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CENTRAL COORDINATION OF BUCCAL AND PEDAL NEURONAL ACTIVITY IN THE POND SNAIL *LYMNAEA STAGNALIS*

BY M. A. KYRIAKIDES AND C. R. McCROHAN

Department of Physiological Sciences, University of Manchester, Manchester M13 9PT, UK

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Summary

Cyclical synaptic inputs were recorded from identified giant neurones and neuronal cluster cells in the pedal ganglia of *Lymnaea stagnalis*. They occurred in phase with rhythmical inputs to buccal ganglion motoneurones, which have been shown to originate from interneurones of the buccal central pattern generator for feeding. In pedal neurones, the cyclical inputs were mainly inhibitory, and occurred predominantly during the radula retraction phase of the feeding cycle.

Tonic depolarization of higher-order interneurones in the feeding system, to activate the buccal central pattern generator, led to the onset of cyclical inputs to pedal neurones. These inputs were abolished after cutting the cerebrobuccal connectives, supporting the hypothesis that they originate from the buccal ganglia. The possible role of these inputs in coordinating foot and body wall movements with the buccal feeding rhythm is discussed.

Introduction

Coordination among different motor centres in the central nervous system (CNS) is essential for the orderly production of behavioural output. In vertebrates, centrally generated rhythmic motor outputs for behaviour patterns such as respiration and locomotion are closely coordinated, though these motor patterns may also occur independently (Viala & Freton, 1983; Miller & Schomburg, 1985). In invertebrates, interneurones have been identified whose synaptic connections lead to coordinated output from central pattern generators controlling different aspects of behaviour. For example, the IVN neurone of the lobster ensures an appropriate relationship between rhythmic motor output to pyloric and gastric regions of the gut, in response to proprioceptive input (Sigvardt & Mulloney, 1982). In the gastropod *Pleurobranchaea*, suppression of the withdrawal response during feeding is mediated by inhibition of cerebral withdrawal

Key words: *Lymnaea stagnalis*, molluscan CNS, neural coordination, feeding, locomotion, motor programme.

neurones by buccal–cerebral corollary discharge neurones, which carry information about feeding motor output from the buccal ganglia to the withdrawal motor centre (Davis *et al.* 1980).

Although central coordination between motor centres responsible for different behavioural outputs clearly exists, examples such as those described above, in which underlying cellular mechanisms have been elucidated, are scarce. The CNS of the pond snail, *Lymnaea stagnalis*, provides a good opportunity to study such neural mehanisms. It has been extensively mapped (Benjamin, Rose, Slade & Lacy, 1979; Slade, Mills & Winlow, 1981; M. A. Kyriakides, C. R. McCrohan, N. Syed & W. Winlow, in preparation), including functional mapping of the neural network responsible for feeding motor output (Benjamin, 1983; McCrohan, 1984*a*,*b*; Elliott & Benjamin, 1985*a*,*b*) and, to some extent, those neurones responsible for producing locomotory output (Haydon, 1982; Haydon & Winlow, 1986; Winlow & Haydon, 1986).

Rhythmic feeding activity in *Lymnaea* is generated by an identified network of central pattern generating interneurones which are located in the buccal ganglia (Elliott & Benjamin, 1985*a*). Differential excitatory and inhibitory postsynaptic inputs from this central pattern generator lead to coordinated bursting activity in motoneurones in the buccal and cerebral ganglia, innervating the buccal mass, salivary glands, gut and lips (Benjamin & Rose, 1979; Rose & Benjamin, 1979, 1981; McCrohan, 1984*a*). The buccal central pattern generator comprises three main interconnected subnetworks of interneurones – N1, N2 and N3 interneurones (Elliott & Benjamin, 1985*a*) – which fire consecutively during each feeding cycle; N1 interneurones during radula protraction, N2 interneurones during radula scraping, and N3 during food ingestion/swallowing. Inputs from each of the subnetworks can be individually recognized whilst recording intracellularly from identified buccal and cerebral motoneurones.

Locomotion in *Lymnaea* is effected either by the activity of cilia on the sole of the foot or by rhythmical muscular contractions of the foot, accompanied in both cases by cyclical movements of the shell and column (Kaiser, 1960; Jones, 1975; Winlow & Haydon, 1986). Motoneurones innervating the body wall and column have been identified as those of the D and F clusters in the pedal ganglia (Slade *et al.* 1981; Winlow & Haydon, 1986). During spontaneous locomotion, these pedal motoneurones receive rhythmical synaptic inputs leading to bursts of spikes which are associated with contractions of the column and body wall and cyclical movements of the shell. Additionally, cells of the pedal E clusters, which have axonal projections to the foot, may also be involved in the production of locomotory motor output (W. Winlow & N. Syed, personal communication). However, the central pattern generator responsible for producing these inputs has not yet been located.

Behavioural observations of *Lymnaea* have shown that cyclical radula movements are correlated with head movements and with the rate of forward locomotion (Dawkins, 1974). In addition, it was observed that radula scraping does not take place at the same time as a reversal in the direction of side to side

head movements. In this study we used the isolated CNS of *Lymnaea* to examine the detailed relationship between activity and synaptic inputs of neurones involved in the central control of feeding, locomotion and other body movements. We show that pedal ganglion neurones, some of which are responsible for neck and body wall contractions, receive inhibitory synaptic inputs which occur at the same time as, and probably arise from, the buccal central pattern generator for feeding. These results provide a basis for studies of the functional relationship between two motor systems and the underlying neural mechanisms which coordinate their activity.

Materials and methods

Pond snails, *Lymnaea stagnalis*, weighing 2–6 g were obtained from animal suppliers (Blades Biological, Cowden, Kent) or collected from ponds in Stockport, Cheshire. They were kept in aerated tap water at 20°C and fed on lettuce.

Experiments were carried out on the isolated CNS which was pinned out by its attached nerves in a recording chamber and bathed in a Hepes-buffered saline (Benjamin & Winlow, 1981) at 20°C. The periganglionic sheath overlying the cells to be recorded was stripped using fine forceps. The cerebral commissure was cut when recording from the pedal ganglia; otherwise all interganglionic connectives were left intact except during specific lesioning experiments.

Intracellular recordings were made using glass microelectrodes containing either a 4 mol l^{-1} potassium acetate solution (resistance 15–35 MΩ) or a 3% Lucifer Yellow CH solution in 1 mol l^{-1} lithium chloride (resistance 30–70 MΩ). Prior to use, the electrode was dipped in black waterproof ink to facilitate viewing of the tip. Signals were preamplified by a probe near the preparation and conveyed to a ×10 modified Soffe amplifier incorporating a bridge balance circuit. They were displayed on an oscilloscope and stored on tape; permanent records were made using a Gould four-channel pen-recorder. Current injected (usually less than 1 nA) was measured using a current to voltage converter placed between the indifferent electrode and earth. Extracellular recordings from nerves were carried out using suction electrodes.

After electrophysiological recording, cells in the pedal ganglia were ionophoretically injected with Lucifer Yellow, using 1 nA, 500 ms hyperpolarizing pulses at 1 Hz for up to 1 h, occasionally interrupted by single 20 nA, 100 ms hyperpolarizing pulses. The preparation was then fixed for at least 2 h in Stewart's fixative (Stewart, 1978) before dehydrating in alcohol and clearing in methyl salicylate. The morphology of neurones was drawn using a drawing tube attachment on a compound epifluorescence microscope.

Results

Cyclical inputs to pedal neurones

Simultaneous intracellular recordings from identified buccal and pedal neurones revealed the presence of cyclical, compound synaptic inputs which occurred at the

same time in cells of both ganglia. In the buccal neurones, these inputs were identifiable as those produced by interneurones of the buccal feeding rhythm generator (Rose & Benjamin, 1981). For example, buccal retractor motoneurones (4-cells) receive consecutive inhibitory inputs from buccal N1 and N2 interneuronal networks during each feeding cycle. These are followed by a post-inhibitory rebound burst of action potentials during the second retraction phase (Rose & Benjamin, 1981). The cyclical inputs were not present in all pedal cell types, being confined to a small number of identifiable cell clusters and certain giant cells. They were found in three pedal cell clusters (PeE, PeF and PeM clusters; Slade *et al.* 1981; M. A. Kyriakides, C. R. McCrohan, N. Syed & W. Winlow, in preparation), in nine pedal giant neurones (R/LPeD1, R/LPeD2, R/LPeD3, LPeD5 and R/LPeD10) and in a newly identified putative pedal interneurone. Fig. 1 shows the location of these cells in the CNS of the snail as well as the positions of some of the buccal and cerebral neurones responsible for the production of feeding motor output. Table 1 lists the pedal neurones in which the cyclical inputs were observed, and summarizes their properties. The polarity of the inputs was mainly inhibitory. They most frequently occurred during the N2 phase of buccal central pattern generator activity, less often during the N1 phase, but never during the N3 phase. Table 1 also summarizes the percentage of 'feeding' preparations in which each pedal cell type was found to receive the rhythmic inputs. Since the isolated CNS does not always generate spontaneous rhythmic motor output for feeding but may be quiescent for indefinite periods (Elliott & Benjamin, 1985*a*), preparations were only included if the buccal central pattern generator was rhythmically active at the time of recording (so-called feeding preparations). In addition, Table 1 lists the nerves in which the pedal cells' axons are found (from M. A. Kyriakides, C. R. McCrohan, N. Syed & W. Winlow, in preparation), thus providing some information about their possible function (cf. Dorsett, 1986).

Cluster neurones

The largest group of pedal neurones receiving cyclical inputs in phase with those on buccal feeding motoneurones was the pedal F cluster (PeF). This cell cluster extends from the dorsal to the ventral surface of the pedal ganglia (Fig. 1) and was, therefore, subdivided into dorsal (F_d) and ventral (F_v) cells. 80 % of F_d cells and 75 % of F_v cells in feeding preparations received the inputs. However, as cells within each cluster were not individually identifiable, these percentages did not distinguish between whether each individual cell received inputs only 75 % of the time or whether most of the cells within the cluster received the inputs all of the time whereas some cells never received them. It was evident from continuous recordings, however, that the same individual cell within a cluster might or might not receive cyclical inputs at different times. The timing and polarity of cyclical inputs to F cells was variable (Table 1). However, the majority of F cells received inhibitory synaptic inputs, the most common sequence being a single, compound inhibitory input during the N2 phase of buccal interneuronal activity (Table 1). Also recorded from some F_d cells (10 %) was a consecutive sequence of two

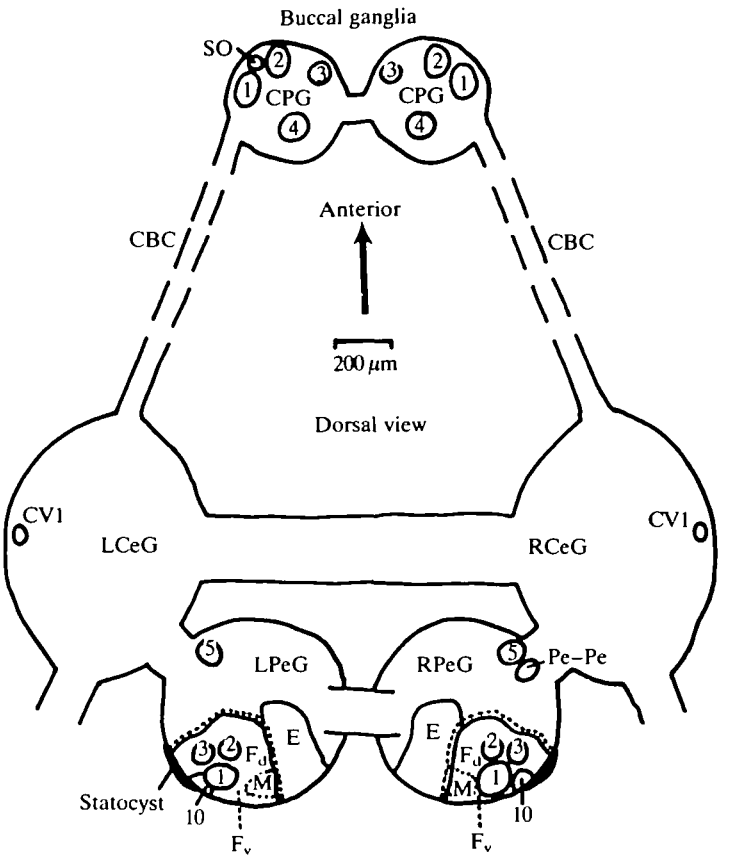

Fig. 1. Diagrammatic representation of the dorsal surfaces of the buccal, cerebral and pedal ganglia of *Lymnaea stagnalis*, showing the locations of the somata of giant pedal neurones (numbered 1, 2, 3, 5 and 10) and the pedal–pedal neurone (Pe–Pe), and the position of pedal cell clusters (solid lines: dorsal E, F_d; dotted lines: ventral F_v, M), all of which receive cyclical inputs in phase with activity in the buccal feeding rhythm generator (CPG). Also shown are the locations of the somata of buccal and cerebral higher-order interneurones (buccal slow oscillator, SO, and cerebral ventral 1, CV1) and four types of buccal motoneurone (1–4), all of which are involved in the production of feeding motor output (Benjamin, 1983). All neurones consist of bilaterally symmetrical pairs apart from the buccal SO of which there is only one, the pedal 2 cells which possess different axon morphologies, and the pedal 1 cells of which the right 1 cell is larger, contains dopamine and has a different axon morphology from the serotonin-containing left 1 cell (M. A. Kyriakides, C. R. McCrohan, N. Syed & W. Winlow, in preparation). CBC, cerebrobuccal connective; LCeG, left cerebral ganglion; LPeG, left pedal ganglion; RCeG, right cerebral ganglion; RPeG, right pedal ganglion.

inhibitory inputs at the same time as N1 and N2 phases of buccal activity (Fig. 2A). Detailed analysis of this sequence showed that the first input was delayed for about 1·5 s after the onset of N1 inputs to buccal neurones, although the second (N2

Table 1. *Polarity and phase of cyclic feeding inputs received by identified pedal ganglion neurones*

Cell type	Phase and polarity of cyclical inputs	% of feeding preparations receiving inputs		Axonal projections*
E cluster	N2 (i)		70 ($N = 10$)	Ipsilateral pedal nerves
F_d cluster (dorsal)	N2(i)	45	80 ($N = 20$)	Ipsilateral cervical nerves
	N1(i), N2(i)	10		
	N1(e), N2(i)	10		
	N1(e)	10		
	N1(i)	5		
F_v cluster (ventral)	N2(i)	38	75 ($N = 8$)	Ipsilateral pedal nerves
	N2(e,i)	38		
M cluster	N2(i)		88 ($N = 8$)	Ipsilateral pedal nerves
RPeD1	N1(e)		20 ($N = 10$)	Right parietal and visceral nerves
LPeD1	N1(e), N2(i)		50 ($N = 8$)	Left parietal and visceral nerves
RPeD2	N2(i)		44 ($N = 9$)	Contralateral inferior cervical nerve
LPeD2	N2(i)	40	50 ($N = 10$)	Left parietal nerve
	N1(e), N2(i)	10		
R/LPeD3	N2(i)		46 ($N = 13$)	Ipsilateral cervical nerves
R/LPeD5	N1(e), N2(i)		10 ($N = 10$)	Ipsilateral superior pedal nerve
R/LPeD10	N2(i)	69	94 ($N = 16$)	Contralateral columellar nerve
	N1(i), N2(i)	25		

Right and left PeD1 and 2 cells are listed separately as they possess different anatomical and physiological properties, whereas the 3, 5 and 10 cells are symmetrical with their contralateral partners.

e, excitatory postsynaptic potential; i, inhibitory postsynaptic potential; N1, N2, consecutive phases of buccal feeding cycles.

* Taken from M. A. Kyriakides, C. R. McCrohan, N. Syed & W. Winlow, in preparation.

phase) input occurred with a much shorter latency after the onset of N2 inputs in the buccal ganglia (i.e. less than 0·3 s, Fig. 2B). Also common amongst ventral F_v cells, but absent on the dorsal side, was a biphasic input during the N2 phase of excitation followed by inhibition (Fig. 3). Dorsal F_d cells possess a single ipsilateral axon projection in either the superior or inferior cervical nerve, and ventral F_v cells a single ipsilateral axon projection in the inferior pedal nerve (Table 1; M. A. Kyriakides, C. R. McCrohan, N. Syed & W. Winlow, in preparation). The cervical

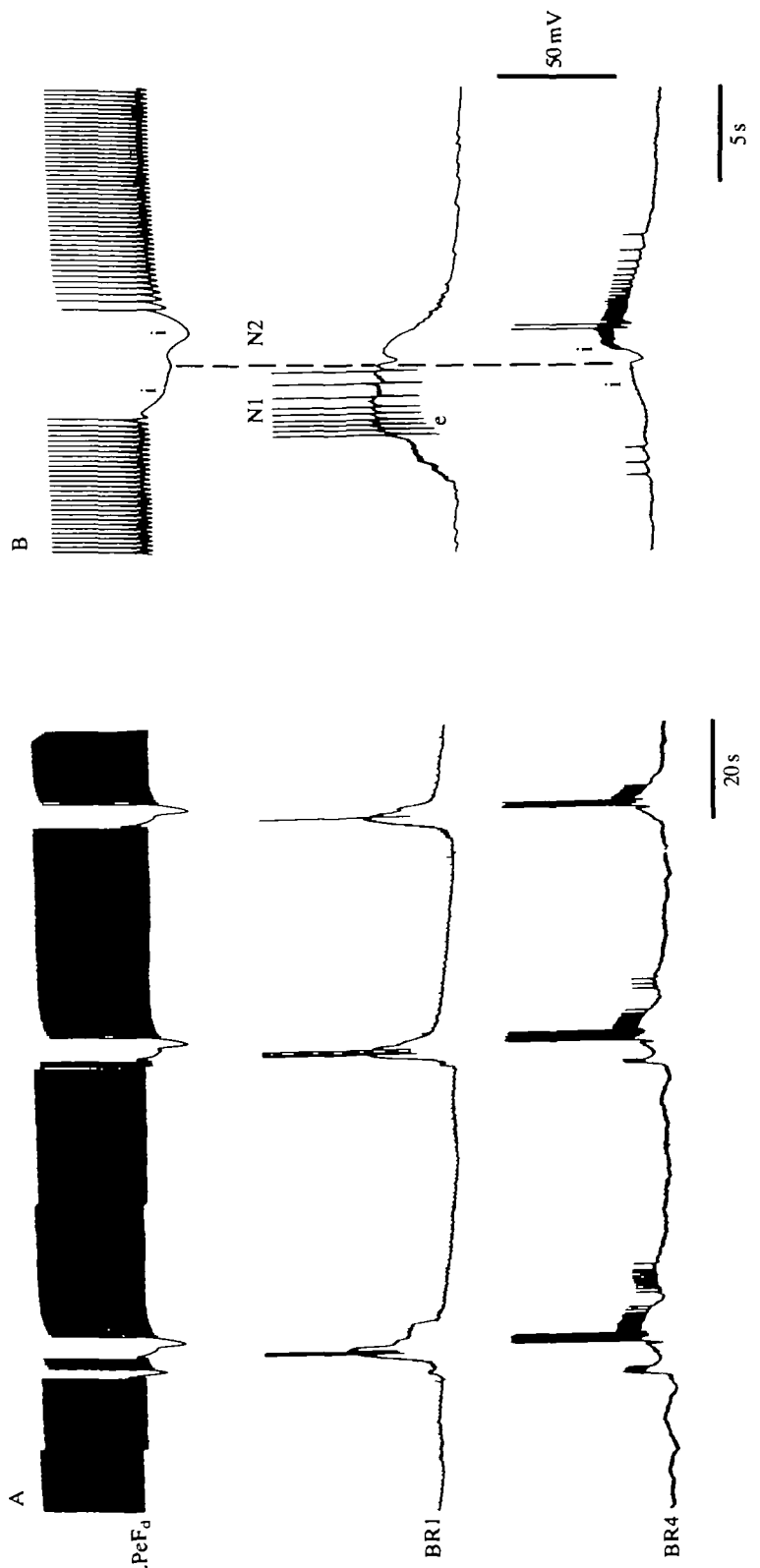

Fig. 2. Cyclical inputs to dorsal pedal F cluster motoneurones occur at the same time as feeding inputs to buccal motoneurones. (A) A left dorsal pedal F cluster motoneurone (LPeF$_d$) receives two successive compound inhibitory inputs at the same time as identified N1 and N2 feeding inputs to buccal motoneurones (BR1 receives excitatory N1 input; BR4 receives successive inhibitory inputs from N1 and N2 interneuronal networks). (B) The first F cell input is delayed for about 1·5 s after onset of N1 input to buccal neurones, whereas the following input to the F cell occurs less than 0·3 s after onset of N2 input to buccal neurones. e, compound EPSP; i, compound IPSP.

nerves enter the neck muscle of the snail and are known to innervate the dorsal longitudinal muscle which shortens the head–foot (Ferguson & Benjamin, 1985). The pedal nerves innervate the foot.

Another large cluster of pedal neurones that received inputs in phase with inputs to buccal neurones were the pedal E cells (PeE, Fig. 1), which project ipsilaterally into the foot *via* one or more of the pedal nerves (Table 1; M. A. Kyriakides, C. R. McCrohan, N. Syed & W. Winlow, in preparation). All pedal E cells which received the cyclical inputs (i.e. 70 % of cells in feeding preparations) received a single inhibitory input at the same time as N2 inputs to buccal motoneurones (Fig. 4).

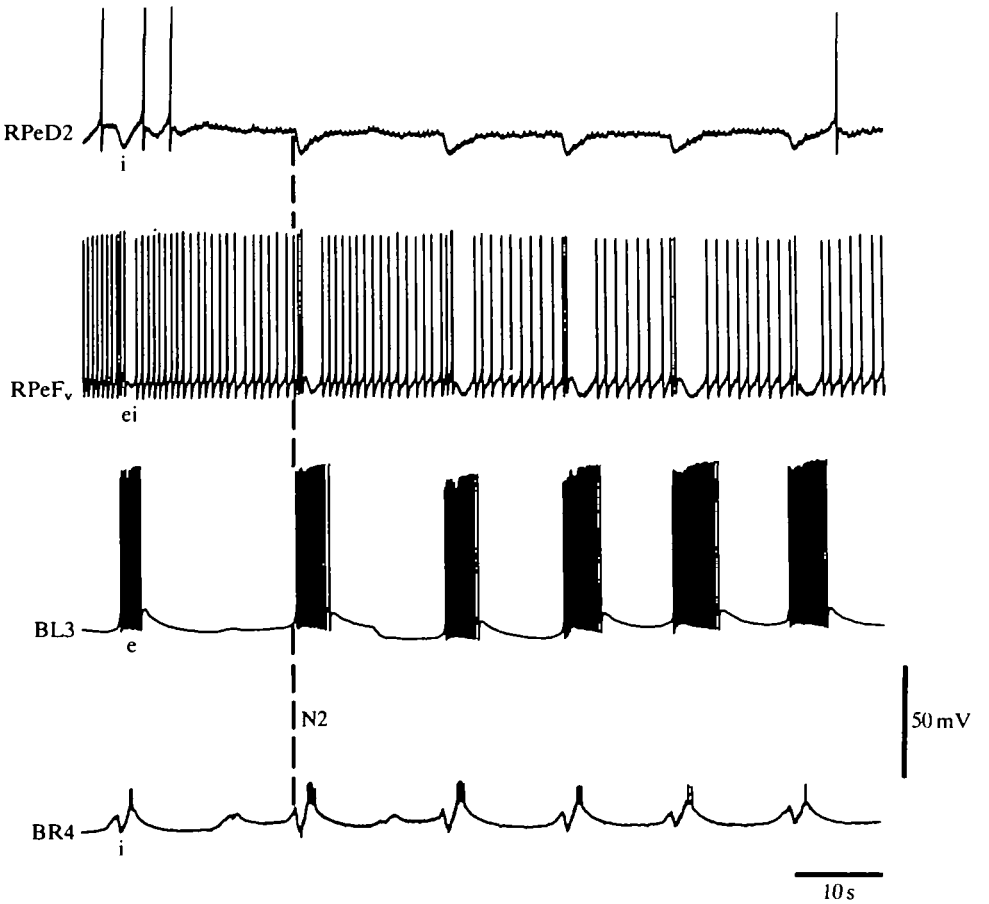

Fig. 3. Cyclical inputs to a ventral pedal F motoneurone and the right pedal dorsal 2 cell (RPeD2) occur at the same time as identified inputs to buccal feeding motoneurones. The right ventral pedal F cluster motoneurone (RPeF$_v$) receives a biphasic postsynaptic input of excitation followed by inhibition (ei), whilst the RPeD2 cell receives a single inhibitory input (i), all at the same time as N2 input to buccal motoneurones (BL3 and BR4).

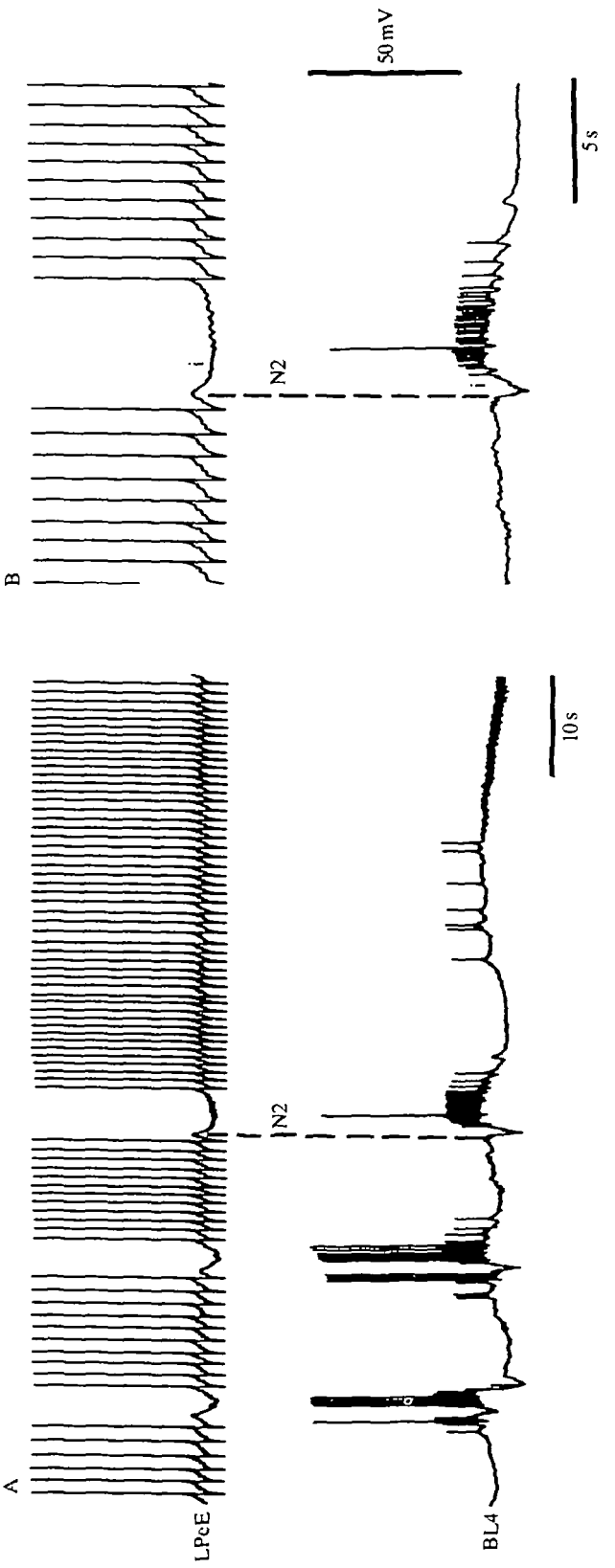

Fig. 4. Cyclical inputs to pedal E neurones occur at the same time as feeding inputs to buccal motoneurones. (A,B) A left pedal E cluster neurone (LPeE) receives a single compound inhibitory input (i) at the same time as N2 inhibitory input to a buccal 4 cell (BL4).

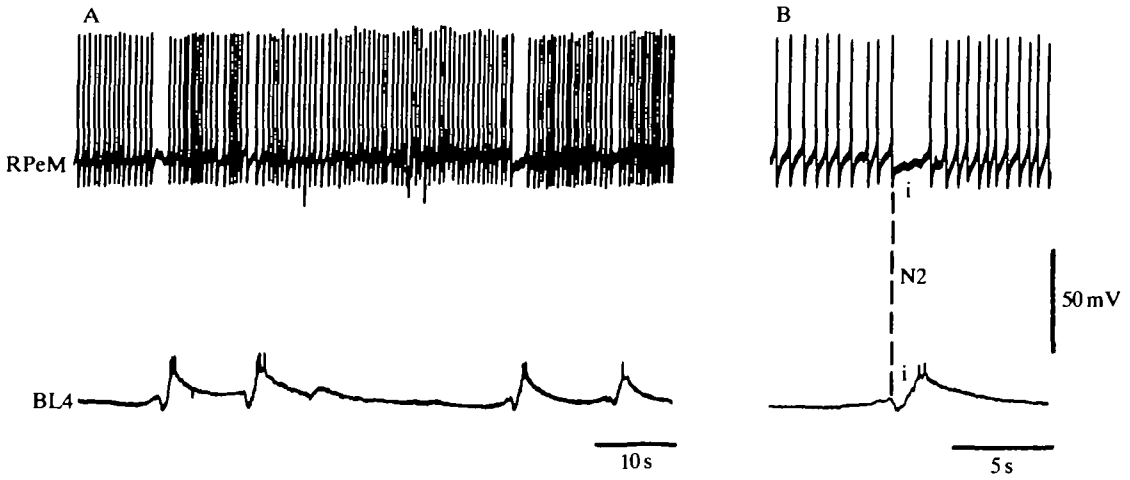

Fig. 5. Cyclical inputs to pedal M neurones occur in phase with inputs to buccal feeding motoneurones. (A) A right pedal M cluster neurone (RPeM) receives a weak inhibitory input at the same time as N2 input to a buccal motoneurone (BL4). (B) Little delay is seen between onset of inhibitory input (i) to RPeM and N2 inhibitory input to BL4.

The third cluster of pedal cells that received the cyclical inputs was the pedal M cluster (PeM, Fig. 1). This is a small group of cells confined to the ventral surface of the pedal ganglia, whose axons project ipsilaterally down one or both inferior and medial pedal nerves (Table 1; M. A. Kyriakides, C. R. McCrohan, N. Syed & W. Winlow, in preparation). As was the case for pedal E cells, all M cells received a single inhibitory input at the same time as N2 input to cells in the buccal ganglia (Fig. 5). This input was less obvious than on other cells and in some cases was seen only as a slight reduction in action potential frequency.

Other pedal cell clusters examined for the presence of cyclical inputs were the pedal A, C, D, G, I, L and N clusters (Slade *et al.* 1981; M. A. Kyriakides, C. R. McCrohan, N. Syed & W. Winlow, in preparation). However, no cyclical inputs related to the buccal rhythm were found in these cells.

Giant neurones

Nine identifiable pedal giant neurones received cyclical inputs at the same time as those in buccal neurones. These are summarized in Table 1, and illustrated for the RPeD2 neurone in Fig. 3. The only giant cells that received these inputs consistently were the R/LPeD10 cells which received inhibitory N2 phase inputs in 94 % of feeding preparations (Table 1; Fig. 6). All the others received cyclical inputs in only 50 % or less of such preparations (Table 1). This suggests that these inputs may not be essential for the generation of normal firing activity in these pedal giant neurones. More evidence to support this suggestion comes from the observation that cyclical inputs to some pedal giant neurones were weak and did not produce any significant effect on their pattern of firing. Such a neurone was

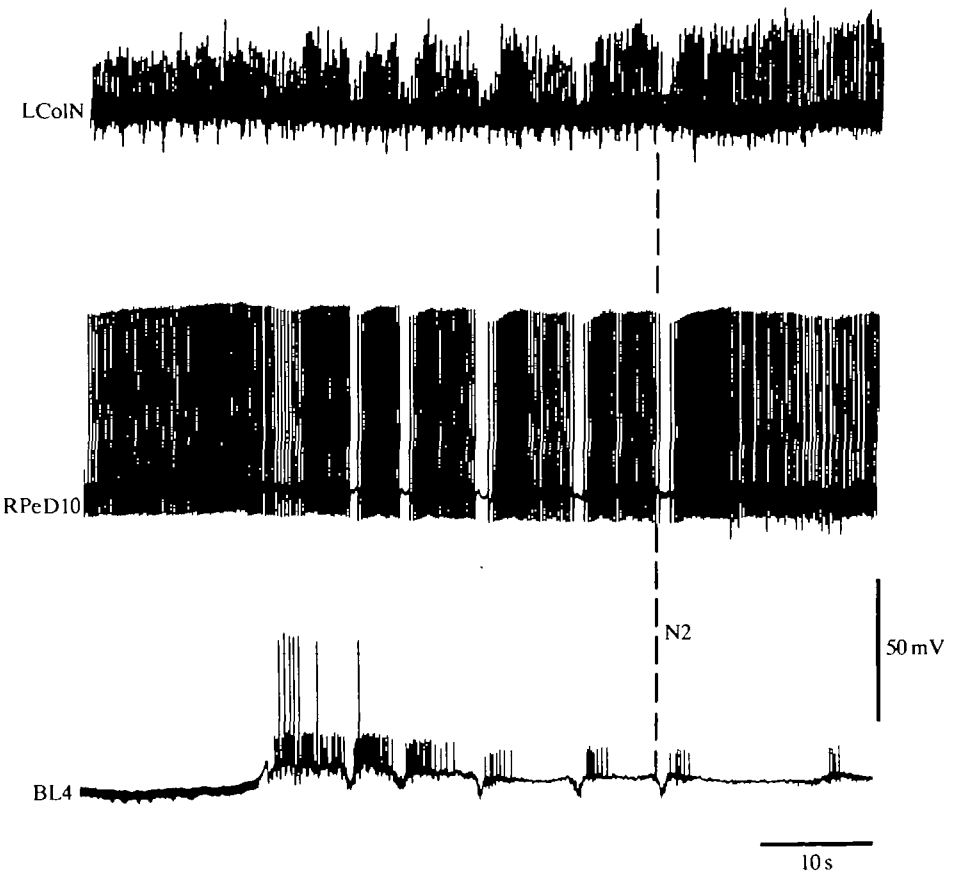

Fig. 6. Cyclical inputs to pedal neurones often produce a marked effect on nerve root activity. The right pedal dorsal 10 cell (RPeD10) receives a compound inhibitory input at the same time as N2 phase inhibitory input to a buccal motoneurone (BL4). This is reflected by a simultaneous reduction of activity in the left columellar nerve (LColN).

right pedal dorsal 1 (RPeD1) which is a single giant dopamine-containing cell (Winlow, Haydon & Benjamin, 1981). RPeD1 received weak excitatory inputs at the same time as rhythmic inputs to buccal cells, but these inputs had little or no effect on its firing pattern (Fig. 7A). The inputs to RPeD1 were shown to be excitatory since they depolarized the cell and did not inhibit action potential generation (Fig. 7A). Furthermore, injection of hyperpolarizing current into the cell caused an increase in their amplitude (Fig. 7B).

Despite the relatively weak effect of the cyclical inputs on some pedal neurones, especially the giant neurones, recordings of efferent activity in nerves arising from the pedal ganglia demonstrated a strong rhythmicity in nerve activity, in phase with feeding motor output from the buccal ganglia (Fig. 6). This indicates that the rhythm can make an important contribution to the pattern of motor output from the pedal ganglia.

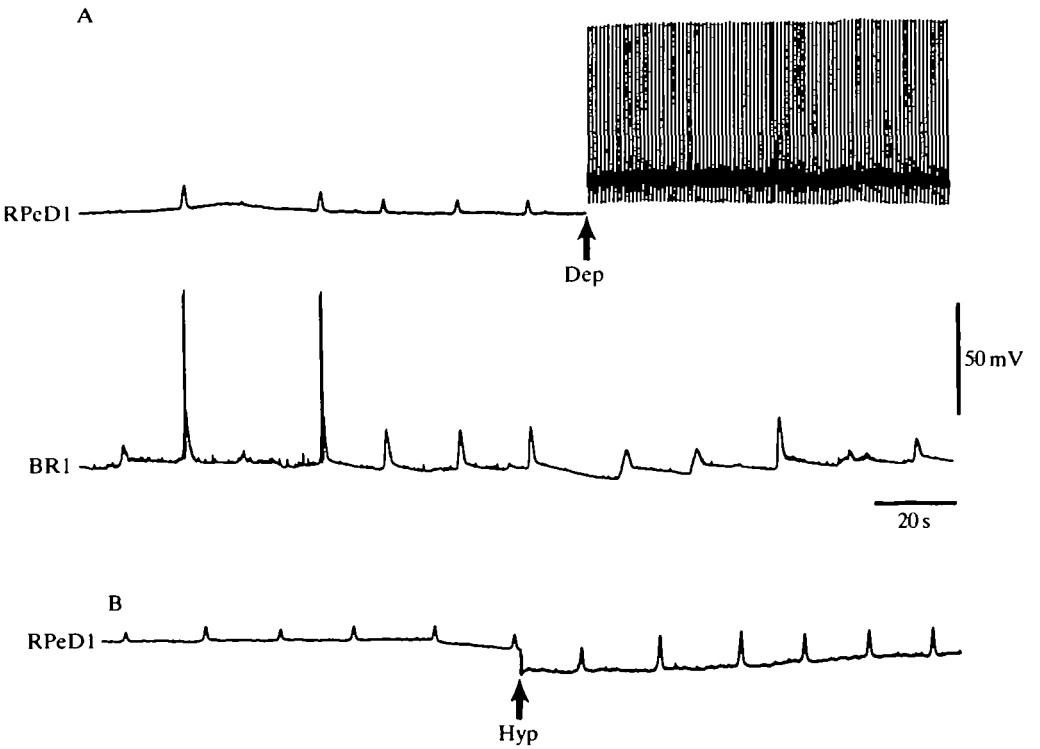

Fig. 7. Cyclical inputs to some pedal neurones produce little effect on their pattern of firing. (A) The right pedal dorsal 1 cell (RPeD1) receives weak inputs at the same time as rhythmic inputs to a buccal motoneurone (BR1), but these inputs have no noticeable effect on its firing pattern when depolarized (Dep). Inputs to RPeD1 were classified as excitatory since they depolarize the cell and do not inhibit action potential generation. (B) Injection of hyperpolarizing current into the cell (Hyp) causes the inputs to increase in amplitude.

Cells in other ganglia were investigated for inputs in common with rhythmic buccal activity (e.g. visceral dorsal 1 cell, visceral F cluster cells, parietal A cluster cells, parietal B cluster cells, parietal dorsal 1 cell, pleural D cluster cells: Benjamin & Winlow, 1981; Haydon & Winlow, 1982), but none was found.

Origin of cyclical inputs

Rhythmic feeding motor output can be initiated and maintained by tonic depolarization of one of two types of higher-order interneurone (buccal slow oscillator interneurone, SO, Rose & Benjamin, 1981; cerebral ventral 1 interneurone, CV1, McCrohan, 1984*b*; Fig. 1). Both these cell types are thought to produce their effects by acting directly on interneurones of the buccal central pattern generator (Benjamin, 1983). The cyclical inputs to pedal neurones were also initiated and maintained by activation of these higher-order interneurones. Tonic depolarization of SO or CV1 led to the initiation of feeding motor output in

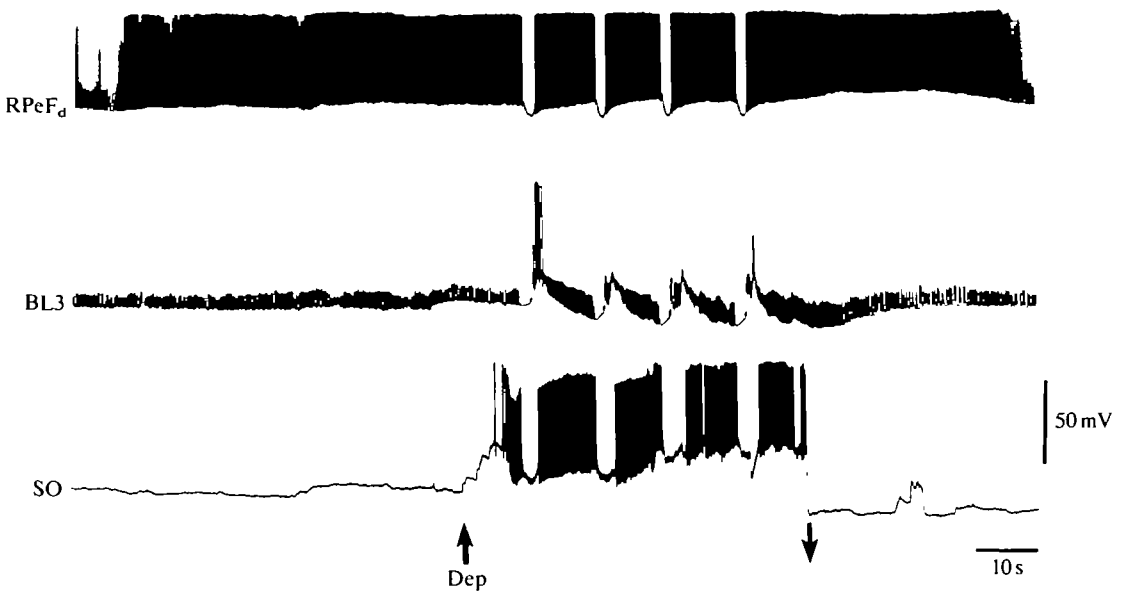

Fig. 8. Initiation of simultaneous cyclical inputs to pedal and buccal motoneurones by activation of the buccal slow oscillator interneurone (SO). Maintained depolarization of the SO (between arrows) initiates and maintains cyclical feeding inputs to a buccal motoneurone (BL3), accompanied by simultaneous inhibitory inputs to a pedal motoneurone (RPeF$_d$), in a previously non-rhythmic preparation.

buccal motoneurones in a previously non-rhythmic preparation (Figs 8, 9A). Cyclical inputs to buccal motoneurones were accompanied by simultaneous inhibitory inputs to pedal neurones, and by rhythmical inputs to SO or CV1 due to feedback from the buccal central pattern generator (Benjamin, 1983). When depolarization of the interneurone was terminated, the rhythmical activity in both buccal and pedal neurones ceased (Figs 8, 9A).

The ability of both the SO and CV1 interneurones to initiate and maintain common inputs to pedal neurones suggests that these inputs are due to activity in the buccal central pattern generator rather than, for example, a putative cerebral oscillator which might be entrained by the buccal central pattern generator (see McCrohan, 1984*b* for discussion). Cyclical inputs were abolished in pedal neurones by cutting the cerebrobuccal connectives, though the buccal motoneuronal rhythm still persisted (Fig. 10). Tonic depolarization of CV1 after severing the cerebrobuccal connectives had no effect on the pedal neurones (Fig. 9B), although CV1 was able to initiate cyclical inputs in both buccal and pedal neurones before the cerebrobuccal connectives were cut (Fig. 9A). These results support the hypothesis that the rhythmical inputs to the pedal neurones originate from and depend upon the activity of the buccal central pattern generator, rather than merely being coordinated in time with the buccal rhythm.

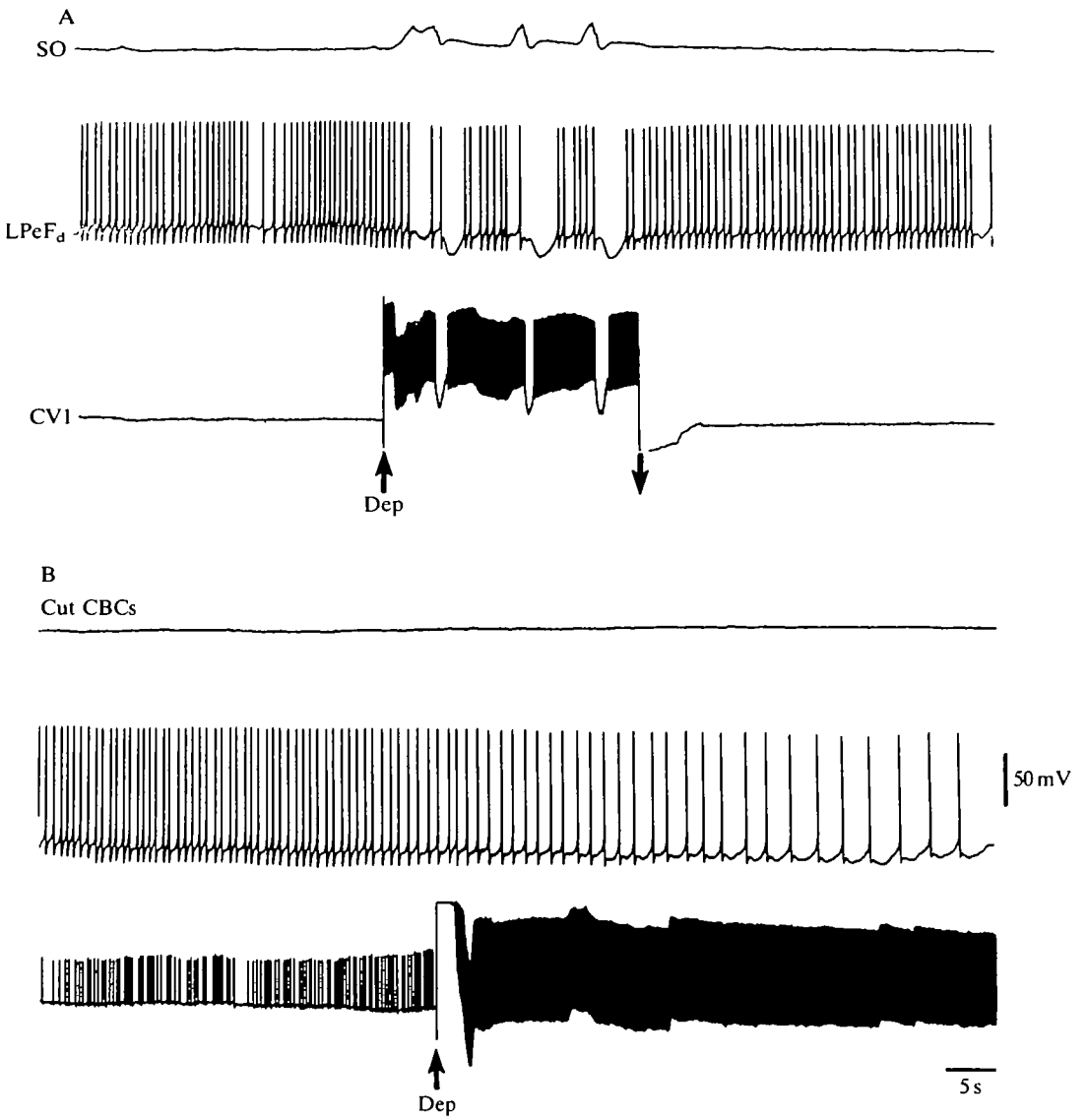

Fig. 9. Initiation of simultaneous cyclical inputs to pedal and buccal neurones by activation of cerebral ventral 1 interneurone (CV1) is blocked by severing the cerebrobuccal connectives (CBCs). (A) Depolarization of the CV1 (between arrows) initiates and maintains simultaneous cyclical inputs to the buccal slow oscillator interneurone (SO) and a pedal motoneurone (LPeF$_d$) in a previously non-rhythmic preparation. (B) After cutting both CBCs, maintained depolarization of the CV1 (arrow) no longer evokes rhythmic activity in either buccal or pedal neurones, making it unlikely that the inputs to pedal cells originate from another rhythm generator outside the buccal ganglia.

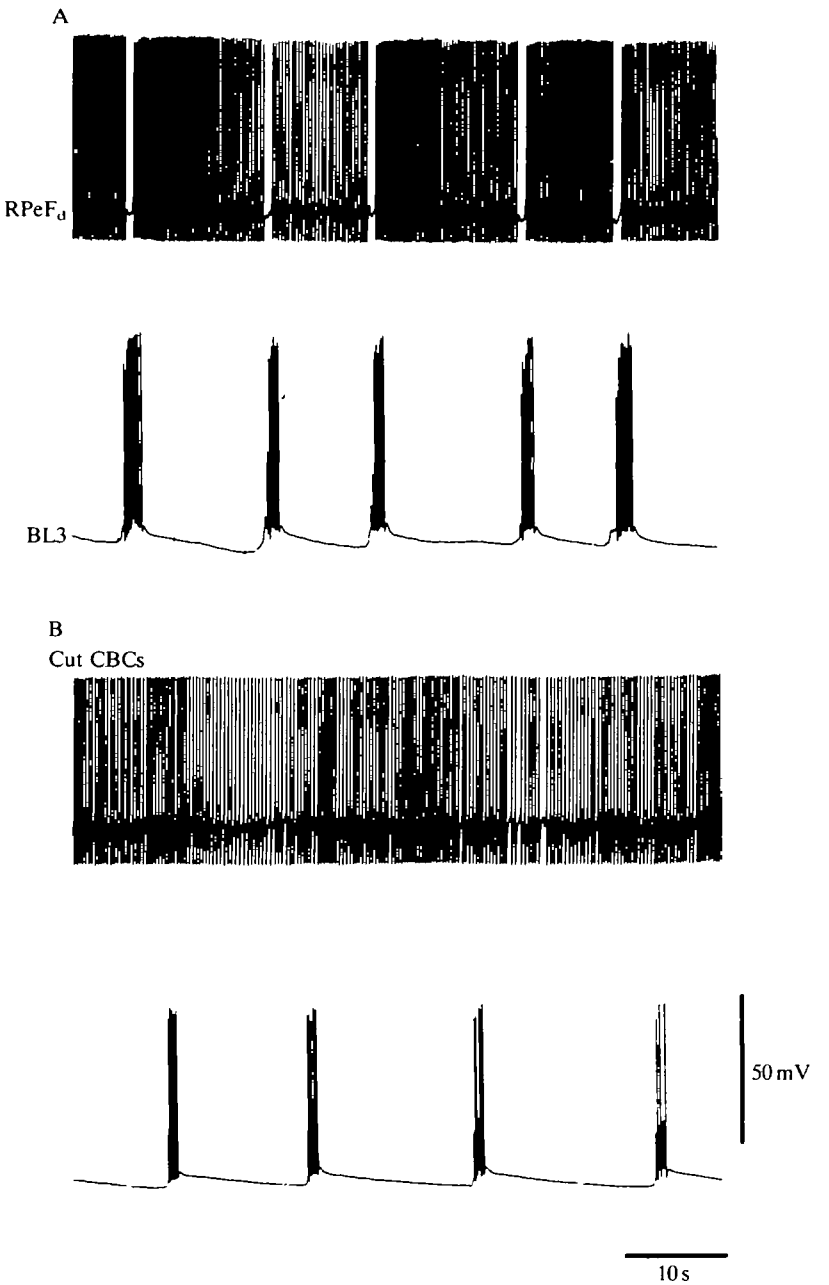

Fig. 10. Simultaneous cyclical inputs to pedal and buccal neurones originate from the buccal ganglia. (A) Simultaneous cyclical inputs recorded in pedal (RPeF$_d$) and buccal (BL3) motoneurones. (B) Cyclical inputs continue in the buccal motoneurone after severing the cerebrobuccal connectives (CBCs); they are abolished in the pedal motoneurone.

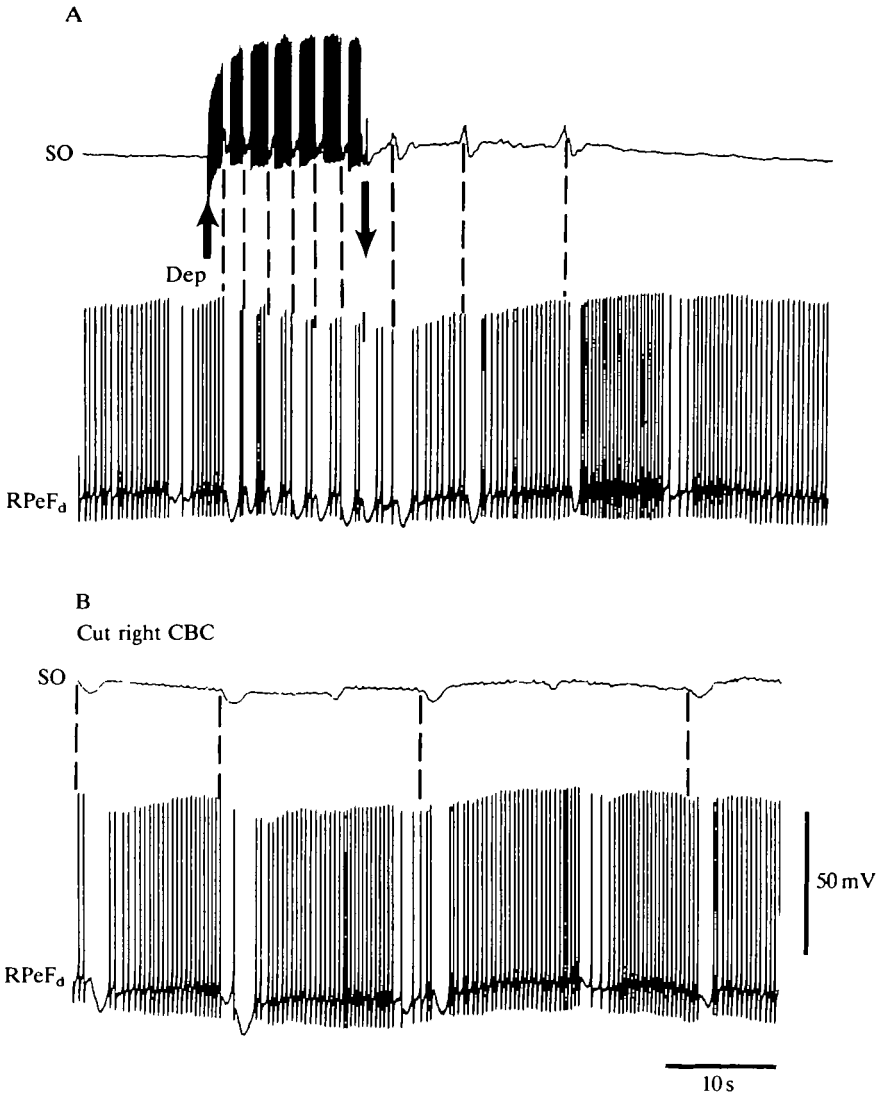

Fig. 11. Unilateral lesioning of a single cerebrobuccal connective (CBC) does not abolish cyclical inputs to the pedal ganglia. (A) Tonic depolarization (between arrows) of the buccal slow oscillator interneurone (SO) leads to the initiation of cyclical inputs in a right pedal motoneurone ($RPeF_d$). (B) These inputs continue in both cells even after severing the right CBC, the ipsilateral route for transmission of inputs from the buccal ganglia to the pedal motoneurone.

Experiments involving selective lesioning of the cerebrobuccal, cerebropleural, cerebropedal or pleuropedal connectives showed that the cyclical inputs to the pedal neurones, originating in the buccal ganglia, travelled through the cerebral ganglia and into the pedal ganglia *via* the cerebropedal connectives. Unilateral lesioning of a single cerebrobuccal connective did not abolish cyclical inputs to either the ipsi- or the contralateral pedal ganglion (Fig. 11). A pedal–pedal

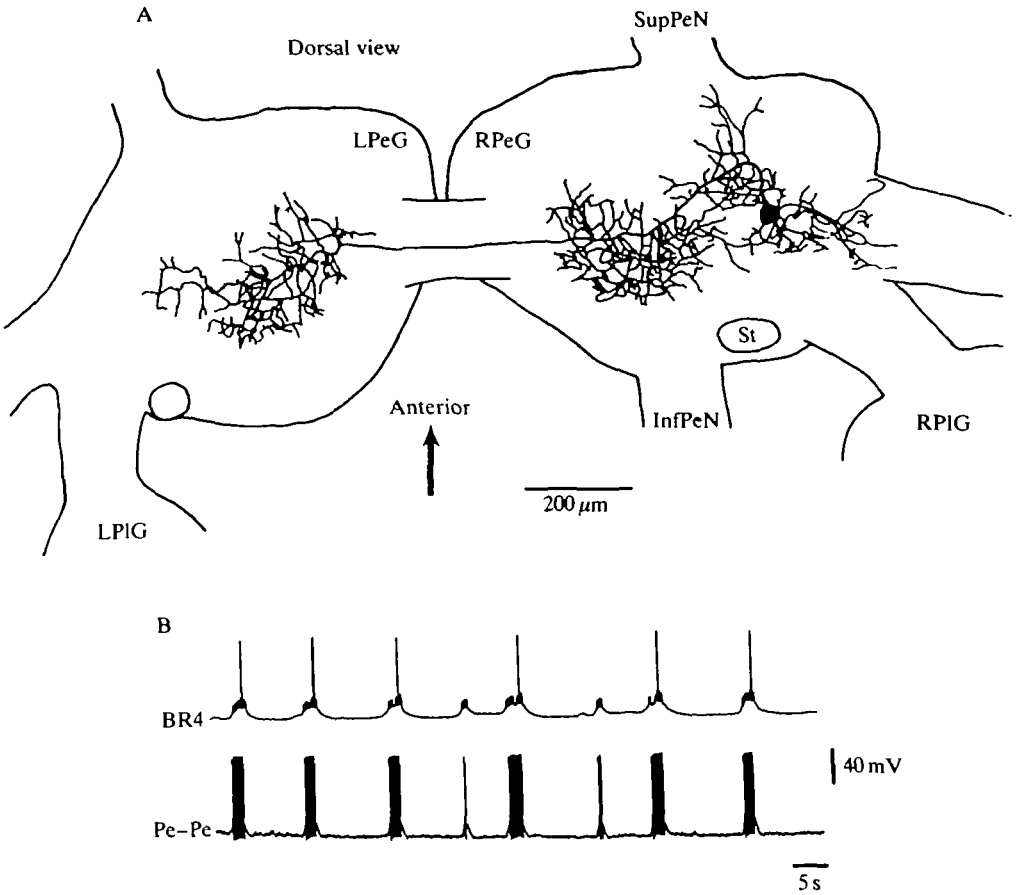

Fig. 12. A candidate putative interneurone for transmission and coordination of cyclical inputs between the two pedal ganglia (LPeG, RPeG). (A) Lucifer Yellow reconstruction of a pedal–pedal neurone with widespread dendritic branching and varicosities in both ganglia. (B) The same cell (Pe–Pe) shows little spiking activity except for strong bursts at the same time as buccal central pattern generator inputs to a buccal motoneurone (BR4). InfPeN, inferior pedal nerve; LPlG, left pleural ganglion; RPlG, right pleural ganglion; St, statocyst; SupPeN, superior pedal nerve.

neurone was identified which has interneuronal morphology, with widespread dendritic branching in both ganglia (Fig. 12A). This cell showed little spiking activity except for strong bursts in phase with rhythmic buccal activity (Fig. 12B) and could, therefore, be responsible for coordinating the rhythm between the two pedal ganglia. This neurone was the only pedal cell type found to receive strong excitatory inputs, leading to bursting activity in phase with the feeding rhythm. Unlike many of the giant neurones, this neurone was not identifiable purely on the basis of soma location. It was recorded only a small number of times, always in the right pedal ganglion, and showed consistent morphology and activity patterns. The possibility that it has a symmetrical partner in the left pedal ganglion cannot be

ruled out. More detailed information is required to establish any possible interneuronal role for this cell in coordinating rhythmic pedal activity.

Discussion

The results presented here demonstrate the presence of cyclical synaptic inputs which are apparently common to cells in the buccal, cerebral and pedal ganglia of *Lymnaea*. The inputs to pedal cells occur at the same time as identifiable inputs to buccal and cerebral neurones and, therefore, are likely to originate from N1 and N2 interneuronal networks of the buccal central pattern generator normally responsible for producing feeding motor output (Benjamin, 1983). Evidence for this is provided by the observation that cyclical inputs to cells in all three ganglia may be initiated, terminated and modulated *via* activity in higher-order interneurones (SO and CV1 cells) which are known to activate the buccal central pattern generator (Benjamin, McCrohan & Rose, 1981; McCrohan, 1984*b*). Furthermore, when the buccal central pattern generator is isolated from the rest of the CNS by severing the cerebrobuccal connectives, the cyclical inputs to pedal cells are abolished even though the buccal neurones still receive them.

The buccal and cerebral neurones recorded in this study are known to be directly involved in the generation of feeding motor output (Benjamin, 1983; McCrohan, 1984*a*,*b*). However, no such role has been shown for any of the cells in the pedal ganglia. Not all pedal cell types receive the rhythmical inputs but, of those that do, some have been shown to be locomotor motoneurones or to have activity correlated with locomotory motor output (pedal E and F cluster cells and LPeD2; Winlow & Haydon, 1986). Although the precise roles of the remaining pedal neurones are not known, their axons mainly project into the cervical, columellar or pedal nerves which are known to innervate the body wall and neck (as well as the tentacle and lip), column and foot, respectively (Dorsett, 1986). Contractions of these regions are necessary for effecting locomotion (Winlow & Haydon, 1986), whole-body withdrawal (Ferguson & Benjamin, 1985) and egg-laying behaviour (Jansen & Maat, 1985).

The role of the cyclical inputs to pedal neurones is, as yet, unknown. They are sometimes absent in a feeding preparation, suggesting that they are not essential to the normal functional output of these cells. As shown for the giant dopamine cell, RPeD1, the cyclical inputs often have no marked effect on neuronal output in terms of spike frequency, but seem only to provide the cell with phasic information about ongoing activity in the buccal ganglia. Such rhythmic information might allow the pedal neurones to modify their output, which would still be largely determined by inputs from another, independent, motor centre (e.g. the locomotory pattern generator; Winlow & Haydon, 1986). A mechanism of this sort has been proposed for the coordination of limbs during locomotion in the cat (Halbertsma, 1983; Miller & Schomburg, 1985). Each limb is thought to be controlled by a separate central pattern generator, whose output is modified in

response to information received about the activity and phase of the other three pattern generators.

A more direct role for cyclical feeding inputs to identified pedal neurones cannot, however, be ruled out. Two possible explanations can be put forward to account for the phenomenon. First, the presence of mainly inhibitory inputs to pedal neurones, coincident with N1 and N2 phases of the buccal feeding cycle, suggests a role for these inputs in suppressing activity in pedal neurones at the precise time that the radula is in contact with the substrate. Movement of head or foot at this time might have the adverse effect of disrupting the rasp, making it less effective. During the N3 (swallowing) phase, and also between feeding cycles, the snail would be free to locomote or move its head to another place to feed; no inputs to pedal neurones were recorded coincident with N3 interneuronal inputs to buccal neurones. Indeed, removal of inhibition of pedal circuitry between rasps could have the advantage of increasing the likelihood that the snail would locomote and thus prevent it from rasping again in exactly the same place. One activity known to be suppressed during rasping in the intact animal is the periodic head reversal movement (Dawkins, 1974). This movement causes the snail to rasp in a zig-zag pattern, thus increasing the area of substrate that is sampled as the animal moves forward.

A second hypothesis involves an even more direct role for the cyclical inputs to pedal neurones, in the generation of feeding behaviour. It is possible that cyclical inputs to pedal neurones, reinforced by sensory input in the intact animal, cause coordinated contraction/relaxation of the body wall, column and foot in phase with the buccal rhythm, thus aiding ingestion of food by providing appropriate head and body movements in time with protraction and retraction of the buccal mass. This might even allow for other, food-related, activities such as manipulation of food into the mouth by the foot and lips. The latter activity has been observed in *Lymnaea*, and has also been reported for other gastropods such as *Aplysia* (Jahan-Parwar & Fredman, 1979). However, as mentioned earlier, an essential role for cyclical pedal neuronal activity during feeding seems unlikely, though it is possible that the cyclical inputs to pedal neurones occur more reliably in the intact animal. Future experiments using more intact preparations will enable us to assess the importance and relevance of the results described here for whole animal behaviour.

The present study provides strong evidence that the rhythmical inputs to pedal neurones originate, either directly or indirectly, from the N1 and N2 interneuronal networks of the buccal central pattern generator. A morphological study of N1 and N2 interneurones (Elliott & Benjamin, 1985*a*) showed that N1, but not N2, interneurones have axonal projections to the cerebral ganglia *via* the cerebrobuccal connectives. However, these were not traced as far as the pedal ganglia. It seems likely, therefore, that an indirect pathway of buccal–cerebral–pedal coordinating interneurones carries phasic information from the buccal rhythm generator to other ganglia. An indirect polysynaptic pathway would provide scope for modulation of the degree to which the buccal and pedal motor centres are

coordinated at a given time (e.g. in response to sensory input). It would also help to account for the failure of transmission of cyclical information from buccal to pedal ganglia that was sometimes observed, and for the variable delay in onset of cyclical inputs to pedal neurones compared with those in buccal neurones. Interganglionic coordinating interneurones carrying rhythmic information about buccal motor output have been described in detail for another gastropod species, *Pleurobranchaea californica* (buccal–cerebral interneurones, BCIs; Cohan & Mpitsos, 1983*a*,*b*). In *Lymnaea*, a candidate interneurone for cerebral–pedal coordination is the cerebral CV2 neurone (McCrohan, 1982) which has its cell body in the cerebral ganglion, projects to the pedal neuropile, and bursts rhythmically in phase with the buccal motor rhythm.

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References

BENJAMIN, P. R. (1983). Gastropod feeding: behavioural and neural analysis of a complex multicomponent system. In *Neural Origin of Rhythmic Movements* (ed. A. Roberts & B. Roberts), pp. 159–193. Cambridge: Cambridge University Press.

BENJAMIN, P. R., MCCROHAN, C. R. & ROSE, R. M. (1981). Higher order interneurones 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.

BENJAMIN, P. R., ROSE, R. M., SLADE, C. T. & LACY, M. G. (1979). Morphology of identified neurones in the buccal ganglia of *Lymnaea stagnalis*. *J. exp. Biol.* **80**, 119–135.

BENJAMIN, P. R. & WINLOW, W. (1981). The distribution of three wide-acting synaptic inputs to identified neurones in the isolated brain of *Lymnaea stagnalis* (*L.*). *Comp. Biochem. Physiol.* **70A**, 293–307.

COHAN, C. S. & MPITSOS, G. J. (1983*a*). The generation of rhythmic activity in a distributed motor system. *J. exp. Biol.* **102**, 25–42.

COHAN, C. S. & MPITSOS, G. J. (1983*b*). Selective recruitment of interganglionic interneurones during different motor patterns in *Pleurobranchaea*. *J. exp. Biol.* **102**, 43–57.

DAVIS, W. J., VILLET, J., LEE, D., RIGLER, M., GILLETTE, R. & PRINCE, E. (1980). Selective and differential avoidance learning in the feeding and withdrawal behaviour of *Pleurobranchaea californica*. *J. comp. Physiol.* **138**, 157–166.

DAWKINS, M. (1974). Behavioural analysis of co-ordinated feeding movements in the gastropod *Lymnaea stagnalis L.* *J. comp. Physiol.* **92**, 255–271.

DORSETT, D. A. (1986). Brains to cells: the neuroanatomy of selected gastropod species. In *The Mollusca*, vol. 9 (ed. A. O. D. Willows), pp. 101–187. London: Academic Press.

ELLIOTT, C. J. H. & BENJAMIN, P. R. (1985*a*). Interactions of pattern-generating interneurons controlling feeding in *Lymnaea stagnalis*. *J. Neurophysiol.* **54**, 1396–1411.

ELLIOTT, C. J. H. & BENJAMIN, P. R. (1985*b*). Interactions of the slow oscillator interneuron with feeding pattern-generating interneurons in *Lymnaea stagnalis*. *J. Neurophysiol.* **54**, 1412–1421.

FERGUSON, G. P. & BENJAMIN, P. R. (1985). Whole-body withdrawal of the pond snail *Lymnaea stagnalis*. *Soc. Neurosci. Abstr.* **11**, 513.

HALBERTSMA, J. (1983). The stride cycle of the cat: The modelling of locomotion by computerized analysis of automatic recordings. *Acta physiol. scand. (Suppl.)* **521**, 1–75.

HAYDON, P. G. (1982). An electrophysiological study of the nervous control of locomotion of *Lymnaea stagnalis* (*L.*). Ph.D. thesis, University of Leeds, UK.

HAYDON, P. G. & WINLOW, W. (1982). Multipolar neurones of *Lymnaea stagnalis*. I. Multiple spike initiation sites and propagation failure allow neuronal compartmentalization. *J. comp. Physiol.* **147**, 503–510.

HAYDON, P. G. & WINLOW, W. (1986). Shell movements associated with locomotion of *Lymnaea* are driven by a central pattern generator. *Comp. Biochem. Physiol.* **83**A, 23–25.

JANSEN, R. F. & MAAT, A. T. (1985). Ring neuron control of columellar motor neurons during egg-laying behaviour in the pond snail. *J. Neurobiol.* **16**, 1–14.

JAHAN-PARWAR, B. & FREDMAN, S. M. (1979). Neural control of locomotion in *Aplysia*: role of the central ganglia. *Behav. neural Biol.* **27**, 39–58.

JONES, H. D. (1975). Locomotion. In *Pulmonates*, vol. 1 (ed. V. Fretter & J. Peake), pp. 1–32. London: Academic Press.

KAISER, P. (1960). Die Leistungen des Flimmerepithels bei der Fortbewegung der Basommatophoren. *Z. wiss. Zool.* **162**, 368–393.

MCCROHAN, C. R. (1982). Properties of two cerebral neurones whose activity is related to feeding motor output in *Lymnaea stagnalis* (*L.*). *J. moll. Stud.* **48**, 225–226.

MCCROHAN, C. R. (1984*a*). Properties of ventral cerebral neurones involved in the feeding system of the snail *Lymnaea stagnalis* (*L.*). *J. exp. Biol.* **108**, 257–272.

MCCROHAN, C. R. (1984*b*). Initiation of feeding motor output by an identified interneurone in the snail, *Lymnaea stagnalis*. *J. exp. Biol.* **113**, 351–366.

MILLER, S. & SCHOMBURG, E. D. (1985). Locomotor coordination in the cat. In *Co-ordination of Motor Behaviour* (ed. B. M. H. Bush & F. Clarac), pp. 201–220. Cambridge: Cambridge University Press.

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. (1981). 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.

SIGVARDT, K. A. & MULLONEY, B. (1982). Sensory alteration of motor patterns in the stomatogastric nervous system of the spiny lobster *Panulirus interruptus*. *J. exp. Biol.* **97**, 137–152.

SLADE, C. T., MILLS, J. & WINLOW, W. (1981). The neuronal organisation of the paired pedal ganglia of *Lymnaea stagnalis* (*L.*). *Comp. Biochem. Physiol.* **69**A, 789–803.

STEWART, W. W. (1978). Functional connections between cells as revealed by dye-coupling with a highly fluorescent napthalimide tracer. *Cell* **14**, 741–759.

VIALA, D. & FRETON, E. (1983). Evidence for respiratory and locomotor pattern generators in the rabbit cervico-thoracic cord and for their interactions. *Expl Brain Res.* **49**, 247–256.

WINLOW, W. & HAYDON, P. G. (1986). A behavioural and neuronal analysis of the locomotory system of *Lymnaea stagnalis* (*L.*). *Comp. Biochem. Physiol.* **83**A, 13–21.

WINLOW, W., HAYDON, P. G. & BENJAMIN, P. R. (1981). Multiple postsynaptic actions of the giant dopamine-containing neuron RPeD1 of *Lymnaea stagnalis*. *J. exp. Biol.* **94**, 137–148.