

## PROPERTIES OF VENTRAL CEREBRAL NEURONES INVOLVED IN THE FEEDING SYSTEM OF THE SNAIL, *LYMNAEA STAGNALIS*

By C. R. McCROHAN

*Department of Zoology, University of Manchester, Manchester, M13 9PL*

*Accepted 13 September 1983*

### SUMMARY

Four identified neurone types (CV3, 7, 5 and 6), located in the ventral cerebral ganglia of *Lymnaea stagnalis*, are described. These cells have axonal projections in one or more of the nerves innervating the lips. In addition, they show rhythmic synaptic inputs leading to strong burst activity in phase with cyclic output from the buccal ganglia, suggesting a role in the control of the oral aperture during feeding. The innervation of lip muscle by one of the cell types (CV7) is confirmed electrophysiologically. The relationship of rhythmic activity in CV cells with that in the buccal feeding system is discussed.

### INTRODUCTION

The neural control of rhythmic buccal movements in the pond snail *Lymnaea stagnalis* has been studied in detail (Benjamin & Rose, 1979; Rose & Benjamin, 1979, 1981*a,b*). Attention has centred on the control of buccal mass and radula movements. Motoneurones controlling coordinated protraction and retraction of the buccal mass are located in the buccal ganglia (Rose & Benjamin, 1979). Rhythmic activity is imposed upon these motoneurones by a presynaptic network of three types of identifiable buccal interneurone, whose interconnections are such as to produce a coordinated output (Rose & Benjamin, 1981*b*). Studies of other gastropod species have revealed more or less similar arrangements of motoneurones and interneurones in the buccal ganglia (e.g. *Helisoma*, Kaneko, Merickel & Kater, 1978; *Tritonia*, Bulloch & Dorsett, 1979).

It has been generally assumed that rhythmic buccal motor output underlies feeding behaviour. However, recent work on *Pleurobranchaea*, a carnivorous gastropod, suggests that the buccal motor pattern may be responsible for a number of different behavioural sequences, including feeding, regurgitation and rejection (McClellan, 1982*a*). Studies in freely-moving animals, semi-intact and isolated preparations showed that rhythmic buccal motor output was common to all three of these behaviour patterns, the only clear difference between them involving changes in frequency and intensity of the rhythm; for example, during the active (vomiting) phase of regurgitation, the buccal rhythm had a shorter cycle time (McClellan, 1982*b*). The main

differences in motor activity underlying the three behaviour patterns seemed to involve the extrinsic buccal muscles which control the jaws and lips (McClellan, 1982*a,b*). For example, motor output in the mouth nerve, innervating the lips, was found to be phasic during swallowing (feeding) and also during the writhing phase of regurgitation. However, during the vomiting phase it became largely tonic and unpatterned (McClellan, 1982*b*).

McClellan's work raises interesting questions concerning the control of motor patterns by the central nervous system. It appears that, in *Pleurobranchaea* at least, a single motor pattern generator in the buccal ganglia is used to provide similar, but not identical, motor sequences of the buccal mass, that may underlie a number of different behaviour patterns. In addition, coordination between motor centres in the cerebropleural ganglia (which contain neurones innervating the extrinsic buccal muscles: Davis, Siegler & Mpitsos, 1973; Lee & Liegeois, 1974) and the buccal ganglia must exist. Such coordination would require flexibility in terms of pattern and phase relationships in order to achieve different behavioural sequences. Therefore questions concerning interganglionic coordination mechanisms and selective behavioural 'recruitment' using the same neural machinery have emerged, and are only beginning to be answered (Cohan & Mpitsos, 1983*a,b*; also see Discussion).

*Lymnaea* is an omnivore, feeding mainly on plant material, but also on detritus and carrion (Graham, 1955; Bovbjerg, 1968). Studies so far suggest that behaviour patterns involving the feeding apparatus are much less varied than those found in *Pleurobranchaea*. However, in addition to rhythmic feeding movements, *Lymnaea* will show egestion responses to non-food material that has been taken into the mouth (Bovbjerg, 1968). It would seem, therefore, that an investigation of the neural mechanisms underlying coordinated movements of lips and buccal mass in *Lymnaea* could provide some answers to the questions posed by the *Pleurobranchaea* studies, but in a much simpler system. In addition, such information will allow a consideration of comparative aspects of motor control in two gastropods which differ markedly in lifestyle and feeding habits.

The present work was intended to provide basic information about the neural control of the lip musculature. Muscles of the lips in *Lymnaea* contract in phase with the cyclic movements of the radula. In a study involving behavioural and anatomical observations, Hubendick (1956) described the arrangement and activity of muscles in the lips which are involved in opening and closing of the mouth in *Lymnaea*. He described several muscle types which control the size of the oral aperture appropriately for protraction and retraction of the radula. The lips are innervated by three pairs of lip nerves, all of which have their roots in the ventral lobes of the cerebral ganglia. It therefore seemed likely that, as in *Pleurobranchaea*, the motoneurones innervating the lip musculature would be located in the cerebral ganglia rather than the buccal ganglia. Furthermore, it would be expected that these cells would show a pattern of rhythmic activity phase-locked to that of cells in the buccal ganglia, which are known to control buccal mass movements during feeding.

This study describes four identifiable types of probable lip motoneurones in the cerebral ganglia of *Lymnaea*, and the patterns of synaptic input that drive their activity. The possible source of these inputs is discussed, including a consideration of the cerebrobuccal coordination mechanism.

## MATERIALS AND METHODS

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

Two types of preparation were used. For experiments involving only intracellular recordings, the preparation consisted of the isolated brain with attached buccal ganglia. For experiments involving muscle recording, a semi-intact preparation was used. This comprised the brain and buccal ganglia, the buccal mass (pinned appropriately to reveal the lip muscle under study), the front portion of the foot, and the body wall and musculature around the oral aperture. The buccal nerves and lip nerves were left intact.

Experiments were carried out at 20 °C in a HEPES-buffered saline (McCrohan & Benjamin, 1980a). The preparation was pinned in a recording chamber and covered with saline. The parts of the brain to be recorded 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<sub>2</sub>SO<sub>4</sub> 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 measured by means of an I-V converter placed between the indifferent electrode and earth. Extracellular recordings from muscles were made using suction electrodes in the conventional way. Signals were stored on tape and displayed on either a storage oscilloscope or a pen recorder.

Intracellular injection of the fluorescent dye Lucifer Yellow was used to reveal the morphology of individual neurones. After electrophysiological recording with a Lucifer Yellow filled electrode, 5 nA, 500 ms hyperpolarizing pulses were passed at 1 Hz for 5–8 min. Material was then fixed, embedded in wax, and sectioned at 10 µm. The morphology of neurones was reconstructed from serial sections using a *camera lucida* attachment on the microscope.

## RESULTS

*Identification of CV neurones*

The four cerebral neurone types (CV3, 7, 5 and 6) described here were identified using combinations of the following morphological and electrophysiological criteria. (The use of one criterion alone was not usually sufficient.)

*Location of soma on the ganglion surface*

The positions of the CV cell bodies in the left ventral cerebral lobe of *Lymnaea* are shown in Fig. 1. [The cerebral giant cells (CGCs, McCrohan & Benjamin, 1980a) and CV1 cells (Benjamin, McCrohan & Rose, 1981) are also indicated.] Cell bodies of CV7, 5 and 6 are found as a mixed group in the ventral lobe and there are two or more of each cell type present in each ganglion. CV3 has a more dorsal location, and up to two CV3 cells have been recorded in each ganglion. Cell body diameters for all four cell types vary from 20–40 µm in living preparations. Symmetrical populations of CV

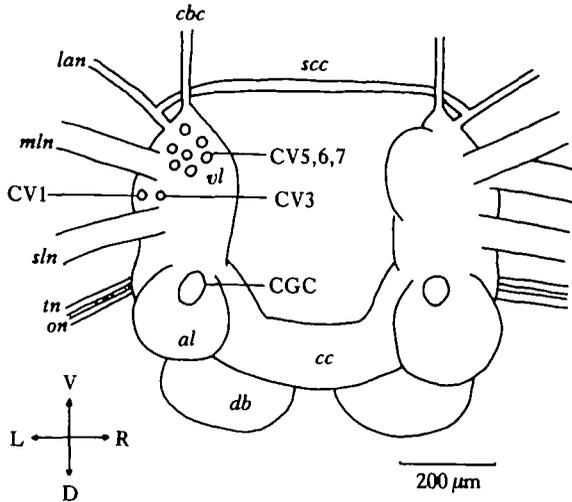


Fig. 1. View of the anterior surfaces of the cerebral ganglia of *Lymnaea stagnalis*, showing the locations of CV cell bodies, and the positions of the nerve roots. *al*, anterior lobe; *cbc*, cerebrobuccal connective; *cc*, cerebral commissure; *CGC*, cerebral giant cell; *D*, dorsal; *db*, dorsal body; *L*, left; *lan*, labial artery nerve; *mln*, median lip nerve; *on*, optic nerve; *R*, right; *scc*, subcerebral commissure; *sln*, superior lip nerve; *tn*, tentacle nerve; *V*, ventral; *vl*, ventral lobe.

cells were found and recorded in both ganglia. However, owing to an extra lobe containing many small neurones and found only on the right side, most recordings were made from the left ganglion which allowed easier access to the cells being studied.

### Electrical activity

CV cells were penetrated and their electrical activity was recorded at the same time as monitoring activity from identified 'feeding' motoneurons in the buccal ganglia (Benjamin & Rose, 1979). About 80% of isolated brain preparations show no spontaneous rhythmic motor output, as monitored from buccal motoneurons. In such preparations ('non-feeding' preparations) the spontaneous recorded activity in each CV cell type varied predictably. However, the four CV neurone types could not be distinguished by this criterion alone.

In preparations in which rhythmic feeding motor activity was recorded from buccal motoneurons ('feeding' preparations – about 20% of preparations), it was found that all four CV neurone types showed spontaneous rhythmic synaptic inputs, in time with those to the buccal motoneurons during the 'feeding' cycle (Benjamin & Rose, 1979; Rose & Benjamin, 1981a). Benjamin & Rose (1981b) described how the patterns of burst activity shown by buccal motoneurons are due to synaptic inputs which arise from three coupled subnetworks of buccal interneurons. Two of these subnetworks (N1 and N2) produce consecutive phases of synaptic input to the motoneurons, occurring during radula protraction and the first phase of retraction respectively. The third subnetwork (N3) provides inputs to the motoneurons, usually during retraction, which are seen as a series of short-duration postsynaptic potentials (PSPs). N1, N2 and N3 inputs may be excitatory or inhibitory on the different follower cells, and thus it is possible, by recording the activity of an identified buccal motoneuron, to monitor the different phases of synaptic input during the cycle ('feeding' inputs). In

In this study, the timing of cyclic inputs was monitored on buccal 3- and 4-group motoneurones. Buccal 4-group cells are known to receive two consecutive phases of inhibitory input during the cycle. These originate from the N1 and N2 interneuronal subnetworks. They are followed by a post inhibitory rebound burst of spikes during retraction. This burst is sometimes fractionated by a series of N3 inhibitory inputs. Buccal 3-cells receive inhibitory N1 followed by excitatory N2 input. This leads to bursting during retraction. (See Benjamin & Rose, 1979; Rose & Benjamin, 1981a for details of synaptic inputs to buccal motoneurones during the 'feeding' cycle.)

Synaptic inputs, seen as summed PSPs which occur at the same time as N1, N2 and N3 inputs to buccal motoneurones, were recorded in the CV neurones. The timing and polarity of these inputs assisted in CV cell identification. For convenience in the present paper, the 'feeding' inputs to CV neurones will be termed 'N1', 'N2' and 'N3' inputs, though their exact origin is unknown (see Discussion).

### *Cell morphology*

After electrophysiological recording, CV neurones were injected intracellularly with the dye Lucifer Yellow. The locations of fine branching and axonal projections further enabled the identification of cell types. All four cell types showed axonal projections in one or more of the three paired lip nerves, the superior and median lip nerves and the labial artery nerve (Fig. 1). These nerves innervate muscles around the mouth (Carriker, 1945; Lacaze-Duthiers, 1872) and have their roots in the cerebral ganglia.

The following section describes the morphology and electrical properties of each of the four CV cell types. The cells fall into two groups, based on their location in the cerebral ganglia, and their burst activity during the 'feeding' cycle: (i) CV3, located more dorsally in the ganglion, shows spike activity mainly during the retraction phase of the cycle, and during the periods between cycles; (ii) cell types CV7, CV5 and CV6 are located as a group more ventrally, and tend to burst during the protraction phase. These three cell types are distinguishable, however, particularly on the basis of their morphology. The four identified types were found repeatedly from one preparation to another.

### *Properties of CV neurones*

#### *CV3 anatomy*

CV3 cell bodies are located on the cerebral ventral lobe surface, between the roots of the superior and median lip nerves (Fig. 1). Each CV3 cell has a single axon which leaves the ganglion *via* the labial artery nerve (Fig. 2A). Some fine branching is seen in the cerebral neuropile.

#### *CV3 electrical properties*

In a 'non-feeding' preparation (one in which N1, N2 and N3 synaptic inputs to buccal motoneurones are not present), CV3 cells fire continuously with occasional interruptions of spike activity which are due to groups of large (approximately 5 mV), short duration (<100 ms) inhibitory synaptic inputs, apparently not shared by buccal motoneurones (Fig. 2B). In a 'feeding' preparation (Fig. 2C), CV3 receives synaptic

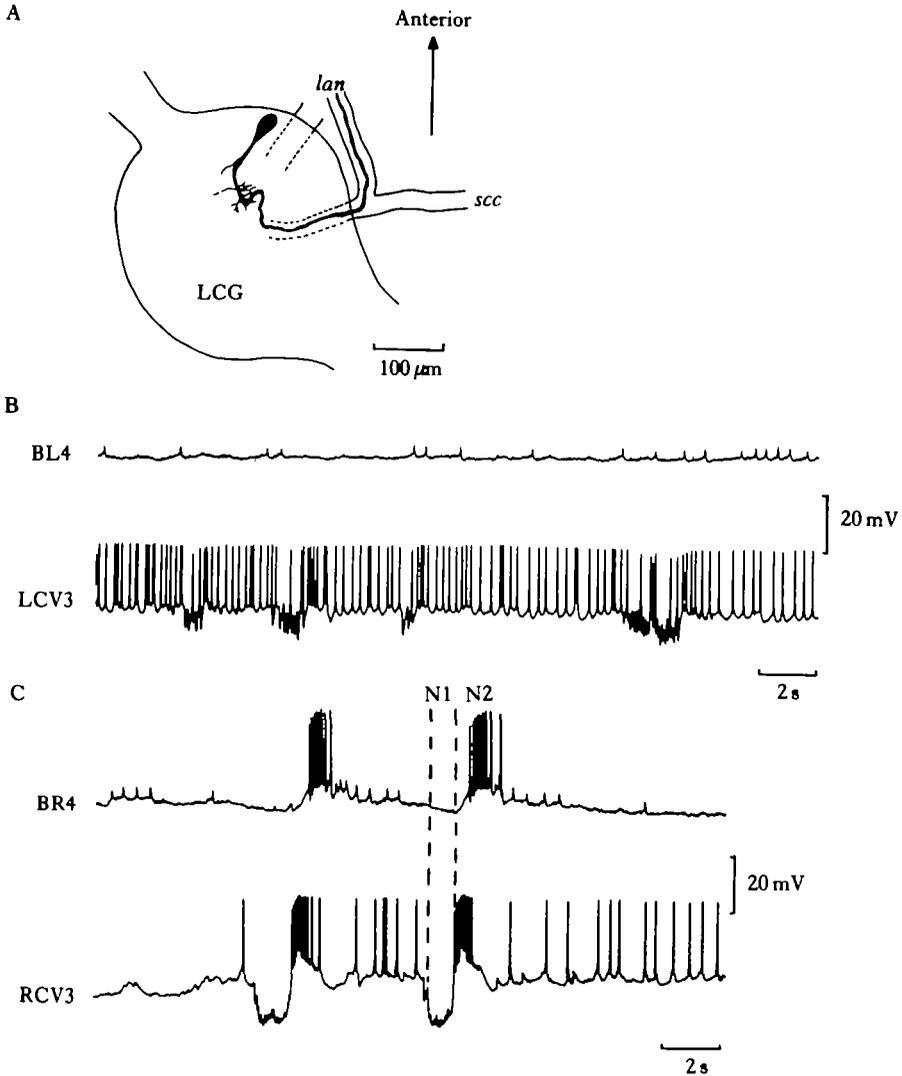


Fig. 2. Anatomy and electrical activity of CV3 cells. (A) Reconstruction of Lucifer Yellow filled left CV3, showing fine branching in cerebral neuropile and single axon in the labial artery nerve. LCG, left cerebral ganglion; *lan*, labial artery nerve; *scc*, subcerebral commissure. (B) Activity of left CV3 cell in 'non-feeding' preparation. Continuous spiking is interrupted by groups of large, short-duration inhibitory inputs, not shared by a buccal 4-group motoneurone (BL4). (C) Activity in right CV3 cell in relation to 'feeding' cycles monitored on a 4-group cell (BR4). CV3 receives 'N1' inhibition followed by 'N2' excitation leading to a burst of spikes.

inputs which appear to be synchronous with the N1 and N2 inputs to buccal motoneurons. The 'N1' input to CV3 is inhibitory, and superimposed on it are phasic (possibly unitary) PSPs which resemble those seen in a 'non-feeding' preparation (Fig. 2B). During the N2 phase, CV3 shows a burst of action potentials (Fig. 2C). This appears to be due to excitatory synaptic input rather than a post inhibitory rebound effect, since it still occurs when a CV3 cell is hyperpolarized to a level at which the 'N1' inhibition is at its reversal potential (Fig. 3). Furthermore, during

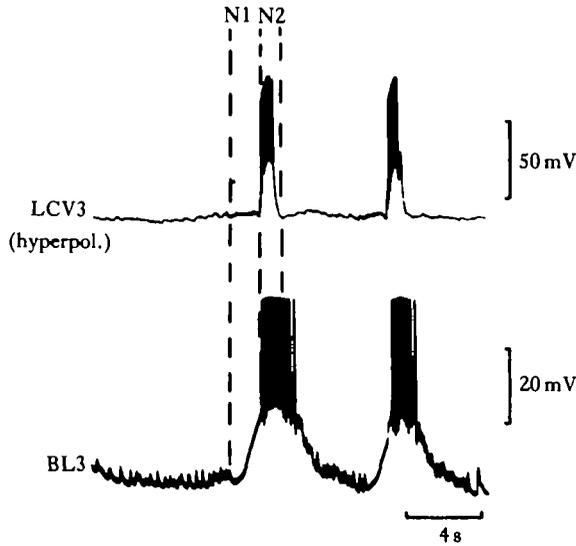


Fig. 3. Effects of hyperpolarizing current injection on 'N1' and 'N2' inputs to left CV3. N1 and N2 inputs are monitored on a left buccal 3-cell (BL3). Following hyperpolarization of CV3 to the reversal potential for its 'N1' inhibition, 'N2' excitation persists at increased amplitude (cf. Fig. 2C).

hyperpolarization, the amplitude of the 'N2' depolarizing potential is increased (from its normal size of about 10 mV to 20 mV for the cell in Fig. 3) suggesting that it is a chemical PSP.

At least two CV3 type cells were shown, by paired recordings, to be present in each cerebral ganglion, but no mutual synaptic connections were demonstrated.

CV3 cells differ from the other three neurone types described here, in that they fire regularly in the absence of 'feeding' inputs, and also burst during the retraction phase (N2) of the cycle, rather than during protraction.

#### *CV7 anatomy*

CV7 cells occur as a population of five or six cells in each cerebral ganglion. The cell bodies are located on the ganglion surface between the roots of the median lip nerve and the cerebrobuccal connective (Fig. 1). Each CV7 cell has a single axon which travels dorsally in the ganglion and then exits *via* the superior lip nerve (Fig. 4A). Some fine branching is seen in the cerebral neuropile.

#### *CV7 electrical properties*

In a 'non-feeding' preparation, CV7 cells are usually silent, though they may show occasional action potentials. In a 'feeding' preparation, synaptic inputs are seen on CV7 cells at the same time as N1 and N2 identified inputs to buccal motoneurons (Fig. 4B). The CV7 cells receive excitatory 'N1' input, resulting in a burst of spikes, followed by a compound inhibitory postsynaptic potential (IPSP) during the N2 phase, when the burst terminates (Fig. 4B,C). Current injection reveals these inputs more clearly, and shows them to behave like conventional chemical PSPs (Fig. 5).

Depolarization decreases the amplitude of the 'N1' input and increases the size of the 'N2' hyperpolarization. Hyperpolarization has the reverse effects.

CV7 cells receive an additional synaptic input during the feeding cycle – a series of summing excitatory postsynaptic potentials (EPSPs) preceding the 'N1' input (Fig. 4C, arrows). The source of these EPSPs is unknown, but they sometimes lead to firing in CV7 before the onset of the 'N1' input.

CV7 cells in the same ganglion are coupled by non-rectifying electrotonic junctions. Application of long-duration square pulses of depolarizing or hyperpolarizing current to one CV7 cell results in an attenuated but similar response in a second CV7 cell, recorded in the same ganglion. There is no obvious delay in the onset of the postsynaptic response compared with that in the current-injected cell (Fig. 6A,B). Coupling of this sort was seen in all pairs of CV7 cells penetrated in the same ganglion. Action potentials in one CV7 cell occur at the same time as either a spike or a small depolarization (probably an electrotonic EPSP) in other CV7 cells (Fig. 6A,B,C). The presence of such EPSPs occurring synchronously in two CV7 cells, in the absence of a spike in either, suggests that the two cells are receiving common inputs, possibly from another CV7 cell in the network (Fig. 6C).

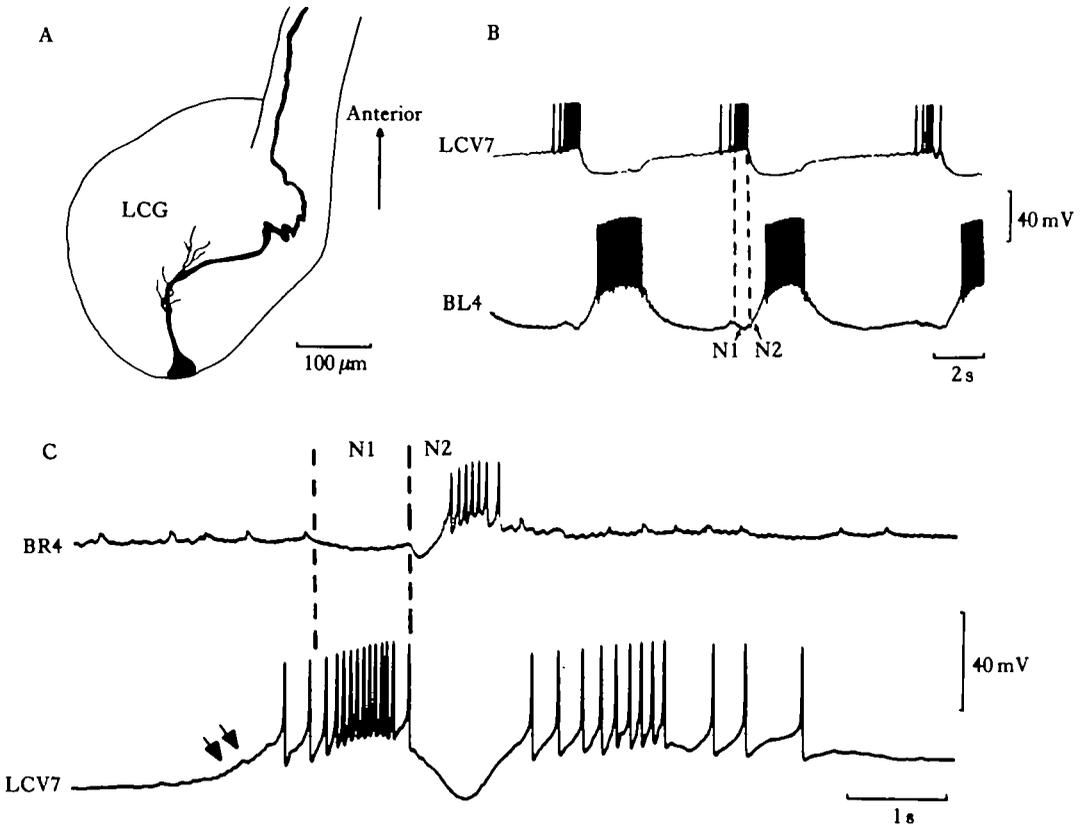


Fig. 4. Anatomy and firing patterns of CV7 cells. (A) Reconstruction of Lucifer Yellow filled left CV7. The single axon exits *via* the superior lip nerve. LCG, left cerebral ganglion. (B) (C) Firing patterns of left CV7 cells in relation to burst activity in buccal 4-group motoneurons (BL4 and BR4). Timing of N1 and N2 inhibitory inputs to 4-group cells is indicated. CV7 receives excitatory 'N1' and inhibitory 'N2' inputs. Additional pre-N1 excitation to CV7 is also present (arrows in C). Rebound activity in CV7 following 'N2' inhibition is sometimes observed (C).

To summarize, CV7 cells occur as an electrotonically coupled network whose coupling could help to produce similar firing activity in all the cells (cf. 4-group cells, Benjamin & Rose, 1979). In 'non-feeding' preparations, CV7 cells are usually inactive. When buccal motoneurons are showing rhythmic 'feeding' activity, CV7 cells show a protraction burst of action potentials owing to excitatory 'N1' input. This is followed by inhibitory 'N2' input.

#### CV5 anatomy

CV5 cells project to the periphery in a single lip nerve, but differ from the other three cell types described here in having a projection to the subcerebral commissure, which connects left and right cerebral ganglia ventrally (Fig. 1). The single axon crosses the cerebral neuropile and enters the labial artery nerve. Here it branches into two, one branch continuing to the periphery, the other entering the subcerebral commissure (Fig. 7A). The CV5 axon in the subcerebral commissure was never seen to enter the contralateral ventral lobe, though this may have been due to insufficient dye in the fine process.

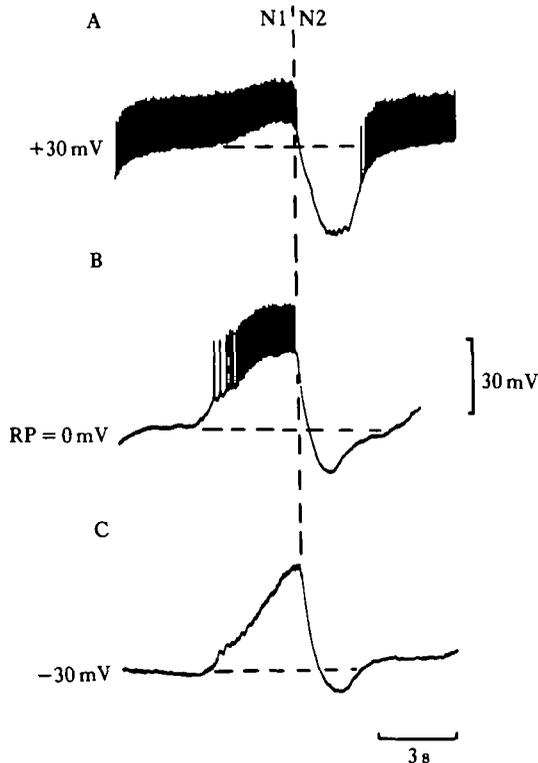


Fig. 5. Effects of current injection on 'N1' and 'N2' inputs to a CV7 cell. CV7 cell was (A) depolarized by approximately 30 mV, (B) recorded at resting potential (RP), and (C) hyperpolarized by approximately 30 mV. (The level of polarization was measured within the limits of accuracy of the bridge-balance system in the preamplifier. Current injected was less than 1 nA.) Amplitudes of 'N1' and 'N2' inputs to CV7 cells change, in response to membrane polarization, appropriately for chemical EPSPs and IPSPs respectively.

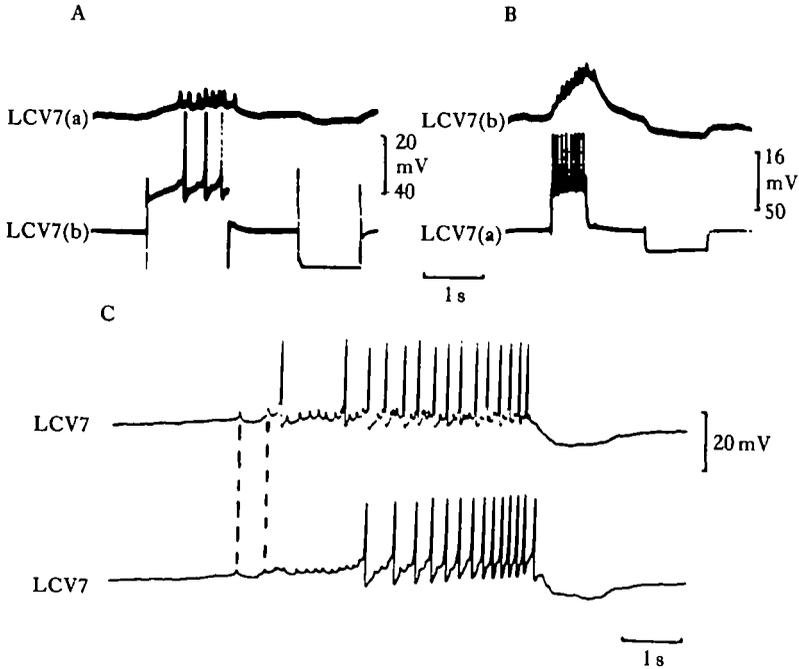


Fig. 6. Electrotonic coupling between CV7 cells. (A) (B) Two CV7 cells (a and b) were simultaneously recorded. The bridge in the preamplifier was balanced for each electrode, to allow passage of current whilst recording fairly accurately any resulting changes in membrane potential. Injection of depolarizing and hyperpolarizing pulses into either cell leads to smaller but similar responses in the other. Spiking in one cell also produces subthreshold depolarizing potentials in the other. The junction is non-rectifying, with a coupling ratio of approximately 0.25. (C) Activity of two CV7 cells, recorded simultaneously in the same ganglion. Cells show synchronous spikes and EPSPs. Dotted lines indicate synchronous EPSPs in the absence of spiking.

### CV5 electrical properties

Like the CV7 cell type, CV5 cells are usually inactive in a 'non-feeding' preparation. However, in a preparation whose buccal motoneurons show rhythmic bursts, the CV5 cells also show strong rhythmic burst activity. During the N1 phase of the feeding cycle they receive a large (20–25 mV) depolarizing compound PSP which leads to a strong burst of action potentials. This is followed by a marked 'N2' inhibitory input, which terminates the burst (Fig. 7B).

CV5 cells therefore resemble CV7 cells in their synaptic inputs during the cycle. These lead to bursting during radula protraction.

### CV6 anatomy

CV6 cell bodies are located in the same area in the cerebral ventral lobe as the CV7 and CV5 cells; that is, between the roots of the median lip nerve and the cerebrobuccal connective. This cell type has two peripheral axonal projections, one in the median lip nerve and a second in the much finer labial artery nerve (Fig. 8A).

### CV6 electrical properties

In a 'non-feeding' preparation, CV6 cells are silent, but in a 'feeding' preparation,

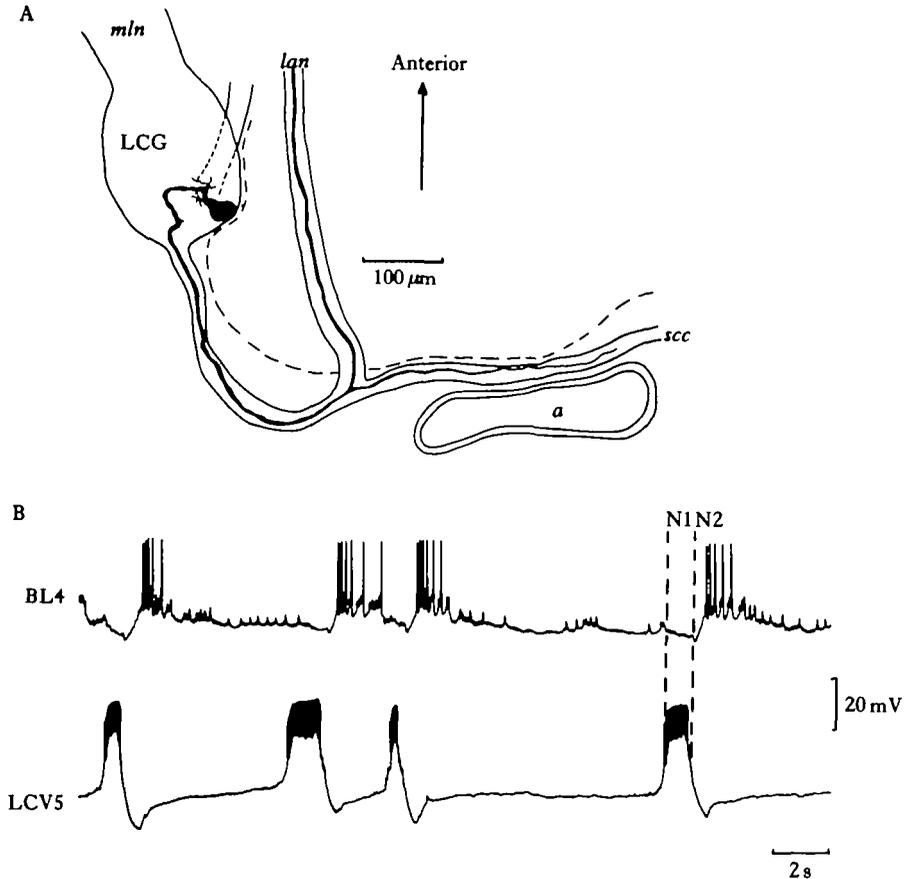


Fig. 7. Anatomy and electrical activity of CV5 cells. (A) Reconstruction of Lucifer Yellow filled CV5, showing fine branching in the cerebral neuropile, and axonal branches in the labial artery nerve and subcerebral commissure. Dashed line indicates extent of connective tissue sheath surrounding the brain. LCG, left cerebral ganglion; *lan*, labial artery nerve; *mln*, median lip nerve; *scc*, subcerebral commissure; *a*, aorta. (B) Electrical activity in CV5 in relation to burst activity of a buccal 4-group motoneurone (BL4). N1 and N2 synaptic inputs to BL4 are indicated for one cycle. CV5 bursts strongly during N1 and is inhibited during N2.

synaptic inputs can be recorded from CV6 cells, which correspond to the N1, N2 and N3 synaptic inputs to buccal motoneurons (Fig. 8B). CV6 receives a compound excitatory 'N1' input, and fires a strong burst. The burst is apparently terminated by inhibitory 'N2' input. A third synaptic input on CV6 occurs at the same times as N3 input to a 4-group cell during its rebound burst. It is a periodic input, occurring as a series of inhibitory waves which fractionate the 4-cell burst into groups of spikes (Rose & Benjamin, 1981a). These inhibitory waves occur at the same time as small waves of inhibitory input to CV6 (Fig. 8B). CV6 was the only cell type of the four described here to display these 'N3' inputs.

A further synaptic input to CV6 is that preceding the 'N1' excitation. As seen on CV7 cells, apparently unitary EPSPs occur on CV6 (Fig. 8B), which sum and can lead to excitation of CV6 prior to the onset of 'N1' excitatory input. It is not known whether this pre-'N1' input to CV7 and CV6 cells has the same origin.

The CV6 cells, in summary, have peripheral projections in two different lip nerves. They receive synaptic inputs during the 'feeding' cycle, which lead to a burst of spikes during radula protraction (N1).

*The relationship of CV neurone activity to that in lip musculature*

The cell types CV3, 7, 5 and 6 are presumed to be motoneurons because of their peripherally projecting axons, and their strong rhythmic burst activity in phase with that in buccal motoneurons, in an isolated brain preparation, where no sensory influences could be acting. The cells all project to the lip musculature, suggesting some role in the control of lip movements. This section provides evidence supporting this hypothesis for one of the CV neurones, CV7.

The innervation of the lip musculature is complex (Carriker, 1945; Elo, 1938; Lacaze-Duthiers, 1872), and knowledge of the nerve along which a cell's axon projects is not sufficient to predict its target muscle (see Discussion). However, comparison of the phases of activity of the different CV cells with the timing of activity in different muscles allows some prediction of the likely targets. Hubendick (1956) and Carriker (1946) described in detail the muscles involved during opening and closing of the mouth in *Lymanaea*, and the times at which they contract. During the protraction

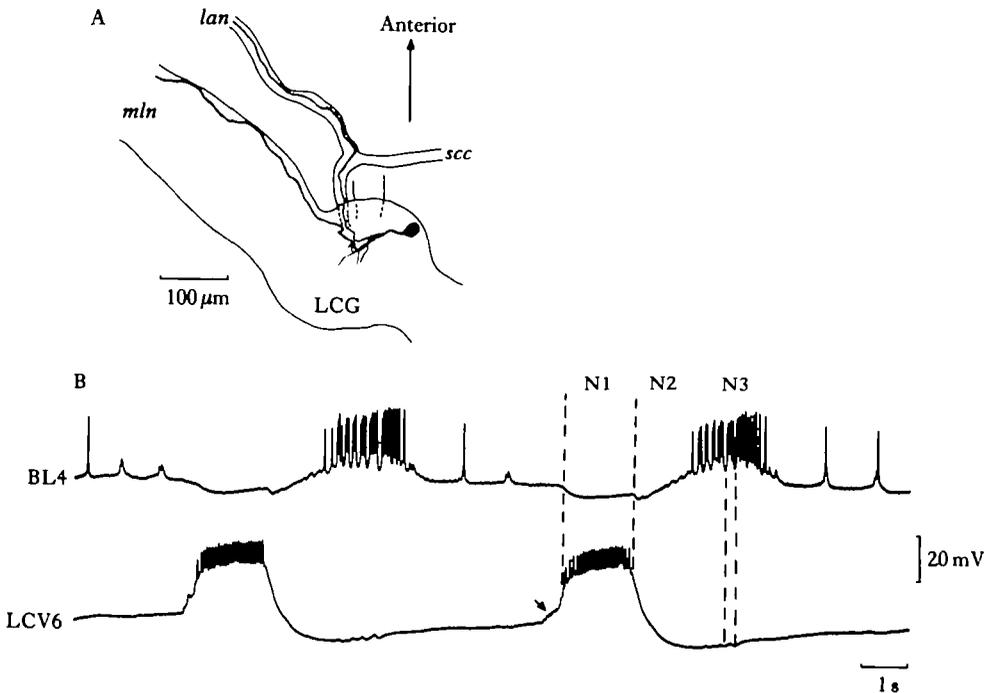


Fig. 8. Anatomy and electrical activity of CV6 cells. (A) Reconstruction of Lucifer Yellow filled left CV6, showing fine branching in the cerebral neuropile, and axonal projections in the median lip nerve and labial artery nerve. *lan*, labial artery nerve; LCG, left cerebral ganglion; *mln*, median lip nerve; *scc*, subcerebral commissure. (B) Electrical activity in CV6, in relation to N1, N2 and N3 inputs to a buccal 4-group cell (BL4). CV6 bursts due to 'N1' excitation, which is preceded by pre-N1 excitatory input (arrow). At the same time as 'N2' inhibition, additional inhibitory inputs to CV6 are asynchronous with N3 inhibitory inputs to BL4 (dotted lines).

Phase (corresponding to N1; Rose & Benjamin, 1981b), the oral aperture opens owing to the action of the suboral and dorsomandibular dilators and the labial retractor muscle. This is the time at which CV7, 5 and 6 cells are bursting. During the latter stages of the cycle (retraction, N2), when the CV3 cells burst, the mouth closes again by action of the labial sphincter muscle and relaxation of the labial retractor.

The suboral dilator (involved in mouth opening during protraction; Hubendick, 1956) is a discrete muscle lying between the body wall and the ventral buccal mass, and is therefore easy to locate for recording purposes. Recordings of activity in this muscle, together with a CV7 cell show that it is indeed active during the protraction phase (Fig. 9A). Furthermore, CV7 cells were found to produce 1:1 spike activity in suboral dilator muscle when firing spontaneously or when made to fire by current injection (Fig. 9B). The latency between spikes recorded in the cell and those in the muscle was about 10 ms. Hence it appears that CV7 cells do innervate a muscle appropriate to cause mouth opening at the correct phase (protraction), when CV7 cells are normally active.

Muscle activity when the mouth closes (during the retraction phase) is more difficult to record. The labial sphincter (the only muscle actively involved; Carriker, 1946) exists as a number of fine diffuse fibres around the oral aperture, as also does the labial retractor muscle (Carriker, 1945). Attempts to record labial sphincter activity during the retraction phase were unsuccessful. Suction electrode recordings from the region of this muscle revealed only activity during protraction, which was

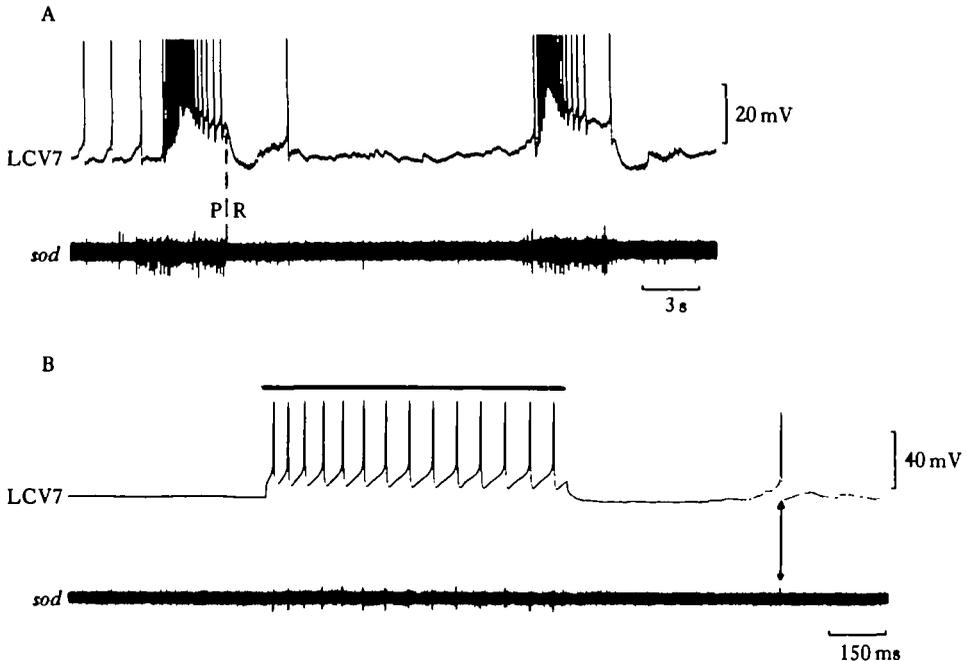


Fig. 9. Innervation of suboral dilator muscle (*sod*) by CV7 cells. (A) CV7 and *sod* both show spike activity during the protraction phase (P) but stop bursting during retraction (R). (B) Action potentials in CV7, evoked by current injection (indicated by bar) are followed 1:1 by spikes in *sod* muscle. Spontaneous spike in CV7 also leads to spike in *sod* (arrow).

presumably due to the labial retractor muscle (a mouth opener muscle; Hubendick, 1956). Because of the difficulties in recording labial sphincter muscle activity it was not possible to confirm the role of CV3 (which is active during retraction, and also in a 'non-feeding' preparation) as a mouth closer motoneurone.

#### DISCUSSION

The results presented here show that there are indeed neurones located in the ventral cerebral ganglia of *Lymnaea* whose morphologies indicate that they may be motoneurons innervating the muscles of the lips. All possess a relatively simple central morphology and one or two large peripheral axons that leave the ganglion along one or more of the lip nerves. In addition, these neurones show an appropriate pattern of cyclic burst activity for the control of lip muscles, which occurs in phase with the rhythmic activity that may be recorded from identified 'feeding' motoneurons in the buccal ganglia. In the case of one of the cerebral cell types (CV7) it was possible to demonstrate physiologically the innervation of lip musculature by recording 1:1 cell and muscle potentials.

There are difficulties involved in demonstrating motoneuronal function for most of the CV neurones, which stem from the complex anatomy of the system. Firstly, the pattern of innervation of muscles is confused. Branches from both the superior and median lip nerves innervate the lips around the oral aperture (Carriker, 1945). These fine branches form a network within the lip tissue so that their areas of innervation overlap (Elo, 1938). Thus axons travelling in either the superior or median lip nerve (or the labial artery nerve; Lacaze-Duthiers, 1872) could reach any of the muscles controlling the oral aperture (i.e. the suboral and dorsomandibular dilators, the labial retractor and the labial sphincter muscle; Carriker, 1945). It is not therefore possible to predict the target muscle from the nerve by which a CV cell leaves the ganglion.

Secondly, some of the lip muscles (e.g. the labial sphincter and labial retractor) exist as diffuse groups of fibres within the body wall, and may overlap. It is therefore difficult to record with certainty from some specific muscles (see Results).

Despite the absence of direct evidence, due to problems outlined here, it seems most likely that cell types CV3, 5 and 6 (like CV7) are motoneurons involved in control of lip musculature. However, the possibility that these cells have another role cannot be ruled out, though this seems unlikely. Sensory neurones, for example, would not be expected to receive strong modulatory inputs in an isolated brain preparation, nor would they show so much spontaneous spiking activity.

The interesting question arising from this work is that of the origin of the 'N1', 'N2' and 'N3' synaptic inputs to the CV cells. How is rhythmic activity in buccal and cerebral motoneurons coordinated? Inputs with timing similarly related to the N1 and N2 phases of the feeding cycle have also been described in cerebral ganglion interneurons, the cerebral giant cells (McCrohan & Benjamin, 1980*b*) and cell CV1 (Benjamin *et al.* 1981). The simplest explanation is that the 'N1', 'N2' and 'N3' inputs originate directly from the N1, N2 and N3 interneurons in the buccal ganglia. This would require that axonal projections of at least some of these interneurons enter the cerebral ganglia *via* the cerebrobuccal connectives. It is not known whether such processes do exist. An indirect coupling by way of intermediate interneurons, which

Receive inputs from the buccal N1, N2 and N3 interneuronal subnetworks and then synapse with the CV cells seems less likely. Phasic 'feeding' inputs were never observed in buccal motoneurones in the absence of similar phase-locked inputs to CV cells. If such indirect connections were present, it would be expected that occasional weakening or failure of coupling could occur. In fact coupling is so strong that even the individual components of the fractionated N3 input are tightly phase-locked between the two ganglia. An alternative explanation is that the cerebral ganglia contain their own rhythm-generating network of interneurons. This would be tightly phase-locked with the buccal network owing to the presence of ascending and/or descending connections in the cerebrobuccal connectives. Davis *et al.* (1973) originally suggested that, in *Pleurobranchaea*, separate 'feeding' oscillators were contained in the brain and buccal ganglia. Each of these oscillators, when isolated from the other, would be capable of generating a rhythmic motor output in its associated nerve roots. The oscillators were thought to be coupled by ascending Corollary Discharge and Efference Copy interneurons in the cerebrobuccal connectives. However, a more recent study (Cohan & Mpitsos, 1983*a,b*) indicates that the generation of a rhythmic output from the brain (associated with feeding behaviour) may in fact depend on a sustained cyclic input from the buccal ganglia *via* buccal-cerebral interneurons, and that the brain in isolation cannot generate a 'feeding' rhythm. Similarly, in *Lymnaea*, it has not been possible to demonstrate a cerebral rhythm generator. Phasic inputs to CV neurones, similar to the 'feeding' inputs described here, were never recorded in preparations in which the cerebrobuccal connectives had been severed. Also, phase coupling would have to be very precise to produce the closely timed pattern of N3 inputs described above.

It appears, therefore, that the mechanism for cerebrobuccal coordination is probably similar in *Lymnaea* and *Pleurobranchaea*, a single buccal rhythm generator providing output to cells of both the buccal and cerebral ganglia. In *Pleurobranchaea* the system is flexible, owing to the heterogeneity of the population of buccal-cerebral interneurons (BCIs) that are thought to coordinate the two ganglia. Cohan & Mpitsos (1983*b*) suggest that activity in different types of BCI can lead to the differences in motor output which underlie the various buccal behaviour patterns such as feeding and regurgitation. It is not known whether a similar system is present in *Lymnaea*. Further investigation of this question would require studies in intact or semi-intact animals, to determine whether the presumed 'feeding' output observed in isolated nervous systems actually represents neural correlates for a number of distinguishable behaviour patterns, as in *Pleurobranchaea*. It would be expected, however, that the system in *Lymnaea* is much simpler, involving a smaller range of different behavioural sequences.

I thank Paul Benjamin and Steve Soffe for advice and comments; also the SERC and the Royal Society for financial support.

#### REFERENCES

- BENJAMIN, P. R., McCROHAN, C. R. & ROSE, R. M. (1981). Higher order interneurons which initiate and modulate feeding in the pond snail *Lymnaea stagnalis*. *Adv. physiol. Sci.* **23**, 171–200.

- BENJAMIN, P. R. & ROSE, R. M. (1979). Central generation of bursting in the feeding system of the snail, *Lymnaea stagnalis*. *J. exp. Biol.* **80**, 93–118.
- BOVBERG, R. V. (1968). Responses to food in lymnaeid snails. *Physiol. Zool.* **41**, 412–423.
- BULLOCH, A. G. M. & DORSETT, D. A. (1979). The integration of the patterned output of buccal motoneurons during feeding in *Tritonia hombergi*. *J. exp. Biol.* **79**, 23–40.
- CARRIKER, M. R. (1945). Morphology of the alimentary system of the snail *Lymnaea stagnalis appressa* Say. *Trans. Wis. Acad. Sci. Arts Lett.* **38**, 1–88.
- CARRIKER, M. R. (1946). Observations on the functioning of the alimentary system of the snail *Lymnaea stagnalis appressa* Say. *Biol. Bull. mar. biol. Lab., Woods Hole* **91**, 88–111.
- COHAN, C. S. & MPITSOS, G. J. (1983a). The generation of rhythmic activity in a distributed motor system. *J. exp. Biol.* **102**, 25–42.
- COHAN, C. S. & MPITSOS, G. J. (1983b). Selective recruitment of interganglionic interneurons during different motor patterns in *Pleurobranchaea*. *J. exp. Biol.* **102**, 43–57.
- DAVIS, W. J., SIEGLER, M. V. & MPITSOS, G. J. (1973). Distributed neuronal oscillators and efference copy in the feeding system of *Pleurobranchaea*. *J. Neurophysiol.* **36**, 258–274.
- ELO, J. E. (1938). Das nervensystem von *Lymnaea stagnalis* (L.). *Lam. Ann. Zool. Soc. Zool. Bot. Fenn. Vanamo.* **6**, 1–40.
- GRAHAM, A. (1955). Molluscan diets. *Proc. malac. Soc. Lond.* **31**, 144–159.
- HUBENDICK, B. (1956). The eating function in *Lymnaea stagnalis* (L.). *Ark. Zool.* **10**, 511–521.
- KANEKO, C. R. S., MERICKEL, M. & KATER, S. B. (1978). Centrally programmed feeding in *Helisoma*: Identification and characteristics of an electrically coupled premotor neurone network. *Brain Res.* **146**, 1–21.
- LACAZE-DUTHIERS, H. DE (1872). Du systeme nerveux des mollusques gasteropodes pulmones aquatiques et d'un nouvel organe d'innervation. *Archs Zool. exp. gén.* **1**, 437–500.
- LEE, R. M. & LIEGEOIS, R. J. (1974). Motor and sensory mechanisms of feeding in *Pleurobranchaea*. *J. Neurobiol.* **5**, 545–565.
- MCCLELLAN, A. D. (1982a). Movements and motor patterns of the buccal mass of *Pleurobranchaea* during feeding, regurgitation and rejection. *J. exp. Biol.* **98**, 195–211.
- MCCLELLAN, A. D. (1982b). Re-examination of presumed feeding motor activity in the isolated nervous system of *Pleurobranchaea*. *J. exp. Biol.* **98**, 213–228.
- MCCROHAN, C. R. & BENJAMIN, P. R. (1980a). Patterns of activity and axonal projections of the cerebral giant cells of the snail, *Lymnaea stagnalis*. *J. exp. Biol.* **85**, 149–168.
- MCCROHAN, C. R. & BENJAMIN, P. R. (1980b). Synaptic relationships of the cerebral giant cells with motoneurons in the feeding system of *Lymnaea stagnalis*. *J. exp. Biol.* **85**, 169–186.
- ROSE, R. M. & BENJAMIN, P. R. (1979). The relationship of the central motor pattern to the feeding cycle of *Lymnaea stagnalis*. *J. exp. Biol.* **80**, 137–163.
- ROSE, R. M. & BENJAMIN, P. R. (1981a). Interneuronal control of feeding in the pond snail *Lymnaea stagnalis*. I. Initiation of feeding cycles by a single buccal interneurone. *J. exp. Biol.* **92**, 187–201.
- ROSE, R. M. & BENJAMIN, P. R. (1981b). Interneuronal control of feeding in the pond snail *Lymnaea stagnalis*. II. The interneuronal mechanism generating feeding cycles. *J. exp. Biol.* **92**, 203–228.