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CHEMICAL AND TACTILE INPUTS TO THE *LYMNAEA* FEEDING SYSTEM: EFFECTS ON BEHAVIOUR AND NEURAL CIRCUITRY

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SUMMARY

Transfer of snails from distilled water to solutions of sucrose or maltose stimulated feeding behaviour. As the concentrations were increased from 10^{-5} mol l^{-1} to 10^{-1} mol l^{-1} the proportions of snails showing feeding movements and the median rate of feeding both increased until at 10^{-3} mol l^{-1} they reached a plateau. At higher concentrations (10^{-1} mol l^{-1}), maltose, but not sucrose, reduced the proportion of feeding snails and median feeding rate to values occurring at 10^{-5} mol l^{-1}. Increases in median feeding rate were due to an increase in the regularity of the feeding rhythm rather than increases in the maximum rates of feeding.

Sugars and tactile stimuli were applied to putative sensory structures (interior of the buccal mass, lips and tentacles) in two types of semi-intact preparation whilst intracellular recordings were made from feeding motoneurones (1, 3, 4, 5 and 7 cells) and higher-order interneurones (Cerebral Giant Cells, CGCs, and the Slow Oscillator, SO). Both types of sensory stimuli applied to the lip–tentacle preparation produced strong activation of the CGCs but only long latency weak excitation of motoneurones with no clear initiation of synaptic inputs from central pattern generating (CPG) interneurones. In a more complex lip–tentacle–buccal mass preparation the evoked responses to sugars were comparable to those in the intact snail. Initiation of motoneuronal activity or increased frequency of activity was observed. This was due to increased CPG synaptic inputs as well as activation of a modulatory interneurone, the SO. Multiple sensory pathways from the periphery, present only in the lip–tentacle–buccal mass preparation, were necessary for sensory initiation of the feeding rhythm. The results support the hypothesis that multimodal sensory inputs are likely to be involved in the initiation or modulation of feeding in *Lymnaea* and act at several levels in the system.

INTRODUCTION

Chemical stimuli can initiate both the appetitive and consummatory phases of feeding in gastropod molluscs (see reviews by Croll, 1983; Benjamin, 1983), and

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amino acids and carbohydrates are amongst the more specific components of food likely to be involved. Natural food stimuli also include a mechanical component, and in *Aplysia* (Rosen, Weiss & Kupfermann, 1982) and *Tritonia* (Audesirk & Audesirk, 1979), cross-modality integration of chemical and tactile stimuli is required for initiation of normal ingestion movements.

This paper investigates the role of sensory input in the control of feeding movements carried out by the buccal mass of the snail *Lymnaea stagnalis*. The main emphasis is on chemical stimuli but the effects of tactile stimulation are also investigated. Sucrose and maltose were used because they initiate changes in rhythmic feeding movements in behavioural and electrophysiological experiments (Goldschmeding & Jager, 1973; Rose & Benjamin, 1979). These sugars also attract snails of the related genus *Biomphalaria* (Thomas, 1985). To examine the behavioural role of sugars we measured the 'spontaneous' feeding rate of starved snails and then compared these with snails immersed in sugars of various concentrations. Electrophysiological experiments on semi-intact preparations were then carried out to see which components of the circuits underlying feeding (Benjamin, 1983) were affected by sensory inputs.

In *Lymnaea stagnalis*, the feeding rhythm consists of three phases: protraction of the radula (P) and two phases of retraction (R1, R2), which correspond to rasping and swallowing movements. This rhythm is generated by the interactions of a network of premotor interneurones (N1, N2, N3) (Rose & Benjamin, 1981*b*; Elliott & Benjamin, 1985*a*). Each interneurone fires in only one phase of the rhythm, in the sequence:

$$N1 \rightarrow N2 \rightarrow N3 \rightarrow N1 \ldots$$

which corresponds to the behaviour:

$$P \rightarrow R1 \rightarrow R2 \rightarrow P \ldots$$

because of the connections from the premotor interneurones to the motoneurones (cell types 1–10). For example, the 1 cell is active during protraction (Rose & Benjamin, 1979) and this is excited by the N1 interneurone. On the other hand the 3 cells are active in the rasping phase of protraction and are inhibited by the N1 cells but excited by the N2 interneurones (Rose & Benjamin, 1979, 1981*b*). The 4 cells (and an electrically coupled cluster of cells: 4CL) are swallowing phase motoneurones and these are inhibited by N1 and N2 interneurones, but fire in their correct phase due to post-inhibitory rebound (Rose & Benjamin, 1981*b*; Benjamin & Rose, 1979). Records from all these motoneurones are presented in this paper because their large size facilitates long-term recordings despite the movements of the buccal mass and because their synaptic inputs provide a monitor of the activity in the pattern generating network. However, we have also recorded from two higher-order, modulatory interneurones, the slow oscillator (SO), and the cerebral giant cells. The SO is a single cell located in one buccal ganglion or the other which is capable of initiating, accelerating or maintaining the rhythmical feeding activity (Rose & Benjamin, 1981*a*; Elliott & Benjamin, 1985*b*). The CGCs are paired cells in the

cerebral ganglia which also modulate the feeding rhythm, mainly serving to increase the strength of motoneuronal bursts (McCrohan & Benjamin, 1980*a,b*).

We will show that sugars stimulate feeding movements in the intact snail and cause a corresponding increase in rhythmic activity of motoneurones in the semi-intact preparations. Underlying this is an increase in activity in the central pattern generating (CPG) network while the cerebral giant cells, CGCs, and the slow oscillator, SO, are also excited. Tactile inputs also evoke activity in motoneurones but in a phasic way. In the semi-intact preparations, the effectiveness (latency and strength of response) of the sensory input in evoking feeding rhythms in motoneurones depends on the type of preparation used. The use of different types of preparation allowed us to evaluate the role of a variety of nervous pathways in mediating the motor responses to sensory stimulation. A final question concerns the role of substances likely to inhibit feeding in the snail. Results with quinine showed that this substance inhibited both the feeding movements of the intact snail and also the rhythmic activity of the CPG in the semi-intact preparation.

MATERIALS AND METHODS

Behavioural observations of feeding responses to sugars

Specimens of *Lymnaea stagnalis*, obtained from animal suppliers, were kept in aerated tapwater and fed *ad libitum* on lettuce. Prior to testing with sugars in both behavioural and electrophysiological experiments, the snails were removed to smaller tanks and starved for 2–3 days. Pre-handling prevented withdrawal responses which occurred if snails were transferred to the experimental chamber without prior disturbance. The base of the experimental dish consisted of a concave mirror so that, with illumination of the dish from above, feeding movements could be monitored visually. The feeding movements consisted of cycles of mouth opening and closing with accompanying extension and backward rotation of the radula of the buccal mass. The occurrences of mouth opening and closing were permanently recorded by pressing a switch connected to a pen-recorder. The temperature of the fluid in the experimental chamber was kept constant at 20 ± 1 °C by circulating constant-temperature water through a jacket surrounding the experimental dish, because temperature is an important factor in determining the feeding rate of *Lymnaea* (Dawkins, 1974). This particular temperature was chosen because it approximates to the temperature in the experimental chambers used for electrophysiology.

Preliminary experiments in which a number of sugars were tested showed that sucrose (D form, B.D.H. Analar) and maltose (Sigma) were particularly effective in stimulating feeding movements and these sugars were used in both behavioural and electrophysiological experiments. Five different concentrations of sucrose and maltose were prepared (10^{-1}–10^{-5} mol l^{-1} made up in distilled water) and each concentration was tested separately in order of increasing concentration on individual marked snails from a group of 18. Only one test was performed each day. For comparison, quinine (1 % or 2 % solution of quinine sulphate, Sigma) was tried as a substance likely to inhibit feeding. Feeding movements were recorded for up to

10 min after immersion of the snails in a test solution or distilled water and tapwater controls.

Electrophysiology

Neuronal recording

To investigate how the patterns of electrical activity leading to feeding movements in *Lymnaea* were induced by the application of sugars, we recorded intracellularly from a variety of neurones known from previous work to be involved in feeding in semi-intact preparations. The effects of tactile stimuli and application of quinine (see above) were also examined. Recordings were made from one or more neurones at a time and the preparations maintained in HEPES buffered *Lymnaea* saline (content given in Benjamin & Winlow, 1981, except that glucose was omitted from the standard formula). Electrodes were drawn from 2 mm capillary tubing (Clark Electromedical) and filled with 1 mol l^{-1} lithium sulphate or saturated potassium sulphate, giving tip resistances of 30–80 MΩ. Signals were amplified with Neurolog or Bioelectric FET amplifiers and fed directly to a pen-recorder.

The semi-intact preparations

In preliminary experiments a variety of semi-intact preparations was tried. These had to allow recording of feeding interneurones and motoneurones as well as providing putative sensory structures and nerve pathways to allow the effect of sugar solutions and other stimuli to reach the central neurones involved in rhythm generation. We had no precise knowledge of the sensory structures likely to be involved in the initiation of feeding in *Lymnaea*, although a variety of data from freshwater snails and other gastropods (reviewed by Croll, 1983) suggested that the lips, tentacles and buccal mass cavity were likely to be involved. Perfusion of sugars through the cavity of the buccal mass alone, by cannulation, proved to be ineffective in activating feeding and the lips in particular needed to be stimulated for feeding activity to be affected by sensory input. Two basic types of semi-intact preparations were eventually used and these both included the lips and tentacles.

Lip–tentacle preparations (Fig. 1A)

Goldschmeding & Jager (1973) showed that one of the pairs of nerves innervating the lips and anterior part of the head, the median lip nerves, were necessary for feeding to be initiated in *Lymnaea*. This result led us to develop our first type of semi-intact preparation which consisted of the lips and tentacles (with some other head tissue) separated into two halves by cutting down the midline and removing most of the foot. These were connected to the cerebral ganglia *via* the left and right median lip nerves. All other peripheral nerves were cut. The buccal ganglia were left connected to the rest of the brain *via* the paired cerebrobuccal connectives (Fig. 1A) but the buccal mass was absent. This meant that the only pathway to the buccal ganglia was *via* the cerebral ganglia and cerebrobuccal connectives. Sugar solutions of the same type as used for the behavioural experiments were added to the left or

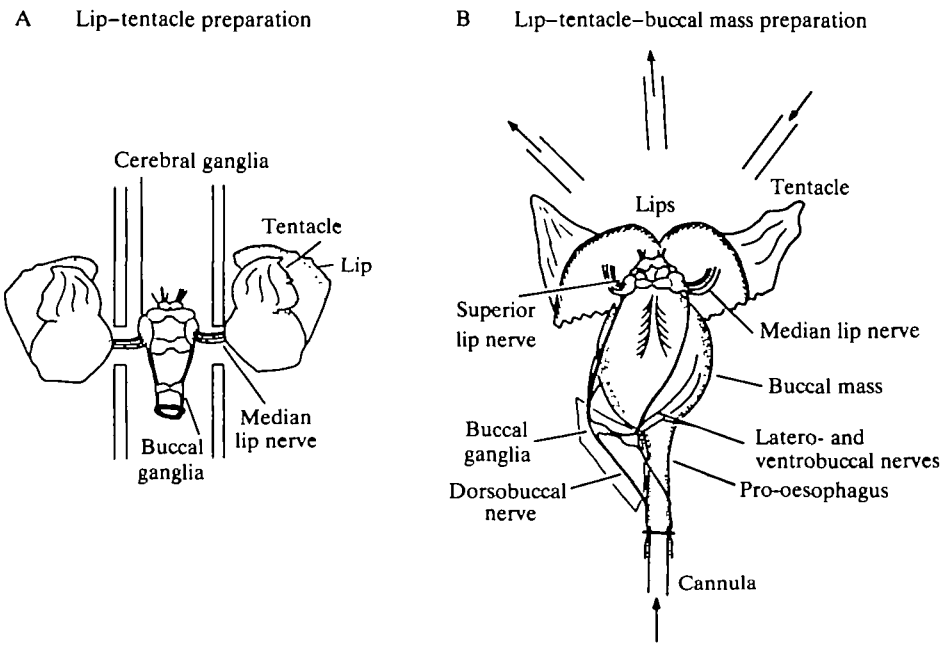

Fig. 1. Semi-intact preparations used for electrophysiology. (A) Lips and tentacles were divided into left and right halves and mounted in separate chambers on either side of a central chamber containing the brain and buccal ganglia. Only the median lip nerves connected the cerebral ganglia to the periphery. (B) Lips, tentacles and buccal mass were retained as a single piece together with nerves to the periphery from cerebral ganglia (superior and median lip nerves) and buccal ganglia (laterobuccal, ventrobuccal and dorsobuccal nerves). Sugars were pumped *via* a cannula placed in the pro-oesophagus through the cavity of the buccal mass and out over the surface of the lips and *via* a second tube directly onto the lips. Two further tubes were used to remove the sugar solutions. The buccal ganglia were pinned to a Sylgard platform.

right (or both) lip–tentacles located in the lateral segments of the experimental dish (Fig. 1A) by simply pipetting solutions onto the putative sensory surfaces kept damp with saline. Tactile stimuli were applied by stroking with a fine brush. Vaseline was used to separate the neural structures and lip–tentacles into three chambers, preventing direct stimulation of central neurones, although there was some possibility that the nerve trunks in the lateral chambers might have been directly affected.

Seventy-one experiments were carried out using this preparation. Responses in the cerebral ganglion CGCs were strong and consistent to both chemical and tactile stimuli but only rarely was the central pattern generator activated strongly enough to recall the characteristic feeding activity in buccal motoneurones reported by Benjamin & Rose (1979). This led to the development of a second type of preparation which included the buccal mass and a more complete pattern of nervous innervation, and was much more successful in obtaining activation of the feeding rhythm.

Lip–tentacle–buccal mass preparation (Fig. 1B)

This was a much more complex preparation of the anterior part of the snail, in which the buccal mass, lips and tentacles were retained. All the anterior body

innervation from the cerebral ganglia was retained including the paired median and superior lip nerves. The main nerves innervating the buccal mass and pro-oesophagus (the paired laterobuccal, ventrobuccal and dorsobuccal nerves, see Benjamin, Rose, Slade & Lacy, 1979) which arise directly from the buccal ganglia were also present. The inclusion of all these structures in one chamber (without separation of left and right halves as in Fig. 1A) meant that a more sophisticated method of chemical perfusion using peristaltic pumps had to be developed. One cannula was inserted and ligatured into the pro-oesophagus and test solutions or saline were pumped by a peristaltic pump through the cavity of the buccal mass and out across the lips. Solutions were also pumped directly across the lips using a second channel. Two other tubes were used to suck solutions away from the mouth and out of the experimental chamber, thus preventing perfusion of the solutions containing test substances over the ganglia. In fact direct application of sucrose and maltose (but not quinine) to central neurones had no obvious effect on electrical activity.

The presence of the buccal mass meant that feeding movements (as opposed to egestion movements sometimes caused by inserting the cannula into the oesophagus) could be visually observed and recorded on an event recorder. In a few experiments a micropipette was placed over the muscle surface to record muscle movements directly. A change in potential accompanied each movement, presumably due to a mechanical occlusion of the electrode tip.

In the 91 experiments using the second type of preparation most were recordings from buccal motoneurones but we also made the SO a particular focus of attention. Long-term recordings from this small neurone were difficult in the semi-intact preparation and were usually achieved only if the posterior part of the buccal mass was restrained by a Sylgard bridge.

RESULTS

Behaviour

Feeding in *Lymnaea* was stimulated by sugars in the environment. In distilled water 30 % of snails showed 'spontaneous' feeding movements, but even 10^{-5} mol l^{-1} sucrose was sufficient to raise the proportion of feeding snails to 60 % (Fig. 2A, filled circles). As the sugar concentration was increased so also did the proportion responding, reaching a plateau of approximately 90 % at 10^{-3} mol l^{-1} sucrose (Fig. 2A). Not only did sucrose stimulate feeding in previously quiescent snails but it also increased the feeding rate in spontaneously feeding snails. In distilled water this was 1 bite min^{-1} but with addition of sucrose it rose from 7·5 bites min^{-1} at 10^{-5} mol l^{-1} to 16 bites min^{-1} at 10^{-2} mol l^{-1} (Fig. 2B, filled circles). These maximum rates corresponded to those reported by Dawkins (1974) when *Lymnaea* was feeding on algal films.

With maltose in the experimental dish, the same phenomena were observed (Fig. 2A,B, open circles). The concentration-dependent effects of both sugars were not significantly different (Wilcoxon interval test, $N = 89$, $P > 0{\cdot}05$) except

at 10^{-1} mol l^{-1}, where maltose (but not sucrose) inhibited feeding. Although at 5×10^{-2} mol l^{-1} maltose, 95 % of snails showed feeding movements with a median rate of 14·7 bites min^{-1}, in 10^{-1} mol l^{-1} maltose this occurred in only half the snails, the median feeding rate being 7·2 bites min^{-1}. Thus 10^{-1} mol l^{-1} maltose was only as effective as 10^{-5} mol l^{-1} maltose.

The detailed patterns of feeding movements in tapwater were compared with those in 10^{-2} mol l^{-1} sucrose (Fig. 3). This showed that although the median rate of feeding increased in sucrose, there was no simple change from the lower level of activity to the higher one. In tapwater there were periods of activity (Fig. 3A) when instantaneous feeding rates were as high as in sucrose but over a period of several minutes this varied considerably and gaps of several seconds without any feeding movements occurred. In sucrose (Fig. 3B) the feeding movements of the same snails were more regular and this gave the overall increase in median feeding rates already described. Quantitative analysis of the raw data of Fig. 3A showed this difference in regularity of the rhythms much more clearly (Fig. 3C). In tapwater the instantaneous feeding rate was significantly more variable than in sucrose (variance ratio,

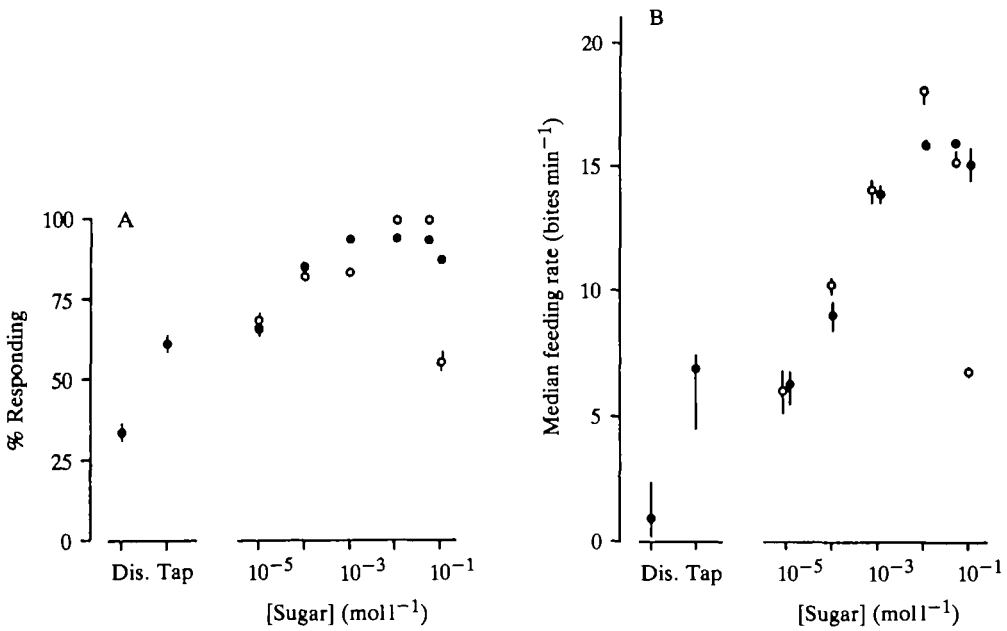

Fig. 2. The behavioural response of *Lymnaea stagnalis* to sugars. The same group of 18 snails was placed in distilled water (Dis.), Brighton tapwater (Tap) or a solution of sucrose (solid circles) or maltose (open circles) and feeding movements recorded during the first minute. (A) The percentage of snails which made feeding movements. The vertical bars indicate ±1 standard error. Where no bars are shown then this is less than the diameter of the points. (B) The median feeding rate (all snails included). The vertical bars indicate the 33 % confidence interval of the mean, calculated using a computer procedure that corresponds to the Wilcoxon signed rank test (Minitab Statistics Package). (Note that ±1 standard error normally corresponds to this 33 % confidence interval.) Increasing sugar concentrations resulted in more snails responding and at higher feeding rates, except for 10^{-1} mol l^{-1} maltose.

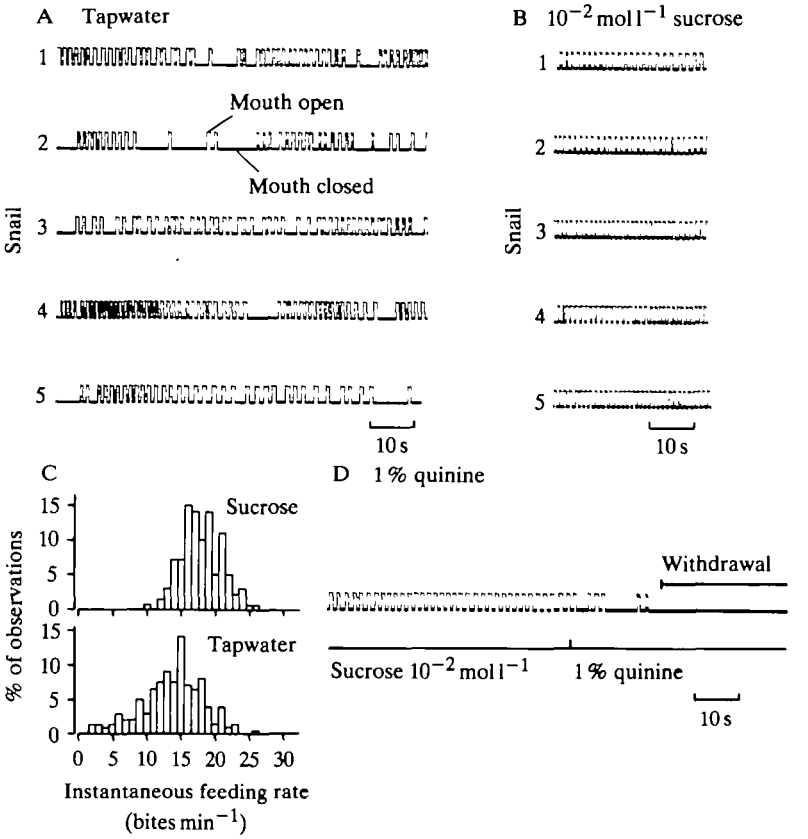

Fig. 3. The feeding behaviour of *Lymnaea* in tapwater, 10^{-2} mol l^{-1} sucrose and 1 % quinine. The same five snails were placed in Brighton tapwater (A) or 10^{-2} mol l^{-1} sucrose (B) and each feeding movement was recorded. In tapwater feeding was irregular and bouts of feeding were often interspersed with pauses but in sucrose the feeding rate was much more uniform at 15–20 bites min^{-1}. (C) shows the data in (A) and (B) plotted to show the frequency of occurrences of instantaneous feeding rates. (D) shows the cessation of feeding caused by pipetting 1 % quinine close to a snail immersed in sucrose; after 20 s the snail started to withdraw into its shell.

$F = 2{\cdot}06$, $P < 1\,\%$) but the maximum feeding rates did not differ (26 bites min^{-1}). In sucrose, feeding rates of less than 13 bites min^{-1} (half the maximum feeding rate) were observed rarely, but they constitute over one-quarter of all observations in tapwater. Thus with tapwater there were many more occurrences of low instantaneous feeding rates reflecting the long gaps between bites seen in Fig. 3A. These results show that sugar not only increases feeding rate but also increases its regularity.

Fig. 3D shows that quinine is a very powerful inhibitor of feeding movements in *Lymnaea*. High frequency feeding movements in 10^{-2} mol l^{-1} sucrose were rapidly inhibited by 1 % quinine. This was followed by a withdrawal of the snail into its shell (Fig. 3D).

This analysis of the behavioural effects of sugars and quinine on the whole animal allowed us to select concentrations of sugars likely to initiate feeding in the semi-intact preparation used for electrophysiology. We mostly used 10^{-1} mol l^{-1} sucrose

but also maltose at 10^{-2} mol l^{-1} and 10^{-1} mol l^{-1} concentrations. Quinine (1 %) was also tried, to see if spontaneous electrical activity could be inhibited.

Electrophysiology

Lip–tentacle preparations

Neurones recorded in semi-intact preparations showed a variety of spike activity in the absence of any applied sensory stimulation as reported previously (Benjamin & Rose, 1979; Rose & Benjamin, 1979). Motoneurones could be silent (Fig. 6A) show bursts (Fig. 6B) or tonic activity (Fig. 7A) in the absence of applied sensory stimulation. Equally, the SO sometimes had a stable, constant membrane potential (Fig. 6C), but at other times showed rhythmically occurring synaptic inputs and occasional spikes (Fig. 8A, arrow). The CGCs, however, showed much less variation and fired tonically in almost all preparations (Figs 4, 5).

CGCs. This pair of giant cells located in the cerebral ganglia showed the most striking responses of all the cells recorded in the lip–tentacle preparation. They showed short latency (less than 1 s), excitatory responses to both chemical and mechanical stimulation (Figs 4, 5). The tactile response was most reliable, occurring in 24 out of 27 preparations, whilst chemically-mediated responses to 10^{-1} mol l^{-1} sucrose were seen in 16.

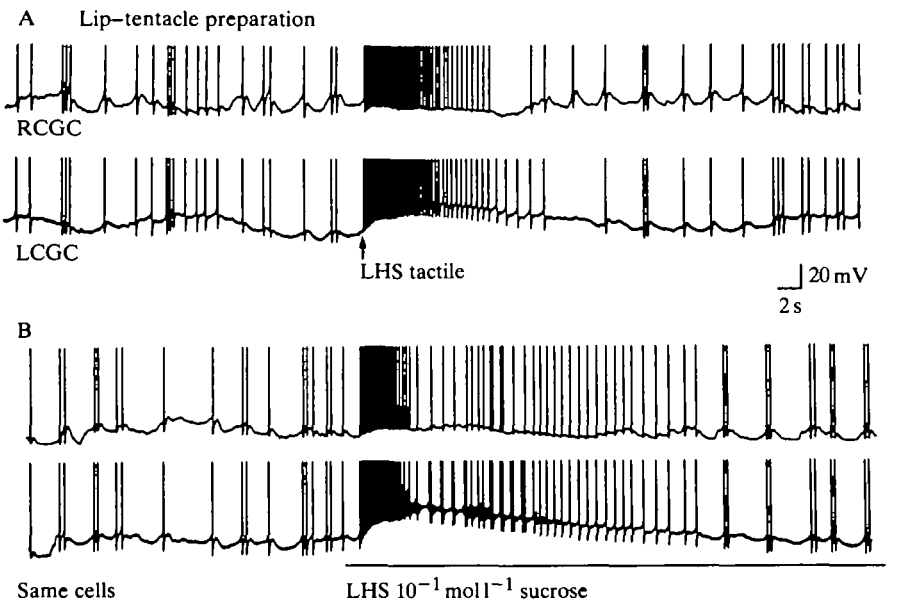

Fig. 4. Responses to sensory stimulation of left (L) and right (R) Cerebral Giant Cells (CGCs) recorded in the lip–tentacle preparation. (A) Transient tactile stimulation of the left-hand side (LHS) lip with a soft paint brush. (B) Stimulation of the LHS lip with a 10^{-1} mol l^{-1} sucrose solution applied from a pipette. Both types of stimuli excited the cells with responses greater in the ipsilateral cell. Sucrose-induced responses were stronger and longer lasting than those produced by a single brush stroke.

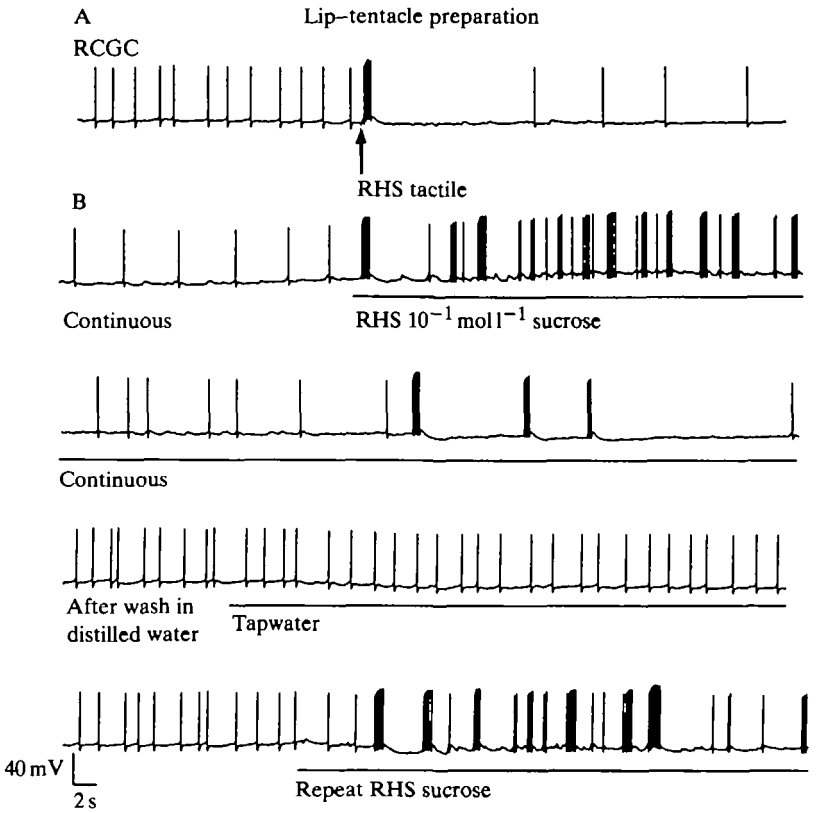

Fig. 5. Burst activity induced in a right Cerebral Giant Cell (RCGC) by application of sucrose to the lip–tentacle preparation. (A) shows a single burst of spikes following a single brush stroke applied to the lips on the right-hand side (B). This was followed by continuous RHS perfusion of sucrose which first induced a sequence of bursts that gradually waned to single spiking or occasional bursts. Initial background single spiking returned after distilled water application. No response to tapwater was observed but a repeated application of sucrose again induced bursting.

A single brush stroke applied to the lips or tentacles evoked a burst of spikes lasting from 2 s (Fig. 5A) up to 18 s (Fig. 4A). Simultaneous recordings from left and right CGCs showed that responses lasted longer in the cell ipsilateral to the site of stimulation compared with the contralateral one (Fig. 4A). Underlying this was a depolarizing wave which was initially higher in amplitude and greater in overall duration in the ipsilateral CGC. Many of the spikes in the contralateral cell could be due to the strong electrotonic coupling of the cells in the buccal ganglia (McCrohan & Benjamin, 1980*a*).

Chemical stimuli were applied to the bath and left for periods of more than 1 min. This caused a longer lasting increase in activity than a single mechanical stimulus (compare Fig. 4B with Fig. 4A), but even with continuous applications of the sugar, increases in spike rate gradually decreased with time (after 30–40 s in Fig. 4B). Part of the initial depolarizing response to chemical application was probably due to mechanical effects caused by pipetting the solution onto the lips but the greater initial

response and its persistence compared with the purely mechanical stimulus showed that the chemical stimulus was having an important effect of its own. This is confirmed in Fig. 5 where the depolarization resulting from the application of sucrose was compared with a control application of tapwater which produced no response. In Fig. 4 the stronger depolarizing effect on the ipsilateral CGC caused a breakdown in the normal 1:1 firing of left and right CGCs (due to electrotonic coupling, cf. McCrohan & Benjamin, 1980*a*).

Chemical stimulation usually just increased the tonic firing rate of the CGCs but in a few preparations (3 out of 16) burst activity was induced. This was particularly clear in the preparations shown in Fig. 5 where the effects of tapwater and mechanical stimulation were compared with 10^{-1} mol l^{-1} sucrose in the same cell. Tactile stimulation caused a strong burst of spikes and activity tended to be partially inhibited until sucrose was applied. This induced bursts, doublets or triplets which gradually reduced in frequency with time. Washing with distilled water brought activity back to control levels and further addition of tapwater caused no obvious change in frequency or pattern of activity. Finally, sucrose was reapplied and again induced burst activity showing that the response was reliable.

Motoneurones and SO. Over 70% of motoneurones (3, 4 and 4 CL cells, $N = 29$) and three out of five SO interneurones responded to chemical and tactile stimuli. Sucrose at 10^{-1} mol l^{-1} concentration induced spike activity in silent motoneurones (Fig. 6A) or increased activity in already firing motoneurones (Fig. 6B), but the latency to the responses was much greater (25–35 s) than for the CGCs (about 1 s). Feeding patterns were only rarely induced: instead the cells were steadily depolarized (Fig. 6A,B). Although this produced some burst activity in silent preparations (Fig. 6A) or increase in spike frequency (Fig. 6B), in neither example shown in Fig. 6A,B did the three cells show the pattern of bursting characteristic of feeding in the semi-intact preparation reported by Rose & Benjamin (1979). In the silent 3 cell of Fig. 6A, 10^{-1} mol l^{-1} sucrose caused irregular bursts of varying duration. Certainly during these bursts, occasional inhibitory or excitatory inputs occurred which resembled 3 cell N1 and N2 inputs but the usual sequences of N1 > N2 > N3 inputs (shown at the beginning of the top trace in Fig. 6B) never occurred. In Fig. 6B the cell was already active and the 3 cell was showing weak but typical rhythmical feeding activity with the usual CPG inputs. Sucrose (10^{-1} mol l^{-1}) caused a strong, steady depolarization of the cell which produced more intense bursts, but the normal pattern of activity was disrupted. There was no obvious straightforward increase in CPG frequency. Fig. 6C shows one of the few examples where weak activation of the central pattern generator occurred. In this case a 3 cell was recorded with an SO and 10^{-1} mol l^{-1} maltose was added to the lips. The overall level of activity in the 3 cell of Fig. 6C was much less than with sucrose shown in Fig. 6A,B, but some weak burst activity was seen in the 3 cell, with a few spikes in the SO cell, after a delay of about 20 s (in Fig. 6C there is also a much shorter latency response in both cells, probably due to mechanical stimulation). In both cells synaptic inputs were appropriate for the normal activation of the CPG. It was interesting that during the chemically-induced part of the response the SO was the

first to show activity following the chemical stimulus. This is likely to be due to synaptic input from the N1 interneurones which excite the SO (Elliott & Benjamin, 1985*b*).

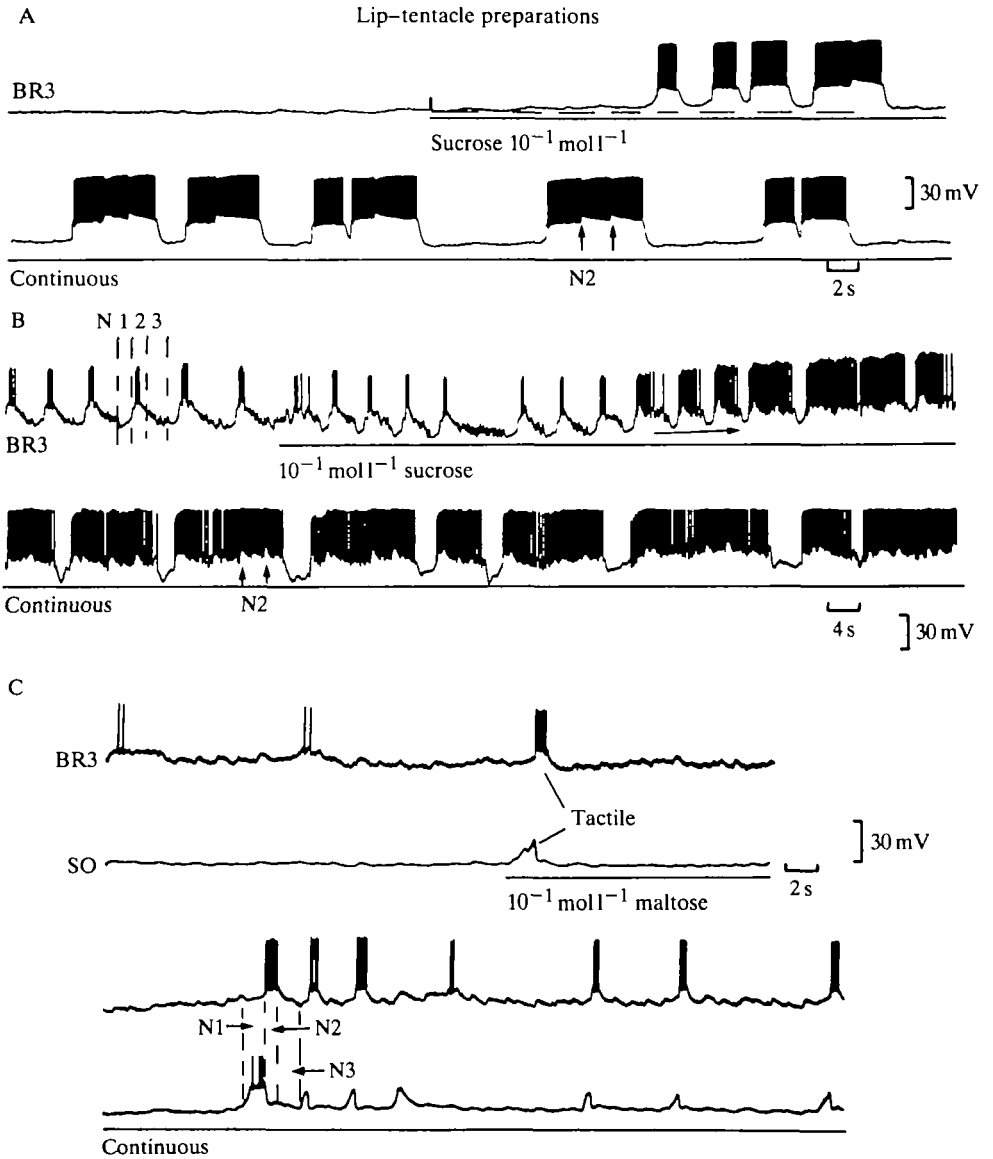

Fig. 6. Motoneuronal and Slow Oscillator (SO) responses to sugar and tactile stimuli in the lip–tentacle preparation. Sucrose induced bursts of spikes in a quiescent (A) or already active (B) 3 cells (BR3). This resulted from a general depolarization of the cells (dashed line in A, horizontal arrow in B) rather than changes in activity in CPG interneurones. (C) is one of the few examples where limited activation of the CPG occurred. This initial response in the 3 cell and SO is a mechanical one due to the application of sugar solution to the preparation. Later maltose induced bursts of spikes or synaptic inputs to both cells which were due to CPG inputs (N1, N2, N3).

Mechanical stimulation of the lips and tentacles produced short latency phasic excitation of motoneurones. This is shown for the 4 cells in Fig. 7A. If the excitation of 4 cells was compared with the CGCs recorded at the same time it could be seen that the period of excitation coincided (Fig. 7A). As the CGCs directly excite the 4 cells by monosynaptic connections (McCrohan & Benjamin, 1980*b*), the depolarization of the 4 cells could well be due to activation of the CGCs first and their subsequent excitation of the 4 cells. Fig. 7B shows from a separate experiment that injection of RCGC with a step of depolarizing current caused coactivation of the LCGC and excitation of the 4 cells recorded at the same time. Thus the 4 cell excitation shown in Fig. 7A can be accounted for by synaptic effects from the CGCs. However, tactile inputs also appeared to have access to the buccal ganglia CPG interneurones because Fig. 6C showed that the initial tactile response caused by application of sugar to the lips produced a smooth wave on the 3 cell followed by a sharp depolarization leading to spikes. Synchronized with this was a depolarization of the SO, followed by inhibition. N1 interneurones characteristically produce such smooth waves in the 3 cell and excite the SO, while N2 interneurones produce strong excitation of the 3 cell and inhibit the SO (Elliott & Benjamin, 1985*b*) so that it appears that one cycle of N1 and N2 inputs was induced by tactile input.

Another example of SO depolarization due to probable tactile stimulation is shown in Fig. 8A (arrowed). Again it appears that N1 input from CPG interneurones is responsible and in this case spikes were evoked. The example is particularly interesting because the tactile response (due to the initial application of quinine solution) is opposite in effect to that finally produced by chemical stimulation. Thus N1 input is probably enhanced by tactile effects but all CPG input (including N1) is inhibited by the longer latency chemical effect (see next paragraph). It appears that mechanical stimulation can excite several types of interneurones (CGCs and CPG interneurones) which in turn influence feeding motoneurones. Direct synaptic effects from mechanoreceptors to motoneurones may also occur but these seem unlikely on the basis of the present evidence.

Both sugars and tactile input caused excitatory effects on 3 cells and 4 cells and SO but quinine had a dramatic and opposite effect. This is shown in the example of Fig. 8, where a 3 cell and SO were recorded at the same time. In this spontaneously active preparation, the 3 cell was showing strong burst activity with the usual N1, N2, N3, CPG inputs. The SO was usually inactive although occasional spikes occurred during N1. Addition of 2% quinine to the right side lip–tentacle (Fig. 8A) caused a partial inhibition of the 3 cell's activity and a slowing in the CPG rhythm on both 3 cell and SO. In Fig. 8B right-hand side applications of quinine were continued but in addition quinine was added to the left side. In this case activity in the 3 cell was completely inhibited and CPG activity ceased in both cells. However, in both Fig. 8A and 8B the inhibitory effect of quinine was only temporary and only lasted for a maximum of 35–40 s. An interesting feature of the SO records is that recovery from inhibition was accompanied by more regular spikes in the SO during the N1 phase of the feeding cycle. The latency of the inhibitory response to quinine

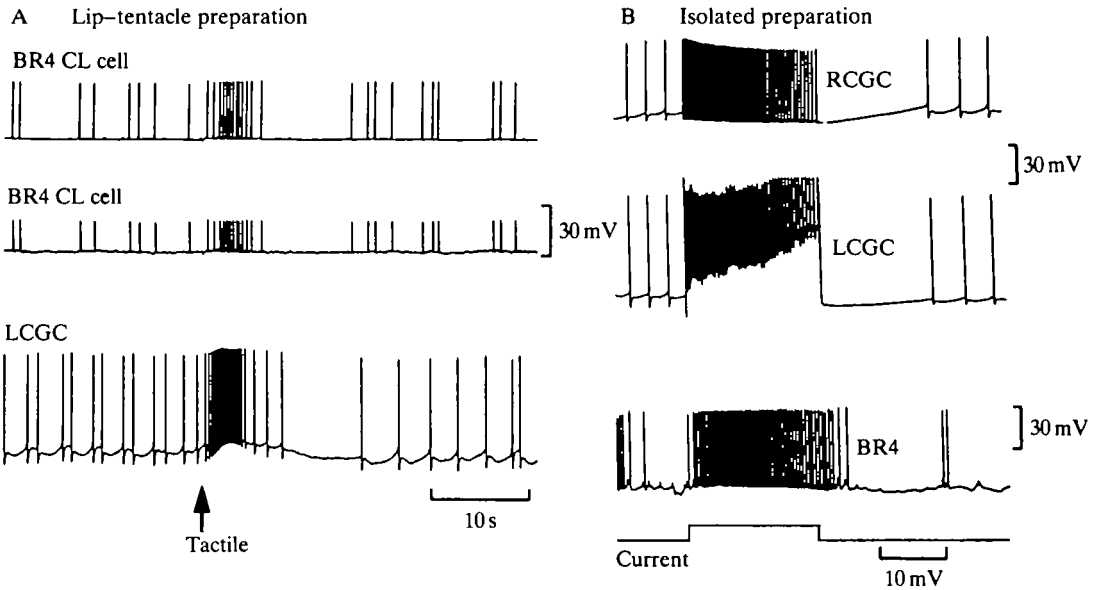

Fig. 7. (A) Tactile response to a brush stroke applied to the lips in a left Cerebral Giant Cell (LCGC) and two motoneurones (4 CL cells) (BR4 CL) in the lip–tentacle preparation. A burst of spikes was induced in all three cells. (B) Direct activation by current injection of the LCGC induced a similar burst of spikes in the 4 cell (BR4) and in the opposite right (R) CGC. This can be explained by the monosynaptic excitatory synapse between CGCs and 4 cells (McCrohan & Benjamin, 1980*b*).

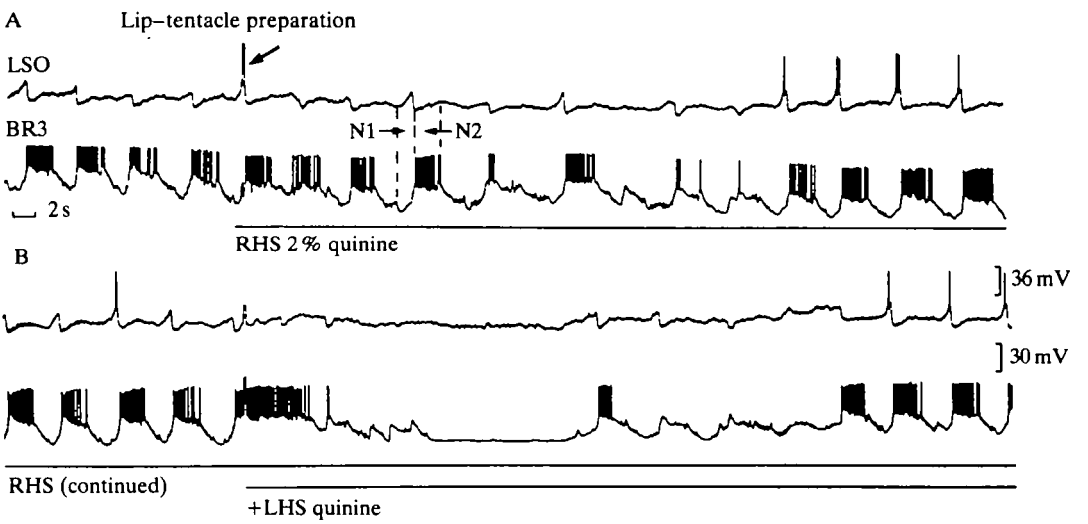

Fig. 8. Inhibition of a spontaneous feeding rhythm by 2% quinine in the lip–tentacle preparation. (A) Right-hand side (RHS) application of quinine to the lips and tentacles caused a partial inhibition of bursting in the 3 cell motoneurone (BR3) and a slowing of CPG synaptic inputs to the Slow Oscillator (SO). (B) Addition of quinine to the lip–tentacle preparation on the left-hand side (LHS) together with continued application to the RHS completely inhibited bursting in the 3 cell with a clear absence of CPG inputs (N1 and N2 marked in A) in the 3 cell and SO. Later in the record bursting in the 3 cell recommenced with single spikes also occurring in the SO during the N1 phase of the CPG rhythm.

(10–15 s) was less than for sugars (25–35 s) but still much greater than for mechanical stimuli (1 s).

We conclude from this section that the median lip nerves *via* their projection to the cerebral ganglia alone cannot mediate the strong effects on feeding movements caused by sugars in the intact snail, despite the strong excitatory effects caused on the CGCs. Sugars tended to cause a general depolarization of the motoneurones, but changes in burst frequency with increases in underlying CPG activity rarely occurred. Mechanical stimuli could excite motoneurones strongly, probably by indirect effects on interneurones. The most significant effect was excitation of the CGCs, but this was insufficient to turn on the feeding rhythm. Quinine was the most effective chemical stimulus used in the lip–tentacle preparation and its ability to inhibit CPG activity appeared to be responsible for inhibition of feeding movements occurring in the intact snail.

Lip–tentacle–buccal mass preparation

Addition of sugars to this preparation produced much more striking changes in activity which were at least qualitatively similar to those occurring in the behavioural experiments. Sugars perfused through the buccal mass and across the surface of the lips induced an increase in the rhythmic contractions of the buccal mass and burst activity in motoneurones (Figs 9, 10). We mainly examined the details of this increase in motoneurone activity but we also showed that the SO was excited under the same circumstances.

Motoneurones. Sucrose (10^{-1} mol l^{-1}) had excitatory effects on 70% of preparations. In already active preparations it increased the frequency of the feeding rhythm (Fig. 9A,B, 5 cell, Fig. 10A) and examination of the synaptic potentials showed that the usual sequence of CPG inputs was present. In completely quiescent preparations CPG inputs and bursting activity in motoneurones were induced *de novo* by application of sugar (Fig. 10B). An intermediate situation occurred in some preparations where some CPG activity was present before sugar perfusion but it was not accompanied by firing in the motoneurone. Here sucrose induced an increase in frequency of the feeding rhythm and a burst accompanied each cycle of CPG input (Fig. 9B, 4 cell).

Changes in burst activity occurred within a few seconds of the start of sucrose perfusion unlike the lip–tentacle preparation where 25–30 s elapsed before effects were seen (see Fig. 6). In experiments where maltose and sucrose were compared in the same preparation sucrose always produced stronger effects than maltose. In Fig. 10B only 10^{-1} mol l^{-1} sucrose could induce rhythmic spiking in the 4 CL cell and 7 cell and maltose at 10^{-1} mol l^{-1} was ineffective. This is similar to the differential effects produced by these sugars shown in behavioural experiments (Fig. 2).

One consistent feature of the response to sucrose was the gradual waning of the response with time with continuous sugar perfusion (Fig. 9B). The stimulating effects of sucrose lasted for a maximum of 2 min but with repeated sequences of

perfusion and wash the responses were often much shorter. This waning of response was not seen in the behavioural experiments except for an initial slight decrease in frequency that occurred in some snails (see Fig. 3B, snail 4).

Another difference between the intact snail and the semi-intact preparation used here was that the maximum frequency of the burst rhythm to 10^{-1} mol l^{-1} sucrose was usually much less (4–5 bursts min^{-1} compared with 16–18 bites min^{-1} in Fig. 2). Occasionally, with the buccal mass completely unrestrained, rhythms within the range shown by the intact snail were seen and an example of this is shown in Fig. 10A. Here instantaneous bursting rates of between 10 and 17 bursts min^{-1} occurred in the first 30 s of sucrose application, although this again decreased with

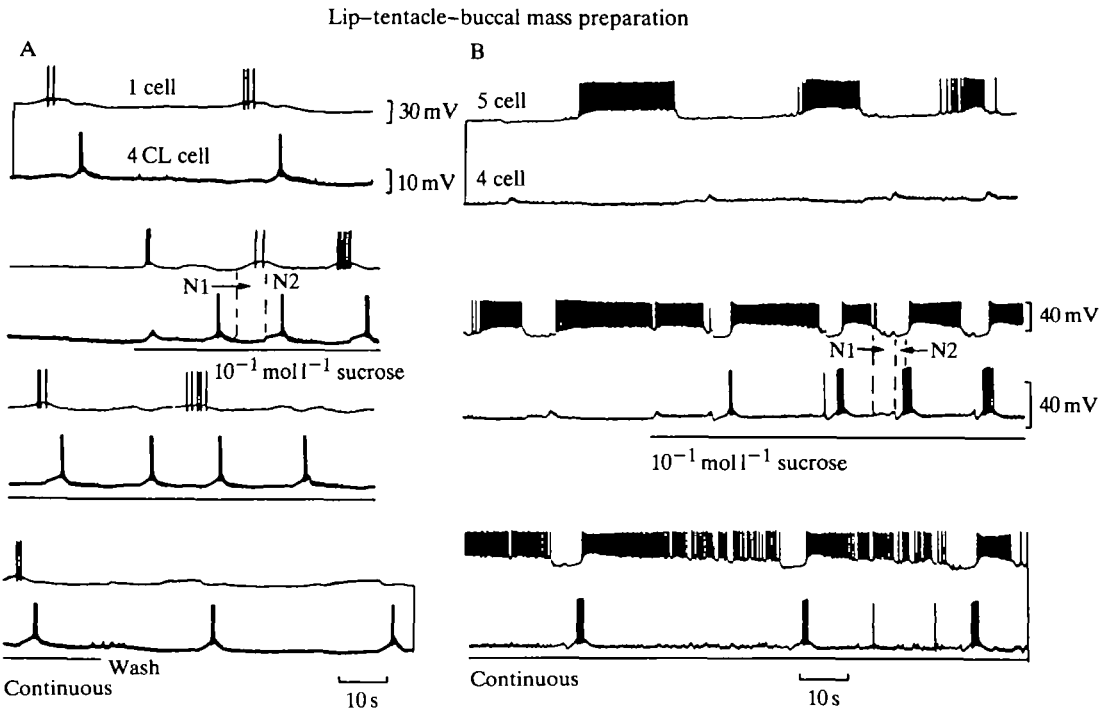

Fig. 9. Activation of the feeding rhythm in motoneurones by the application of 10^{-1} mol l^{-1} sucrose to the lip–tentacle–buccal mass preparation. (A) In this spontaneously active preparation the burst frequency of the two motoneurones (1 cell and 4 CL cell) was increased about threefold by the perfusion of sucrose. Underlying this was an increase in frequency of the rhythm of synaptic inputs (N1 and N2) from CPG interneurones. Washing reduced the frequency of the rhythm to prestimulus levels. (B) Sucrose activated the silent 4 cell and increased the frequency of bursting of the 5 cell. Again an increase in the frequency of the underlying CPG rhythm was responsible for the change in spike activity of the motoneurones. Note that although the 4 cell was initially silent it did show spontaneous inputs from the CPG, which were ineffective in generating spikes. Small depolarizing potentials are probably electrotonic EPSPs due to spikes in other more active 4 CL cells (Benjamin & Rose, 1979).

time during continuous perfusion. The stimulatory effect of sucrose was very striking in this preparation and a saline wash reduced activity and CPG inputs considerably.

Mechanical responses also occurred in about 70% of preparations. These were evoked by a bubble passing through the perfusion system following a change of solution and preceded any effect from the chemical stimulus. For instance, the first strong burst of spikes after sucrose began to be perfused in the 1 cell of Fig. 9A was probably due to a mechanical effect. In general, mechanical responses were similar to those in the lip–tentacle preparation. Thus short latency, phasic excitatory responses were seen in 4 cells, 3 cells and 1 cell. Again it seemed likely that interneurones like those of the CPG were being activated rather than the motoneurones themselves. Evidence for this is shown in Fig. 11 where mechanical stimulation excited the 3 cell but caused an inhibitory wave in the 7 cell. This would be expected if the N2 cells of Rose & Benjamin (1981*b*) or Elliott & Benjamin (1985*a*) were being excited.

The SO. In a small number of experiments ($N = 9$) we were able to record the SO long enough to test thoroughly its response to chemical and tactile stimuli. In six preparations it was shown that the SO was depolarized by both chemical and tactile input (Fig. 12A,B), and in two of these rhythmic activity was evoked (Fig. 12C,D). A single tactile stimulus caused a strong phasic depolarizing response upon which spikes (truncated by the pen-recorder) were superimposed (Fig. 12B). Perfusion of 10^{-1} mol l^{-1} sucrose depolarized the SO but in this preparation (shown in Fig. 12A) no spikes in the SO or CPG synaptic inputs on either cell were obtained. The 4 cell did fire fairly continuously during sucrose perfusion, perhaps due to CGC activation (see Fig. 7B). This could also account for the depolarization seen on the SO because the CGCs are also known to excite the SO weakly (Benjamin, McCrohan & Rose, 1981). In the preparation shown in Fig. 12C, rhythmic spike activity was induced in the SO and rhythmic synaptic inputs also occurred on the motoneurone (7 cell) recorded at the same time. It is very clear in this record that activity in the SO occurs before (or at least at the same time as) the CPG activity begins on the 7 cell. (Note that there are no direct synaptic connections between the SO and 7 cells; Rose & Benjamin, 1981*a*.) After the first burst in the SO, only a weak N2 inhibiting input occurred but after the second and third SO burst clear N1, N2 and N3 inputs were obvious on both cells. This suggested that the SO was being activated by sugar at an early stage of the changes induced in the feeding system. However, weak activation of the feeding rhythm in motoneurones did not necessarily require SO activity because in Fig. 12D the CPG input to the 4 CL cell and SO (one cycle of N2 inhibition on both) occurred before any spike activity in the SO and this was accompanied by rhythmic buccal mass movements (top trace).

It should be noted that current injection into the SO in the partially-restrained preparation cannot drive the system as fast as in the isolated preparations (Rose & Benjamin, 1981*a*; Elliott & Benjamin, 1985*b*). The minimum interval between synaptic inputs to the 4 CL cell of Fig. 12E was 6 s and this was with very strong current injection into the SO. This compares with the minimum period of 3 s in

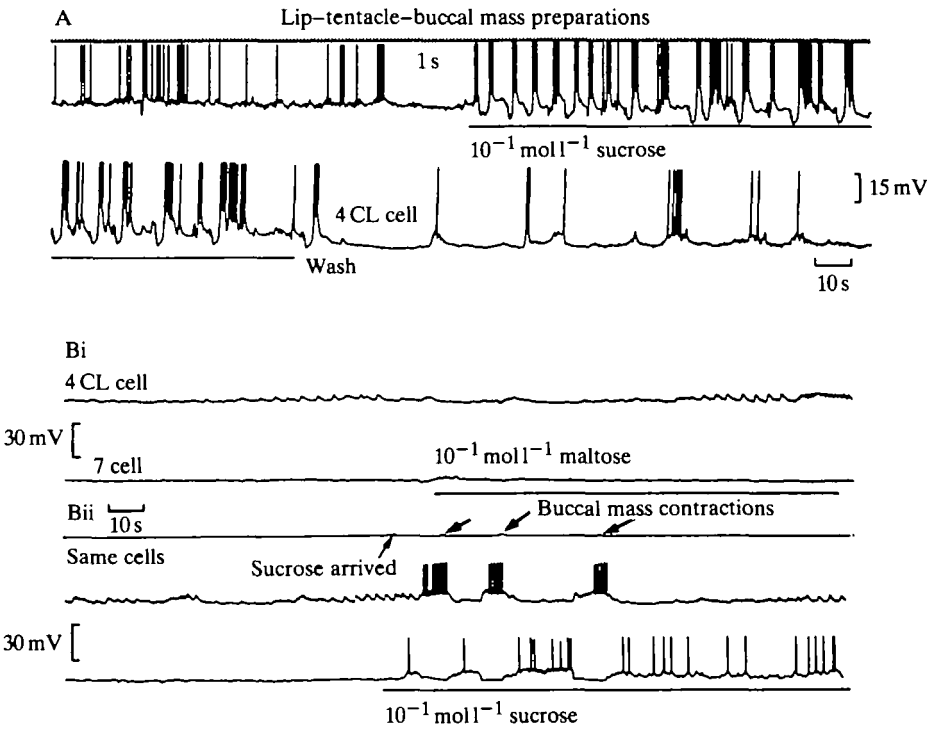

Fig. 10. High frequency rhythm in motoneurones induced by 10^{-1} mol l^{-1} sucrose (A) and differential effects of maltose and sucrose (B) on the lip–tentacle–buccal mass preparation. (A) Sporadic non-rhythmic activity in this 4 CL cell was converted to strong bursting by sucrose in this completely unrestrained preparation. Particularly prominent were strongly hyperpolarizing N2 inputs which disappeared on washing with saline. (B) 10^{-1} mol l^{-1} maltose failed to initiate feeding bursts in 4 CL and 7 cells whereas 10^{-1} mol l^{-1} sucrose in the same preparation induced a short sequence of bursting with characteristic synaptic inputs from CPG interneurones, together with contractions of the buccal mass (Bii) (no movements of the buccal mass were seen in Bi).

the sucrose-stimulated intact snail (Fig. 2) and SO-stimulated isolated CNS preparations (Rose & Benjamin, 1981*a*; Elliott & Benjamin, 1985*b*). This accords with the idea that restraining the buccal mass may partially inhibit the ability of the feeding circuitry to respond to sucrose. High frequency responses were only seen in completely unrestrained lip–tentacle–buccal mass preparations (Fig. 10A).

We can conclude from this second type of preparation that both motoneurones and the SO are excited by stimuli known to induce feeding movements in the intact snail. Furthermore, rhythmic movements of the buccal mass (e.g. Fig. 12D) actually accompanied burst activity in the cells. Motoneurone activation or increases in burst frequency were associated with increased cycling of the CPG interneurones whose effects could be indirectly monitored on the motoneurones. The main effects of both tactile stimulation and sucrose appeared to be on the CPG interneurones, but SO activation could also occur at the same time.

DISCUSSION

The role of sensory input in eliciting feeding in Lymnaea

Behaviour of the intact snail

Sucrose and maltose both induced feeding movements in *Lymnaea*, whereas quinine inhibited feeding even in the presence of high sugar concentrations. Three main changes were observed in snails kept in sugar solutions at concentrations above 10^{-5} mol l^{-1} compared with distilled water or tapwater: (a) an increase in the proportion of snails showing feeding movements; (b) an increase in the median rate of feeding; (c) an increase in the regularity of feeding (when tapwater was compared with sugar). (a) and (b) were shown to be affected by the concentrations of sugars used so that as these increased both parameters of feeding increased fairly linearly from 10^{-5} up to 5×10^{-2} mol l^{-1}. In sucrose, increasing the concentration from 5×10^{-2} to 10^{-1} mol l^{-1} produced little change, but for maltose this appeared to produce an inhibition of both the proportion of snails feeding and the mean feeding rate. At such high concentrations of sugar, we should not be surprised if direct osmotic effects begin to compete with the chemosensory input. Since sucrose and maltose are equiosmolar, we should suppose that the inhibition due to maltose indicates a difference in binding to unidentified receptors or in transport rates.

We were surprised to find that the stimulatory effects of 10^{-5} mol l^{-1} sucrose or maltose (whether measured as the proportion of snails feeding or the median feeding rate) were the same as that of unfiltered tapwater. The effects of tapwater could be

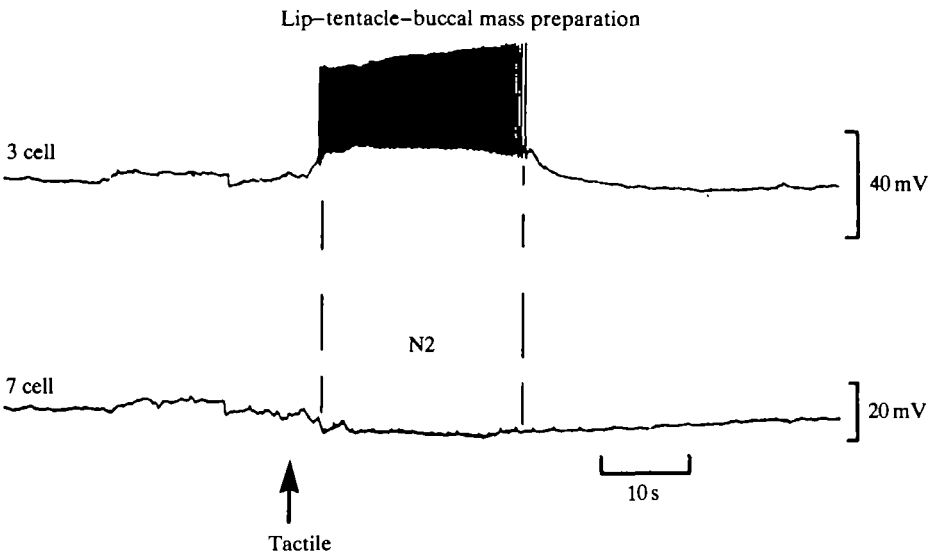

Fig. 11. Mechanical response of two motoneurones in the lip–tentacle–buccal mass preparation. The tactile stimulus induces a wave of depolarization in the 3 cell and inhibition in the 7 cell. These correspond to synaptic inputs normally received from the N2 CPG interneurones suggesting that this interneuronal type has been first excited by the tactile stimulus.

due to dissolved organic compounds, salts or bacteria which stimulated the feeding system. Even in distilled water, 30% of the hungry snails showed a few feeding movements and we suggest that the snails are sampling the environment in anticipation of finding food. Slade (1981) saw similar feeding movements even in unstarved *Lymnaea*. These feeding movements in distilled water were spontaneous as far as environmental stimulation was concerned although their occurrence could be explained by an internal state homologous to hunger in vertebrates. 'Feeding' activity seen in buccal neurones or buccal mass muscles in isolated or semi-isolated preparations (Benjamin & Rose, 1979; Rose & Benjamin, 1979) in the absence of sensory stimuli from the environment may underlie the spontaneous feeding movements seen in the intact snail. Recent work (Elliott & Benjamin, 1985*a*) shows that the proportion of isolated ganglia showing spontaneous rhythmic activity is up to 80% in starved snails compared with the previously reported figure of 20% (Benjamin, 1983) for fed snails.

We did not investigate the role of tactile input to the lips or tentacles in eliciting feeding movements in the intact snail but they are likely to be significant given the electrophysiological results discussed below.

Electrophysiological analysis of the semi-intact preparation

The prediction from the whole-animal experiments was that sugars should be capable of initiating or increasing the rate of bursting in feeding motoneurones. Underlying this would be an increase in the activity of premotor CPG interneurones known to drive the motoneurones in the *Lymnaea* feeding system (Rose & Benjamin, 1981*a,b*; Elliott & Benjamin, 1985*a*). Quinine would be expected to inhibit any on-going interneuronal rhythm. These predictions were borne out by the experimental results, particularly in the more complex lip–tentacle–buccal mass preparation. Sucrose, at 10^{-1} mol l^{-1}, could increase activity in rhythm-generating interneurones whose output was monitored indirectly by the records from motoneurones. The normal sequence of inputs reported by Rose & Benjamin (1981*a*) occurred in a variety of motoneurones. Maltose, at 10^{-1} mol l^{-1}, was less effective than sucrose in initiating feeding rhythms but this could be predicted from the behavioural experiments where it was shown that maltose produced only low level activation at this concentration. Quinine (in the lip–tentacle preparation) inhibited on-going rhythms and abolished activity in the CPG. However, certain features of the responses to chemicals were quantitatively different from the intact snail: (a) the maximum frequency of the response to 10^{-1} mol l^{-1} sucrose was lower than in the intact snail; (b) the responses waned with time even when sucrose was continuously perfused over the sensory surfaces.

A partial explanation for the low maximum frequency induced by 10^{-1} mol l^{-1} sucrose lies in the type of preparation used for most experiments. A partially-restrained buccal mass was usually required to enable intracellular recordings to be made during the muscular contractions underlying feeding movements. In the smaller number of experiments where the buccal mass was completely unrestrained, high frequency rhythms similar to those occurring in the intact snail were seen (e.g.

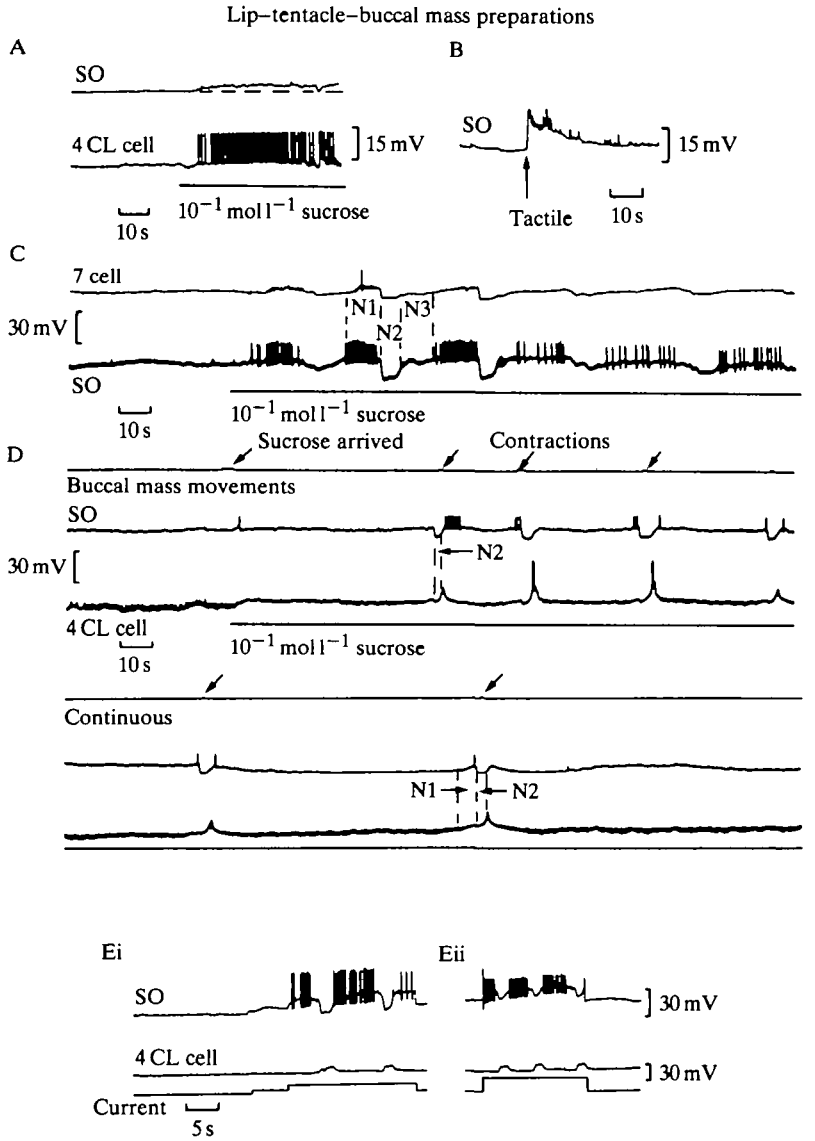

Fig. 12. Responses of the Slow Oscillator (SO) interneurone to tactile and chemical stimuli applied to the lip–tentacle–buccal mass preparation. (A) 10^{-1} mol l^{-1} sucrose depolarized the SO and excited the 4 CL motoneurone without activating the feeding rhythm. (B) Tactile stimulation caused a strong phasic depolarization of the SO with the initiation of truncated spikes (spike peaks lost off top of record). (C) Rhythmic bursting induced in an SO by 10^{-1} mol l^{-1} sucrose. This was accompanied by the usual N1, N2, N3 synaptic inputs on SO and 7 cell motoneurones. (D) Some activation of the SO and 4 CL cell occurred with sucrose perfusion with CPG (N2) inputs occurring on the cells *before* any spikes in the SO. The response waned with time with continuous application of sucrose (bottom pair of traces). Note too the immediate single spike in the SO in response to the arrival of sucrose. (E) The SO was injected with various levels of suprathreshold depolarizing current to assess its ability to activate the CPG rhythm in the semi-intact preparation. Two steps of current were injected in Ei but even with the maximum amount of current injected in Eii the frequency of synaptic inputs to the 4 CL cell was lower than previously reported for the isolated preparation (Rose & Benjamin, 1981*a*; Elliott & Benjamin, 1985*b*).

Fig. 10A) so the stimulation or deformation by the Sylgard bridge may have had an inhibitory effect on the system. This was supported by experiments in which the SO was used to drive the rhythm. Here partially-restrained preparations never reached the frequencies of synaptic inputs or motoneurone bursts reported to occur in the isolated preparation (Rose & Benjamin, 1981*a*; Elliott & Benjamin, 1985*b*). Restraining the buccal mass might provide tonic inhibition from mechanoreceptors.

The waning of the response in semi-intact preparations to sugar and quinine during continuous perfusion is difficult to understand. One obvious difference between the intact and semi-intact preparation is that, in the intact snail, feeding movements presumably result in sugar being taken into the gut and then into the blood system, whereas in the semi-intact snail sugar will only reach at best the cavity of the buccal mass *via* the inserted cannula. This means that, in the semi-intact snail, there may be a lack of internal stimulation which is required for a strong and sustained response to sugars. Susswein, Weiss & Kupfermann (1984) showed that subsatiating levels of seaweed fed to *Aplysia* reduced the latency of the biting response for up to 80 min after the previous exposure to food. This shows that internal stimuli relating to the ingestion of food can have important consequences for the responsiveness of the feeding system to external stimuli.

Tactile stimuli could also initiate activity in buccal motoneurones. Sometimes simple phasic excitation of the motoneurones occurred, particularly in the lip–tentacle preparation, but activation of the CPG was most common with one cycle of synaptic input being produced by each stimulus, indicated by the specific sequences of N1 and N2 synaptic inputs (either excitatory or inhibitory) associated with CPG input on specified motoneurones. We presume that these tactile inputs arising from solid food such as pondweed, on which the snail normally feeds, could enhance the effects of chemical signals present in the food. The enhanced cross-modality effect of linking mechanical and chemical stimuli was indicated by the behavioural experiments of Rosen *et al.* (1982) in *Aplysia*.

Neural basis of CPG activation by sensory input

On the basis of previous work on gastropod molluscs (reviewed by Croll, 1983) we presume that there will be chemoreceptive or mechanoreceptive neurones located either in the periphery or CNS which carry the sensory information to the feeding neurones, but we know nothing of these cells in relation to feeding in *Lymnaea*. The sensory input could be directly activating the rhythm-generating CPG interneurones described by Rose & Benjamin (1981*b*) and/or these could be indirectly excited by higher-order interneurones (such as the CGCs or the SO: McCrohan & Benjamin, 1980*a,b*; Rose & Benjamin, 1981*a*).

It seems unlikely that the CGCs are mainly responsible for initiating bursting in feeding motoneurones because from previous experiments they appear unable to initiate a rhythm in these cells. They were excited by both sugar and tactile sensory stimulation but this would only enhance their supposed modulatory role in the feeding system (McCrohan & Benjamin, 1980*b*). The SO, an interneurone capable

of initiating feeding bursts in motoneurones, was also excited by tactile and chemical inputs and could play an important role in mediating the sugar-induced responses. However, CPG activity could be induced in CPG interneurones before or in the absence of SO activity. Admittedly this occurred with the CPG active at low frequency (e.g. Fig. 12D), so we cannot be sure that the SO activation is not absolutely necessary for a strong, sustained response, but it seems most likely on the basis of the present evidence that sensory input is acting at several points in the system; at least at the level of CPG interneurones and higher-order interneurones. We cannot rule out the possibility that the motoneurones are also being directly affected by sensory input but so far we can account for changes in motoneuronal activity on the basis of input from CPG interneurones and higher-order interneuronal effects. An example of the latter was that CGC excitation appeared to account for the phasic spike activity in 4 cells (retractor motoneurones) due to tactile stimulation (Fig. 7). The simplest hierarchical models for *Lymnaea* feeding discussed by Benjamin (1983), where sensory input directly excited the SO alone, seem unlikely on the basis of the present experiments.

Nerve pathways mediating the sensory responses

Results from the two types of semi-intact preparation used here show that multiple sensory pathways from peripheral sensory structures are likely to be required for normal sensory activation of the feeding system in *Lymnaea*.

One pair of nerves, the median lip nerves projecting to the cerebral ganglia, appeared from the work of Goldschmeding & Jager (1973) to mediate most of the sucrose-induced feeding movements in *Lymnaea* but in the present experiments the lip–tentacle preparation (consisting of lips, tentacles and left and right median lip nerves) proved incapable of regularly mediating activation of the feeding rhythm. The median lip nerves project directly to the cerebral ganglia (Fig. 1A) so it was perhaps not surprising that strong chemical and tactile activation of the CGCs occurred. However, only rarely did sucrose induce regular rhythmic bursting activation of buccal motoneurones and any effects had longer latencies than the inputs seen in the CGCs. The exceptions to this were the tactile responses whose effects on buccal motoneurones were seen within a few seconds of application of the stimulus. Some of these effects were probably due to the CGC connections directly to the motoneurones (the CGCs monosynaptically excite 3 cells and 4 cells, two types of motoneurones, McCrohan & Benjamin, 1980*b*). Limited tactile activation of the CPG interneurones also occurred and this must be mediated *via* sensory neurones or relay interneurones projecting to the buccal ganglia in the cerebrobuccal connectives which were still present (Fig. 1A).

Local input from nerves innervating the buccal mass (the laterobuccal and ventrobuccal nerves) was also incapable of mediating full sensory responses. This was not the case with the related snail *Helisoma* where perfusion of the buccal cavity with wheat germ caused rhythmic bursting activity in motoneurones in a preparation consisting solely of buccal ganglia and nerves projecting from the buccal ganglia to the buccal mass (Horwitz & Senseman, 1981). Thus local sensory pathways from the

buccal mass to the buccal ganglia feeding centre appear less important in *Lymnaea* than in *Helisoma*.

Only when all the nerves innervating both the buccal mass (from the buccal ganglia) and anterior head structures (from the cerebral ganglia) were present did sucrose induce short latency, chemo-activation of the feeding system. This occurred in the lip–tentacle–buccal mass preparation which retained the paired superior and medial lip nerves, laterobuccal, ventrobuccal, dorsobuccal nerves and the single postbuccal nerves (as described by Benjamin *et al.* 1979).

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