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CEREBRAL INTERNEURONES CONTROLLING FEEDING MOTOR OUTPUT IN THE SNAIL *LYMNAEA STAGNALIS*

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Summary

1. The cerebral ventral 1 (CV1) interneurones of *Lymnaea* occurred as a population of at least three in each ganglion, all with similar morphologies. Steady depolarization of a CV1 cell led to initiation and maintenance of rhythmic feeding motor output from the buccal ganglia.

2. CV1 interneurones produced facilitating excitatory postsynaptic potentials in N1 interneurones of the buccal central pattern generator for feeding. Connections with N2 interneurones were not found.

3. The CV1 population could be separated into two subgroups. $CV1_a$ received strong synaptic feedback in phase with the buccal rhythm, leading to strong bursting during generation of feeding motor output. $CV1_b$ received only weak feedback, and often fired continuously when depolarized.

4. Unitary inhibitory postsynaptic potentials were characteristic of all CV1 neurones, but were only visible in $CV1_b$ when it was depolarized. These inputs are thought to arise indirectly from the buccal central pattern generator.

5. The CV1 population is probably homologous with similar neurones in other gastropod species.

Introduction

The feeding system of gastropod molluscs is a widely used model for studies of the neural bases of rhythmic behaviour. Neural networks responsible for generation of rhythmic buccal activity have been described in terms of their cellular and synaptic properties (Benjamin, 1983). A class of neurones responsible for initiation of rhythmic activity has been characterized for a number of species (Gillette *et al.* 1982; Delaney and Gelperin, 1985; Elliott and Benjamin, 1985*b*). Since these neurones can determine whether the feeding system is active they may play a role in 'decision-making' within the network. Detailed studies of these neurones could therefore provide an insight into the cellular mechanisms of behavioural selection or switching. Indeed, interneurones which initiate feeding motor output in *Pleurobranchaea californica* (Davis *et al.* 1983) are thought to play a central role in mediating some of the changes in neuronal excitability which underlie behavioural plasticity.

Key words: gastropod feeding, central pattern generator, higher-order interneurone.

The feeding system of the pond snail *Lymnaea stagnalis* is one of the best described in terms of its cellular components and synaptic interactions (Benjamin, 1983). The central pattern generator (CPG) for feeding is located in the buccal ganglia and comprises three main subnetworks of interneurones (N1, N2 and N3) which fire successively during each cycle of buccal activity (Elliott and Benjamin, 1985*a*). These interneurones impose three successive phases of synaptic input on buccal and cerebral motoneurones which innervate muscles of the buccal mass and lips (Rose and Benjamin, 1979; Benjamin, 1983; McCrohan, 1984*a*). Three types of higher-order interneurone have been described which play a role in initiation, maintenance and modulation of rhythmic feeding motor output. These are the serotonergic cerebral giant cells (CGCs; McCrohan and Benjamin, 1980*a*), the buccal slow oscillator (SO) cell (Rose and Benjamin, 1981*a*; Elliott and Benjamin, 1985*b*) and the cerebral ventral 1 (CV1) cells (McCrohan, 1984*b*). The CGCs are mainly concerned with modulation of rhythmic buccal activity, though they are capable of initiating feeding motor output (McCrohan and Benjamin, 1980*b*; McCrohan and Audesirk, 1987). The SO and CV1 interneurones have similar actions on the buccal CPG; steady depolarization of either SO or CV1 leads to initiation and maintenance of rhythmic feeding motor output from the buccal ganglia (Rose and Benjamin, 1981*a*; McCrohan, 1984*b*). The SO and CV1 cells in turn receive cyclical synaptic inputs which are phase-locked with the buccal rhythm. These two higher-order interneurones are apparently not connected synaptically (McCrohan, 1984*b*). There exist, therefore, two independent routes by which the buccal CPG may be activated. The SO is a single, unpaired neurone located in either the left or right buccal ganglion (Elliott and Benjamin, 1985*b*). It exerts its effect on the buccal CPG mainly *via* an excitatory connection which leads to 1:1 facilitating excitatory postsynaptic potentials (EPSPs) in N1 interneurones following spikes in the SO (Elliott and Benjamin, 1985*b*). CV1 cells are located in the cerebral ganglia and project *via* the cerebrobuccal connective to the buccal ganglia (McCrohan, 1984*b*). We previously identified a single CV1 neurone in each cerebral ganglion and described the ability of CV1 to initiate feeding motor output (McCrohan, 1984*b*). However, the synaptic mechanism by which CV1 exerts this effect was not known. In this paper, we describe the finding of a population of CV1 neurones in each cerebral ganglion, which can be divided into two subgroups based on differences in the intensity of identified synaptic inputs. We also describe the postsynaptic action of CV1 cells on interneurones of the buccal CPG. It is likely that the CV1 population is homologous with similar populations of higher-order interneurones in the feeding systems of the marine opisthobranch *Pleurobranchaea californica* (Gillette *et al.* 1982) and the terrestrial pulmonate *Limax maximus* (Delaney and Gelperin, 1985).

Materials and methods

Specimens of *Lymnaea stagnalis* were obtained from suppliers (Blades Biological, Kent) and maintained in tanks on a diet of lettuce. Experiments were carried

out on the isolated central nervous system (CNS) pinned in a recording dish under Hepes-buffered saline (McCrohan and Benjamin, 1980*a*) at room temperature. The part of the nervous system to be recorded from was treated with Protease (Type XIV, Sigma) for about 5 min and then washed thoroughly. The outer connective tissue sheath was removed using fine forceps. Intracellular recordings were made using thin-walled glass microelectrodes with resistances in the range 15–25 MΩ when filled with 3 mol l^{-1} potassium acetate. The preamplifiers incorporated a bridge circuit to allow current injection through the recording electrode. Current injected was monitored using a current to voltage converter placed between the indifferent electrode and earth. Signals were stored on tape and displayed using an oscilloscope and pen-recorder.

For intracellular dye injection, electrodes were filled with a 6% solution of Lucifer Yellow CH (Sigma). Dye was ionophoresed using 500 ms pulses of hyperpolarizing current (1–2 nA) at 1 Hz for 5–15 min. The preparation was fixed in 4% formaldehyde in phosphate buffer for at least 2 h. Material was then cleared in methyl salicylate, observed using an epifluorescence microscope, and drawn with the aid of a drawing tube attachment.

Activity in the buccal CPG for feeding was monitored by recording identified cyclical synaptic inputs from pattern-generating interneurones to buccal feeding motoneurones. For example, buccal retractor motoneurone 4 is known to receive successive compound inhibitory inputs from N1 and N2 interneurones; it then bursts on the rebound from this inhibition. Buccal motoneurone 1 receives excitatory input from N1 interneurones, leading to a burst of spikes (Benjamin, 1983). The central pattern-generating interneurones were identified by their synaptic effects on identified buccal motoneurones, and by their ability to reset the phase of an existing rhythm. CV1 interneurones were identified using three criteria outlined previously (McCrohan, 1984*b*): (i) ability to initiate and modulate rhythmic feeding motor output; (ii) receipt of rhythmic synaptic inputs in phase with activity in the buccal CPG; and (iii) morphology.

Results

Paired intracellular recordings and dye injections revealed at least three CV1 neurones in each cerebral ganglion. Their cell bodies were located in the 'V' formed by the superior and median lip nerves which have their roots in the ventral cerebral lobes. The detailed morphology (Fig. 1) was similar for all individuals ($N = 27$). The cell soma was approximately 20 μm in diameter. A single axon crossed the cerebral neuropile, travelled to the ipsilateral buccal ganglion *via* the cerebrobuccal connective, and then crossed into the contralateral buccal ganglion where it terminated with fine branching. A few fine branches were also found in the ipsilateral buccal ganglion. There was extensive neuritic branching in both ventral and dorsal cerebral neuropile. In particular, one or more long neurites extending into the origin of the cerebral commissure was always present.

Steady depolarization of a single CV1 neurone led to initiation and maintenance

of rhythmic feeding motor output in a previously non-rhythmic preparation (Fig. 2). The main, N1 and N2, phases of the feeding cycle were clearly recognizable as successive synaptic inputs to identified feeding motoneurones (e.g. N1 and N2 inhibitory inputs to a 4-cell, Fig. 2; see Rose and Benjamin, 1981*b*). As described by McCrohan (1984*b*), CV1 interneurones receive excitatory input during the N1 phase of the cycle and inhibitory input during the N2 phase (Fig. 2).

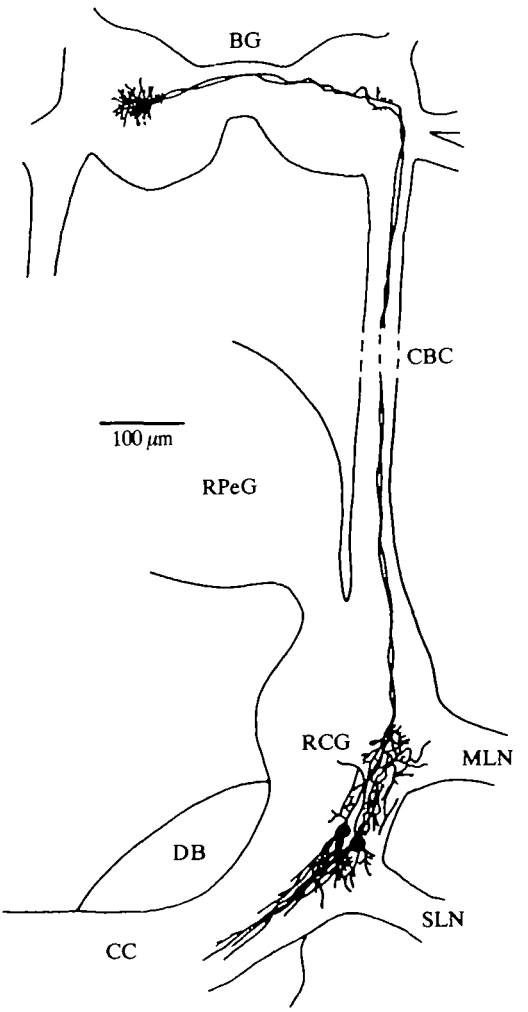

Fig. 1. Morphology of two CV1 interneurones, injected with Lucifer Yellow, in the same preparation. Cell bodies are located between the roots of the superior and median lip nerves. The axons project to the ipsi- and contralateral buccal ganglia. Fine neuritic branching is present in cerebral and buccal neuropiles. BG, buccal ganglia; CBC, cerebrobuccal connective; CC, cerebral commissure; DB, dorsal body; MLN, median lip nerve; RCG, right cerebral ganglion; RPeG, right pedal ganglion; SLN, superior lip nerve.

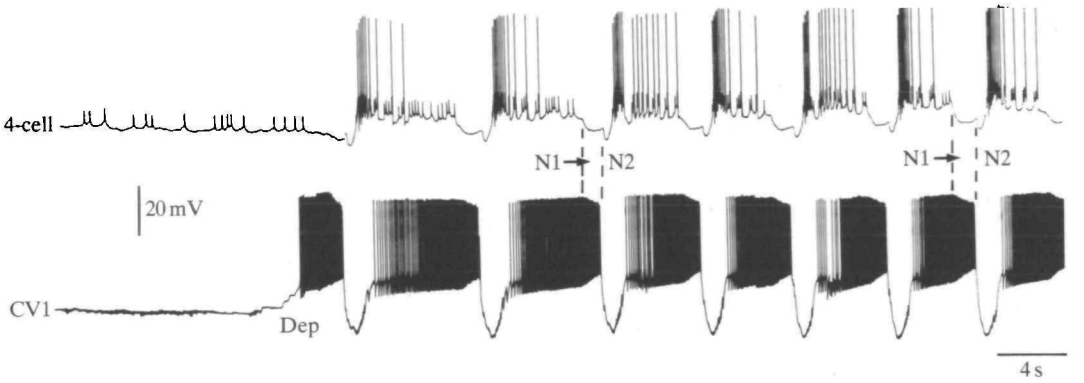

Fig. 2. Initiation of rhythmic feeding motor output by CV1. Steady depolarization of CV1 leads to rhythmic activity and bursting in buccal retractor motoneurone 4 and in CV1 itself. Identified N1 and N2 interneuronal inputs from the buccal CPG are indicated. Motoneurone 4 gives bursts following successive inhibitory inputs from N1 and N2 interneurones. CV1 receives excitatory input during the N1 phase, and inhibitory input during the N2 phase of each cycle.

Thus, it is possible to identify the occurrence and phase of rhythmic feeding motor output by monitoring the activity of CV1 alone.

CV1 neurones produced 1:1, facilitating EPSPs in N1 interneurones of the buccal CPG. N1 interneurones were identified mainly by their postsynaptic effects on identified buccal motoneurones (Rose and Benjamin, 1981*b*). For example, they produced 1:1 IPSPs in a 4-cell (Fig. 3A), but led to a compound EPSP in a 1-cell (Fig. 3B). Depolarization of CV1 to produce a burst of spikes led to a large compound EPSP in N1 cells (Fig. 3C). On a faster time base, this could be seen to consist of 1:1 facilitating EPSPs, which were indistinguishable from background noise at the start of the compound EPSP but increased to an amplitude of more than 4.5 mV after 150 action potentials in the CV1 cell (Fig. 3D). Evidence that the connection between CV1 and N1 is monosynaptic was provided by the constant latency of about 10 ms between spikes in CV1 and EPSPs in N1 (Fig. 3E). The CV1 cells had no direct synaptic effect on N2 interneurones of the CPG.

Although the CV1 neurones were morphologically indistinguishable, they could be separated into two subgroups depending on their pattern of firing in response to steady depolarizing current. These two types were clearly separable, though the physiological relevance of the distinction is not known. We have called the subgroups $CV1_a$ and $CV1_b$. Paired recordings from the same ganglion revealed either two $CV1_b$ cells, or one $CV1_a$ and one $CV1_b$. Pairs of $CV1_a$ cells on the same side were never found. Furthermore, on no occasion was a CV1 neurone observed to change its pattern of activity from $CV1_a$-type to $CV1_b$-type or *vice versa*. It seems likely, therefore, that the separation of CV1 neurones into these subgroups is a real one, and that the CV1 population consists of one $CV1_a$ and at least two $CV1_b$ on each side. The most obvious difference between the two cell types is their

response to maintained depolarization. When injected with steady depolarizing current, both were capable of initiating rhythmic feeding motor output in a previously non-rhythmic preparation, or of increasing the frequency of an existing rhythm (Fig. 4). However, the firing pattern of $CV1_a$ was strongly phasic (Fig. 4A), whereas that of $CV1_b$ was more or less tonic, with only weak frequency modulation in phase with the feeding rhythm; firing rate slowed at the onset of the N2 phase of the feeding cycle (Fig. 4B). The $CV1_b$ cells did, however, generate rhythmic bursts of activity when the buccal rhythm was activated by depolarization of a $CV1_a$, or when the CPG was spontaneously active (Fig. 4). Fig. 5 shows the

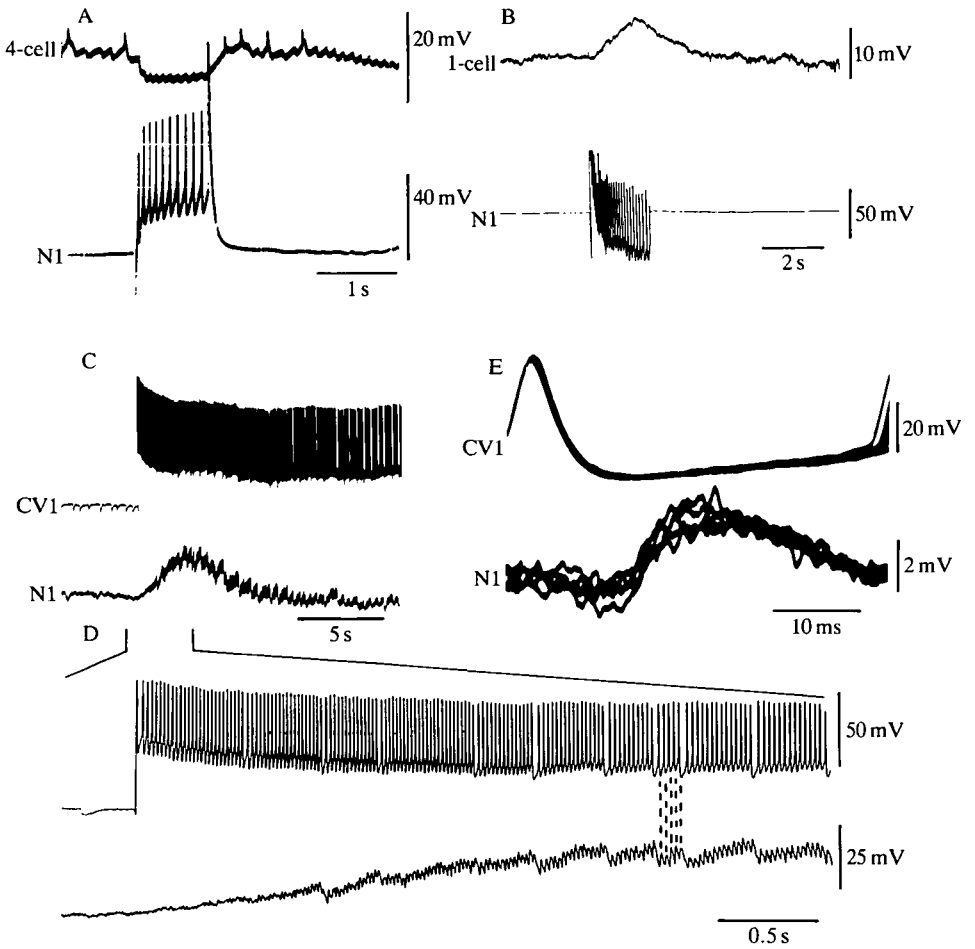

Fig. 3. CV1 produces 1:1 facilitating excitatory inputs in N1 interneurones of the buccal CPG. N1 is identified by its actions on buccal motoneurones. Here it produces 1:1 IPSPs in a 4-cell (A), and a compound EPSP in a 1-cell (B). (C) An evoked burst of spikes in CV1 leads to a large compound EPSP in N1. (D) On a faster timebase, the compound EPSP in N1 is seen to consist of 1:1 unitary potentials, which increase in amplitude following successive spikes in CV1. (E) Five superimposed spikes in CV1 produce constant-latency 1:1 EPSPs in N1. Latency is about 10 ms.

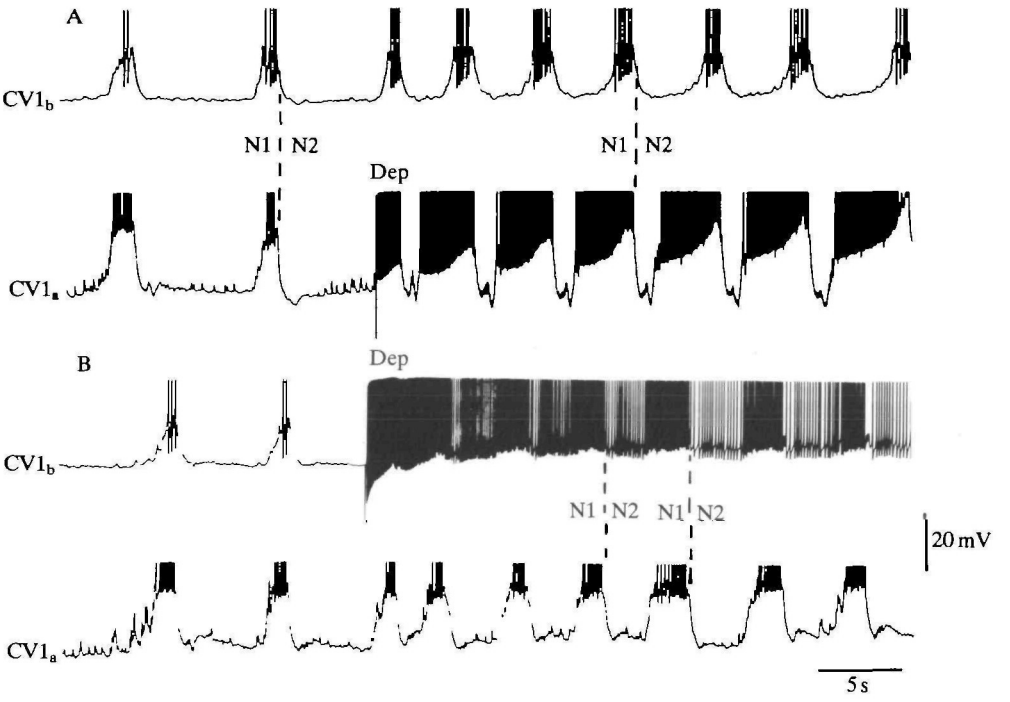

Fig. 4. Responses of $CV1_a$ and $CV1_b$ cells to steady depolarization. Maintained depolarization of $CV1_a$ (A) or $CV1_b$ (B) cells, in the same preparation, produces increased frequency of rhythmic activity in the buccal CPG, monitored as cyclical inputs to CV1 neurones themselves. Cyclical inputs produce strong bursting in a $CV1_a$ cell when it is depolarized, but only weak modulation of firing rate in a similarly depolarized $CV1_b$ cell. N1 and N2 phases of synaptic activity are indicated for some cycles.

effect of injecting measured amounts of depolarizing current into $CV1_a$ and $CV1_b$, in a preparation that was spontaneously generating rhythmic feeding motor output. In the absence of current injection into either cell, both generated rhythmic bursts of activity in response to cyclical inputs in phase with N1 and N2 interneuronal activity of the buccal CPG (Fig. 5B,C). When progressively depolarized, $CV1_a$ produced a progressive increase in frequency of the buccal rhythm (from 6.9 cycles min^{-1} to 12.9 cycles min^{-1} for the preparation shown in Fig. 5). Progressive depolarization of $CV1_b$ also led to increased frequency of the rhythm. However, the maximum frequency obtained for a given amount of current injected (9.3 cycles min^{-1} with 1.5 nA) was lower than that obtained with $CV1_a$. This indicates that $CV1_b$ has a weaker effect on the buccal CPG than $CV1_a$, possibly owing to the tendency of $CV1_b$ to fire more tonically when strongly depolarized (Fig. 5C).

$CV1_a$ and $CV1_b$ receive apparently common synaptic inputs (Fig. 6). At resting potential, inputs to the two cell types appeared to differ in some respects (Fig. 6A). The cells received a simultaneous compound EPSP (*e* on Fig. 6), which

occurred in a cyclical fashion. However, $CV1_a$ also received a series of repeated, probably unitary, IPSPs (*i*) which were not visible in $CV1_b$ at this membrane potential. These IPSPs reversed and disappeared when $CV1_a$ was hyperpolarized (Fig. 6B); the EPSPs increased in amplitude, and inputs to the two cells appeared identical. When $CV1_b$ was depolarized, unitary IPSPs, 1:1 with those in $CV1_a$, became visible (Fig. 6C). However, these were much smaller in amplitude than

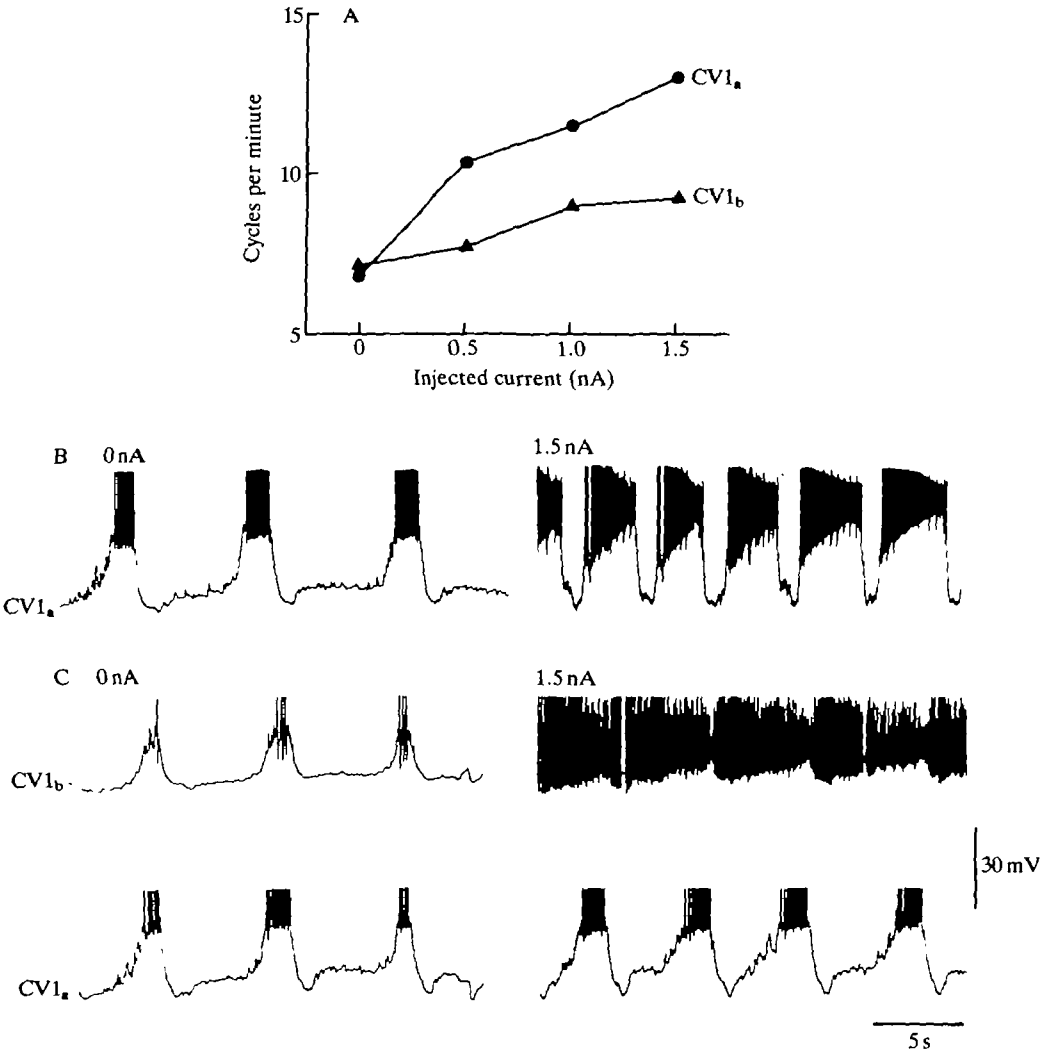

Fig. 5. Frequency modulation of the buccal rhythm by CV1. Using a preparation in which a rhythm occurred spontaneously, a $CV1_a$ or $CV1_b$ cell was depolarized using known amounts of current (0–1.5 nA). (A) For both cells, progressive depolarization leads to an increase in frequency of the rhythm, which is greater for $CV1_a$ than for $CV1_b$ cells. (B,C) Sample recordings from the data plotted in (A). At high levels of depolarization (1.5 nA), the $CV1_a$ cell bursts strongly (B), whereas the $CV1_b$ cell fires continuously (C).

those in $CV1_a$, and were not visible when $CV1_b$ was at resting potential. The $CV1_a$ and $CV1_b$ interneurones thus received common excitatory and inhibitory inputs. However, the latter were much smaller in $CV1_b$.

The cyclical compound EPSPs in CV1 neurones (Fig. 6) occurred during the N1 phase of the buccal feeding rhythm and arose directly or indirectly from the N1 interneuronal network (McCrohan, 1984*b*). The unitary IPSPs have been de-

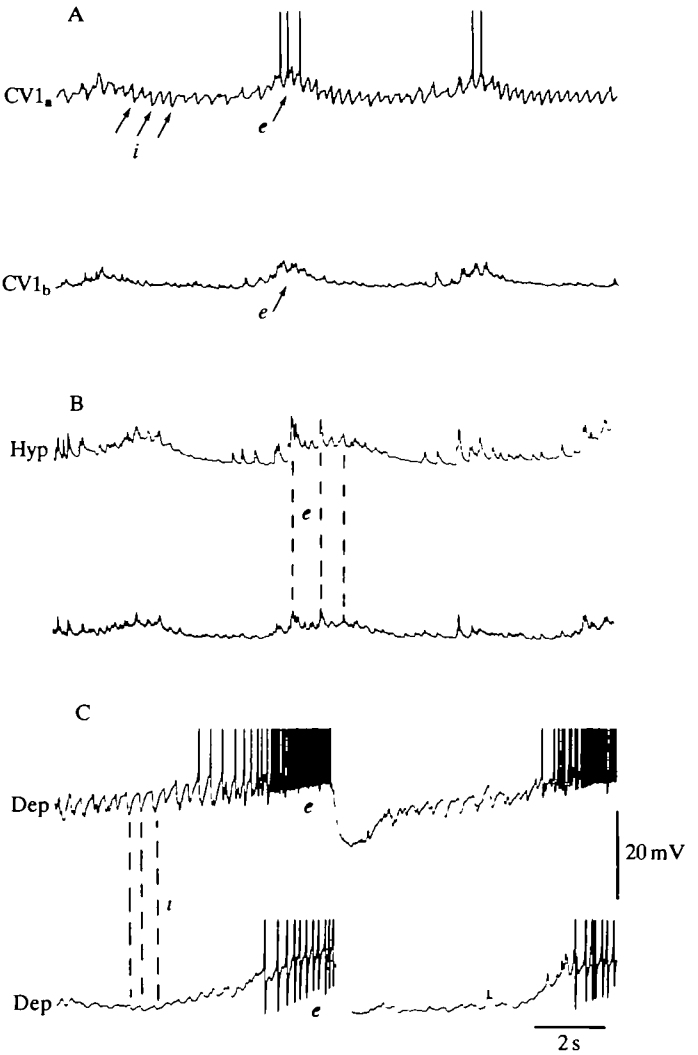

Fig. 6. Common synaptic inputs to $CV1_a$ and $CV1_b$ cells. (A) Both cells at resting potential. Apparently common excitatory inputs (*e*) are present; unitary IPSPs (*i*) in the $CV1_a$ cell are not visible in the $CV1_b$ cell. (B) When the $CV1_a$ cell is hyperpolarized, the IPSPs disappear; excitatory inputs increase in amplitude and postsynaptic activity in the two cells looks identical. (C) Both cells depolarized. Unitary IPSPs in the $CV1_a$ cell increase in amplitude. Small unitary IPSPs appear in the $CV1_b$ cell which are 1:1 with those in the $CV1_a$ cell.

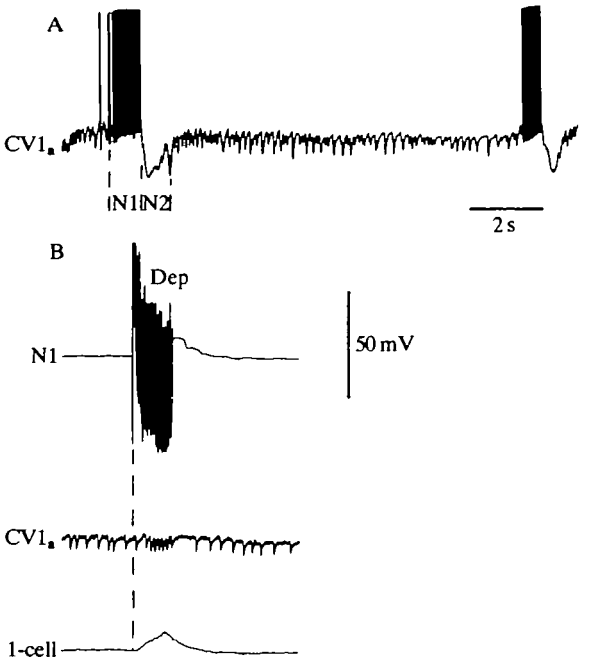

Fig. 7. (A) Unitary IPSPs occur in $CV1_a$ cells between feeding cycles, and may sum to produce a compound IPSP during the N2 phase of the buccal rhythm. (B) An evoked burst of spikes in a buccal N1 interneurone leads to a compound EPSP in buccal motoneurone 1, and also to increased frequency of unitary IPSPs in a $CV1_a$ cell. N1 interneurones are known to excite N2 interneurones (Elliott and Benjamin, 1985*a*).

scribed previously (McCrohan, 1984*b*), but their origin was unknown. They occurred at frequencies of up to $8\,s^{-1}$ both in non-rhythmic preparations and in between feeding cycles in rhythmic preparations (Fig. 7A). Two pieces of evidence suggest that these inputs arise, directly or indirectly, from the N2 interneuronal network, and that they sum to produce a compound IPSP in CV1 cells during the N2 phase of the feeding cycle (Fig. 7A). First, the unitary IPSPs occurred as very weak inputs to $CV1_b$ cells, whereas they could reach up to 8 mV in $CV1_a$. This correlates with the appearance of N2-phase compound inhibitory inputs in $CV1_b$ and $CV1_a$; these reached up to 25 mV in amplitude in a depolarized $CV1_a$ cell, whereas they only caused a slight decrease in firing rate in a similarly depolarized $CV1_b$ (see Fig. 4). A second observation in support of an N2 origin for the unitary IPSPs is illustrated in Fig. 7B. An evoked burst of spikes in an N1 interneurone caused a compound EPSP in a buccal 1-cell, as expected (Rose and Benjamin, 1981*b*), and also an increase in frequency of unitary IPSPs in $CV1_a$. N1 interneurones are known to excite N2 interneurones (Elliott and Benjamin, 1985*a*), which would lead to increased N2 input to CV1 cells. It should be noted that depolarization of a single N1 interneurone was not sufficient to cause a significant compound EPSP in the $CV1_a$ cell in Fig. 7B; the compound EPSP

which occurs during the N1 phase of the buccal cycle presumably arises from a population of N1 interneurones.

One final point should be noted. In this study, CV1 cells were identified according to the three criteria outlined by McCrohan (1984*b*) and in the Materials and methods section of the present paper. During the course of our work, several cells were found in a similar location which fulfilled the second and third criteria (receipt of rhythmic synaptic inputs during the feeding cycle, and morphology), but which did not appear to affect the buccal CPG. It is possible that these cells form part of a separate population of cerebral–buccal interneurones with a different function. However, it is also possible that CV1 cells are not always capable of initiating or modulating feeding, depending, perhaps, on the initial state of activity of the preparation. Further work would be required to resolve this question.

Discussion

The CV1 population in *Lymnaea* bears many similarities to cerebral interneurones in the feeding system of *Pleurobranchaea californica* (paracerebral neurones, PCNs; Gillette *et al.* 1982; Kovac *et al.* 1982) and *Limax maximus* (cerebral–buccal interneurones, CBs; Delaney and Gelperin, 1985; King *et al.* 1987). In all three species, these cerebral–buccal interneurones have similar locations and morphologies, can elicit rhythmic feeding motor output from the buccal ganglia, and show activity and synaptic inputs which are phase-locked with the buccal rhythm. It is reasonable, therefore, to conclude that these cells are probably homologous in the different species. Both the PCNs and the CBs are central elements in current studies of the cellular bases of associative conditioning (Davis *et al.* 1983; Gelperin, 1986), and are therefore of great interest from both a comparative and a general point of view.

Previous work (McCrohan, 1984*b*) suggested that the source of rhythmic inputs to CV1 neurones during the feeding cycle was the N1 and N2 interneurones themselves. This would imply that both these cell types have axonal projections to the cerebral ganglia *via* the cerebrobuccal connective. Elliott and Benjamin (1985*a*) have subsequently described the morphologies of the buccal CPG interneurones. The N1 interneurones do project, *via* the contralateral cerebrobuccal connective, into both cerebral ganglia; the axon passes through the ventral cerebral neuropile, making a direct connection with CV1 a likely possibility. However, Elliott and Benjamin (1985*b*) have already pointed out the difficulty of demonstrating a monosynaptic connection between an N1 interneurone and a follower cell. Stimulation of one N1 cell usually leads to activity in the whole population, owing to their electrotonic coupling, so that 1:1 PSPs cannot be distinguished. It may be relevant, however, that phasic excitation of PCNs of *Pleurobranchaea* during proboscis eversion (equivalent to protraction when N1 interneurones are active) arises from neurones with cell bodies located not in the buccal ganglia but in the cerebropleural ganglia, whose activity is phase-locked

with the buccal rhythm (Kovac *et al.* 1983). Whether or not N1 neurones make direct contact with CV1 neurones, their morphology makes them good candidates for coordinating rhythmic activity in buccal and cerebral ganglia. The N2 interneurones do not project to the cerebral ganglia (Elliott and Benjamin, 1985*a*). Any N2-phase compound inhibitory inputs to CV1 must therefore occur *via* a polysynaptic pathway. The present paper suggests that these inputs are due to summation of unitary inhibitory inputs that are present in non-rhythmic preparations. Similar inhibitory inputs have been described in the PCNs; these sum to provide strong inhibition of PCNs during proboscis withdrawal (equivalent to retraction), and are termed cyclic inhibitory potentials (CIPs; Gillette *et al.* 1982). The origin of the CIPs is located in the cerebropleural ganglia (Gillette *et al.* 1982). The neurone(s) which produces inhibitory N2-phase input to CV1 cells could be similarly located, possibly receiving phasic information about feeding cycles from the N1 interneurones. However, a buccal origin for these inputs cannot be ruled out at present. The CIPs of *Pleurobranchaea* are of particular interest because they provide the tonic inhibition of the PCNs that is seen following food avoidance conditioning (Davis *et al.* 1983). The similar 'N2' inputs to CV1 may also be modifiable and warrant further study. The presence of two subgroups of CV1 interneurones, in which the strength of these inputs differs, may provide a useful means of determining their role. Any alteration in their frequency or occurrence in response to sensory input or previous experience would be expected to have more effect on the activity and output of $CV1_a$ than on $CV1_b$ interneurones. Thus, although the separation of CV1 neurones into two types may not have any functional significance in terms of their ability to initiate feeding motor output, it could represent a useful tool for assessing the importance of N2-phase inhibitory inputs to the CV1 population.

This paper reports for the first time the synaptic mechanism by which CV1 neurones can exert their effect on the feeding pattern generator. CV1 produces facilitating EPSPs in N1 interneurones of the buccal CPG. These EPSPs are similar to those seen in N1 following activation of another higher-order interneurone in the feeding system, the buccal slow oscillator cell (SO; Rose and Benjamin, 1981*a*; Elliott and Benjamin, 1985*b*). It appears therefore that both interneurones activate the CPG by a similar mechanism. However, SO also produces facilitating IPSPs in the N2 interneurones (Elliott and Benjamin, 1985*b*). No connection was demonstrated from CV1 to N2 interneurones. This difference in output may reflect subtly different roles for CV1 and SO in the initiation and maintenance of rhythmic buccal activity.

The presence of a direct synaptic connection between CV1 and N1 interneurones, and also of synaptic inputs to CV1 neurones in phase with the buccal rhythm, raises the possibility that CV1 neurones are themselves an integral part of the CPG for feeding. Indeed, the strong rhythmic feedback to CV1 means that changes in CPG activity would significantly affect the activity of CV1, which in turn influences the CPG. In a previous paper (McCrohan, 1984*b*), CV1 interneurones were assigned a higher-order, or 'command', function. Their location in

the cerebral rather than the buccal ganglia supports this view. Furthermore, the buccal CPG is capable of generating rhythmic motor output when isolated from the cerebral ganglia (Kyriakides and McCrohan, 1988), showing that CV1 neurones are not required for the buccal CPG to function. It must be borne in mind, however, that it is not always easy, or even useful, to define precisely a given neurone's role in a 'motor hierarchy', particularly in a system in which strong feedback loops are present (see Davis, 1985, for further discussion).

We have divided the CV1 population into two subgroups, based on their response to steady depolarization. Both can activate the buccal CPG, but $CV1_b$ cells receive much weaker N2-phase inputs, so that their firing activity is less phasic than that of $CV1_a$ cells during generation of rhythmic motor output. The PCNs of *Pleurobranchaea* also fall into two subgroups, called 'phasic' and 'tonic' (Kovac *et al.* 1982). Both can initiate rhythmic activity, though the phasic PCNs produce a stronger and faster rhythm. However, unlike $CV1_a$ and $CV1_b$, the two PCN types are also distinguishable morphologically. Phasic PCNs are larger and monopolar; tonic PCNs are smaller and multipolar. They also show differences in firing rates, spike threshold and input resistance (Kovac *et al.* 1982). More significantly, tonic PCNs do not receive CIPs and their firing activity during rhythmic feeding motor output is always tonic. $CV1_b$ neurones are therefore probably not equivalent to tonic PCNs. The cerebral–buccal (CB) interneurones of *Limax* are individually identifiable owing to differences in morphology and in their input and output properties, and some are more effective than others in activating the buccal rhythm (Delaney and Gelperin, 1985; King *et al.* 1987; K. Delaney and A. Gelperin, personal communication). It is suggested that these different CB types play subtly different roles in the control of feeding motor output; for example, selectively enhancing activity in different parts of the bite cycle (K. Delaney and A. Gelperin, personal communication). While species differences are to be expected, it is possible that further cerebral–buccal interneurones will be discovered in *Lymnaea*, which can be separated on the basis of morphological, electrophysiological and functional criteria.

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