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IDENTIFIED NEURONES INVOLVED IN THE CONTROL OF RHYTHMIC BUCCAL MOTOR ACTIVITY IN THE SNAIL *ACHATINA FULICA*

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Summary

In the land snail *Achatina fulica*, it has been suggested that two pairs of cerebral neurones, ventral cerebral distinct neurones (v-CDNs) and C1 neurones, and a pair of buccal motoneurones (B1s) are involved in the control of rhythmic motor activity (RMA) in the buccal ganglia. These neurones, when tonically fired by depolarizing current injection, could individually initiate and maintain RMA in previously quiescent isolated ganglia. The rhythm elicited by v-CDN persisted for several cycles after the firing of v-CDN stopped, while that elicited by C1 or B1 ceased immediately after the firing of these neurones stopped. RMA also occurred spontaneously and could be induced by labial nerve stimulation in a reduced preparation. Nevertheless, such rhythms were not always accompanied by the firing of v-CDN, C1 or B1. Thus, the firing of these neurones appears to be sufficient, but not essential, for rhythm generation in the experimental conditions.

Taste stimulation of the lip in semi-intact preparations often induced RMA in the buccal ganglia. However, v-CDN and B1 were not tonically excited by the stimulation. It seems unlikely that v-CDN and B1 are critical elements in the generation of the feeding rhythm. C1 responded to taste stimuli with excitation after RMA had begun, suggesting that C1 is involved in the taste-induced buccal rhythm.

Introduction

In invertebrates such as the leech, some arthropods and several gastropod molluscs, the neural bases of central pattern generation underlying rhythmic behaviours have been well investigated (Gillette *et al.* 1980; Nagy and Dickinson, 1983; McCrohan, 1984; Brodfuehrer and Friesen, 1986*a*,*b*,*c*). In order to understand the neural mechanisms involved, it is important to identify and characterize the neurones that have a command-like function in the generation of the rhythmic behaviour in question. In the pond snail *Lymnaea stagnalis*, three types of higher-

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order interneurones which have command-like functions have been identified (Rose and Benjamin, 1981*a*; McCrohan, 1984; McCrohan and Audesirk, 1987). It has been suggested that, among these neurones, cerebral giant cells are important in controlling the feeding motor output in the whole animal (McCrohan and Audesirk, 1987). In the leech *Hirudo medicinalis*, identified trigger neurones, Tr1 and Tr2, are thought to contribute to the normal activation of swimming initiated by mechanosensory stimulation (Brodfuehrer and Friesen, 1986*c*).

The pulmonate snail *Achatina fulica* feeds by rhythmic movement of the buccal apparatus. The rhythm is generated in the buccal ganglia, which contain many identified motoneurones. Modulation of buccal muscle contraction by a serotonergic cerebral neurone, the ventral cerebral distinct neurone (v-CDN), has also been investigated (Yoshida and Kobayashi, 1991). However, mechanisms involved in the initiation of rhythmic buccal activity have not yet been studied. Rhythmic feeding movements of *A. fulica* are induced by food stimulation of the lip and tentacle. To understand further the feeding behaviour of *A. fulica*, it is important to ascertain which neurones mediate the sensory information that drives rhythmic motor output from the buccal ganglia. The command-like neurones that can initiate rhythmic motor activity are candidate mediators.

The aims of the present experiments were to identify the neurones that have a command-like function in the initiation of rhythmic buccal activity of *A. fulica* and to examine the physiological functions of these neurones.

Materials and methods

African giant snails, *Achatina fulica* Férussac, used in the present experiments, were captured in Okinawa and transported by air to Hiroshima. The animals were reared at 24°C in moist containers and fed on sweet potatoes. Four types of preparations were used: the isolated buccal ganglia; the buccal ganglia with the cerebral ganglia; the cerebral and buccal ganglia with the lips connected *via* the labial nerves; and the isolated lip with the stumps of the labial nerves.

After dissection in normal saline (see below), the sheath of connective tissue covering the ganglia was softened by protease treatment (Sigma type XV: 12.5 mg ml^{-1}) for 5–10 min. The preparation was then washed with normal saline and pinned onto a chamber covered with silicone resin. When cerebral ganglionic neurone C1 was to be penetrated, the sheath of the ventral surface of the cerebral ganglion was first surgically removed. In the experiments in which the ganglia–lip preparation was used, the experimental chamber was separated into two compartments (ganglia and lips) by a partition with a slit through which the labial nerves passed. The slit was sealed with silicone grease. The ganglia and lips were continuously perfused independently with normal saline.

Solutions for the chemical stimulation of the lips were prepared from carrot, apple and sweet potato. The materials were crushed and filtered using Dismic 13 CP (Advantec: pore size, 0.45 μm) to obtain their juices. The juices were stored at −15°C. They were diluted to half of the original concentration with normal

saline just prior to use. To stimulate the lips with the test solutions, the perfusate of the lip compartment was switched from normal saline to the test solution. There was no detectable tactile response to the replacement of this solution.

Extracellular recording from buccal or labial nerves was performed using a glass suction electrode. Intracellular recordings of neurones were made with glass microelectrodes filled with 3 mol l^{-1} potassium acetate or 5% (w/v) Lucifer Yellow CH (lithium salt) in distilled water and with resistances ranging from 10 to 35 MΩ. Current was injected into the neuronal cell body through the recording electrode or through another electrode inserted into the same cell. The data were stored on FM tape for later analyses, and permanent records were made using a pen recorder.

To examine the neuronal morphology after physiological identification, Lucifer Yellow CH was injected through the recording electrode by passing hyperpolarizing pulses lasting 500–700 ms (8 nA, 1 Hz, 20 min). After overnight incubation at 4°C, the preparation was fixed in 4% formaldehyde. It was then dehydrated in an alcohol series, cleared in methylbenzoate, and examined with a fluorescence microscope. The stained neurone was photographed and drawn.

The composition of normal saline was as follows (in mmol l^{-1}): NaCl, 61; KCl, 3.3; $CaCl_2$, 10.7; $MgCl_2$, 13; glucose, 5; Hepes, 10 (pH 7.5). In order to reduce synaptic transmission, some preparations were perfused with a high-Mg^{2+} and low-Ca^{2+} solution of the following composition (in mmol l^{-1}): NaCl, 33; KCl, 3.3; $CaCl_2$, 3.56; $MgCl_2$, 39; glucose, 5; Hepes, 10 (pH 7.5). All physiological experiments were performed at 20–25°C.

Results

In the present experiments, three pairs of neurones that showed command-like ability in the initiation of rhythmic motor activity (RMA) in the buccal ganglia of *Achatina fulica* were characterized. RMA was recognized by rhythmic activity of buccal motoneurone B1 or by rhythmic bursts of activity in the buccal nerves. One pair of these neurones, C1s, was newly identified in the cerebral ganglion. The other two pairs of neurones, the ventral cerebral distinct neurones (v-CDNs) and B1s, have been identified previously and partially characterized (Ku *et al.* 1985; Yoshida and Kobayashi, 1991). Isolated buccal ganglia of *A. fulica* usually showed RMA spontaneously for tens of minutes to several hours after dissection from the animal. However, the rate of RMA declined gradually and in some preparations ceased completely. A preparation showing rhythmic activity of less than 0.2 cycles min^{-1} was regarded as a quiescent preparation.

B1

A pair of B1s has been identified by Yoshida and Kobayashi (1991) as excitatory motoneurones of the radula retractor and the outer muscle of the buccal mass. The B1 neurones are located symmetrically on the caudal surface of the buccal ganglia and can be easily identified from their position and size (Fig. 1). Each B1 has

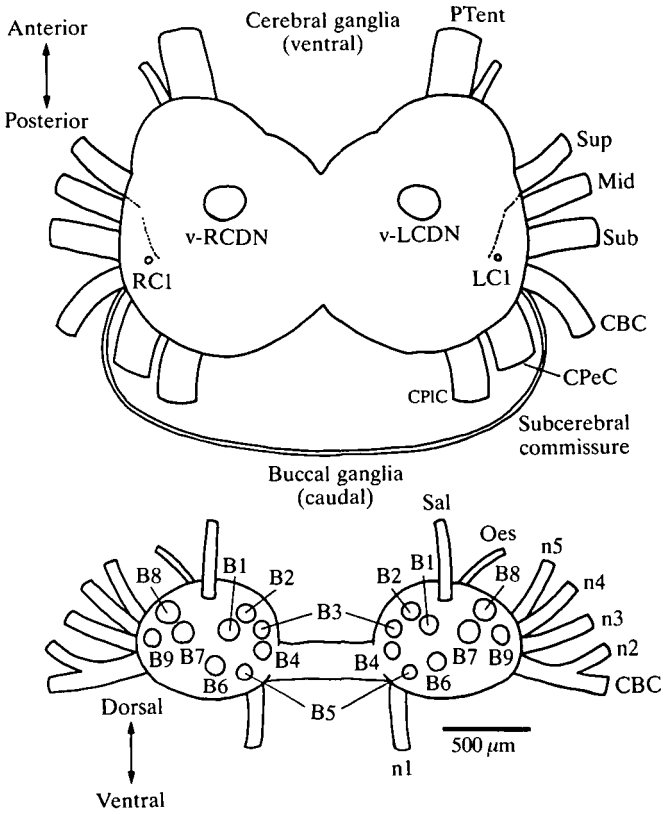

Fig. 1. Schematic drawing of the cerebral and buccal ganglia showing the positions of identified neurones. The caudal margins of intraganglionic tracts of the supralabial nerves are indicated by dotted lines. Sup, supralabial nerve; Mid, midlabial nerve; Sub, sublabial nerve; CBC, cerebrobuccal connective; n1–n5, buccal nerves 1–5; LC1, left C1; RC1, right C1; v-LCDN, ventral left cerebral distinct neurone; v-RCDN, ventral right cerebral distinct neurone; PTent, posterior tentacular nerve; CPeC, cerebropedal connective; CPlC, cerebropleural connective; Sal, salivary nerve; Oes, oesophageal nerve.

axonal projections bilaterally in buccal nerve 1 (n1), buccal nerve 2 (n2) and buccal nerve 3 (n3) and dendritic arborizations in both buccal ganglia. When one B1 neurone was fired by depolarizing current injection, RMA was initiated in previously quiescent isolated buccal ganglia (as revealed by rhythmic activity in the contralateral B1) (Fig. 2A). In 10 of the 11 preparations tested, RMA was maintained while B1 was artificially stimulated to fire at more than 5–10 Hz, but stopped when firing of B1 ceased. In one preparation, firing of B1 could not induce RMA successfully. Both right and left B1s showed identical effects (Fig. 2A). During spontaneous RMA, however, high-frequency firing of B1, which was necessary for the neurone by itself to initiate and maintain the RMA in quiescent preparations, was not observed (Fig. 2B). Thus, continuous firing of B1 seemed to

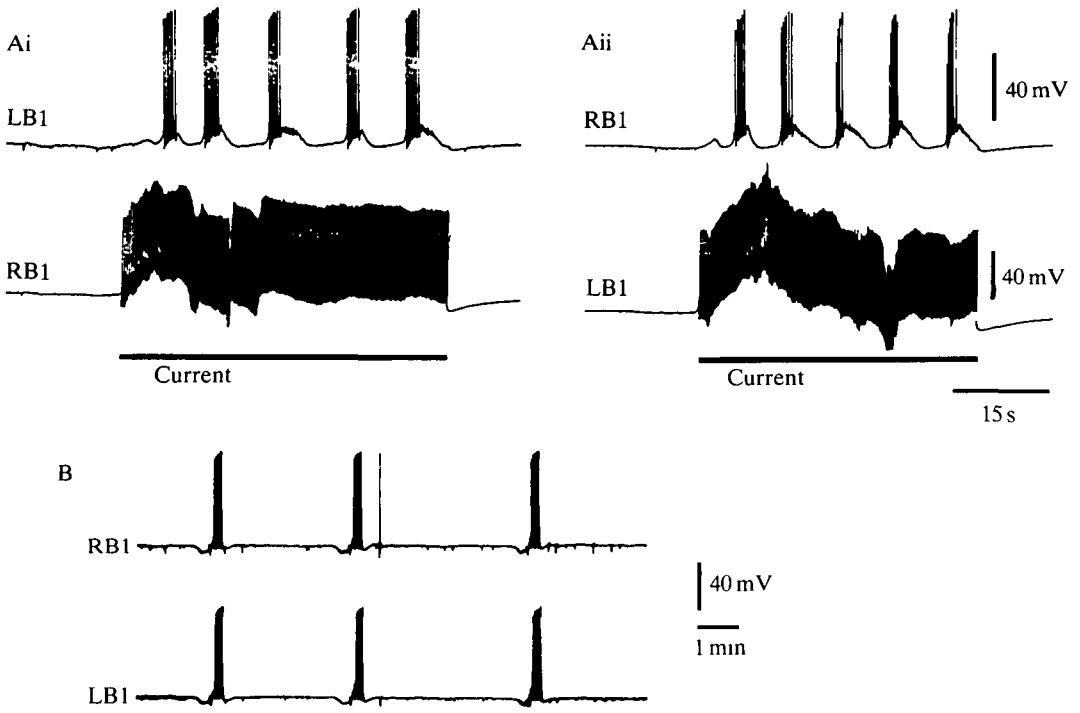

Fig. 2. (A) Simultaneous recordings of the right and left B1 neurones (RB1 and LB1) showing the rhythmic bursts in one B1 caused by high-frequency tonic firing in the contralateral cell. RB1 (Ai) or LB1 (Aii) was made to fire tonically by injecting depolarizing current during the period indicated by the bar under each record. (B) Simultaneous recordings of RB1 and LB1 during spontaneous rhythmic motor activity (RMA).

be sufficient, but was not always necessary, for the maintenance of RMA. B2 (situated just adjacent to B1, see Fig. 1) has similar morphology and peripheral innervation to B1 (Yoshida and Kobayashi, 1991). However, high-frequency firing of B2 caused by depolarizing current injection did not initiate RMA in the isolated quiescent buccal ganglia.

v-CDN

The v-CDN neurones, located on the ventral surface of the cerebral ganglia (Ku *et al.* 1985; see Fig. 1) are serotonergic and have been shown to enhance buccal muscle contraction induced by firing of identified cholinergic motoneurone B4. They also increase the excitability of this motoneurone (Yoshida and Kobayashi, 1991). In addition, v-CDN exerted excitatory effects on another eight pairs of identified buccal neurones with a variety of functions (see Fig. 1). We observed no inhibitory effects of v-CDN.

In earlier experiments (Yoshida and Kobayashi, 1991), electrical coupling between two v-CDNs was not observed. In the present experiments, however (when the subcerebral commissure was left intact), it was found that these

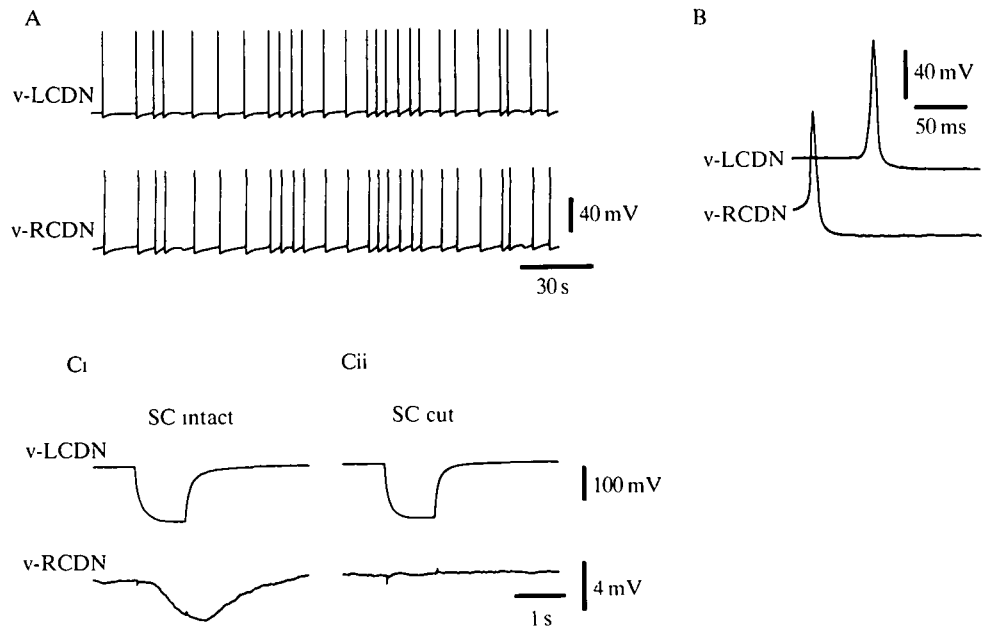

Fig. 3. (A) The 1:1 relationship between spontaneous firing in v-LCDN and v-RCDN. (B) An expanded section of A to illustrate the delay between spikes in v-LCDN and in v-RCDN. (C) The effect of cutting the subcerebral commissure (SC) on electrical coupling between v-LCDN and v-RCDN. In Ci and Cii, hyperpolarizing pulses were injected into v-LCDN through one electrode, while the membrane potentials of v-LCDN and v-RCDN were measured with another two recording electrodes. (Ci) SC intact. Note that the induced potential change in v-RCDN occurs after a delay of approximately 250 ms. (Cii) When SC was cut, the coupling disappeared (no response in lower trace).

neurones were strongly electrically coupled (Fig. 3). The subcerebral commissure (Chase and Tidd, 1991) is very thin and runs along the ganglionic artery. The electrical coupling ratio between v-LCDN and v-RCDN cell bodies was low (approximately 0.02), but the connection at the coupling region appeared to be tight enough to make them behave nearly as a single unit (Fig. 3A). The electrical connection appeared to be made *via* the subcerebral commissure, since the coupling disappeared when the commissure was cut (Fig. 3C). The time delay between an action potential in one v-CDN cell body and that in the contralateral one was about 50 ms (Fig. 3B). This seems reasonable considering the time taken by the action potential to propagate across the subcerebral commissure.

When a hyperpolarizing pulse was applied to one v-CDN cell body, a corresponding change of membrane potential occurred in the contralateral cell body after a delay of 200–300 ms (constant for each preparation) (Fig. 3C). This was observed in all preparations examined (more than five trials each in five

preparations). We attribute this delay in electrotonic conduction to the long time constant of these cells and the distance between recording positions (7–10 mm).

Previous experiments using preparations with a severed subcerebral commissure revealed that v-CDN had axonal projections in all ipsilateral buccal nerves but not in contralateral ones (Yoshida and Kobayashi, 1991). In the present experiments, contralateral projections of v-CDN were examined electrophysiologically using preparations with an intact subcerebral commissure. In these experiments, the ganglia were perfused with high-Mg^{2+} and low-Ca^{2+} solution to reduce synaptic transmission. A 1:1 relationship with constant latency between artificially evoked action potentials in v-CDN and action potentials in the buccal nerve trunks was the criterion used to identify the projections of v-CDN. The contralateral v-CDN was prevented from firing by passing hyperpolarizing current into its cell body. There are two pathways through which the v-CDN could travel to project in the contralateral buccal nerves: one *via* the subcerebral commissure and the other *via* the buccal commissure. Intracellular staining shows that v-CDN does not have a contralateral branch through the main cerebral commissure (Yoshida and Kobayashi, 1991). In all of the 12 preparations tested, v-CDN projected to the contralateral buccal nerves. However, v-CDN action potentials in n2, n3, n4, n5, the oesophageal nerve and the salivary nerve disappeared (with one or two exceptions for each nerve) when the subcerebral commissure was cut, although the projection to n1 remained in 58 % of the preparations. Thus, projections of v-CDN in the contralateral buccal nerves appeared to be provided mainly through the subcerebral commissure.

v-CDN showed a command-like ability to initiate rhythmic activity in the buccal ganglia. In the experiment shown in Fig. 4A, v-CDN was caused to fire tonically by constant current injection. This initiated RMA in the previously quiescent buccal ganglia (recognized by the rhythmic excitation of B1). To maintain RMA, firing of v-CDN at more than 5 Hz was needed in four of the five preparations tested. In one preparation, firing of v-CDN at 2 Hz was sufficient to initiate and maintain RMA. The command-like ability of v-CDN was not significantly changed by cutting the subcerebral commissure (N=3). RMA, once initiated by continuous v-CDN firing, lasted for several cycles after the firing of v-CDN had stopped (Fig. 4A). A shorter high-frequency stimulation of v-CDN did not cause maintained RMA, although a 'single cycle' of the rhythm was often elicited (Fig. 4B).

C1

A pair of cerebral neurones (LC1, RC1) has been newly identified in these experiments, and we suggest that these neurones are involved in the generation of RMA in the buccal ganglia. The C1 neurones' cell bodies (30–40 μm diameter) were located on the ventral surface of the cerebral ganglia, lateral to the intraganglionic tract of the supralabial nerve at the intersection of the intraganglionic roots of the sublabial nerve and the cerebrobuccal connective (Fig. 1). Just posterior to C1 there was a cluster of several neurones that fired spontaneously at a relatively high frequency. C1 could initiate cyclic bouts of action potentials in

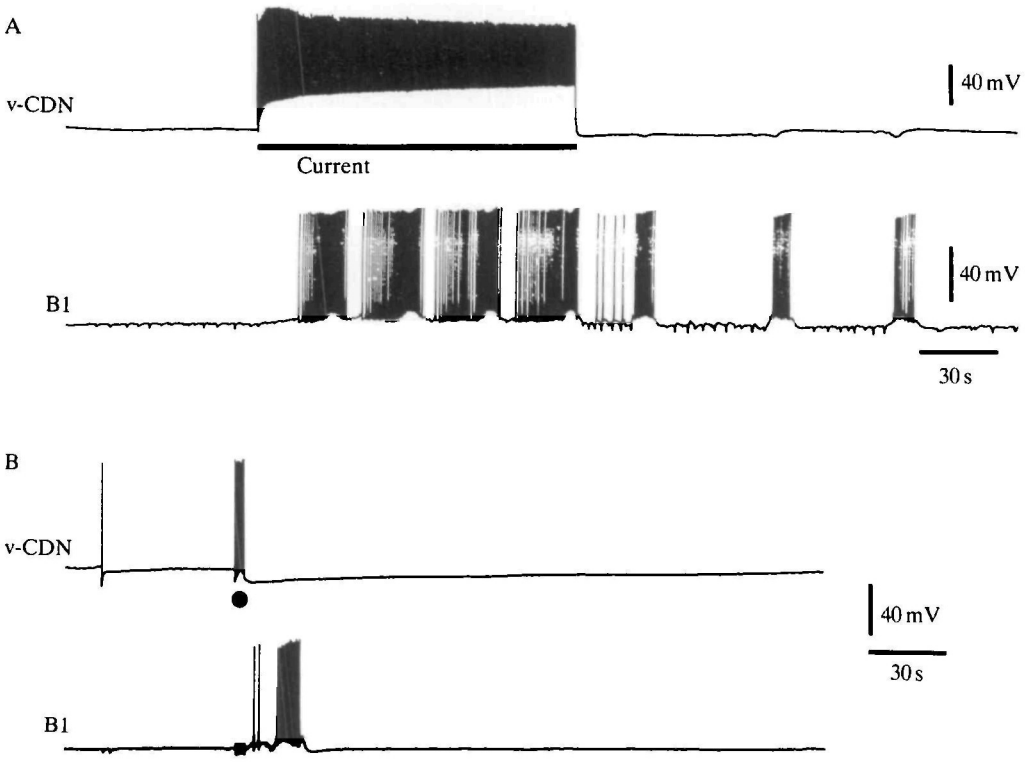

Fig. 4. (A) Simultaneous recordings of v-CDN and B1 showing the generation of RMA caused by v-CDN firing. v-CDN was caused to fire by depolarizing current injection during the period indicated by the bar under the record. (B) A single cycle of RMA (revealed by a burst in B1) was elicited by brief stimulation of v-CDN (dot). v-CDN fired at 12 Hz throughout the 5 s current injection.

buccal motor nerves when it was stimulated to fire at high frequency by depolarizing current injection (Fig. 5A).

When C1 was made to fire at high frequency by depolarizing current injection, RMA was initiated and maintained in the previously quiescent buccal ganglia (as recognized by the cyclic activity of B1) (Fig. 5B). During RMA, C1 was inhibited when B1 was activated. This inhibition was strong enough to stop the high-frequency firing of C1 caused by the applied depolarizing current (Fig. 5B). Such cyclical inhibition in C1 was also observed during artificially evoked continuous firing of B1, which probably induced and maintained RMA in the buccal ganglia (Fig. 5C). Since B1 does not project to the cerebral ganglia, it was clear that B1 did not directly inhibit C1. Thus, this inhibition appeared to be a feedback action from other components of the central pattern generator in the buccal ganglia.

To ascertain the morphology of C1, neurones that satisfied the above criteria (soma position and rhythm-initiating ability) were stained intracellularly with Lucifer Yellow (Fig. 6). C1 had an axon in the ipsilateral CBC in all the

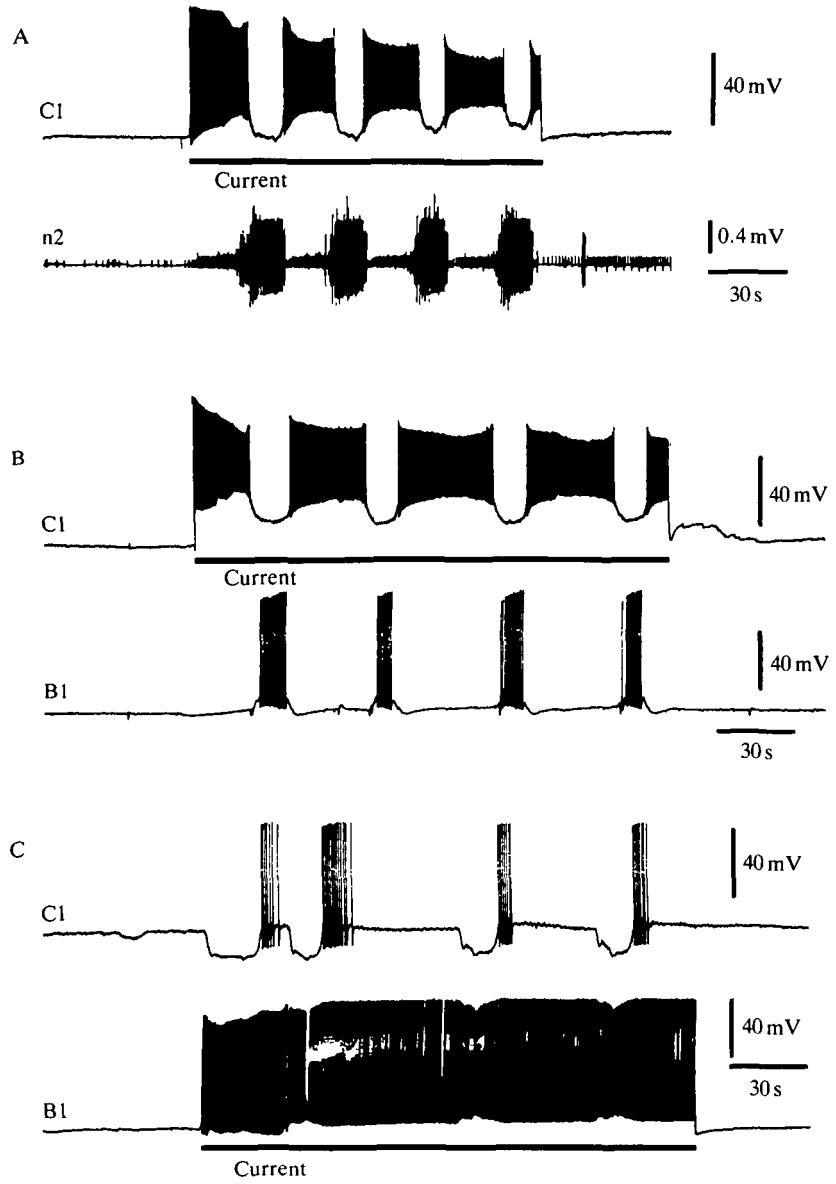

Fig. 5. (A) Cyclical bursts of activity in buccal nerve 2 (n2) elicited by C1 firing. (B,C) Simultaneous recordings of C1 and B1 showing the generation of RMA by the firing of C1 (B) or B1 (C). C1 or B1 was caused to fire by depolarizing current injection during the period indicated by the bar under each record. In B, the imposed firing of C1 is periodically interrupted by inhibitions that coincide with activity in B1. In C, imposed tonic firing of B1 (which presumably initiates RMA) is accompanied by hyperpolarizations in C1 (upper trace).

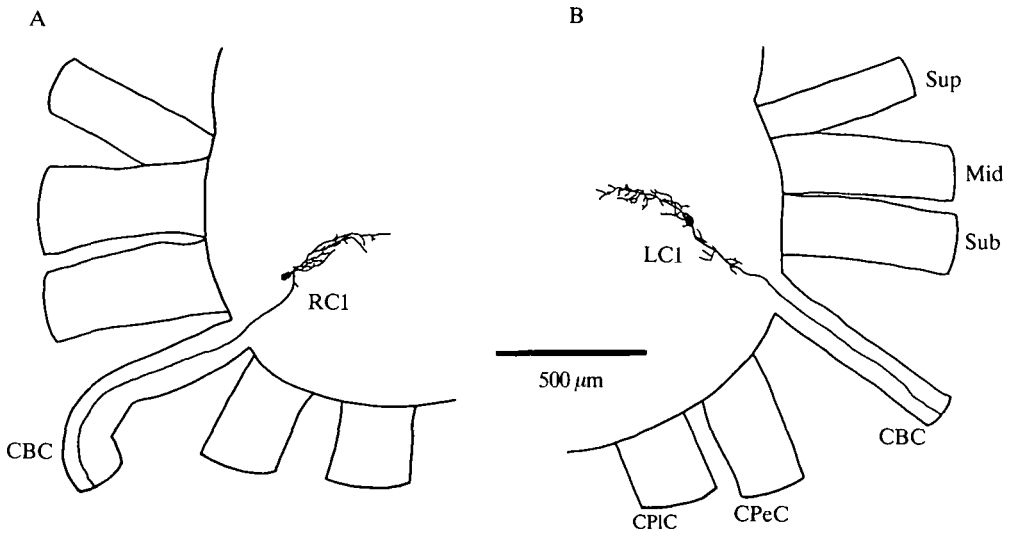

Fig. 6. Morphology of right C1 (RC1) and left C1 (LC1) stained by injection of Lucifer Yellow in different preparations. A ventral view of the cerebral ganglia is shown. Other abbreviations as in Fig. 1.

preparations tested (N=4), and had a dendritic arborization within the ipsilateral cerebral ganglion. There were no contralateral projections through the cerebral commissure. Visualization of the projection of C1 in the buccal ganglia was not successful.

Fig. 7A shows recordings from C1 and buccal nerve 2 during spontaneous RMA. C1 received phase-locked feedback inhibition from the buccal ganglia and fired between the inhibitions. In this preparation (consisting of the buccal ganglia with the cerebral ganglia attached), however, spontaneously occurring RMA was not always accompanied by firing of C1 and v-CDN (not shown). This was consistent with the finding that the isolated buccal ganglia could generate RMA. Buccal RMA could also be induced and maintained by electrical stimulation of the labial nerves. In the case shown in Fig. 7B, stimulation of the supralabial nerve at 2 Hz did not cause continuous firing in C1 and B1, although RMA was initiated in the buccal ganglia. These observations suggested that continuous firing of C1 was sufficient to initiate and maintain RMA, but was not essential. Stimulation of individual labial nerves (except the sublabial nerve, which contains axons of v-CDN) did not induce continuous firing of v-CDN (not shown).

RMA induced by taste stimuli

An isolated lip–nerve preparation could respond to taste stimuli (Fig. 8A). Carrot juice was applied to the lip by perfusion. Chemosensory afferents responded to the taste stimulus with a burst of spikes which was recorded extracellularly using a suction electrode placed over the cut end of the supralabial

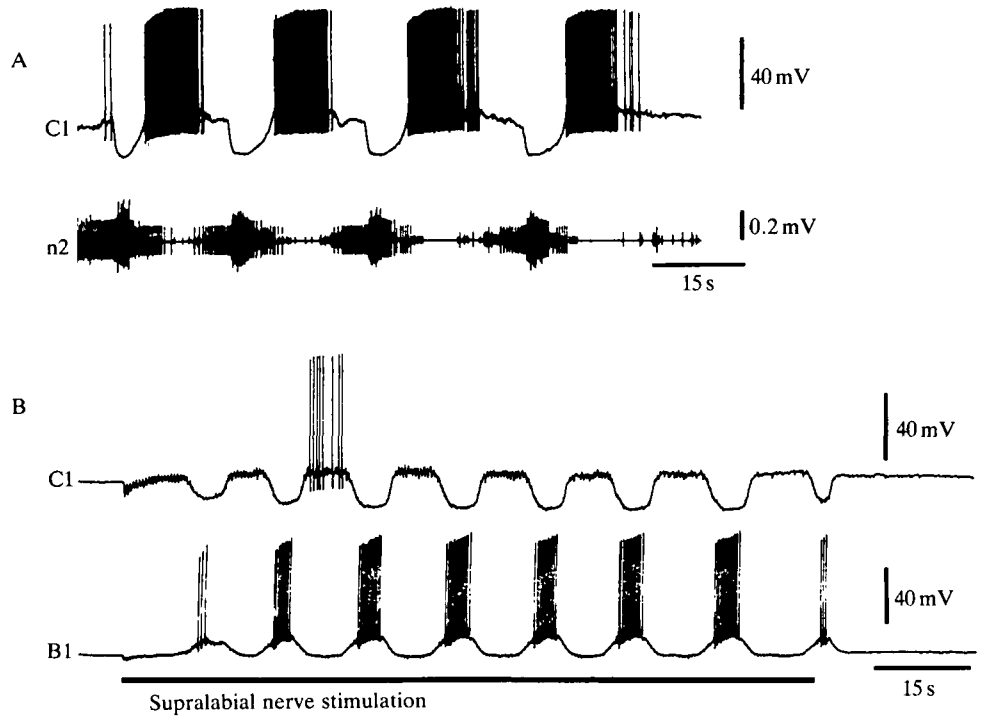

Fig. 7. (A) Simultaneous recordings of C1 and buccal nerve 2 (n2) during spontaneous RMA. (B) Simultaneous recordings of C1 and B1 during RMA elicited by electrical stimulation of the supralabial nerve. The nerve was stimulated at 2 Hz during the period indicated by the bar under the record.

nerve. Similar responses to taste stimuli could be recorded in the other labial nerves. In the lip–ganglia preparation, taste stimuli such as carrot juice and apple juice applied to the lip often induced RMA in the buccal ganglia, although the responsiveness varied from preparation to preparation.

Fig. 8B shows the response of C1 to taste stimulation of the lip. C1 responded to the stimulus with excitation, and received inhibition that might be feedback from the buccal ganglia, in which RMA was generated. This observation suggested that C1 was involved in the taste-induced RMA. The excitation of C1, which was preceded by the burst in n2, persisted for some time after the taste stimulant had been washed out (Fig. 8B). To examine the role of C1 in taste-induced RMA, C1 was prevented from firing by injection of hyperpolarizing current during the taste stimulation of the lip (N=3). There were no apparent differences in the rate or intensity of taste-induced RMA during the hyperpolarizing current injection into C1.

In the experiment shown in Fig. 9A, perfusion of the lip with carrot juice induced RMA in the buccal ganglia (indicated by cyclic bursts in B1 and buccal motor nerve n2). In addition, v-CDN showed phase-locked inhibitory responses

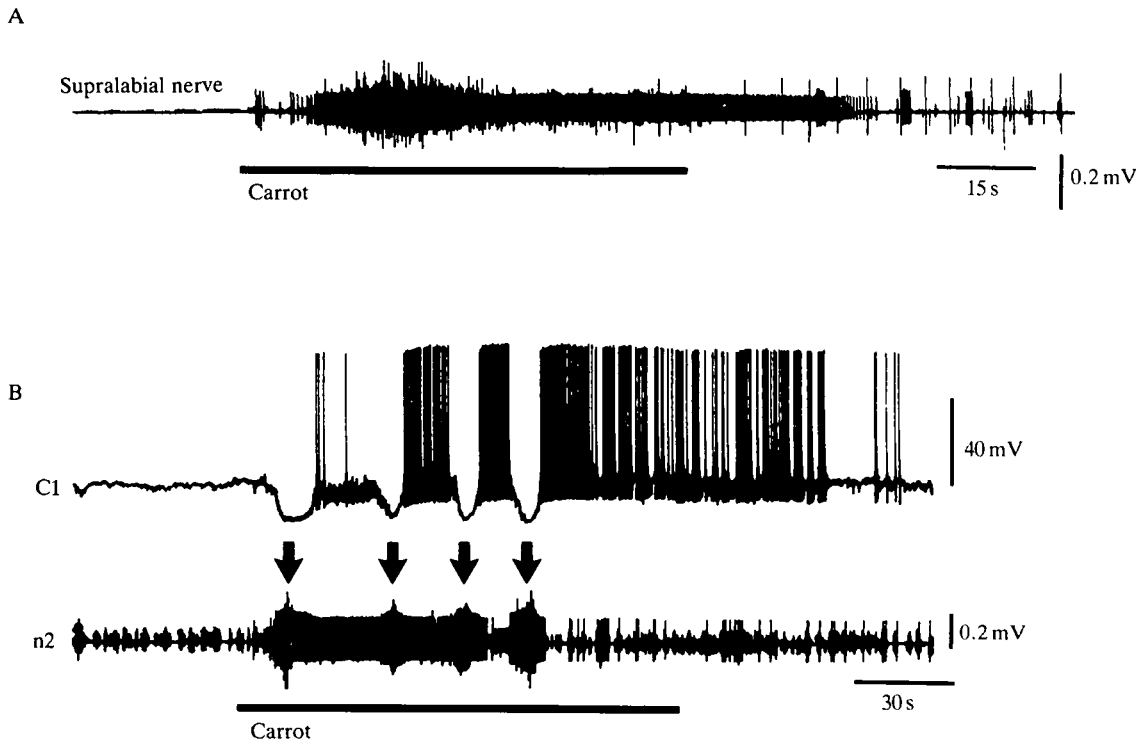

Fig. 8. (A) Extracellularly recorded response of the supralabial nerve to taste stimulation of the lip. Carrot juice was applied to the lip during the period indicated by the bar under the record. (B) Response of C1 and buccal nerve 2 (n2) to taste stimulation of the lip. The stimulation elicited several cycles of RMA that could be recognized as cyclic bursts in n2 (arrows). C1 received periodic inhibition coincident with the activity in n2. Carrot juice was applied to the lip during the period indicated by the bar under the record.

which appeared to be feedback from the buccal ganglia, since such inhibition was never seen in preparations without the buccal ganglia. RMA usually ceased before the stimulus was removed. Responses of v-CDN to taste stimulation of the lip varied among preparations. In the majority of cases (8 of 13 trials in 8 preparations), v-CDN tended to hyperpolarize slightly in response to taste stimuli (Fig. 9A). However, in 2 of 5 trials, v-CDN was slightly excited and, in 3 trials, v-CDN had biphasic responses (not shown).

Firing of B1 was not essential for taste-induced generation of RMA, since the rhythmic activity was generated by taste stimulation of the lip even when both B1 neurones were prevented from firing by hyperpolarizing current injection (Fig. 9B).

When freely moving *Achatina fulica* were eating solid food, such as sliced potato, the rate of radula rasping was about 0.3 Hz. In comparison, the rate of

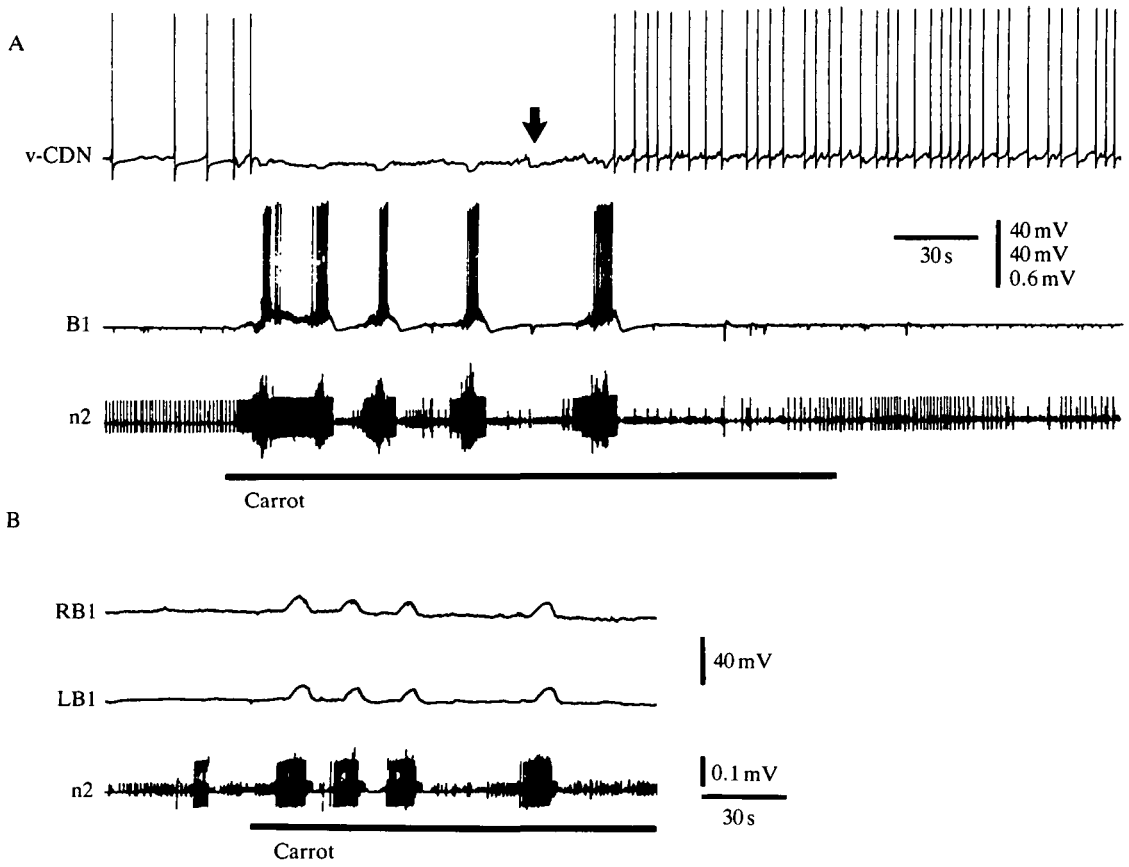

Fig. 9. (A) Responses of v-CDN, B1 and buccal nerve 2 (n2) to taste stimulation of the lip. Several cycles of RMA were elicited by the stimulation. v-CDN received inhibitions synchronized with the bursts in B1. The origin of an extra hyperpolarization in v-CDN (arrow) is not known. (B) Responses of RB1, LB1 and buccal nerve 2 to taste stimulation of the lip. Several cycles of RMA were elicited in n2, while both B1 neurones were prevented from firing by hyperpolarizing current injection. In A and B, carrot juice was applied to the lip during the periods indicated by the bars under the records.

RMA in reduced preparations was considerably lower (<0.13 Hz). However, since taste stimuli that were attractive for intact animals induced RMA when applied to the lip of the isolated preparation, the rhythm observed in experimental conditions might represent, at least in part, the feeding rhythm observed in intact animals.

Discussion

In several invertebrate species, identified neurones that can initiate fixed behaviours such as swimming and feeding have been reported (Getting, 1976; Granzow and Kater, 1977; Brodfuehrer and Friesen, 1986*a*,*b*,*c*; Delaney and

Gelperin, 1990*a*). These command-like neurones may be divided into two categories. One is a group of 'driver' neurones that are required to fire continuously for the maintenance of a patterned activity (Delaney and Gelperin, 1990*a*). The other is a group of 'trigger' neurones that can trigger a cyclic activity that outlasts the triggering stimulus (Brodfuehrer and Friesen, 1986*a*). In the present experiments, two pairs of cerebral neurones and a pair of buccal neurones were found to have a command-like ability in the generation of RMA in the buccal ganglia of *Achatina fulica*. RMA elicited by firing in C1 or B1 continued only during the tonic excitation of these neurones. Thus, C1 and B1 might function as drivers of RMA in the experimental conditions, although the possibility that they are the components of the motor-pattern-generating network cannot be ruled out. In contrast, v-CDN-induced RMA continued for several cycles after the firing of the neurone had stopped.

In gastropods other than *A. fulica*, such as *Lymnaea stagnalis*, *Pleurobranchaea californica* and *Aplysia californica*, buccal ganglionic neurones that can initiate rhythmic activity have also been identified (Gillette *et al.* 1980; Rose and Benjamin, 1981*a*; McClellan, 1983; Susswein and Byrne, 1988; Kirk, 1989). However, the properties of these neurones varied among the species. The slow oscillator interneurone in *L. stagnalis* drove rhythmic activity similar to that seen in normal feeding (Rose and Benjamin, 1981*a*,*b*). In *P. californica*, the ventral white cell (VWC) is thought to be a feeding command cell (Gillette *et al.* 1980), while some research suggests that the VWC elicits vomiting (McClellan, 1983). In addition, in this animal, it has been proposed that a single motor pattern generator in the buccal ganglia controls several rhythmic behaviours involving common buccal structures (McClellan, 1982). It is not clear from the present experiments whether the rhythmic activities induced by B1, C1 or v-CDN are identical. It is now necessary to describe in detail the motor patterns elicited by each type of stimulus.

Are B1, v-CDN and C1 acting as rhythm-initiating neurones in the intact animal? B1 and v-CDN do not appear to be essential for the generation of the feeding rhythm, because they did not fire tonically during the taste-induced RMA in reduced preparations, even though tonic firing was required for them to initiate and maintain RMA by themselves. It seemed unlikely that initiation of RMA was a major function of the v-CDN neurone in the intact animal, since v-CDN tended to respond to taste stimulation with a slight hyperpolarization. Taking the results of our previous report (Yoshida and Kobayashi, 1991) into consideration, it appears likely that v-CDN functions to increase the activities of muscles and buccal neurones subserving a variety of functions. Nevertheless, since B1 and v-CDN could both initiate RMA, they might play a role in the generation of rhythmic activities in certain circumstances. Kemenes *et al.* (1986) suggested that multiple sensory pathways from peripheral sensory structures are required for normal sensory activation of the feeding system in *Lymnaea stagnalis*. In the present experiments, only the lip region was stimulated to induce RMA. Such restricted stimulation may not be sufficient to activate the networks involving B1 or v-CDN.

It has been suggested that v-CDNs are homologous to serotonergic cerebral giant cells in other gastropod species, e.g. the cerebral giant cell (CGC) in *L. stagnalis* and the metacerebral giant cell (MGC) in *Limax maximus* (Yoshida and Kobayashi, 1991). It has been reported that CGC and MGC can initiate RMA in both species, and that a short high-frequency burst of MGC is more effective in initiating RMA than is prolonged firing (McCrohan and Audesirk, 1987; Delaney and Gelperin, 1990*b*). In *A. fulica*, however, a short high-frequency burst in v-CDN did not maintain RMA, although such a burst sometimes induced a 'single cycle' of the rhythm. It has been suggested for *L. stagnalis* that phasic activity in CGCs, which could be elicited artificially by application of food stimuli to the lips and tentacles, is relevant for controlling the feeding motor output in the animal (McCrohan and Audesirk, 1987).

C1 is likely to be involved in taste-induced rhythm generation since it responded to taste stimulation of the lip with bursts of spikes. However, C1 was not always excited by electrical stimulation of the lip nerve, even when RMA was initiated by the same stimulus. In addition, when the lip was stimulated by taste substances, the burst of spikes in buccal nerves preceded firing of C1 (see Fig. 8B). Thus, some neurones or neurone groups other than C1 must fulfil the role of rhythm initiation, while C1 might be involved in maintenance of the rhythm. However, the frequency of firing of C1 elicited by taste stimulation to the lip region did not seem to be sufficient to affect RMA, since the taste-induced RMA was not affected when C1 was prevented from firing by injection of hyperpolarizing current.

Command-like cerebral neurones other than serotonergic cerebral giant cells have also been identified in other gastropod species: cerebral ventral 1 (CV1) in *L. stagnalis* (McCrohan, 1984); paracerebral neurones (PCNs) in *P. californica* (Gillette *et al.* 1978); and cerebral–buccal interneurones (CBIs) in *A. californica* (Rosen *et al.* 1987, 1988, cited in Kirk, 1989). In *L. maximus*, several identified cerebral to buccal interneurones (CBs) were shown to trigger fictive feeding, and the role of CBs in the generation of a taste-induced fictive feeding rhythm has been carefully investigated (Delaney and Gelperin, 1990*a*,*b*,*c*).

It has been suggested that CBIs are homologous to paracerebral neurones and CV1 (Kirk, 1989). McCrohan and Kyriakides (1989) also suggested that CV1 neurones are homologous with PCNs and CBs. These cerebral–buccal interneurones have similar locations and morphologies, can elicit rhythmic motor output from the buccal ganglia and show phase-locked activity and synaptic inputs during the buccal rhythm (McCrohan and Kyriakides, 1989). It is therefore interesting to note that C1 in *A. fulica* is located in a similar region of the cerebral ganglion to these identified cerebral–buccal interneurones of other gastropod species, and that C1 shows similar characteristics in relation to rhythmic buccal motor activity.

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