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FEEDING MOTOR PROGRAMME IN *LIMAX*. II. MODULATION BY SENSORY INPUTS IN INTACT ANIMALS AND ISOLATED CENTRAL NERVOUS SYSTEMS

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SUMMARY

The sources of variability in feeding motor programme (FMP) of the terrestrial slug, *Limax maximus*, were examined in relation to: (1) changes in load on the feeding apparatus; (2) changes in concentration of attractive food stimuli; and (3) changes in satiety signalled by feedback from the gut. These sources of variability, which affect both timing of the central pattern generator for feeding and probability of occurrence of FMP, were compared in intact animals and in isolated brain preparations.

The load on feeding apparatus of intact animals is altered by varying the hardness of their food. An animal will show a higher 'bite cycle' frequency on soft food as compared with hard food. In physiological preparations, weights attached to buccal muscles similarly increase load. Cycle frequency of FMP triggered by food extracts is increased when buccal muscles are unloaded compared with FMP when muscles are loaded.

Increasing the chemostimulant concentration of food results in greater numbers of intact animals feeding for longer periods. Increasing the food extract concentration used to trigger FMP in physiological preparations causes similar increases in feeding duration.

Intact animals use cues from gut distention to indicate satiation and terminate feeding. Inflation of the crop in physiological preparations causes an early termination of feeding activity, along with decreased FMP cycle frequency.

INTRODUCTION

Rhythmic movements of the buccal musculature underlying feeding in the terrestrial mollusc *Limax maximus* are controlled by a central neural network. The output of this central neural network is termed the feeding motor programme (FMP) (Gelperin & Forsythe, 1976; Gelperin, Chang & Reingold, 1978). FMP produced by semi-intact preparations consisting of chemosensitive lips, brains and buccal complex has been shown to be the neural substrate of feeding activity in intact, behaving animals. In previous studies we have shown that expression of the FMP *in vitro* can be strictly controlled by defined chemical stimuli (Gelperin *et al.* 1977, 1978).

In spite of the control of rhythmic feeding movements by a central pattern generator,

meal duration and bite frequency within a meal can vary. A number of internal factors may interact with the chemical attractiveness of a food substance, its physical characteristics, and the level of hunger in an animal to modulate the expression of FMP (Kater & Rowell, 1973; Susswein & Kupfermann, 1973, 1974, 1975*a*, *b*, 1976; Siegler, 1977; Senseman, 1978; Gelperin *et al*, 1977; Reingold & Gelperin, 1978). Such modulation adjusts feeding movements to different types of food and regulates food consumption.

In the present paper, we demonstrate several types of variability in FMP of intact animals. We also show that similar variability can be generated in highly dissected, *in vitro* preparations. Given the ability to record activity in individual cells in the *Limax* central nervous system during FMP (Gelperin & Forsythe, 1976; Gelperin, 1976; Gelperin & Cheng, 1977; Prior & Gelperin, 1977), these studies will lead to an examination of behaviourally relevant modulation of the feeding pattern generator at the cellular level.

MATERIALS AND METHODS

Laboratory cultures of *Limax* were maintained on an artificial diet of autoclaved powdered Purina Lab Chow (500 g), sea sand (100 g), calcium carbonate (10 g) and Vionate Vitamin-Mineral powder (5 g). A 0·24% solution of Tegosept M was added to retard fungal growth. Animals were housed in polyethylene containers lined with moist paper towelling and kept at 19 ± 2 °C, under a 14:10 h day–night light cycle.

Behavioural studies

Observations were made during the dark cycle when *Limax* is normally active (Sokolove *et al.* 1978). Food deprived animals were placed near a pellet of prepared diet, which was attached directly to the arm of a force transducer (Bionix F-200). Animals were allowed to feed *ad libitum* or for about 100 feeding cycles, depending on the experiment. Each cycle of feeding activity was recorded by the force transducer as a deflexion on a moving chart pen recorder (Fig. 1). Each cycle of pen deflexion represented one protraction-retraction cycle for feeding, termed a 'bite cycle'. Data were collected for individual animals and analysed by measuring: (1) instantaneous bite cycle frequency throughout a meal, and (2) the duration of the meal.

Food pellets were made from various concentrations of carrot or potato extract. To determine the effects of hardness on feeding activity, carrot-agar pellets were prepared as follows: 250 g fresh pureed carrot was mixed with 50 ml distilled water and blended into a heated solution consisting of 200 ml distilled water, 0·66 g $CaCO_3$, 0·26 g vitamin-mineral powder, and 6 g (for 3% wt/vol, or 'soft' pellets) or 18 g (for 15% wt/vol, or 'hard' pellets) agar. The mixture was poured into glass tubes, allowed to harden, removed and sliced into uniform pellets.

To determine the effects of food concentration on feeding activity, potato-agar pellets were prepared as follows: 100% potato extract was made of 8 g 'Hungry Jack' potato flakes (Pillsbury Co.), 0·66 g $CaCO_3$, 0·26 g vitamin-mineral powder, in 200 ml distilled water. 50% and 10% solutions were made by diluting this 'stock'

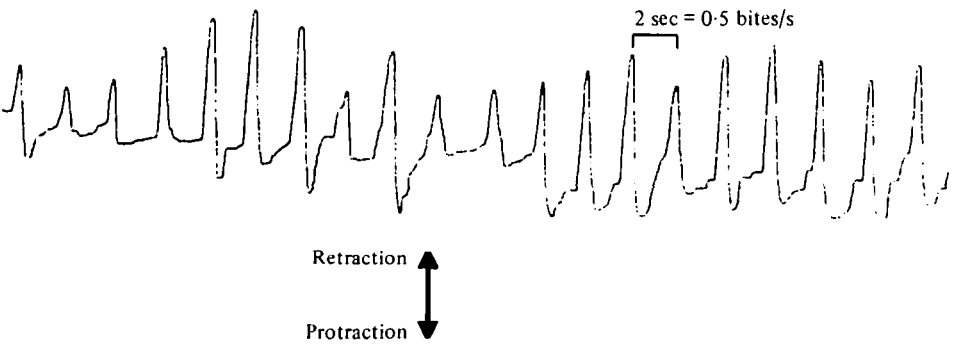

Fig. 1. Segment of feeding activity removed from the middle of a longer feeding bout, and recorded by force transducer and chart recorder, as described in the text. Pen deflexions represent cycles of radula-odontophore protraction and retraction. Each cycle results in the ingestion of small amounts of food. Cycle intervals are measured from peak retraction to subsequent peak retraction, and converted to an instantaneous bite frequency as indicated.

with appropriate amounts of distilled water. All solutions were blended with a 5% (wt/vol) agar solution, allowed to harden, and sliced into uniform pellets.

In vitro *physiological studies*

In vitro preparations, consisting of lips, cerebral, and buccal ganglia, were prepared as described previously (Gelperin *et al.* 1978) and pinned to a sylgard plate. These are called 'lip-brain' preparations. In preparations which included buccal musculature, the buccal mass was split dorso-ventrally through the midline and the two halves also pinned to the sylgard plate (Fig. 2A). The mid-dorsal tooth, radula and odontophore were also split in these preparations. These are called 'lip-brain-buccal mass' preparations (see Gelperin *et al.* 1978, for details of the functional anatomy of the buccal complex). Innervation from the buccal ganglia was left intact to both halves of the buccal mass, via bilateral buccal nerve roots 1, 2, and 3. Extracellular nerve recordings were obtained using polyvinylchloride suction electrodes placed *en passant* on buccal nerves and salivary nerves. FMP was triggered by lip stimulation with standard potato extract delivered as described previously (Gelperin *et al.* 1978). Standard potato extract consisted of 1·0 g 'Hungry Jack' potato flakes (Pillsbury Co.) mixed with 25 ml saline for 5 min, coarse filtered and millipore filtered (0·45 μm pore size). FMP data were filmed directly or tape recorded for later analysis. Feeding activity was recognized on the basis of correlated activity in salivary and buccal nerves. The cycle frequency for FMP was determined by measuring burst onset times of the salivary burster cells. These bilateral, autoactive neurones fire at the rate of one burst per bite during feeding (Prior & Gelperin, 1977; Gelperin *et al.* 1978) and are easily recorded in the bilateral salivary nerves.

To determine the effects of loading of buccal muscles on FMP in a lip-brain-buccal mass preparation, a small hole was made in the medial tooth where it attached to the buccal mass on one side. A fine suture was threaded through the hole and tied to the tooth, and a lightweight container for weights attached to the other end (Fig. 2A). Weights could be added or removed easily. They were applied to only one-half of the split buccal mass in each preparation, and served to increase the load on half of the

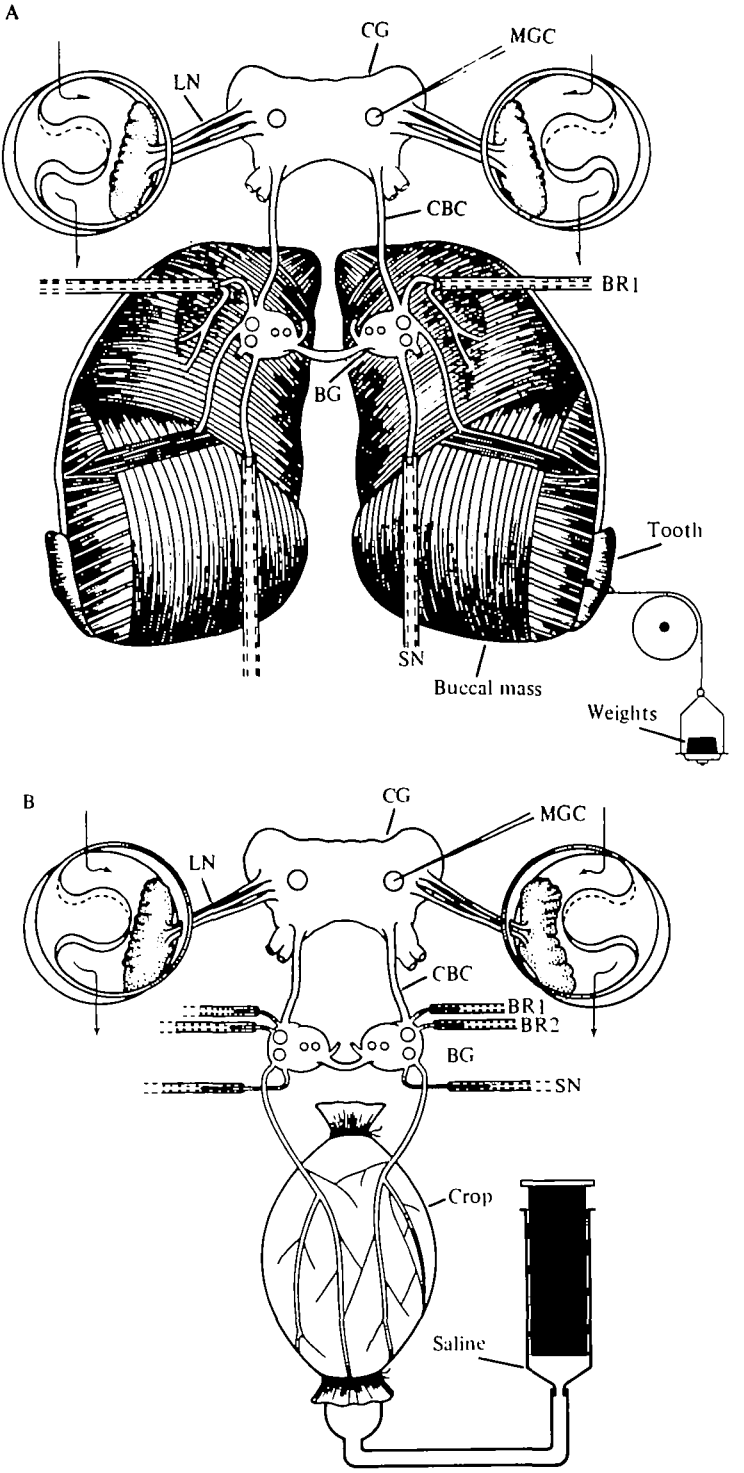

Fig. 2. (A) Schematic of lip-brain-buccal mass preparation. Weight applied to one-half of the buccal musculature provides artificial load during FMP. (B) Schematic of lip-brain-gut preparation. Gut is cannulated and inflated or deflated with saline injections. BG, buccal ganglion; CG, cerebral ganglia; LN, lip nerves; CBC, cerebrobuccal connective; BR1, BR2, first and second buccal nerve roots; SN, salivary nerve; MGC, metacerebral giant cell.

buccal complex. Contraction of buccal musculature during generation of FMP was still possible with weights attached in the manner described.

To study the effects of feedback from the oesophagus and crop on FMP, a lip-brain-gut preparation was used (Fig. 2B) similar to the lip-brain-buccal ganglia preparation but with the addition of the oesophagus and crop. Neural connexions to the oesophagus and crop were maintained by the bilateral gastric nerves originating from the buccal ganglia. A polyvinylchloride cannula was inserted into the crop and secured with a ligature. The oesophagus was ligatured closed. Thus the crop could be emptied and inflated to any desired volume with saline injections through the cannula. FMP was elicited by chemostimulation of lips, as described above, or by tonic electrical stimulation (2 ms duration, 71 s) of the cerebro-buccal connectives. Extracellular electrodes on buccal and salivary nerves were used to monitor feeding activity, and FMP was compared when the gut was deflated and inflated.

RESULTS

The feeding response: intact animals

Attractive food substances cause a slug to approach, evert the lips, and begin rhythmic feeding activity. Feeding movements consist of cycles of rasping, by radula and odontophore protraction and retraction, coupled with coordinated movements of the buccal musculature (Carriker, 1946*a*, *b*; Gelperin *et al.* 1978). Animals will feed on pellets of carrot juice hardened with agar, showing an initial rise in instantaneous bite frequency which plateaus to a relatively constant frequency for the duration of the meal. Fig. 3A shows such a response, representative of over 50 animals (Gelperin *et al.* 1978). Feeding activity on different types of attractive food is very similar.

In vitro *preparations*

In response to brief stimuli of attractive food extracts delivered to chemosensitive lips, rhythmic FMP is generated in an isolated preparation of only lips and brain (Gelperin *et al.* 1978). In cases of vigorous FMP, after an initial rise, the peak frequency attained by such *in vitro* preparations is equivalent to that seen in intact, feeding animals. *In vitro* bouts of FMP tend to be shorter than feeding bouts in intact animals, and show a gradual decrease in the feeding frequency throughout the response (Fig. 3B, representative of eight preparations).

FMP affected by load on buccal complex: behavioural studies

To determine the effects on FMP of changes in load on the feeding apparatus, carrot-agar food pellets of different hardness were presented to intact animals.

Starved individuals were presented with a 15% agar pellet (hard) which was attached to the arm of a force transducer, and allowed to feed for about 100 bite cycles. The pellet was then rapidly replaced (in < 5 s with minimal disturbance to the animal's feeding rhythm) by a 3% (soft) pellet. Another sample of 100 bites was measured on the soft pellet. Instantaneous bite frequency was determined for each diet, and plotted as a function of sequential bite number. Fig. 4 shows a representative result for one animal. Note that the initial rise in feeding frequency (a 'warm up' period) is seen

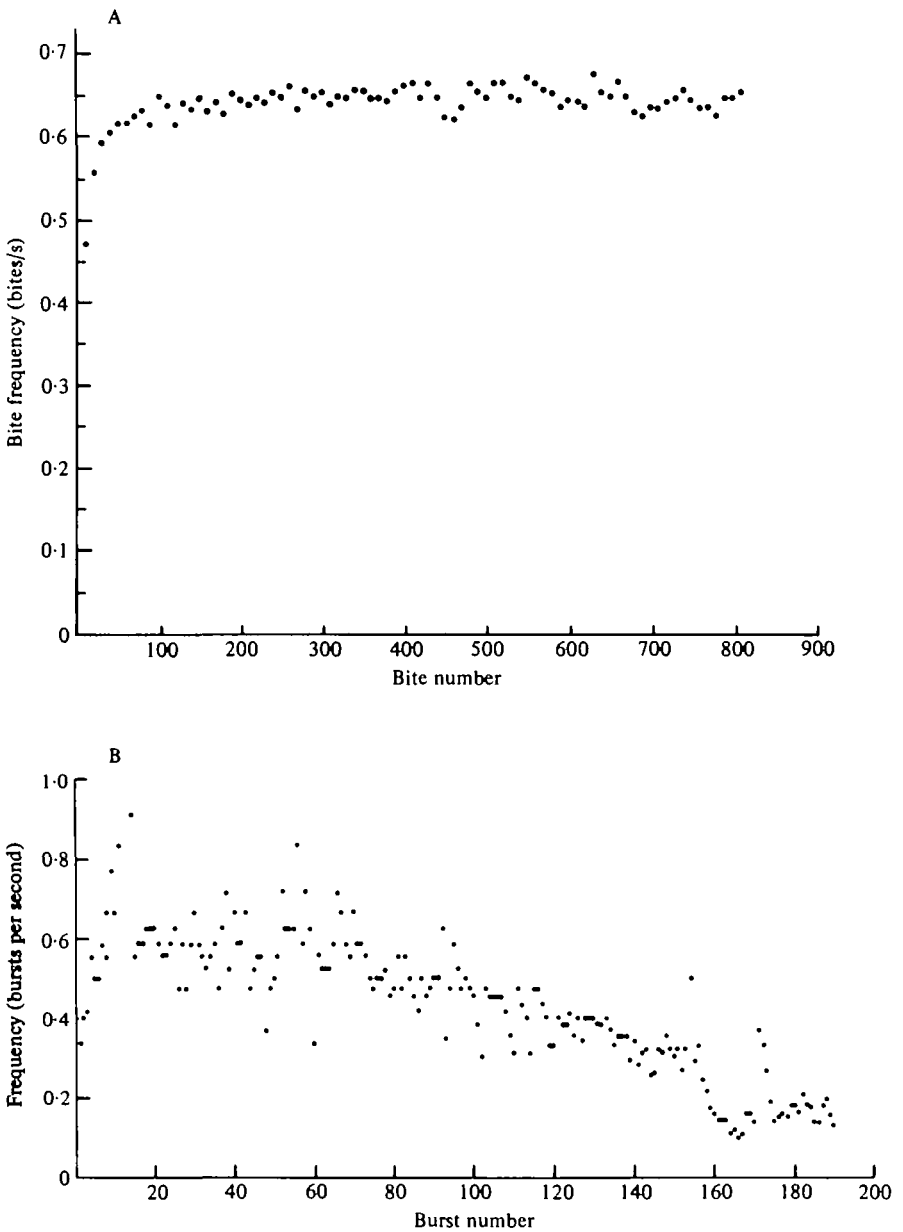

Fig. 3. (A) Bite frequency throughout a single meal on 5% carrot-agar diet, for a single, behaving animal. Each point is the average of 10 sequential bites. (B) Burst frequency (equivalent to FMP frequency) throughout a single feeding bout for a lip-brain preparation. Unlike (A) above, points were not averaged. Carrot extract was delivered to the lips for 30 s to elicit feeding.

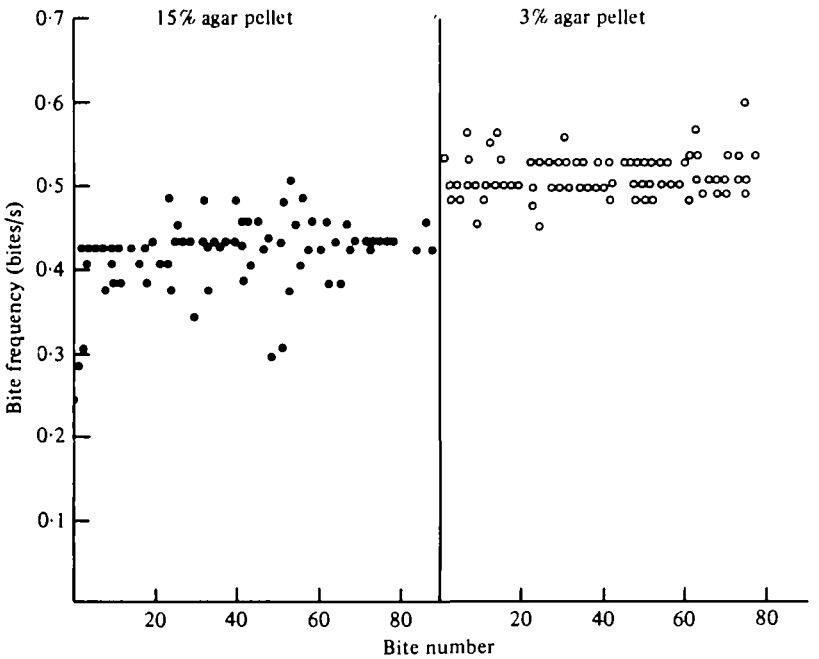

Fig. 4. Instantaneous bite frequency as a function of sequential bite number for one animal. 15% carrot-agar pellet (hard) was presented first, followed by 3% carrot-agar pellet (soft). Feeding frequencies on the soft pellet are statistically higher than on the hard pellet (Mann–Whitney U test, $P < 0{\cdot}01$).

for feeding on the first (15%) pellet presented, but not on the second. This indicates that the 'plateau phase' for feeding was not disturbed by changing the food pellet. Instantaneous feeding frequency is significantly higher ($P < 0{\cdot}01$, Mann–Whitney U test) for the soft (3%) pellet than for the hard (15%) pellet. Similar results were seen in 27 of 31 (87%) animals. In 2 of 31 (6%) animals, frequency on 3% was higher, but not significantly so, and in 2 of 31 (6%) animals, the harder pellet elicited higher instantaneous feeding frequencies than the softer pellet. These results were found to be independent of the order of food presentation. In a series of control experiments, a food pellet was replaced after the initial feeding bout by a second pellet of equivalent hardness. No significant differences in instantaneous feeding frequency were seen for the two pellets of equivalent hardness. Thus, disruption of feeding by replacement of the food pellet had no effect on feeding frequency.

In these experiments, each animal was tested sequentially on the two pellets, one presentation following immediately upon the other. Feeding activity was compared only on different food pellets for individual animals. Each animal thus serves as its own control for each pair of measurements. This is an important control for the variability seen in feeding frequencies between individual animals on a given day (Fig. 5A) and from meal to meal for a given animal on different days (Fig. 5B).

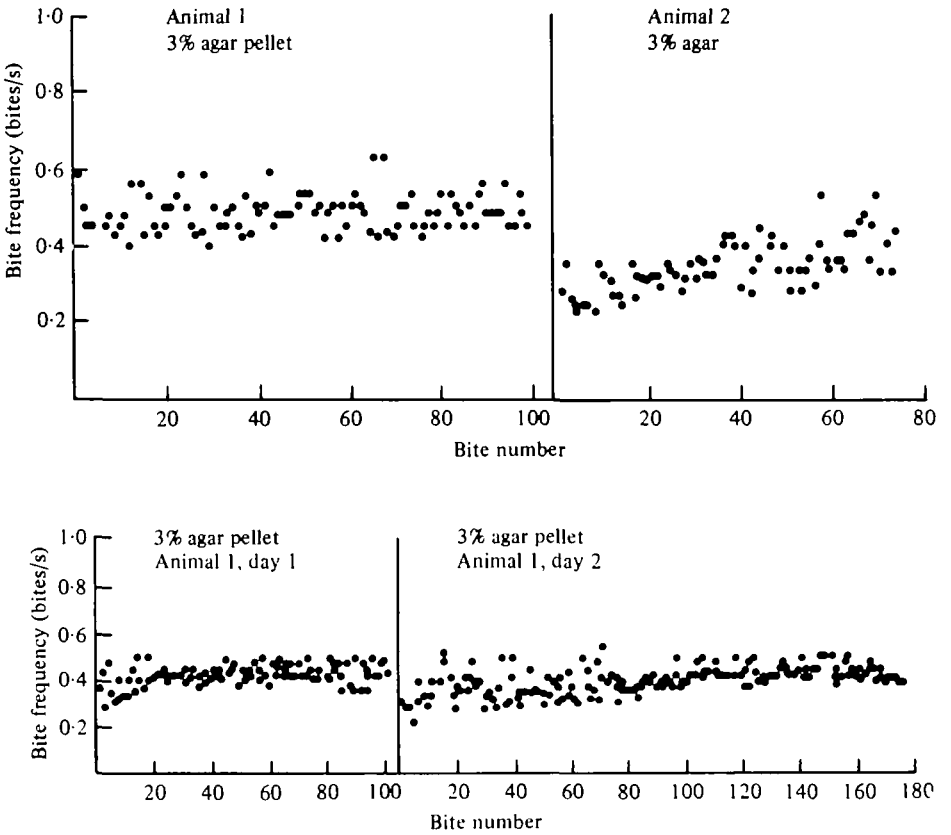

Fig. 5. (A) Feeding frequencies as a function of sequential bite number for two different animals, both feeding on 3% (soft) carrot-agar pellets. (Statistically different at $P < 0{\cdot}01$, Mann–Whitney U test.) (B) Feeding frequencies as a function of sequential bite number for a single animal, but for two different feeding bouts on successive days. In both cases, food pellet was 3% (soft) carrot-agar. (Statistically different, $P < 0{\cdot}01$, Mann-Whitney U test.)

Physiological studies

Loading of the buccal complex in intact animals by food of varying hardness can be approximated in an *in vitro* preparation by attaching weights to the buccal musculature while eliciting FMP. FMP is triggered by chemical extracts delivered to the lips, and causes contraction of buccal musculature which then must work against the artificially imposed load. To test for the effects of such loading, half of the medial tooth and the musculature of half of the buccal complex were loaded reversibly with small weights, as described in materials and methods.

FMP was triggered with standard potato extract and recorded with extracellular suction electrodes on buccal and salivary nerves. Frequency of FMP was monitored and sequential bouts of FMP compared for each preparation in both the loaded and unloaded states.

Fig. 6 shows a representative sequence of feeding bouts triggered by pulses of potato extract. Loading of the left half of the buccal complex resulted in a reduced

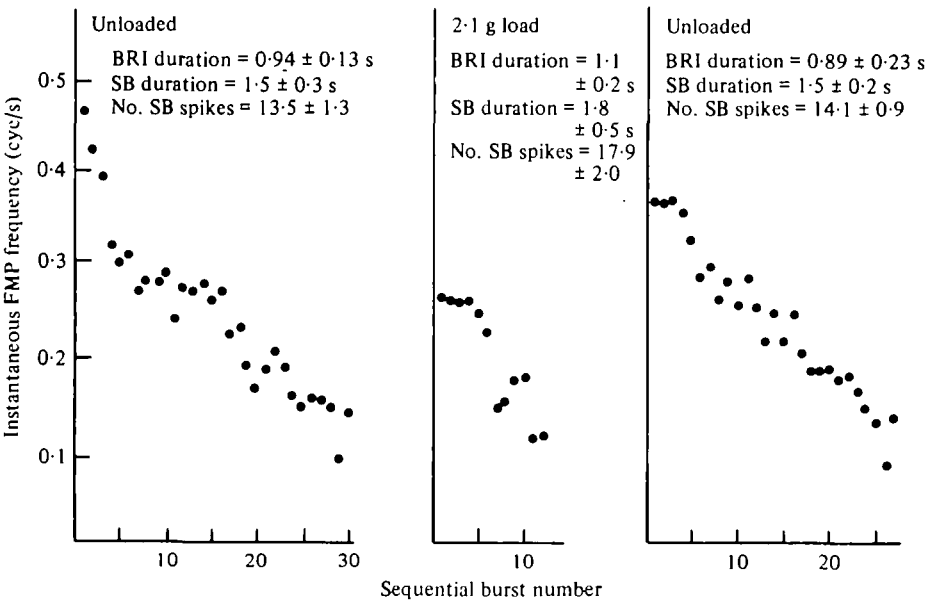

Fig. 6. Sequence of FMP bouts in a single lip-brain-buccal mass preparation. Each sequence was triggered by a 30 s pulse of potato extract to the lips and separated by 15 min during which lips were washed in saline. Alternate feeding bouts were triggered with buccal musculature loaded (see text) with weights in the amount shown. Insets represent mean ± 1 S.D. for nerve root burst durations and numbers of action potentials in the salivary burster, recorded extracellularly. Burst sample sizes are: $N = 30$ (first unloaded FMP bout); $N = 12$ (loaded FMP bout); $N = 27$ (second unloaded FMP bout). BR1, buccal nerve root 1; SB, salivary burster.

peak frequency of FMP when compared with the unloaded state. This reduction in peak FMP frequency is accompanied by small statistically insignificant increases in the mean values of the burst duration recorded in buccal nerve roots and the salivary nerves, and increases in the number of action potentials within each burst of the salivary burster cells (Fig. 6). Out of 12 preparations, 9 showed lower FMP peak frequency when tooth and musculature were loaded than when unloaded, 2 showed lower FMP peak frequency when unloaded and 1 showed no systematic change. Loads below 900 mg rarely produced a change in FMP.

FMP affected by different concentrations of attractive food substances: behavioural studies

The concentration of attractive food substance in an agar-based food pellet was varied by diluting the food extract before mixing into the agar matrix. This results in food pellets of constant hardness, but of different chemostimulative quality (presumably both olfactory and gustatory). The effect of varying the concentration of food substance on both meal duration and instantaneous bite frequency was determined in two sets of experiments.

First, 15 animals were starved and separated arbitrarily into three groups of five animals. Members of the first group were presented with food pellets of 10% potato extract; those of the second, with food pellets of 50% potato extract; and those of the

Table 1

Animal	Food concentration (%)	Amount eaten (g)	No. of bite cycles
1	10%	0·06	23
2	10	0·02	20
3	10	•	—
4	10	•	—
5	10	•	—
6	50	0·45	571
7	50	•	—
8	50	•	—
9	50	0·38	302
10	50	•	—
11	100	0·45	1120
12	100	0·50	1589
13	100	0·53	1441
14	100	0·48	1275
15	100	0·90	> 1600

• Indicates that animals did not eat pellets.

third, with food pellets of 100% potato extract. Each animal was allowed to feed until it turned and moved away from the food. Table 1 indicates that the higher the concentration of attractive food substance in a pellet, the greater the number of animals which will eat, and the greater the number of individual bites in each meal. To ensure that the lack of feeding of some of the animals on 10% and 50% potato extract pellets reflected the attractiveness of those foods and not a refractoriness to feed, animals which did not feed were subsequently given 100% potato extract pellets. Each animal (except no. 10, Table 1) fed vigorously on these pellets of higher potato concentration.

In a second series of experiments, each of 19 animals was fed sequentially, for about 100 bites each, on pellets made from 50% and 100% potato extract (prepared as before). The order of presentation was randomized. Bites were monitored by a force transducer and instantaneous bite frequency throughout the feeding bout was plotted for each food. Fig. 7 shows that, for a single animal, there is no significant difference in bite frequency on pellets of different potato concentration (Mann–Whitney U test, $0{\cdot}21 < P < 0{\cdot}22$). This result was obtained for 16/18 (89%) additional animals; in 2/18 (11%) a significantly higher bite frequency was seen for food of higher concentration ($P < 0{\cdot}01$, Mann–Whitney U test). Thus, while the concentration of an attractive food substance will affect both the number of animals which will eat and the duration of a feeding bout once it has begun, the frequency of bite cycles is equivalent on foods which are of twofold difference in chemostimulant concentration.

Physiological studies

The effect of chemostimulant concentration on FMP was also studied in the lip-brain preparation. Potato extract was prepared (1 g 'Hungry Jack' potato flakes soaked in 25 ml saline, and double filtered, used as 100% stock solution) and diluted from stock to various concentrations. Potato extract was delivered to the lips for 30 s and FMP monitored by recording from buccal and salivary nerve roots. Lips were

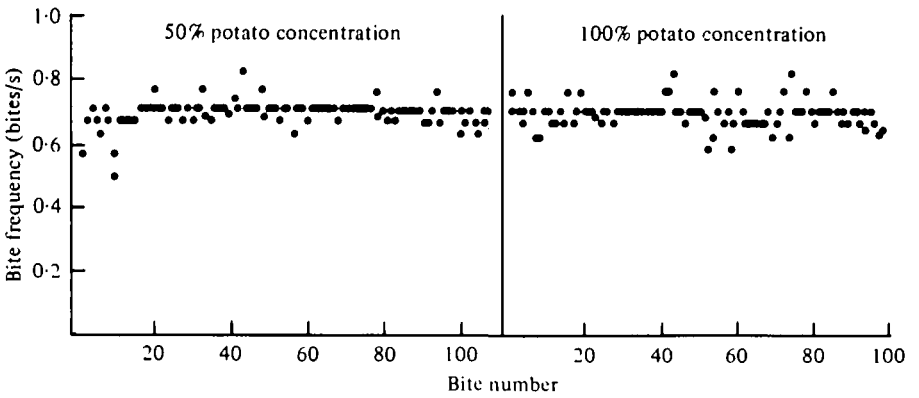

Fig. 7. Bite frequency as a function of sequential bite number for a single animal feeding on 50% potato extract pellet, followed by 100% potato extract pellet.

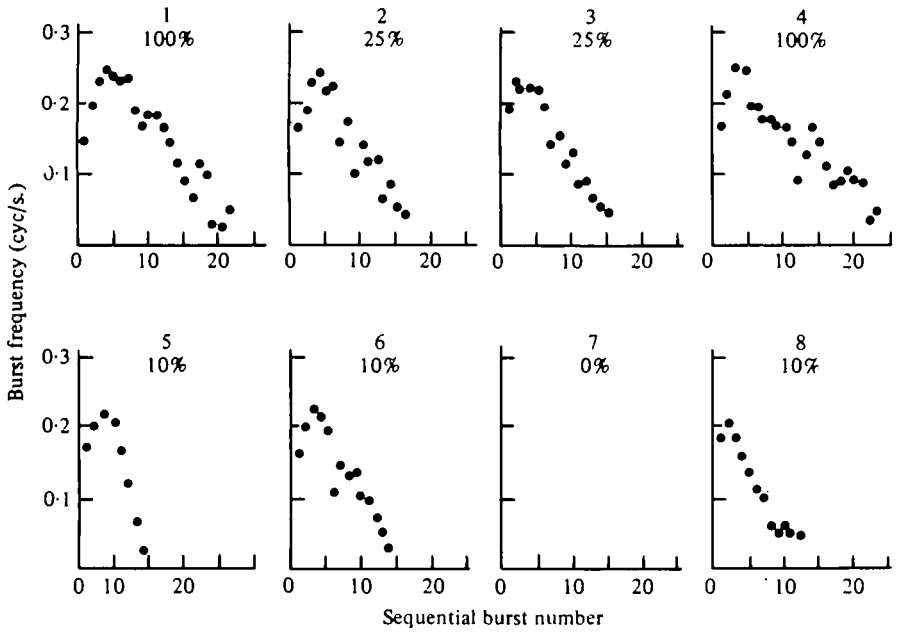

Fig. 8. Instantaneous burst frequency as a function of sequential burst number for several FMP sequences in one lip-brain preparation. Potato extract, of the concentrations shown, was delivered to the lips for 30 s to trigger FMP, and then lips were washed for 20 min between presentations, with fresh saline. 0% represents a test for specificity of the FMP response to food extracts: fresh saline delivery evokes no feeding.

washed in saline and a subsequent presentation of potato extract given after 20 min. Usually, only two or three different stimulus concentrations were presented in sequence to a given preparation, with the order of presentation randomized.

Fig. 8 shows successive trials for one lip-brain preparation responding to different concentrations of potato extract. The peak FMP frequencies on 100%, 25% and 10% concentration potato extract are nearly equivalent, but the duration of feeding bouts is consistently longer on 100% than on 25% and 10% solutions.

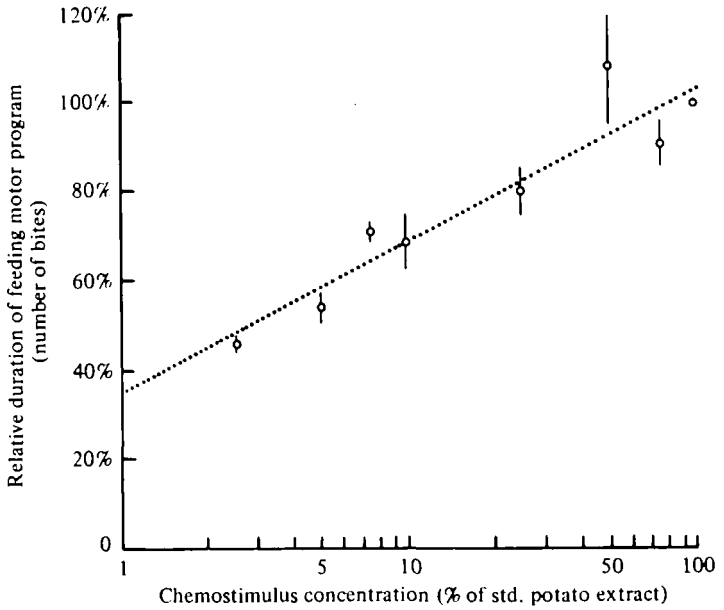

Fig. 9. Relative duration of FMP, plotted as a function of the logarithm of the potato extract concentration, pooled from 11 lip-brain preparations. (See text for pooling procedure.)

In Fig. 9, data from 11 separate preparations are pooled. For each preparation, the number of feeding cycles in response to each of eight stimulant concentrations was averaged. Averages were then normalized to the mean number of feeding cycles on 100% concentration (designated as 100%). The figure shows that the mean number of FMP cycles decreases significantly as the concentration of stimulant decreases (regression line: $R = 0{\cdot}94$; $F\ (7, 80) = 36{\cdot}36$; $P < 0{\cdot}001$, for 2 factor ANOVA test).

Peak FMP frequency, determined by inspection, showed no statistically significant differences as a function of concentration of attractive food substance ($F\ (7, 80) \doteq 2{\cdot}0$).

Thus, changes in concentration of attractive food extracts have similar effects on FMP produced by *in vitro* preparations as on feeding by intact animals: bite cycle frequency remains relatively constant over a range of food concentration presented, while the duration of feeding bouts is greater on foods of higher stimulant concentration.

FMP in response to feedback from the gut: behavioural studies

Termination of feeding activity in slugs can be influenced by chemosensory factors (demonstrated in the previous section) and feedback from the gut (Senseman, 1978; Susswein & Kupfermann, 1976).

Evidence that gut feedback is probably determined by bulk distension was obtained from the following experiment. For 3 days running, slugs were given access to two agar pellets simultaneously which differed in the concentration of feeding stimulant they contained [rat tube feeding diet (RTFD), a commercially available

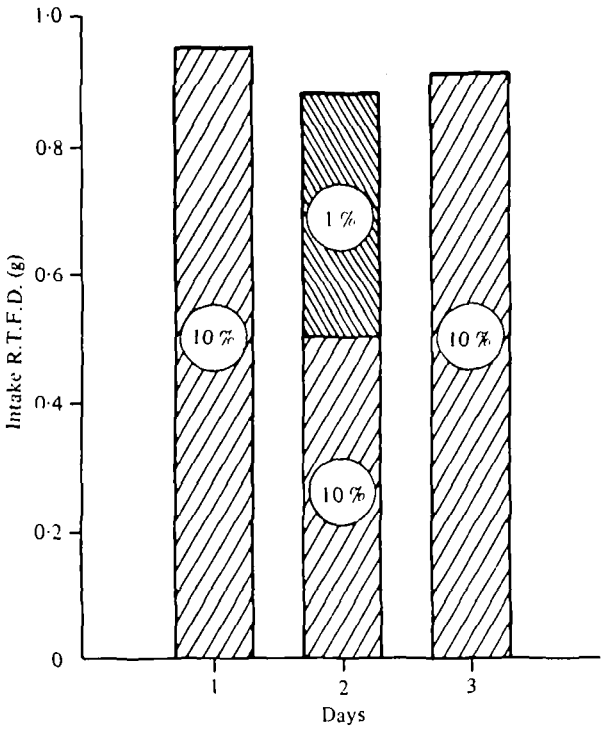

Fig. 10. Total intake of 4 % agar pellets containing either 1 % or 10 % rat tube feeding diet. Pellets containing the two food concentrations were offered simultaneously. Amounts eaten on 10 % RTFD pellets alone are not statistically different from amounts eaten on 1 % plus 10 % RTFD pellets (Mann–Whitney U test).

(ICN Life Sciences Group) artificial diet which is highly attractive and nutritious for slugs (Senseman, 1978)]. The pellets contained either 1% or 10% RTFD, made in a 4% agar matrix. Pellets of each concentration were preweighed, offered side-by-side to individual slugs for a meal (1 h each day), and then reweighed to determine the amount ingested. In the majority of these trials, slugs ate only the higher concentration 10% RTFD pellets. On six occasions, single slugs ate some 1% RTFD pellet plus some 10% RTFD pellet during a 1 h test. We compared the total amount eaten (weight) on days when each of six slugs ate some of both pellets, to the amount eaten by those same slugs the day before and the day after, when only 10% RTFD pellet was eaten. The results are shown in Fig. 10. The amount eaten on days when a slug splits its meal between the pellets is not statistically different from the amount eaten the day before and the day after by that same slug (Mann–Whitney U test, two-tailed). Thus, the total amount eaten (in grams) is also important in terminating a meal, independent of the concentration of the attractive food substance.

Physiological studies

Using the lip-brain-gut preparation, we found that distension of the gut can cause an early termination of FMP.

In Fig. 11, 30 s stimuli with potato extract were presented to the lips every 30 min,

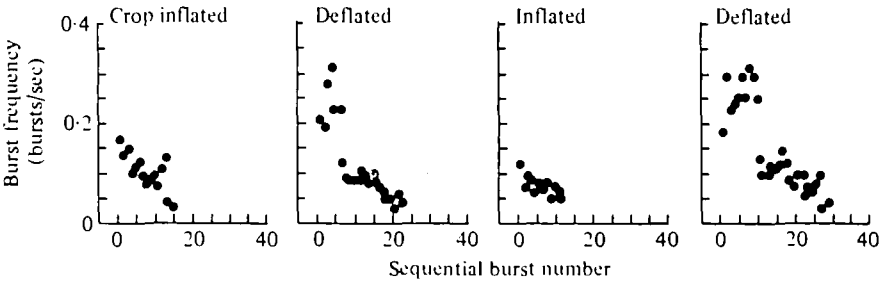

Fig. 11. FMP burst frequency plotted as a function of sequential burst number in four sequences of FMP, for a single lip-brain-gut preparation. Alternate food presentations were accompanied by gut inflation and deflation, as indicated.

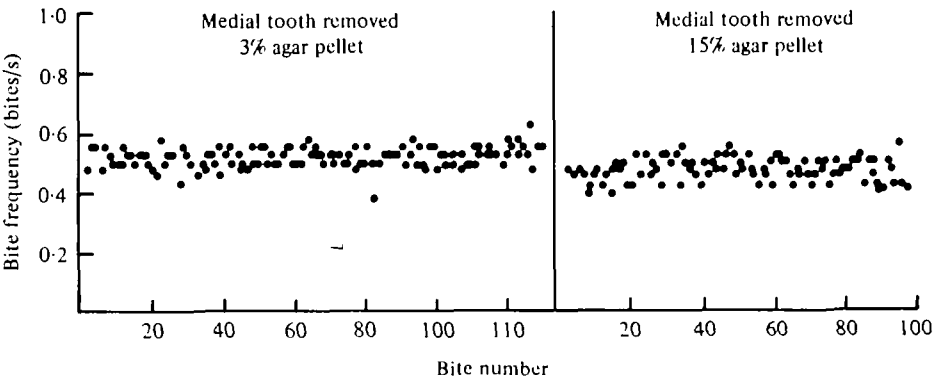

Fig. 12. Bite frequency plotted as a function of sequential bite number for one animal with medial tooth removed. First food presentation was 3 % carrot-agar pellet (soft), followed by 15 % carrot-agar pellet (hard). Data are significantly different, at $P < 0{\cdot}01$ (Mann–Whitney U test), and are not different from sham-operated controls (see text).

with oesophagus and anterior crop alternately inflated and deflated. Both strength (indicated by peak frequency) and duration (number of feeding cycles) were reduced by gut inflation. Similar results were seen in 7 of 9 (78%) preparations.

The ability of standard chemostimuli to elicit FMP is thus affected by the degree of inflation of oesophagus and crop, in a manner analogous to the effects of amount of food ingested on satiation in intact animals.

Medial tooth: a substrate for detecting load on buccal mass during feeding?

Initial behavioural experiments indicated that changes in food hardness had a significant effect on bite cycle frequency (Fig. 4). *In vitro* experiments suggested that loading of the medial tooth produced similar changes in FMP frequency (Fig. 6). To test the role of input from the medial tooth on regulation of FMP frequency in intact animals, the medial tooth was surgically removed in a group of animals. Slugs were anaesthesized with 0·1–0·2 ml of 10 mg/ml succinylcholine chloride in saline, injected into the foot haemocoel (Burton, 1975). In half ($N = 9$), the buccal complex was extruded and the medial tooth surgically removed. In a control group, sham-

Table 2

Variable	Feeding in intact animal	FMP in *in vitro* preparation
Load	Increase food hardness	Increase load on buccal muscle
	↓	↓
	Decrease bite frequency	Decrease FMP frequency and duration
Chemostimulant concentration	Increase food concentration	Increase food concentration
	↓	↓
	Increase meal duration	Increase FMP duration
Gut feedback	Amount of food consumed	Gut inflation
	↓	↓
	Meal termination	Decrease duration and frequency of FMP

operations were performed, in which the buccal complex was extruded, but the tooth left intact ($N = 9$). After 3 weeks, individuals in both groups were fed on soft (3% agar) and hard (15% agar) pellets, in the manner previously described. Soft and hard pellets contained the same concentration of carrot extract as a feeding stimulant. Feeding frequency was monitored and plotted as before. For the experimental animal shown in Fig. 12, feeding frequency on a 3% agar (soft) pellet was still statistically ($P < 0{\cdot}01$) greater than on a 15% agar (hard) pellet. Similar results for 7/9 (78%) experimental animals were seen, regardless of the order of food presentation. In 2/9 (22%) experimental animals, feeding frequency values for the two food types were not statistically different. Post-mortem dissection of the experimental animals confirmed that the medial tooth had not regenerated during the 3-week recovery period. In the control animals, from which the medial tooth was not removed, feeding frequencies recorded during feeding on the soft pellets were again statistically higher ($P < 0{\cdot}01$) than during feeding on the hard pellets.

DISCUSSION

The feeding motor programme in *Limax* is generated by a central neuronal network which has elements in both the cerebral and buccal ganglia (Gelperin & Forsythe, 1976). While FMP can be generated in the absence of any sensory input, normally both external chemosensory input and internal proprioceptive cues are important in modulating feeding activity in intact animals. We have now shown both these types of cues can affect feeding in highly dissected *in vitro* preparations as well (Table 2). Changes in duration of feeding bouts, produced by alterations in chemosensory input and gut feedback, could involve sensory input directly onto feeding motoneurones within the buccal ganglia (Prior & Gelperin, 1977). Changes in FMP frequency, however, as seen in gut distension and buccal mass loading experiments, must involve sensory input onto central neurones involved in the timing of the pattern generator for feeding (Siegler, 1977).

Feeding selectivity in intact animals is based on initial acceptability by olfactory

receptors, followed by acceptability by gustatory receptors. Some foods, such as onion, which might be rejected on the basis of smell are acceptable on the basis of taste if the first sensory modality is by-passed (Kittel, 1956; Gelperin *et al.* 1978). Thus, modulation of feeding by olfactory and gustatory input operate independently. Little is known about the effects of olfactory input on the pattern generator for feeding in slugs. In *Limax*, olfactory input from superior tentacles, caused by chemical or food odours, results in synaptic excitation of the metacerebral giant cells (MGC) (Egan & Gelperin, 1976, 1979). These large, serotonergic interneurones are active during feeding and have modulatory effects on FMP frequency (Gelperin & Chang, 1976). The selective responsiveness to a variety of food odours of tentacle olfactory receptors and subsequent activation of interneurones for feeding have yet to be examined in detail.

The physiological basis for gustatory food selectivity in *Limax* has also not been examined in detail. In the land snail *Helix pomatia*, lip chemoreceptors can be divided into two distinct types, on the basis of ultrastructural data. It has been suggested that such ultrastructural distinctions might form the basis of physiological selectivity for different chemical substances (Benedeczky, 1977).

The present study demonstrates that changes in the hardness of a food substance can result in statistically significant changes in the frequency of feeding cycles in intact *Limax*. Presumably, alteration of food hardness represents a change in load on the feeding apparatus. In a similar study of the banana slug, *Ariolimax californicus*, change in food hardness had little effect on bite frequency during a meal, even though it did alter food 'consumption rate' (meal size/meal duration) and amounts of food eaten per bite (bite volume) (Senseman, 1978). Such a discrepancy between *Limax* and *Ariolimax* may represent a species difference. However, data collected for *Ariolimax* were pooled for many animals, without regard to differences in feeding frequencies between animals, such as we have demonstrated exist in *Limax*. Grouping of feeding frequency data in *Ariolimax*, then, might have obscured significant differences in feeding frequency generated as a function of food hardness. 'Feeding equations' based on such data (Senseman, 1978) may have significance for populations of animals, but can have little predictive value for the behaviour of individual animals.

In *Limax*, behaviourally relevant changes in feeding frequency were approximated in highly dissected preparations by artificially loading the medial tooth and buccal musculature. However, removal of the tooth in intact animals did not prevent shifts in feeding frequency as a function of changes in food hardness. In intact animals, input from other structures [for instance, radula and odontophore, or direct sensory input from buccal musculature (Gelperin *et al.* 1978)] must also have a role in perception of load and regulation of feeding frequency.

Shifts in frequency of cyclic feeding activity have also been detected in *in vitro* preparations of the sea slug, *Pleurobranchaea*, as a result of deafferentation, and as a function of changes in electrical triggering stimuli. As with proprioceptive loading in *Limax*, such changes in feeding frequency are associated with changes in motoneurone burst duration and spike frequency. In *Pleurobranchaea* as well, loading of buccal musculature by distension of the buccal mass may serve to regulate motoneuronal and interneuronal activity (Siegler, 1977), but the means by which sensory input modulates the timing mechanism for feeding cycles is not understood. In the pond

snail *Helisoma*, similar deafferentation studies showed no changes in frequency of feeding rhythms (Kater & Rowell, 1973). However, motoneurone burst durations and spiking frequencies are altered, as in *Limax* and *Pleurobranchaea*. Such changes in motoneurone activity suggest an ability to alter power generated by muscles as they encounter food of different physical characteristics (Kater & Rowell, 1973; Seath, 1977).

In intact, behaving, slugs the chemostimulative quality of the food substance (concentration of attractive food substance) had marked effects on the number of animals which would eat, and on the duration of a meal once feeding had begun. The initial decision on whether or not to eat must have been made at a distance, using olfactory cues, since no contact with the food was made in animals which did not eat. In *Aplysia*, similar distance olfactory discrimination is made by animals which can determine the location of higher food concentrations in a T-maze (Preston & Lee, 1973). Once food has been accepted in *Limax*, if it is of low stimulative concentration, meal durations are short. Thus, gustatory cues are important in maintaining feeding activity, and must be interacting with internal proprioceptive cues (see below) to determine meal duration. Meal duration is also a function of the chemostimulative concentration in *Ariolimax* (Senseman, 1978).

Whereas meal duration may be affected by chemostimulant concentration, frequency of feeding cycles in intact *Limax* is not altered by changes in food stimulant concentration. This is contrary to studies in *Aplysia*, where the chemostimulative concentration of food affects the latency to respond, the inter-bite interval (a frequency measure) and the amplitude of the bite response (Susswein & Kupfermann, 1976). However, to generate a sequence of bite responses in *Aplysia*, food was held just out of reach of an animal. Each successive bite thus represented an attempt to obtain food rather than maintainance of an on-going bout, as was the case with *Limax*.

In highly dissected, *in vitro* preparations of *Limax*, consisting only of lips and brain, different concentrations of food extract had similar effects on FMP as in freely behaving animals. Frequency of rhythmic feeding remained unchanged, but feeding bouts were consistently shorter on food extracts of lower concentration than on those of higher concentration. Using such a highly dissected preparation, it is clear that the length of a feeding bout is in large part determined by the activity of lip chemoreceptors since tentacular olfactory receptors, buccal mass chemo- and mechano-receptors, and gut proprioceptors are removed from the preparation. Whereas input from each of these must contribute to determining duration of feeding activity, primary sensory adaptation or habituation at the level of sensory to inter- or motoneurone interaction certainly play a key role in terminating feeding activity.

The effect of proprioceptive feedback from the gut on meal termination is a well documented phenomenon in both insects (Gelperin, 1967, 1971) and molluscs (Susswein & Kupfermann, 1975*a*, *b*), where gut distention as a result of bulk loading has an inhibitory effect on feeding activity. In *Limax*, this phenomenon can be studied in highly dissected preparations as well as in intact, behaving animals. This raises the possibility of examining the effect of gut distension, via input from the gastric nerves, on activity in motoneurones and interneurones underlying feeding behaviour.

Using behavioural and physiological preparations, we have examined some aspects of feeding behaviour including initiation by chemosensory input, frequency modulation

by load imposition, and regulation of meal duration by both chemical and proprioceptive cues. Each of these is important in modulating the activity of a largely endogenous pattern generator for the feeding behaviour. We have also recently demonstrated a learned modification of FMP (Chang & Gelperin, 1978) analogous to food avoidance learning in the intact animal (Gelperin, 1975). It should be possible to explore the cellular basis of these effects in *in vitro* preparations, in which the behavioural significance of cellular interactions can be determined.

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