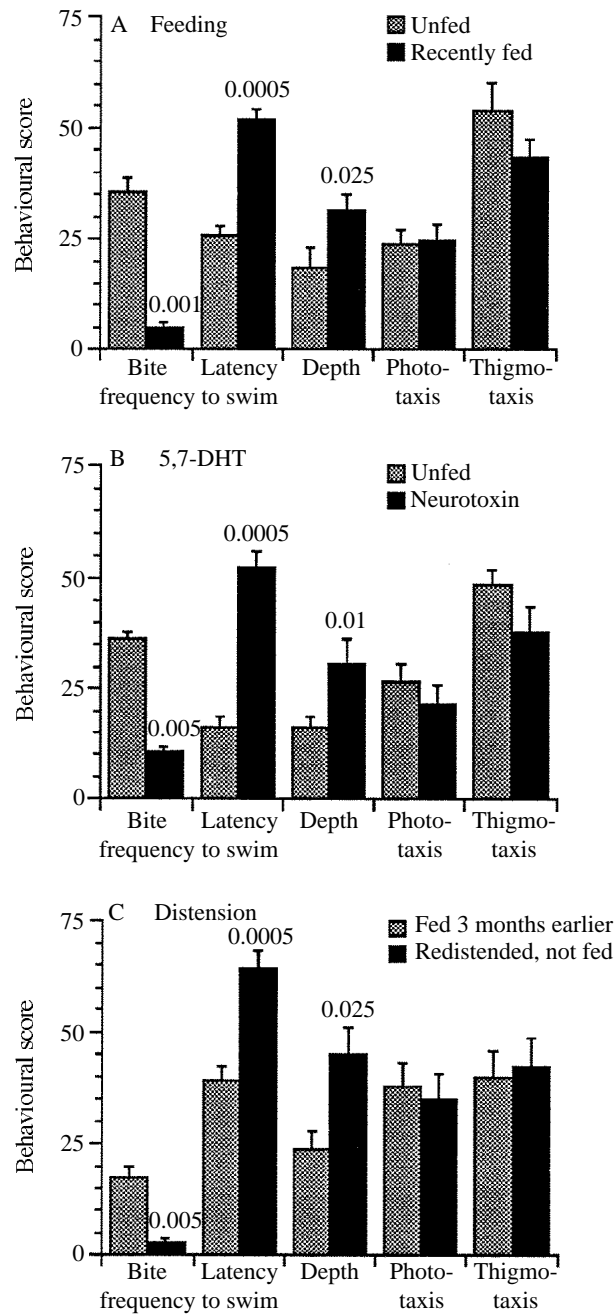


Fig. 2. Similar behavioural alterations elicited by ingestion (A), neurotoxic depletion of ganglionic serotonin (B) or artificial distension of the body wall (C). Behavioural scores are represented as bites min^{-1} ($\times 100$), latency to swim (s), depth in water column (mm) and percentage of trials in which leeches were positively phototactic and positively thigmotactic. The level of significance for *t*-test evaluation of behavioural alteration is given for particular treatments in which $P \leq 0.05$. Values are mean + S.E.M., $N=9-12$ (see text).

pond water and allowed at least 48h to recover before their behaviour was assayed. Serotonin-depleted leeches exhibited a profile of behavioural alterations similar to that observed after ingestion (Fig. 2B). Depth in the water column increased by 90.3% to 30.5 ± 5.7 mm ($P \leq 0.01$). Swim latency increased in toxin-treated animals by 218.3% to 52.1 ± 3.7 s ($P \leq 0.0005$), while biting frequency decreased by 70.7% to 0.11 ± 0.01 min⁻¹ ($P \leq 0.005$). Phototaxis and thigmotaxis were not affected significantly by toxin injection.

Distension inhibits appetitive or ingestive biting behaviour in *Hirudo* (Lent and Dickinson, 1987). In this study we extended the examination of the effects of this stimulus to other putative components of the behavioural state of feeding. Three to four months after feeding, behavioural assays indicated a substantial reversal in behavioural state from that of satiation towards that of hunger in 9 of the 11 leeches fed previously. In these leeches, depth in the water column and swim latency had decreased while biting frequency had increased (Fig. 2C). We filled the crops of these leeches with blood and L-15 medium directly from the needle of a syringe to assess the effects of body wall distension on the feeding-related behavioural state. These distended, but unfed, leeches exhibited alterations closely resembling the effects of feeding or serotonin depletion (Fig. 2C). Depth in the water column increased from 23.9 ± 4.2 mm to 45.2 ± 5.9 mm (89.3% increase; $P \leq 0.025$), swim latency increased from 38.9 ± 3.3 s to 64.3 ± 3.8 s (65.3% increase, $P \leq 0.0005$) and biting frequency decreased from 0.17 ± 0.03 min⁻¹ to 0.03 ± 0.01 min⁻¹ (83.9% decrease, $P \leq 0.005$). Phototaxis and thigmotaxis were not significantly affected by distension.

The results of this study suggest that the transition between the behavioural states of hunger and satiation in the leech is best characterized by alterations of biting frequency and latency to swim in response to water vibrations. These behaviours are consistently and significantly altered by feeding, by neurotoxic depletion of serotonin or by body distension. Geotaxis was similarly affected, but the effect was smaller. Interestingly, in an earlier study (Dickinson and Lent, 1984) in which groups of leeches were tested for geotaxis before and after feeding, the preferred depth after feeding was much greater than that in the present study. Neither phototaxis nor thigmotaxis was affected by any of the treatments in our tests. However, these responses might have feeding-related behavioural significance that was not revealed by the nature of our tests. For example, thigmotaxis, like geotaxis, may have a group-related component rendered undetectable by tests on individuals designed to restrict the effects of multiple variables.

Serotonin depletion or body wall distension is sufficient to produce the behavioural state of satiation in *Hirudo*. Leeches which fed to satiation exhibited behavioural alterations that were very similar to those of leeches which had been injected with 5,7-DHT or whose body walls had been distended without expressing ingestive behaviours. Serotonin has a pivotal role in a number of feeding-related behaviours in *Hirudo* (Lent and Dickinson, 1984). Swim initiation and biting frequency, two behaviours shown by the present study to be correlated in hungry leeches, are markedly influenced by serotonin (Willard, 1981; Lent and Dickinson, 1984). Ingestive behaviour reduces the serotonin content of the leech central nervous system significantly, while body wall distension inhibits firing by the central serotonin neurones (Lent and Dickinson, 1987; Lent *et al.*

1991). These and the present findings support a serotonergic orchestration of the feeding-related behavioural state in *Hirudo*.

Similar alteration of behavioural state by ingestion, neurotoxic depletion or artificial distension suggests a common neural correlate. The serotonin-containing Retzius effector neurones of the leech central nervous system are likely to play an important role in this regulation of behavioural state in the leech. These neurones receive sensory inputs known to affect swimming and biting behaviours (Brodfuehrer and Friesen, 1986; Lent and Dickinson, 1987; Groome and Lent, 1991). Histochemical and HPLC data indicate that Retzius neurones are depleted of serotonin by ingestion or by 5,7-DHT to a greater extent than are the serotonin interneurones (Lent and Dickinson, 1984; Lent *et al.* 1991; Ehrlich *et al.* 1992). In addition, body wall distension has been shown to inhibit impulse activity of Retzius neurones (Lent and Dickinson, 1987), which may therefore regulate appetitive as well as consummatory aspects of the behavioural state of hunger and its transition to satiation with feeding.

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