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INITIATION OF FEEDING MOTOR OUTPUT BY AN IDENTIFIED INTERNEURONE IN THE SNAIL *LYMNAEA STAGNALIS*

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SUMMARY

1. The cerebral ventral 1 (CV1) cells of *Lymnaea* are located in the cerebral ganglia, and have axonal projections to the buccal ganglia.
2. Maintained depolarization of a CV1 neurone leads to initiation, maintenance and modulation of rhythmic feeding motor output from buccal and cerebral ganglia.
3. The CV1 cells receive rhythmic synaptic inputs, in phase with feeding cycles, which probably originate from buccal rhythm-generating interneurones.
4. CV1 cells initiate feeding cycles independently of the buccal slow oscillator (SO) neurone, previously described. The possible roles of CV1 and SO are discussed.

INTRODUCTION

Neural networks underlying rhythmic motor output have been analysed for a number of invertebrate behaviour patterns, such as leech swimming (Friesen, Poon & Stent, 1976), *Tritonia* escape swimming (Getting, Lennard & Hume, 1980) and gastropod feeding (Siegler, Mpitsos & Davis, 1974; Rose & Benjamin, 1981*a,b*). Associated with these neural networks are so-called 'command' neurones, which may initiate, maintain and modulate rhythmic motor output. Thus, for example, experimentally evoked spike activity in cell 204 of the leech (Weeks & Kristan, 1978), cell C2 of *Tritonia* (Getting, 1977) or the paracerebral cells (PCNs) of *Pleurobranchaea* (Gillette, Kovac & Davis, 1982) leads to patterned motor output that is remarkably similar to that observed in preparations showing spontaneous motor output, or those in which motor output has been evoked by sensory stimuli or nerve stimulation. It has been concluded that such neurones play a role in controlling the expression of behaviour, though their activity may not always be necessary for a particular behaviour to occur.

In addition to the initiation of specific behaviour patterns, command neurones may also be involved in regulating the expression of other behavioural sequences. For example, the IVN command cells in the lobster stomatogastric system are known to initiate the pyloric rhythm while also inhibiting the gastric mill complex (Sigvardt & Mulloney, 1982). Clearly, further studies of command neurones will shed more light

Key words: Molluscan feeding, command neurone, motor rhythm.

on the whole question of neural mechanisms for initiation of specific behaviour patterns at particular times.

This paper provides a description of a cell type in the feeding system of the snail, *Lymnaea stagnalis*, which can initiate and maintain rhythmic motor output from the buccal and cerebral ganglia. This cell type, cerebral ventral 1 (CV1) is located in the ventral lobe of the cerebral ganglion. CV1 appears to have direct effects on the feeding rhythm generator in the buccal ganglia, and, in turn, receives cyclic inputs in phase with the buccal rhythm. The relationship of CV1 with a buccal interneurone, the slow oscillator (SO), which can also initiate and maintain the buccal rhythm (Rose & Benjamin, 1981*a*), is examined and discussed. For convenience, rhythmic buccal activity, similar to that described by Rose & Benjamin (1981*a*), will be termed feeding motor output, though the possibility that the rhythm may also underly other related activities cannot be ruled out (McClellan, 1982).

MATERIALS AND METHODS

Specimens of *Lymnaea stagnalis* (L.) were collected from outside ponds, kept in aerated tap water and fed on lettuce.

Experiments were carried out at 20 °C in a HEPES-buffered saline (McCrohan & Benjamin, 1980). The nervous system preparation, consisting of isolated brain with attached buccal ganglia, was pinned in a recording chamber and covered with saline. The parts of the brain from which recordings were to be made were treated with protease (Type V, Sigma) for 10 min and then washed with saline.

Intracellular recordings were made from neurones using glass microelectrodes filled with either 0·5 M-K_2SO_4 or 3 % Lucifer Yellow solution. The preamplifiers incorporated a bridge circuit which enabled fairly accurate monitoring of membrane potential whilst passing current through the microelectrode. Current injected was monitored by means of an I-V converter placed between the indifferent electrode and earth. For depolarization and hyperpolarization of cells, currents injected were less than 3 nA. Signals were stored on tape and displayed using a pen-recorder.

After electrophysiological recording, CV1 cells were injected intracellularly with Lucifer Yellow to confirm identification, using 5 nA, 500 ms hyperpolarizing pulses, at 1 Hz for 5–8 min. Material was then fixed, embedded in wax, and sectioned at 10 μm. Neurone morphology was reconstructed from serial sections using a drawing tube attachment on the microscope.

MONITORING THE FEEDING RHYTHM

The work described in this paper relates the activity of the CV1 cells to the feeding rhythm, which is generated by the buccal ganglia. It was therefore necessary to monitor the occurrence and phase of buccal feeding cycles.

The generation of rhythmic feeding motor output in *Lymnaea* has been described by Benjamin & Rose (1979; Rose & Benjamin, 1979, 1981*a,b*; McCrohan, 1984). Identified buccal and cerebral feeding motoneurones receive three phases of synaptic input during each feeding cycle. These inputs to buccal motoneurones, and probably also to cerebral motoneurones, originate from three coupled subnetworks of identified

buccal rhythm-generating interneurones (N1, N2 and N3 networks; Rose & Benjamin, 1981*a,b*). The N1 and N2 interneurones produce consecutive phases of synaptic input to motoneurones during radula protraction and the first phase of retraction, respectively. N3 interneuronal input to motoneurones follows the N2 input, and occurs as a series of short-duration postsynaptic potentials (PSPs). N1, N2 and N3 inputs may be excitatory or inhibitory on the different follower cells. It is therefore possible, by recording the activity of identified buccal and cerebral motoneurones, to monitor the different phases of synaptic input during the feeding cycle.

Spontaneous cyclic motor output from buccal and cerebral feeding neurones may be recorded from about 20 % of isolated brain preparations ('feeding' preparations).

RESULTS

Identification of CV1 cells

CV1 cells were identified using three criteria, all of which are described in detail in the following sections: (i) ability to initiate and modulate rhythmic feeding motor output from buccal and cerebral ganglia; (ii) presence of synaptic inputs in phase with N1, N2 and N3 inputs to identified feeding motoneurones; (iii) anatomy, including details of axonal projections and fine branching. The cyclic inputs to CV1 will be termed 'N1', 'N2' and 'N3' inputs, though their exact origin is unknown. All CV1 cells studied fulfilled criteria (i) and (ii). All cells subsequently examined morphologically fulfilled criterion (iii).

Anatomy of CV1 cells

The CV1 cells lie on the anterior surfaces of the ventral lobes of the cerebral ganglia. The CV1 soma lies in the 'V' made by the roots of the superior and median lip nerves. Somata are small (approx. 25 μm diameter), and cannot be distinguished from adjacent cells by their appearance alone, in the freshly dissected preparation. However, the small number of cells in this location allows electrophysiological identification of CV1 cells in most preparations. Multiple electrode penetrations in this region have failed to reveal more than one CV1 cell in each cerebral ganglion.

The CV1 cell has a single axon which descends in the cerebral neuropile to the ipsilateral cerebrobuccal connective (Fig. 1). Some fine branching is present in the neuropile near to the cell body. The axon travels *via* the ipsilateral cerebrobuccal connective to the buccal ganglia, where it crosses over and enters the contralateral ganglion. Fine branching is present in the neuropile of both ganglia. Axonal projections of CV1 cells were never found in peripheral nerves, suggesting a purely interneuronal role for this cell type. The morphology of all cells examined was extremely consistent.

Output properties of CV1 cells

Intracellular injection of steady, depolarizing current into a CV1 cell led to initiation and/or modulation of rhythmic bursting in feeding motoneurones (Fig. 2). In 'non-feeding' preparation, maintained depolarization of CV1, sufficient to induce

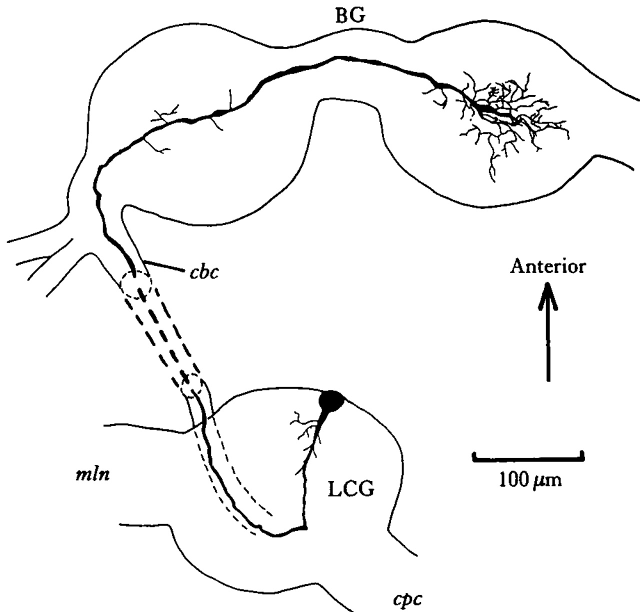

Fig. 1. Anatomy of CV1 cell. Reconstruction of Lucifer Yellow filled left CV1. The single axon projects to the buccal ganglia *via* the ipsilateral cerebrobuccal connective. Fine branching is present in cerebral and buccal neuropiles. BG, buccal ganglia; *cbc*, cerebrobuccal connective; *cpc*, cerebropedal connective; LCG, left cerebral ganglion; *mln*, median lip nerve.

spiking, led to rhythmic bursting activity in buccal motoneurones (Fig. 2A), apparently similar to that observed in a spontaneously 'feeding' preparation. Bursting was maintained for as long as current injection continued. Underlying the bursts in the buccal neurones were the two main phases of buccal interneuronal input (N1 and N2). For the buccal neurone in Fig. 2A (10-cell; Rose & Benjamin, 1981*a*), these inputs are weak N1 excitation followed by a stronger N2 excitation. Despite constant current injection, CV1 also showed bursts in phase with the feeding rhythm. Spiking in CV1 was inhibited during the N2 phase owing to inhibitory synaptic input (described later).

In a spontaneously 'feeding' preparation, rhythmic synaptic inputs to CV1 occurred in phase with feeding cycles (Fig. 2B,C), leading to strong burst activity. Maintained depolarization of a CV1 cell in such a preparation led to an increase in both frequency and intensity of feeding bursts in buccal motoneurones (Fig. 2B,C). The buccal 4-cells of Fig. 2B,C are retractor motoneurones, which receive successive N1 and N2 inhibitory inputs and then burst during retraction owing to postinhibitory rebound (Benjamin & Rose, 1979). In the preparation shown in Fig. 2B, CV1 depolarization led to a drop in cycle period for 4-cell feeding bursts from 3·7 to 3·0 s. In addition, retractor bursts were strengthened from an average 4 spikes per burst to 6. This effect on burst intensity in feeding motoneurones was usually delayed by one cycle after the onset of CV1 stimulation (Fig. 2B,C). In contrast, the effect on burst frequency occurred immediately. A further intensity-modulating effect following CV1 depolarization was seen in a significant number of 'feeding' preparations (Fig. 2C). During the second or third cycle after the onset of CV1 stimulation, a third identifiable synaptic input to buccal motoneurone

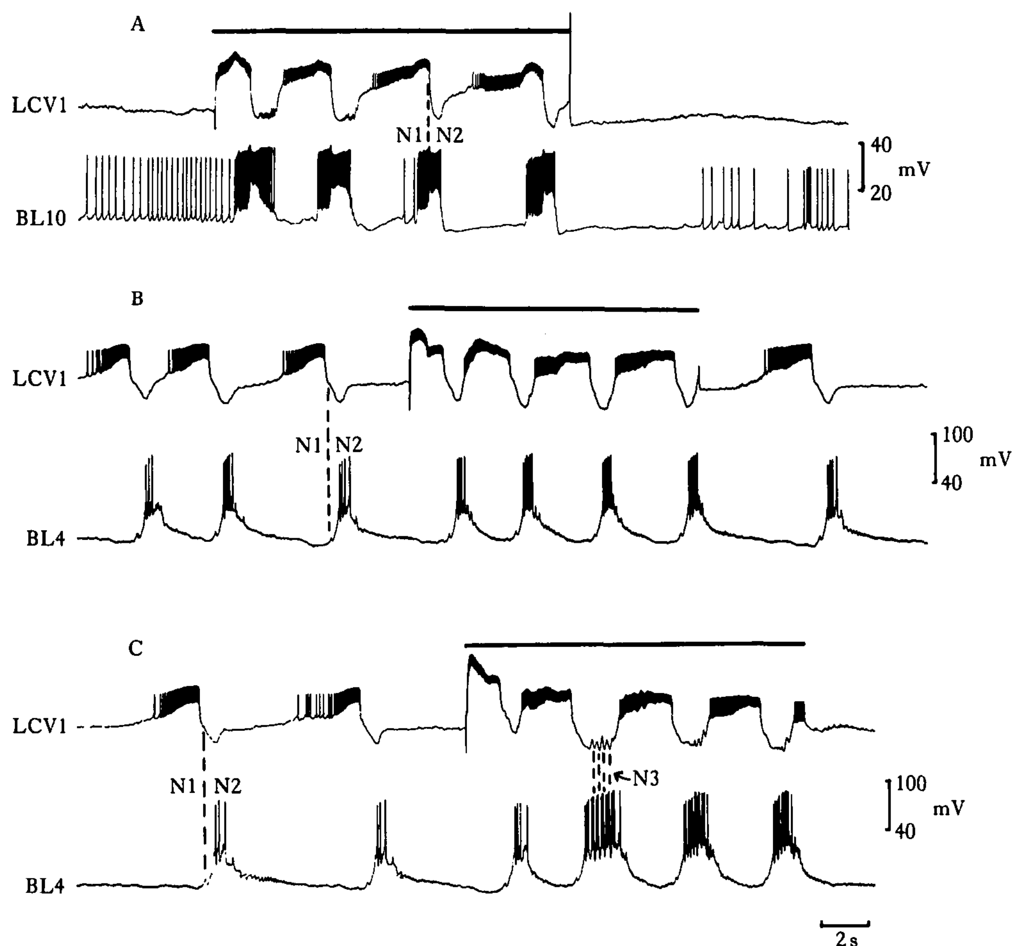

Fig. 2. Effects of CV1 cells on feeding rhythm. (A) Steady depolarization of left CV1 (LCV1), by current injection, leads to rhythmic bursting in buccal motoneurone (BL10) and in LCV1 itself, in a previously quiescent preparation. (B),(C) Maintained depolarization of LCV1 in an already rhythmic preparation leads to increased frequency and intensity of bursting in buccal motoneurones (BL4). N1, N2, N3, phases of buccal interneuronal input to motoneurones. Bars indicate duration of depolarizing current applied to CV1.

(N3) became more apparent. This had the effect of further intensifying 4-cell retractor bursts (Fig. 2C), since N3 inputs to 4-cells are inhibitory and the 4-cell fires on rebound from each N3 input. Inhibitory inputs to CV1, apparently synchronous with N3 inputs to buccal motoneurones, were also present (Fig. 2C). Thus activity in a CV1 cell can initiate cyclic output from the buccal rhythm generator, and in addition it can recruit activity in N3 interneurones in an already rhythmic preparation.

A further property of CV1 cells became apparent during the course of experiments illustrated by Fig. 2. Attempts were made to set CV1 spike frequency at different levels, using current injection, in order to observe quantitative effects on burst frequency and intensity in buccal motoneurones. However, this proved difficult. It was found that levels of current sufficient to elicit spiking usually led immediately to high frequency (30–40 Hz) firing in CV1. Lower frequencies were rarely obtained.

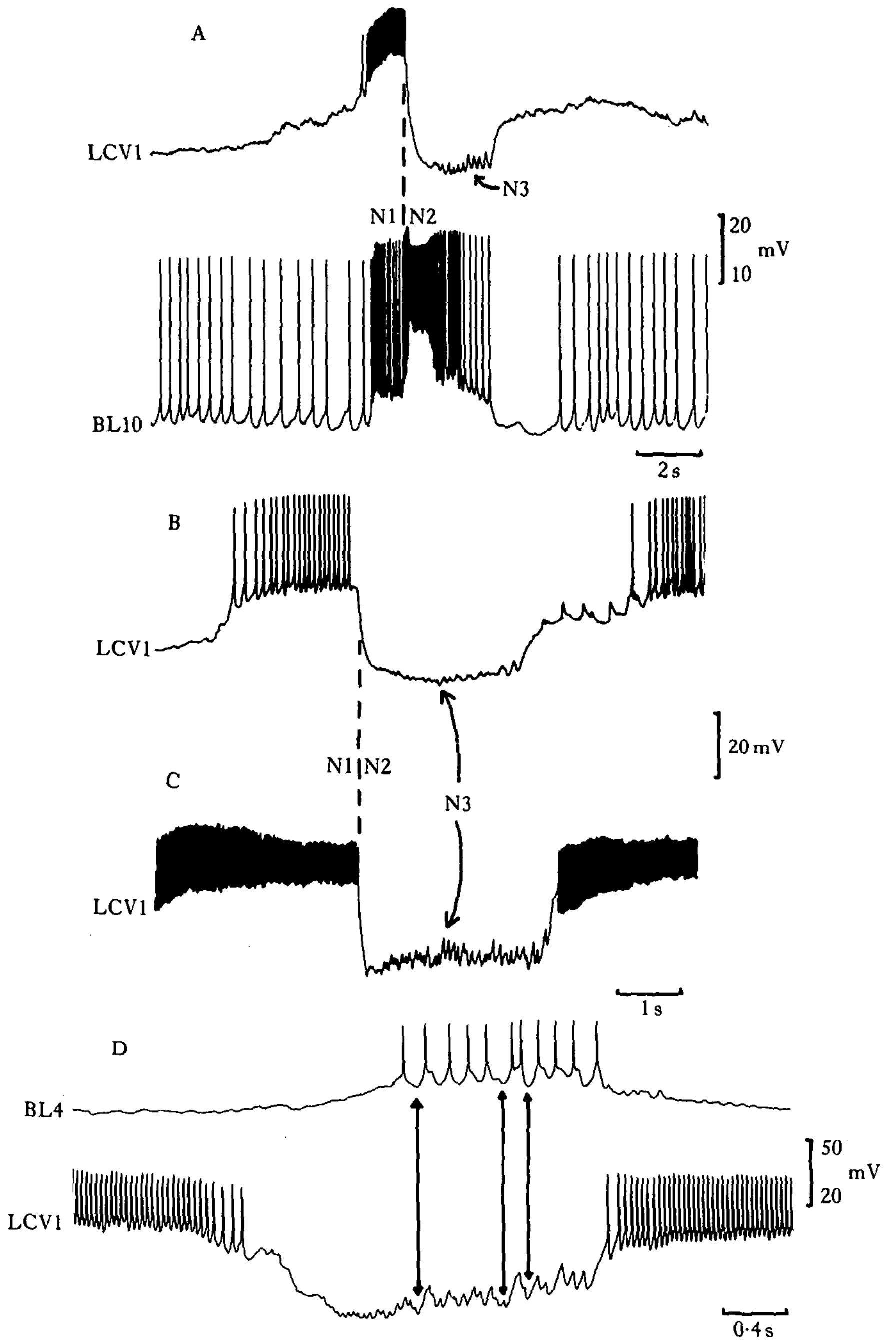

Fig. 3. Rhythmic inputs to CV1 in 'feeding' preparations. (A) Excitatory and inhibitory waves on left CV1 (LCV1) occur at the same time respectively as identified N1 and N2 excitatory synaptic inputs to buccal 10-cell (BL10). A series of N3-phase inhibitory inputs are superimposed on LCV1's 'N2' inhibition. (B) LCV1 recorded at resting potential, showing 'N1' excitation, followed by 'N2' and 'N3' inhibitory inputs. (C) Same cell as in (B), depolarized to reversal potential for 'N1' excitation. 'N2' and 'N3' inputs are increased in amplitude. (D) Larger components of inhibitory N3 inputs (arrows) are apparently synchronous on a buccal motoneurone (BL4) and CV1 (LCV1). Smaller components are not synchronous.

Attempts to increase CV1 spike frequency significantly beyond 40 Hz led to unstable recordings, possibly owing to cell damage in response to unphysiological levels of current injection. This observation suggests that CV1 cells may normally have only a limited range of firing rates, thus limiting the types of effects they may have on the feeding rhythm (see Discussion). However, it is clear that the CV1 cells can modulate burst frequency and intensity in buccal neurones to some extent (Fig. 2B,C).

Synaptic inputs to CV1 cells

The most obvious spontaneous synaptic inputs to CV1 cells were those which occurred in phase with N1, N2 and N3 interneuronal inputs to buccal motoneurones. 'N1' and 'N2' inputs to CV1 were apparently synchronous with N1 and N2 inputs on identified buccal neurones (Fig. 3A). CV1 cells received a compound excitatory 'N1' input (approx. 20 mV), which usually elicited a strong burst of spikes. This burst was terminated by a compound inhibitory 'N2' wave (approx. 15 mV). In addition a series of short duration inhibitory inputs (5–10 mV, 50 ms duration) were sometimes superimposed on the smooth 'N2' inhibition. These occurred at the same time as N3 inputs to buccal motoneurones (Fig. 2C). The nature of 'N1', 'N2' and 'N3' inputs to CV1 was revealed in more detail when their amplitude was compared in a cell, first at normal membrane potential (Fig. 3B) and then depolarized to the reversal potential for the 'N1' input (Fig. 3C). The amplitudes of all three changed appropriately for chemical synaptic inputs.

Previous work (McCrohan, 1984) suggests that 'N1', 'N2' and 'N3' inputs to ventral cerebral (CV) neurones arise directly from N1, N2 and N3 buccal interneurones. N1

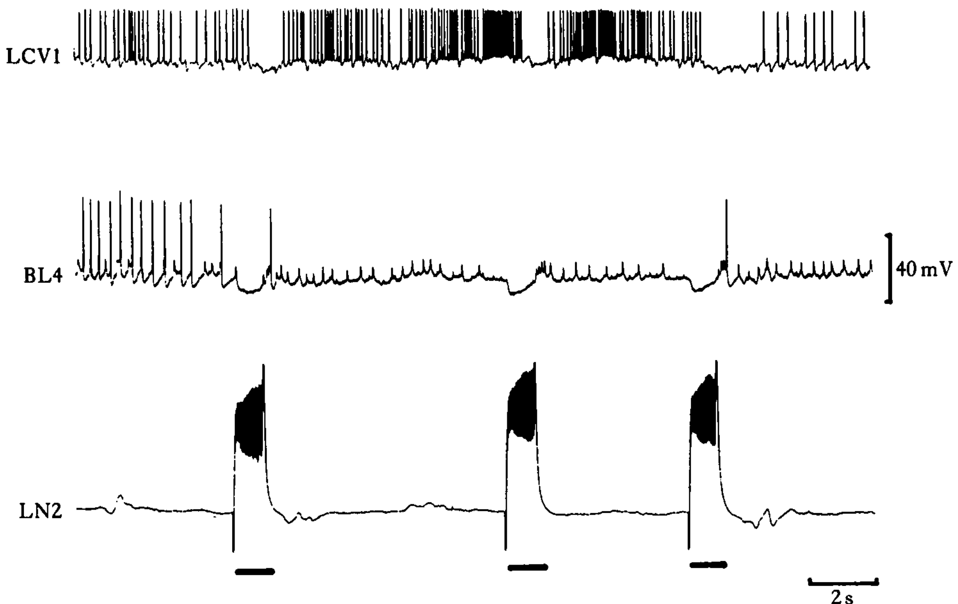

Fig. 4. Input to CV1 from a buccal interneurone. Evoked bursts of spikes (bars) in a buccal N2 interneurone lead to pronounced inhibitory inputs to buccal 4-cell (BL4) and weak inhibition of CV1 cell (LCV1), which becomes apparent after a delay of approx. 0·2 ms. Unusually, LCV1 shows spontaneous tonic spike activity which, however, reveals the weak inhibitory effect more clearly.

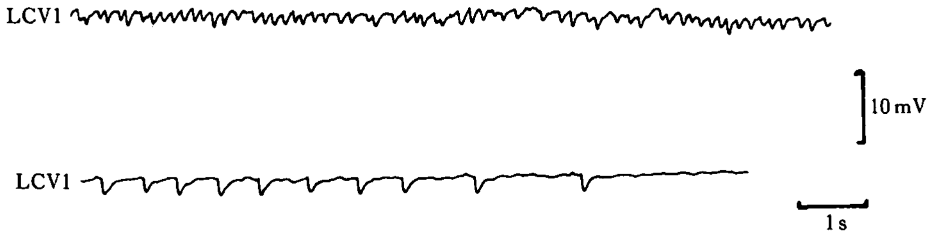

Fig. 5. Inputs to CV1 in 'non-feeding' preparation. Tonic, probably unitary, inhibitory inputs to left CV1 (LCV1) vary in frequency from (top trace) 8 to (bottom trace) less than 1 Hz. Amplitudes are approx. 2 mV.

and N2 inputs usually consist of smooth, compound waves. N3 input, however, is separated into individual, possibly unitary, components. The larger components of N3 inputs to buccal motoneurones and CV1 were synchronous (Fig. 3D, arrows). However, the smaller components were often asynchronous (Fig. 3D), indicating that different elements of the N3 network might be responsible for inputs to cells in the buccal and cerebral ganglia.

Experiments were carried out to test for connections between buccal interneurones and CV1 (Fig. 4). N2 interneurones are identifiable by their postsynaptic effects on buccal motoneurones, and by their synaptic inputs and activity during the feeding cycle (Rose & Benjamin, 1981*b*). N2 interneurones produce 1:1 inhibitory postsynaptic potentials (IPSPs) on 4-group cells, so that a burst of spikes in N2, elicited by current injection, led to a sharp compound inhibitory input on 4-group cells (Fig. 4). After a short delay, a weak inhibitory effect became apparent on CV1 (Fig. 4). The small size of this effect, when compared with a typical spontaneous 'N2' input to CV1, is presumably because experiments of this sort lead to activation of only one element of the interneuronal subnetwork. Similar weak effects on CV1 activity were observed for other buccal interneurones, demonstrating that interneurones of the buccal feeding generator do have appropriate actions on CV1 cells.

Synaptic inputs to CV1 in a 'non-feeding' preparation were usually small and subthreshold. CV1 cells rarely fired in such preparations. The most prominent input was a series of inhibitory, possibly unitary PSPs (approx. 2 mV), which occurred fairly regularly at frequencies between 1 and 8 Hz (Fig. 5). It is possible that the presence of mainly inhibitory inputs to CV1 cells in a 'non-feeding' preparation accounts partly for their inactivity.

Contribution of the cerebral ganglia to rhythmicity in cerebral feeding neurones

The data presented here and previously (McCrohan & Benjamin, 1980; McCrohan, 1984) suggest that rhythmic bursting in ventral cerebral neurones, including CV1, in phase with feeding cycles, is due to synaptic inputs from interneurones of the buccal rhythm generator. However, experiments on 'isolated' brain preparations in which the cerebrobuccal connectives have been cut, show that some degree of rhythmicity may be generated in the cerebral ganglia and/or other parts of the brain, when isolated from the buccal ganglia (Fig. 6). In one such experiment, spontaneous feeding cycles were recorded in a CV1 cell, a buccal retractor motoneurone (BL4) and a CV7 cell in the intact nervous system (Fig. 6A). CV7 cells are lip motoneurones which receiv

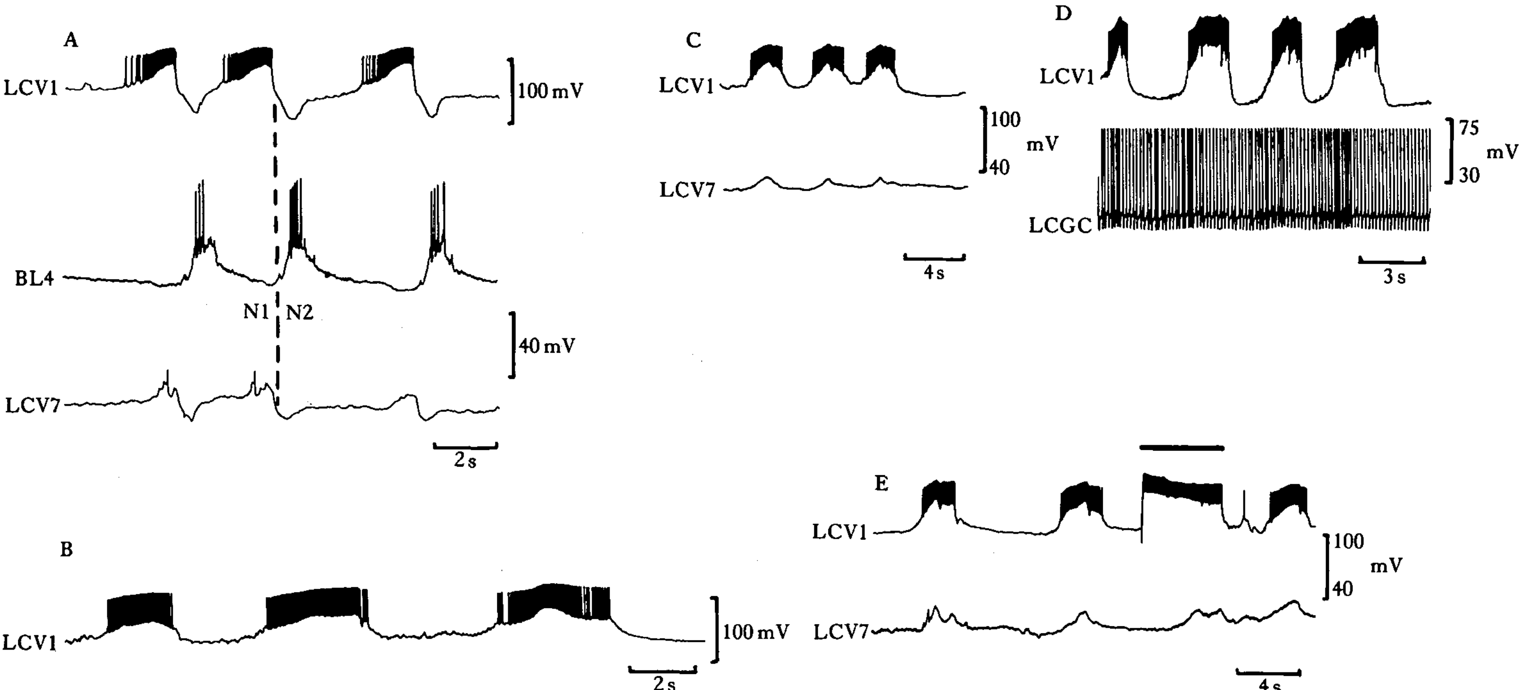

Fig. 6. Rhythmic excitatory inputs to CV1 and other cerebral feeding neurones in the absence of buccal inputs. (A) Rhythmic N1- and N2-phase feeding inputs occur on left CV1 (LCV1), and on buccal and cerebral feeding motoneurones (BL4, LCV7), in intact nervous system. (B),(C),(D) After sectioning both cerebrobuccal connectives, N1- and N2-phase inputs to cerebral neurones are absent. However, synchronous rhythmic excitatory inputs are apparent on cerebral feeding neurones, leading to strong bursting in LCV1 (B,C), waves of depolarization in LCV7 (C), and increased spike frequency in left cerebral giant cell (LCGC, D). (E) Preparation as in B–D. Timing of phasic excitatory inputs to LCV7 is unaffected by burst of spikes in LCV1, evoked by current injection (bar).

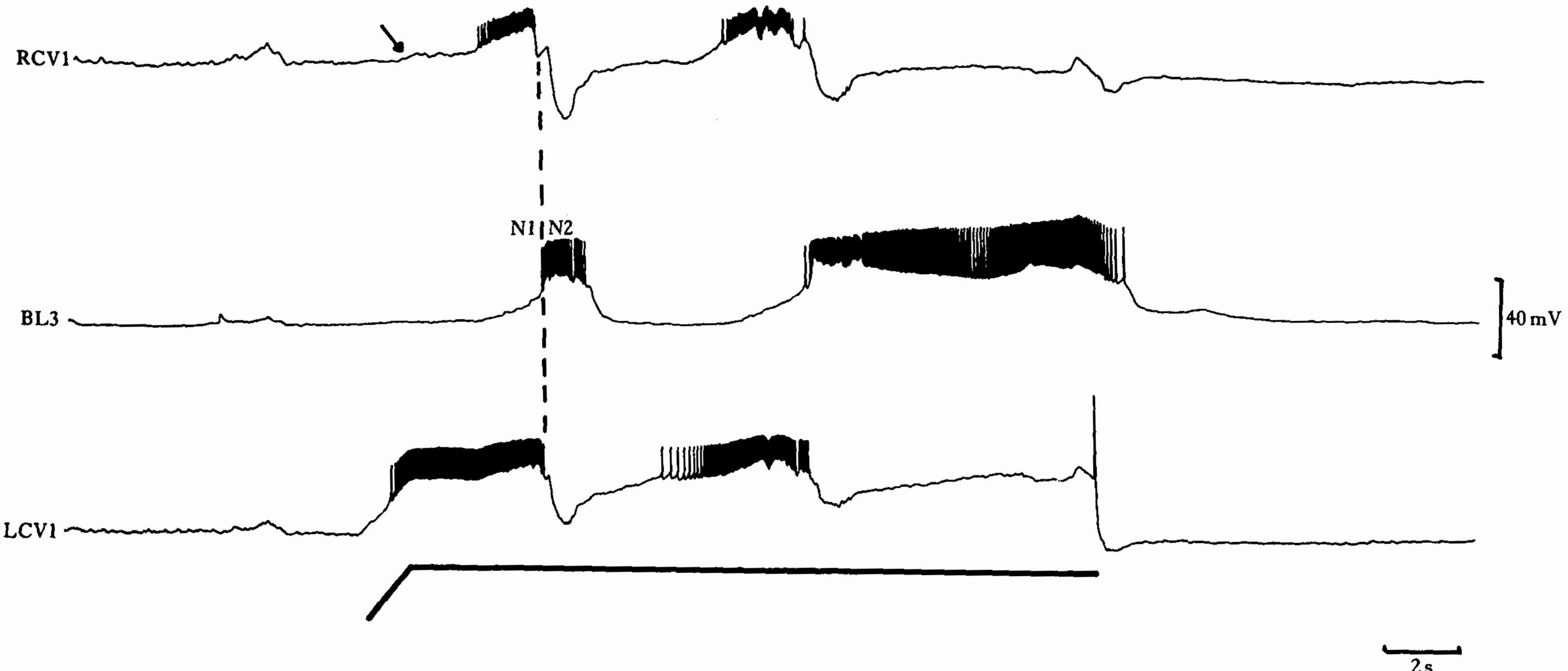

Fig. 7. Relationship between left and right CV1 cells. Depolarization of left CV1 (LCV1, indicated by bar) leads to two feeding cycles, monitored on buccal motoneurone (BL3). Identifiable 'N1' and 'N2' inputs are present on both left and right CV1 cells for both cycles. Arrow indicates slight depolarization of right CV1 (RCV1), following initial excitation of LCV1.

'N1' excitatory followed by 'N2' inhibitory inputs (McCrohan, 1984). After identification of the cells, the electrodes were removed and both cerebrobuccal connectives were cut. The CV1 and CV7 cells were then re-impaled. In this 'isolated' brain preparation, from which the buccal ganglia had been removed, the CV1 cell showed strong rhythmic bursts of spikes (Fig. 6B), with a similar, but slightly longer, period to the feeding bursts recorded previously in the intact brain. However, the obvious 'N1' and 'N2' inputs to CV1 were no longer present, providing more evidence that these originate in the buccal ganglia. The strong bursts in CV1 occurred at the same time as waves of excitation on CV7 and on the cerebral giant cell (CGC) in the same ganglion (Fig. 6C,D). It appears, therefore, that CV1, CV7 and CGC (and possibly other cerebral feeding neurones) receive common rhythmic inputs, with a period similar to that of the buccal feeding rhythm. CV1 itself is not responsible for the generation of these cyclic inputs to CV7 and CGC in the 'isolated' brain. When CV1 was depolarized at mid-cycle to make it burst strongly, the next excitatory wave on CV7 was not brought forward in line with CV1's evoked burst (Fig. 6E), showing that a single CV1 itself cannot reset the rhythm. The source of rhythmic inputs to cerebral feeding neurones in the absence of buccal inputs, is therefore an unidentified one, outside the buccal ganglia.

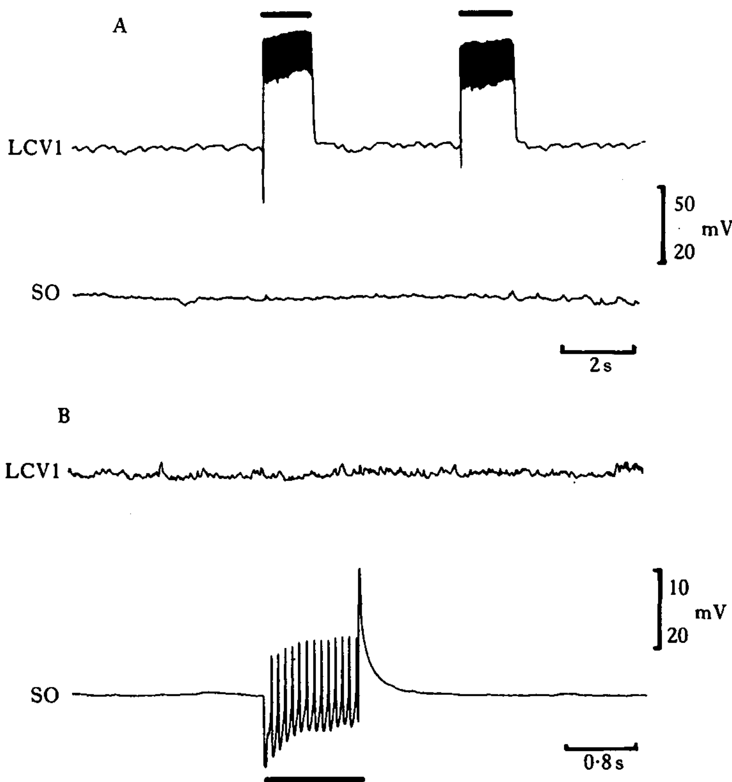

Fig. 8. Absence of direct connections between CV1 and slow oscillator neurone. (A) Strong bursts of spikes in CV1 (LCV1), evoked by current injection (bars), have no effect on membrane potential of slow oscillator neurone (SO). (B) Evoked burst in SO (bar) has no effect on LCV1 membrane potential.

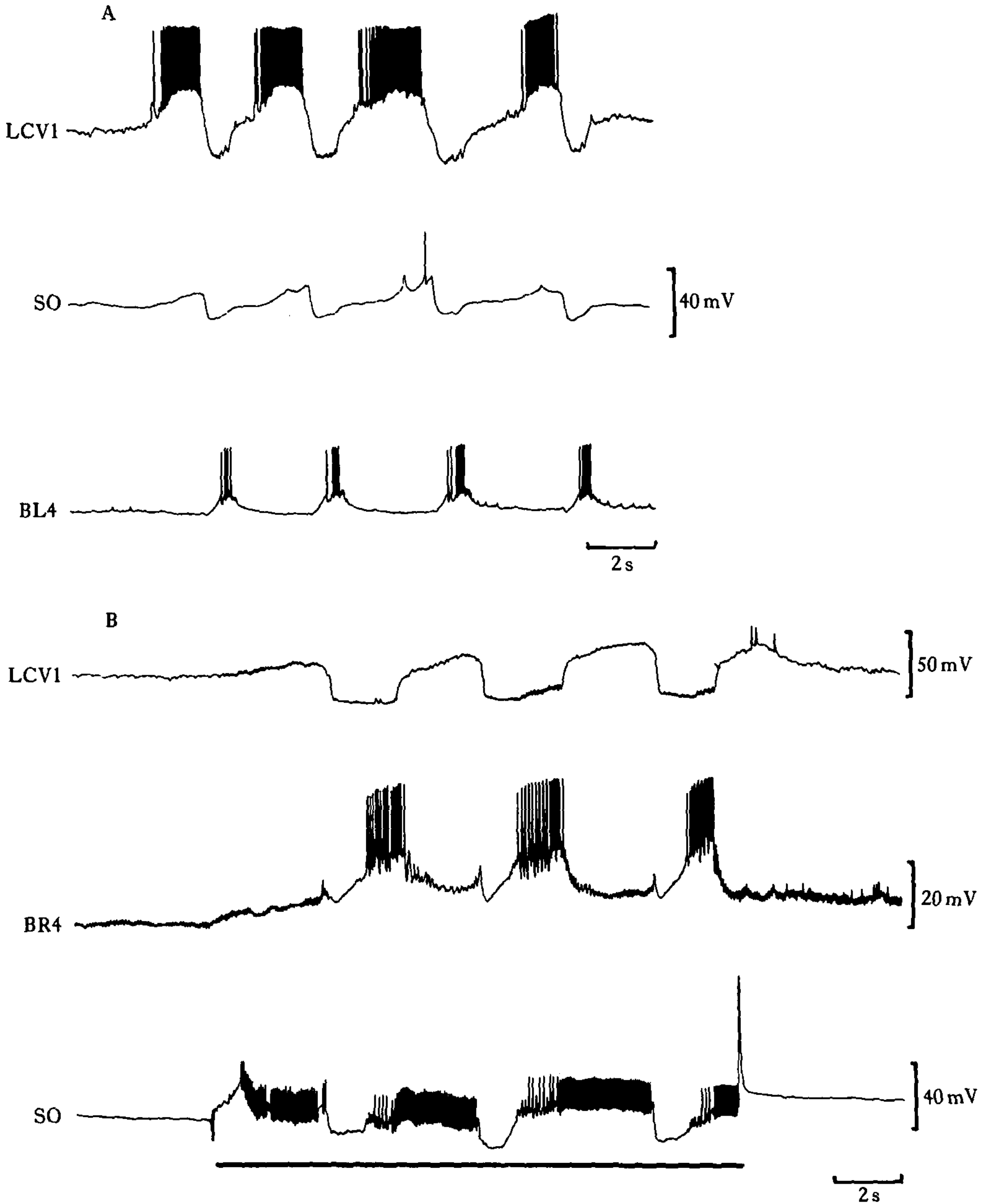

Fig. 9. Rhythmic spike activity in CV1 and slow oscillator neurone seen at different times. (A) Spontaneous feeding cycles, monitored on buccal motoneurone (BL4), are accompanied by rhythmic bursts of spikes in CV1 (LCV1), but slow oscillator (SO) is silent (except for a single spike) despite receiving synchronous rhythmic inputs. (B) Rhythmic feeding bursts in buccal motoneurone (BL4) are evoked by steady depolarization of SO (bar). SO fires bursts of spikes, in phase with feeding rhythm, but LCV1, though receiving synchronous rhythmic inputs, is silent.

Relationships between CV1 cells

Many neurones in the *Lymnaea* feeding system have bilateral connections between cells of the same type in symmetrical pairs of ganglia (Benjamin & Rose, 1979;

McCrohan & Benjamin, 1980). These connections help to maintain a bilaterally symmetrical motor output. Owing to their morphology, any such connections between left and right CV1 cells would be difficult to demonstrate. The connection would probably be located in the buccal ganglia, some distance away from the recording sites in the cell bodies. However, evoked activity in one CV1 cell does have a marked effect on the other, within the period of a single feeding cycle. Maintained depolarization of a CV1 cell in a 'non-feeding' preparation, sufficient to initiate feeding cycles, led to strong bursting in the contralateral CV1 within a single cycle (Fig. 7). In addition, identifiable 'N1' and 'N2' inputs were present on both cells. A small depolarizing effect on the contralateral CV1 cell was apparent as soon as the injected cell began to fire (Fig. 7, arrow). However, it is not possible to say whether the two CV1 cells are connected directly since this effect and certainly the inputs which generate bursting, could be mediated by activation of the buccal rhythm generator.

Relationship of CV1 with the buccal SO cell

The buccal slow oscillator (SO) neurone is a single identifiable interneurone, occurring in either the left or the right buccal ganglion (Benjamin, 1983). Maintained depolarization of SO leads to initiation of buccal feeding cycles. SO can also modulate frequency and intensity of rhythmic buccal output (Rose & Benjamin, 1981*a*). It was thought therefore, that the CV1 cells might have their effect on feeding by activating SO *via* a strong direct excitatory connection. No such connection could be demonstrated. Depolarization of a CV1 cell to produce a short, strong burst of spikes, not prolonged enough to elicit feeding cycles, had no effect on SO membrane potential (Fig. 8A). A reverse connection from SO to CV1 was also absent (Fig. 8B).

Since they do not connect directly with each other, the CV1 and SO cells must activate the buccal rhythm generator independently. Experiments in which CV1 and SO activity were monitored at the same time, indicate that these two cell types may be responsible for switching on and maintaining the rhythm at different times. In the large majority of preparations studied, the buccal rhythm was accompanied by burst activity in either CV1 or SO, but not in both (Fig. 9). Rhythmic inputs to both cell types, in phase with feeding cycles, were always present, but these inputs only led to bursts of spikes in one or other of the two cell types. This may indicate that there are two independent routes by which feeding may be initiated. This question is considered further in the Discussion.

DISCUSSION

The CV1 cells of *Lymnaea* are shown here to have a command function with regard to feeding motor output, at least in the isolated nervous system. Their anatomy is appropriate for cells which have their main output to neurones of the buccal rhythm generator. The evidence suggests that the CV1 cells have direct access to the buccal rhythm generator, allowing them to modify rhythmic motor output within the period of a single feeding cycle. It is probable that, like the slow oscillator (Rose & Benjamin, 1981*b*), CV1 cells act to excite the N1 buccal interneurones, thus initiating the cycle of events in the rhythm-generating network that leads to generation of feeding cycles (Rose & Benjamin, 1981*b*).

CV1 cells receive synaptic feedback, causing them to fire rhythmically in phase with buccal feeding cycles. The source of 'N1', 'N2' and 'N3' inputs to CV cells in *Lymnaea* has been discussed previously (McCrohan, 1984), and is probably the N1, N2 and N3 interneurones themselves. Similar feedback connections between a rhythm-generating network and command cells have been described elsewhere. The paracerebral cells (PCNs) of *Pleurobranchaea* are a population of up to eight neurones in each cerebropleural ganglion. Like CV1, these cells send axons along the cerebrobuccal connective to the buccal ganglia (Gillette *et al.* 1982). During each feeding cycle, the PCNs receive excitatory and then inhibitory inputs, corresponding with proboscis eversion and withdrawal. These inputs are thought to arise chiefly from the buccal rhythm generator (Gillette, Kovac & Davis, 1978). It was suggested that such cycle-by-cycle feedback to modulate command cell activity may act as a self-reinforcement mechanism, enabling rhythmic output to continue beyond the duration of the initial triggering stimulus (Gillette *et al.* 1978).

Another similarity between CV1 and PCNs is the presence of unitary IPSPs in quiescent cells. These were found to be the predominant synaptic inputs in both CV1 and PCN when a feeding rhythm was not occurring (Fig. 5). In *Pleurobranchaea*, these inputs have been studied in detail (Gillette *et al.* 1982). They have been termed cyclic inhibitory potentials (CIPs), and arise from a population of cyclic inhibitory neurones (CINs), at least some of which reside in the brain, outside the buccal ganglia. It is the concerted activity of the CINs which is thought to give rise to the rhythmic compound IPSPs seen during proboscis withdrawal (Gillette *et al.* 1982). Furthermore it was demonstrated that the frequency of CIPs on the PCNs may be increased both by increased activity in the PCNs themselves, suggesting a recurrent inhibitory pathway, and by presentation of food stimuli to a semi-intact preparation (Gillette *et al.* 1982). Similar unitary inhibitory inputs to command cells are seen in the leech swim system. Cell 204 is an intersegmental neurone whose activity can elicit and maintain swimming activity. It bursts during swimming, in phase with the swim rhythm (Weeks & Kristan, 1978). Between swim episodes, cell 204 receives prominent tonic IPSPs (Weeks, 1982). It is possible therefore, that inhibitory inputs, both within and between periods of cyclic activity, are important in determining the activity and final output of command neurones such as CV1, PCN and cell 204. The inhibitory inputs to CV1 (Fig. 5) warrant further study, particularly with regard to their source and possible function.

CV1 cells exhibit many properties which suggest that they may be homologous with the PCNs of *Pleurobranchaea*. However, the complexity of the feeding system in *Pleurobranchaea* appears much greater than that of *Lymnaea*. Only one CV1 cell was demonstrated in each cerebral ganglion of *Lymnaea*, though the possibility that others are present at different locations cannot be ruled out. The eight PCNs are subdivided into two types, phasic and tonic, which receive somewhat different inputs, and may have different though complementary roles (Kovac, Davis, Matera & Gillette, 1982; Kovac, Davis, Matera & Croll, 1983). This difference in complexity for a single cell type in the two species may be a reflection of the variability in behaviour patterns in the carnivorous *Pleurobranchaea*, as against the more stereotyped grazing behaviour of *Lymnaea* (Benjamin, 1983).

The results presented here suggest that CV1 and SO cells in *Lymnaea* have their

effects on the feeding system *via* independent pathways. Several possible explanations arise for this apparent redundancy of command elements for feeding. It is likely that CV1 and SO have access to different types of sensory input. The sensory inputs to these cells have not yet been examined in detail. However, their locations in the central nervous system suggest that SO may receive proprioceptive inputs from the buccal mass, which would be important for the maintenance and modulation of feeding behaviour. CV1, on the other hand, could receive information from tentacles and lips, such as tactile and chemosensory inputs, which would indicate the presence of food. This would emphasize a triggering role for CV1. This view is supported by the fact that SO appears capable of modulating frequency and intensity of feeding motor output over a fairly wide range (Rose & Benjamin, 1981*a*). In contrast, the present work suggests that spike frequencies in CV1 are restricted to a certain range, possibly owing to intrinsic properties of the CV1 cells and/or their connections with the feeding network, thus limiting CV1's ability to modulate the rhythm. This all-or-nothing characteristic of CV1, making it fire either at high frequencies or not at all, would be a useful feature for a neurone concerned mainly with initiation of motor activity.

Another possible explanation for the presence of two command pathways in the *Lymnaea* feeding system is that the CV1 and SO cells may, in fact, control different types of buccal motor output. Unlike *Pleurobranchaea*, in which a buccal rhythm has been shown to underly several different behaviour patterns (McClellan, 1982), the behaviour in *Lymnaea* is more stereotyped. However, in addition to rhythmic feeding movements, *Lymnaea* does show egestion responses to non-food material that has been injested (Bovbjerg, 1968). It is possible that activity in CV1 and SO leads to subtly different buccal motor rhythms, responsible for different behaviour. In *Pleurobranchaea*, the PCNs' activity is specific to feeding motor output and they are silent, for example, during egestion of an unpalatable object (Gillette *et al.* 1982). In contrast, evidence has been presented to suggest that another command cell in *Pleurobranchaea*, the buccal ventral white cell (VWC) is involved in the control of the vomiting rhythm (McClellan, 1983). Investigation of similarly specific roles of CV1 and SO would require studies on a more intact preparation.

The present work revealed the presence of an oscillator outside the buccal ganglia, which imposes rhythmic activity on cerebral feeding neurones. The role of this oscillator is unknown. It is possible that it is entirely overridden when the buccal rhythm generator is active in an intact preparation, since its cycle frequency is lower than that of the buccal rhythm generator. However, it may serve to reinforce the buccal rhythm, as it excites those cerebral feeding neurones which are excited during the N1 phase of the cycle (e.g. CV1, CV7, CGC). A similar brain oscillator has been described for the feeding system of *Pleurobranchaea* (Davis, Siegler & Mpitsos, 1973). However, it appears that in this species the buccal rhythm generator is still of prime importance for the generation of feeding motor output from the brain and buccal ganglia (Cohan & Mpitsos, 1983).

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