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PATTERNS OF ACTIVITY AND AXONAL PROJECTIONS OF THE CEREBRAL GIANT CELLS OF THE SNAIL, *LYMNAEA STAGNALIS*

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SUMMARY

1. The paired cerebral giant cells (CGCs) of *Lymnaea* were studied using electrophysiological and anatomical techniques.

2. A strong electrotonic junction coupling the CGCs is located in the buccal ganglia, several millimetres distal to the cell bodies. This leads to 1:1 spike activity in the two cells.

3. The CGCs receive phasic and tonic inputs which appear to be common to the two cells. Two types of phasic input are synchronous with synaptic input to feeding motoneurones in the buccal ganglia.

4. Morphological studies, using three separate dye injection techniques, confirm the axonal projections shown by electrophysiological methods. The CGCs project ipsilaterally into one lip nerve, the cerebrobuccal connective, and all buccal nerves.

5. Symmetry of anatomy and of firing activity cause the CGCs to function as a single neural unit.

INTRODUCTION

A pair of symmetrical, giant, serotonin-containing neurones have been found in the cerebral ganglia of the pulmonate molluscs *Limax*, *Ariolimax*, *Helix*, *Planorbis* and *Helisoma* (Osborne & Cottrell, 1971; Senseman & Gelperin, 1973; Pentreath, Osborne & Cottrell, 1973; Marsden & Kerkut, 1970; Granzow & Kater, 1977), and the opisthobranch molluscs *Aplysia*, *Tritonia* and *Pleurobranchaea* (Weinreich *et al.* 1973; Gillette & Davis, 1977). These neurones are believed to be homologous in the various species (Senseman & Gelperin, 1973; Weiss & Kupfermann, 1976) and have proved useful in the study of the role of serotonin as a neurotransmitter. In several species the giant cerebral cells have been shown to make mono- and polysynaptic connexions with cells of the buccal ganglia (Cottrell & Macon, 1974; Berry & Pentreath, 1976; Weiss & Kupfermann, 1976; Gerschenfeld & Paupardin-Tritsch, 1974; Gillette & Davis, 1977), suggesting a function in the higher control of feeding.

Sakharov and Zs.-Nagy (1968) have located serotonin in a pair of giant cells in *Lymnaea*, identified by size and position to be the cerebral giant cells (CGCs). This paper examines the electrophysiology and axonal projections of the CGCs in

Lymnaea. It shows that the electrical activity of the two cells is closely related, owing to their electrotonic coupling and receipt of common synaptic inputs, two of which occur at the same time as synaptic inputs to buccal ganglion motoneurones. The following paper (McCrohan & Benjamin, 1980) examines the synaptic connexions between the CGCs and motoneurones of the feeding system in the buccal ganglia of *Lymnaea*, and relates the activity of these cerebral interneurones to the previous description of the feeding cycle (Benjamin & Rose, 1979; Benjamin, Rose, Slade & Lacy, 1979; Rose & Benjamin, 1979).

MATERIALS AND METHODS

Specimens of *Lymnaea stagnalis*, obtained from Gerrard and Haig Ltd, East Preston, Sussex, were kept in aerated tap water at 20 °C and fed on lettuce. Experiments were carried out at 20 °C in a Hepes-buffered saline of the following composition (mM): Na^+, 24·0; K^+, 2·0; Ca^{2+}, 4·0; Mg^{2+}, 2·0; Cl^-, 38·0; $H_2PO_4^-$, 0·1; Na-Hepes, 35·4. Glucose (15 mg/l) was added to this solution. A bicarbonate-buffered snail saline (Winlow & Benjamin, 1976) was adapted to give high Mg^{2+}/low Ca^{2+} and high Mn^{2+} salines, by substituting chloride of the appropriate cation for NaCl. Two types of preparation were used. The isolated preparation (used more often) consisted of the brain plus paired buccal ganglia, with the cerebrobuccal connectives intact. The semi-isolated preparation included the buccal mass and oesophagus with attached brain and buccal ganglia (for more detail see Rose & Benjamin, 1979). The brain was soaked in a protease solution (approx. 0·1%) (Type V, Sigma, London) for about 15 min, washed in saline and then pinned in a recording chamber lined with Sylgard.

Simultaneous intracellular recordings were made from one, two or three cells using glass microelectrodes filled with 0·5 M-K_2SO_4. Electrode resistances ranged from 5 to 20 MΩ. Current could be passed into a cell either through the recording electrode (using the bridge balance system in the preamplifier) or by impaling the cell with a second microelectrode which was used solely for passing current. Current injected was monitored using an *I–V* converter placed between the indifferent electrode and earth.

Glass suction electrodes were used for extracellular recording and stimulation. Conventional amplifying and stimulating equipment was used, and records were displayed on a storage oscilloscope or pen-recorder.

Iontophoresis of Procion Yellow and horseradish peroxidase

Microelectrodes were filled with either 6% Procion Yellow (M-4RS) solution, or 4% horseradish peroxidase (HRP) (Miles) made up in 0·2 M-K_2SO_4. Cells were impaled and 500 ms, 5–25 nA pulses (hyperpolarizing for Procion Yellow, depolarising for HRP) were passed at 1 Hz for about 1 h. The preparation was then kept in saline at 4 °C overnight. Procion-injected preparations were processed as described by Benjamin *et al.* (1979). HRP-injected material was processed by a method similar to that of Lavail & Lavail (1974). Brains were dehydrated, embedded in paraffin wax, and serially sectioned at 15 μm. In both cases the appearance of neurones was reconstructed from serial sections using a *camera lucida*, and some sections photographed.

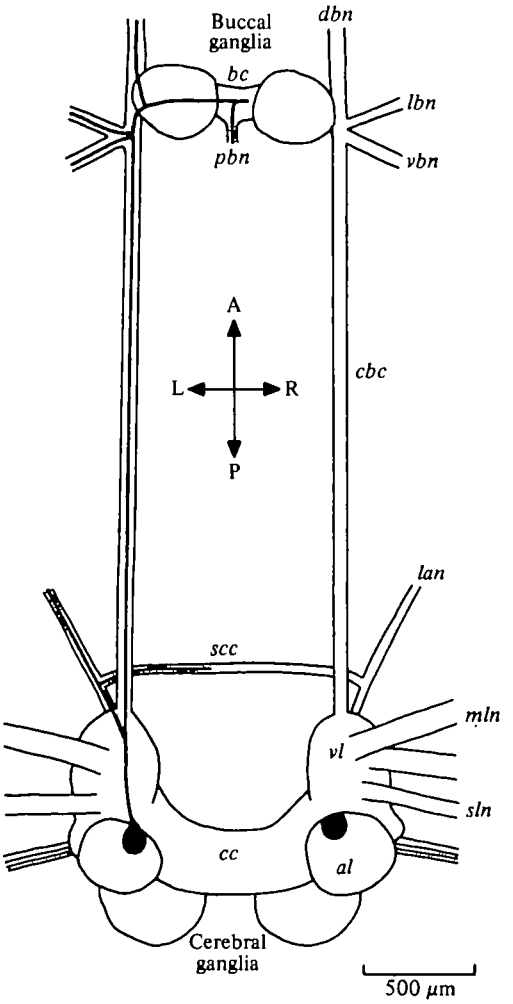

Fig. 1. The cerebral and buccal ganglia of *Lymnaea*, showing the location of the cerebral giant cell bodies within the cerebral ganglia. The axonal projections of the left CGC, as determined by anatomical techniques, are shown. A, Anterior; *al*, anterior lobe; *bc*, buccal commissure: *cbc*, cerebrobuccal connective; *cc*, cerebral commissure; *dbn*, dorsobuccal nerve; L, left; *lan*, labial artery nerve; *lbn*, laterobuccal nerve; *mln*, median lip nerve; P, posterior; *pbn*, postbuccal nerve; R, right; *sln*, superior lip nerve; *scc*, subcerebral commissure; *vbn*, ventrobuccal nerve; *vl*, ventral lobe.

Cobalt chloride back fills

Cut ends of nerves were dipped into a 1 M cobaltous chloride solution and left overnight at 4 °C, the brain being maintained in saline. The preparations were processed as described by Benjamin *et al.* (1979). Whole mounts were drawn using a *camera lucida*, and photographed.

RESULTS

Identification of the cerebral giant cells (CGCs)

The CGCs are a pair of bilaterally symmetrical giant neurones whose cell bodies lie on the ventromedial surface of the anterior lobes of the cerebral ganglia. (These lobes and others in the cerebral ganglia of *Lymnaea* were described by Joosse, 1964). They can be recognized prior to electrode penetration by their large size (80–120 μm, depending on the size of the individual) and fairly constant location on the ganglion surface (Fig. 1). Confirmation of visual identification is obtained after impalement of both neurones, by observing 1:1 spike activity in the two cells (Fig. 2*a*).

Patterns of Activity

The CGCs show a variety of firing patterns (Fig. 2), some of which can be related to the activity of motoneurones of the feeding system (Fig. 2*c*), previously described by Benjamin & Rose (1979). For the purposes of the present paper it is sufficient to say that the burst activity of feeding motoneurones occurs in response to two consecutive phases of synaptic input occurring during Protraction and the first Retraction phase (Retraction 1) of the buccal muscle system. No common synaptic input to motoneurones occurs during Retraction 2. The burst activity of feeding motoneurones varies both between preparations and in the same preparation at different times. About 50% of preparations show some sort of cyclic activity, but only in about 20% is burst activity regular and strongly synchronized. No method has been found, as yet, of triggering such cyclical activity in feeding motoneurones.

A regular single-spiking pattern of activity was often recorded in CGCs, which varied in frequency within the range 0·5–2·0 Hz and was 1:1 in left and right cells (Fig. 2*a*). This occurred in preparations where no obvious bursting of buccal motoneurones occurred ('non-feeding' preparations). In preparations where buccal motoneurones were only bursting sporadically (i.e. synaptic inputs to motoneurones were irregular) the CGCs' firing activity was less regular, with a tendency for spikes to occur in pairs or triplets (Fig. 2*b*). Regular bursting of buccal motoneurones was accompanied by phasic spike activity in the CGCs which, in more pronounced cases, appeared as bursts of spikes. These occurred at the same time as the first (Protraction) phase of synaptic input to motoneurones (which is inhibitory on 4-group cells), and were followed by periods of inhibition lasting up to 3 seconds. To illustrate this, recordings from a CGC and a 4-group neurone are presented in Fig. 2(*c*). 4-group neurones are retractor motoneurones in the feeding system (Rose & Benjamin, 1979) which fire on the rebound following a first (Protraction) phase compound inhibitory post-synaptic potential (i.p.s.p.). Bursts in the CGC occurred at the same time as these compound i.p.s.p.s in the 4-group neurone. Between these regular bursts of spikes the CGC fired in groups of two and three spikes (Fig. 2*c*).

In a few preparations very long bursts of spikes occurred at 10–15 min intervals (Fig. 2*d*), each followed by a period of inhibition.

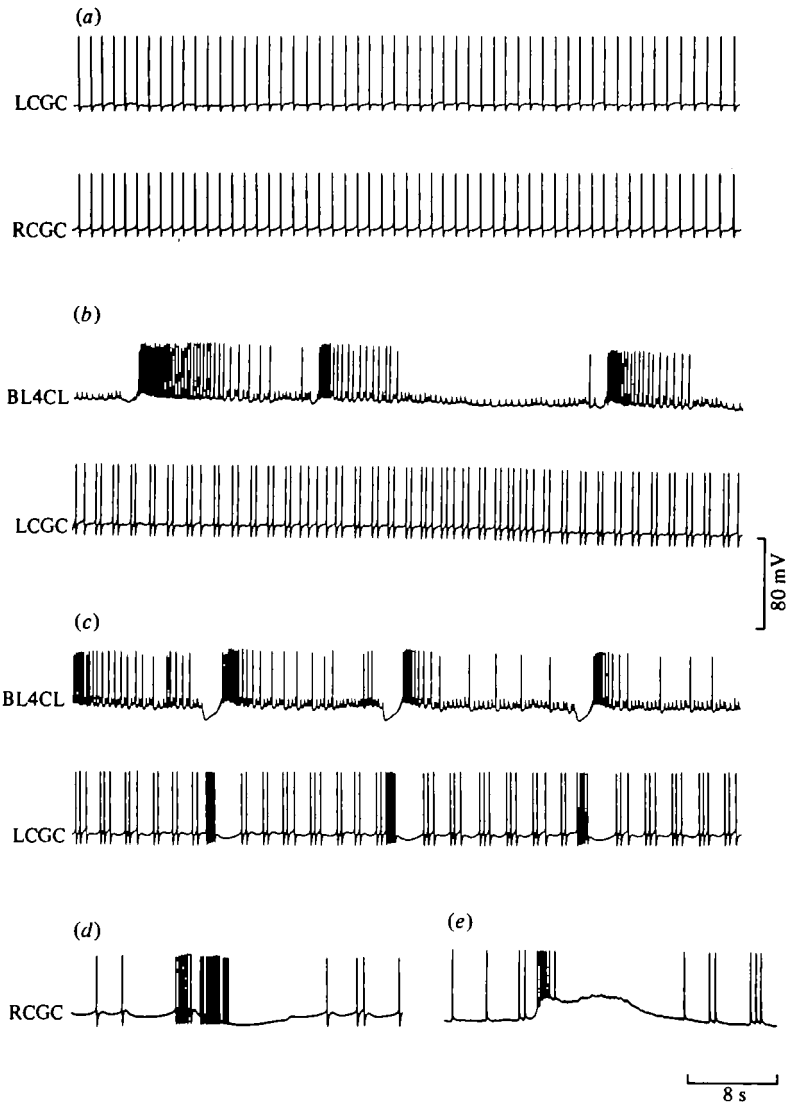

Fig. 2. Patterns of firing activity of cerebral giant cells (CGCs). (*a*) Regular single spiking activity, 1:1 in left and right CGCs (LCGC and RCGC). (*b*) Irregular spiking pattern shown in left CGC, associated with sporadic feeding bursts monitored in a left 4-group neurone (buccal 4 cluster cell, BL4CL). (*c*) Bursts of spikes in the left CGC occurring at the same time as inhibitory inputs to a left buccal 4-group cell (BL4CL), followed by inhibition during the 4 cell bursts. (*d*) An occasional large burst of spikes seen in a right CGC. (*e*) A similar large burst seen in a right CGC, hyperpolarized to reveal the underlying compound e.p.s.p.

Synaptic Inputs to CGCs

The spike activity described in the previous section occurred in the absence of stimulation applied either to central neurones or to peripheral nerve roots. Three major types of synaptic input were observed under these conditions. Two of these are phasic in occurrence and are responsible for the bursts followed by inhibition

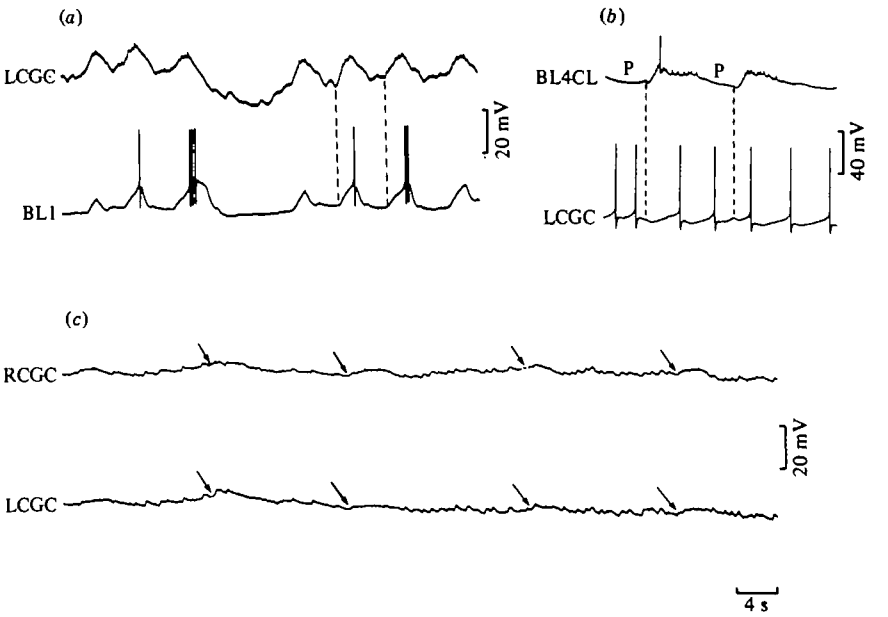

Fig. 3. Synaptic inputs to the CGCs. (*a*) Phasic excitatory synaptic input to a left CGC (hyperpolarized to prevent spiking) synchronous with that on a 1 cell of the left buccal ganglion (BL1). (*b*) Inhibitory inputs to a left CGC synchronous with those on a left buccal 4-group cell (BL4CL). First (Protraction) phase of synaptic input is marked 'P'. (*c*) Tonic input, consisting of unitary e.p.s.p.s, and phasic input (arrowed), synchronous on the left and right CGCs, both hyperpolarized by 40 mV to prevent spiking.

shown in Fig. 2(*c*). The third is a continuous (tonic) input which is probably responsible for the irregular firing seen in Fig. 2(*b*) and the double and triplet spiking seen between bursts in Fig. 2(*c*).

The two phasic inputs appear as excitation followed by inhibition. The excitatory postsynaptic potential (e.p.s.p.) underlying the CGC burst occurs at the same time as both the inhibitory input to the 4-group cell of Fig. 2(*c*) and also the excitatory input occurring in the buccal 1-cell of Fig. 3(*a*). This is consistent with previous work (Benjamin & Rose, 1979) which also showed that these 4-group cell and 1-cell inputs occur at the same time. To see the wave-form of this e.p.s.p. underlying the CGCs' bursts it is necessary to prevent spike activity by application of strong d.c. hyperpolarizing current to both left and right cells. Application of hyperpolarizing current to one cell alone is not sufficient to prevent firing owing to strong electrotonic coupling between the two CGCs (described later). Fig. 3(*a*) shows that the compound e.p.s.p underlying the CGC burst occurs at the same time as that in a 1-cell of the buccal ganglion. The i.p.s.p. which follows this compound e.p.s.p. in the CGC is shown in Fig. 3(*b*). It is again a compound potential and Fig. 3(*b*) shows how it occurs at the same time as the second phase of synaptic input to buccal motoneurones. In some larger 4-group neurones (such as the 4-cluster cell of Fig. 3*b*), in which strong bursts of spikes may not be seen, the i.p.s.p. occurring during Protraction (marked 'P' in Fig. 3*b*) is followed by a short second i.p.s.p. during Retraction 1, just prior to the rebound which may lead to spiking. (This second i.p.s.p. is not see

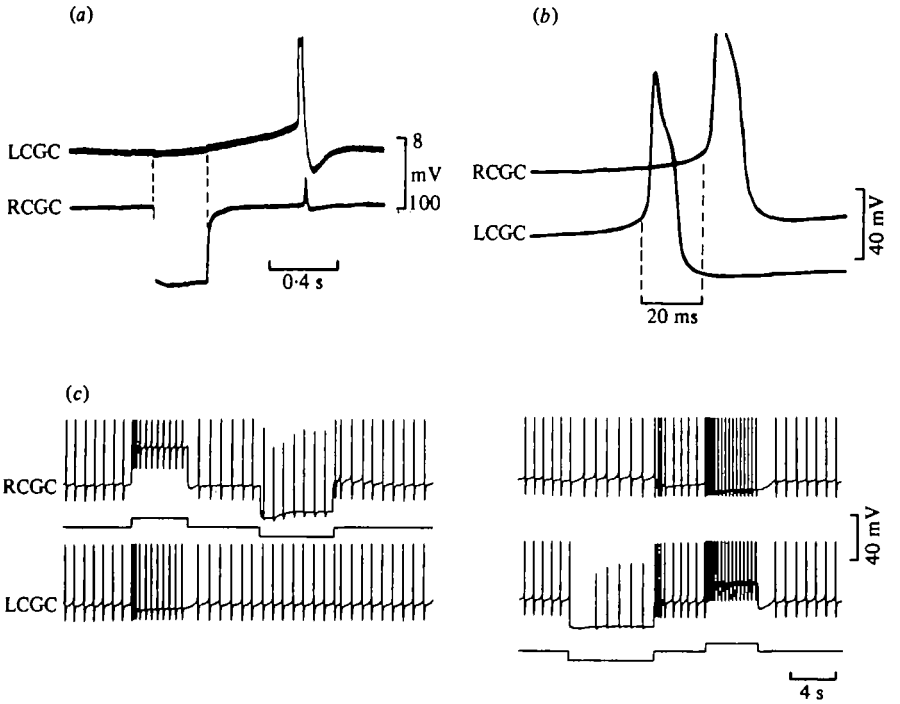

Fig. 4. Electrotonic coupling between the CGCs. (*a*) Application of a square hyperpolarizing pulse (approx. 5 nA) to a right CGC producing a 100 mV change in membrane potential, is accompanied by < 1 mV change, of the same polarity and duration, in the left CGC. Coupling ratio is < 0·01. (*b*) 20 ms delay between onset of spikes in left and right CGCs. (*c*) 1 : 1 firing in the CGCs after application of depolarizing or hyperpolarizing current to either cell, producing changes in firing frequency. Depolarizing and hyperpolarizing pulses are indicated.

in more active 4-group neurones such as that of Fig. 2*c*.) In Fig. 3*b* the timing of the CGC i.p.s.p. is shown to occur simultaneously with the second i.p.s.p. on the 4-group neurone. This compound i.p.s.p. on the CGC is not always apparent as a change in membrane potential, but can be seen as a delay before the onset of the next spike. The significance of this relationship between bursts in the CGCs and the firing of buccal motoneurones will be discussed in the following paper (McCrohan & Benjamin, 1980). It is sufficient to say here that the burst of spikes in the CGCs occurs in the first (Protraction) phase of the feeding cycle, and the period of inhibition in the second (Retraction 1) phase of the cycle as described by Rose & Benjamin (1979).

Tonic synaptic inputs to the CGCs were revealed when spiking was prevented in both cells (Fig. 3*c*). This tonic, low-level input occurred between the dual phasic inputs described in the last paragraph. It is presumed that this continuous low-level synaptic input is responsible for the spike activity seen between bursts in Fig. 2*c*, although it is not possible to relate spike activity to individual p.s.p.s.

A fourth type of input, shown in Fig. 2(*d*, *e*) is a long duration compound e.p.s.p. whose size increases with successive hyperpolarization of the cell. This is responsible for the occasional long burst of spikes seen in CGCs.

Fig. 3(*c*) showed that both left and right CGCs receive similar synaptic inputs,

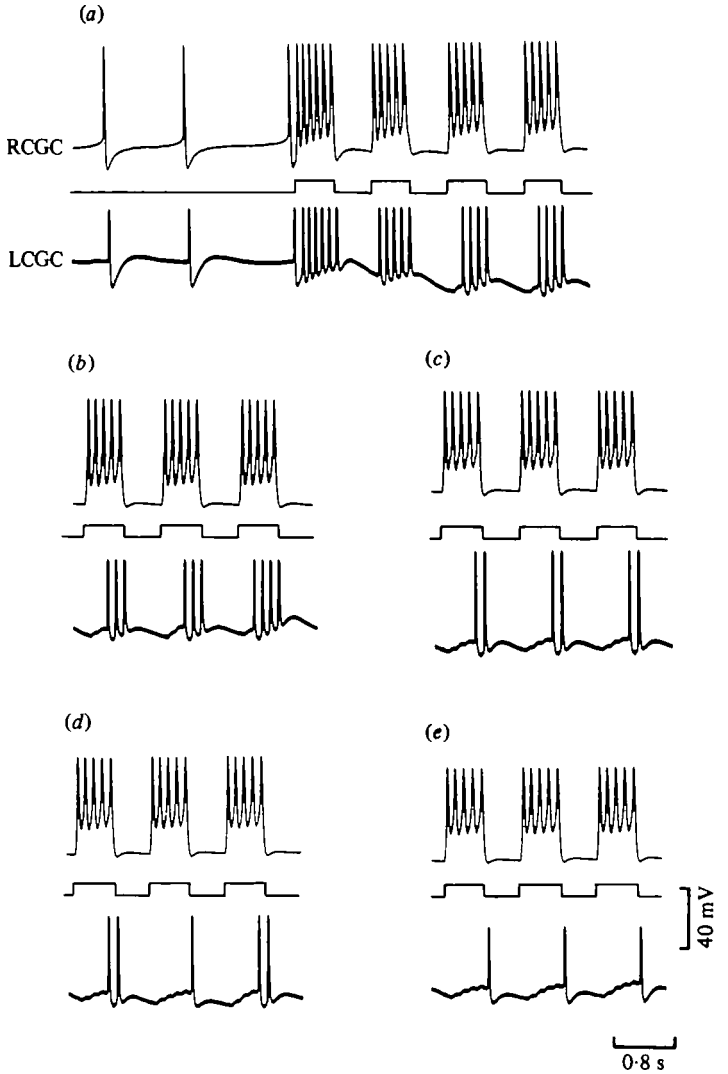

Fig. 5. Absence of fatiguing of the coupling between CGCs. 500 ms pulses of depolarizing current applied to the right CGC at 1 Hz (pulses are indicated), produce high frequency burst of spikes. Recordings are shown at (*a*) 0 s; (*b*) 8 s; (*c*) 40 s; (*d*) 70 s; (*e*) 1000 s, after onset of stimulation. A 1 : 1 postsynaptic response is always present.

apparently arising from a common source. This would aid in producing similar spike activity in the CGCs, but an electrotonic junction connecting the two cells (described in the next section) is probably more important in producing the 1 : 1 firing shown in all preparations containing brain and buccal ganglia (e.g. Fig. 2*a*).

Electrotonic coupling of CGCs

The electrotonic junction connecting the two CGCs was shown to be symmetrical and non-rectifying, with a d.c. coupling ratio, as measured at the cell bodies, of less than 0·01. A square current pulse (approx. 5 nA) passed into either CGC produced

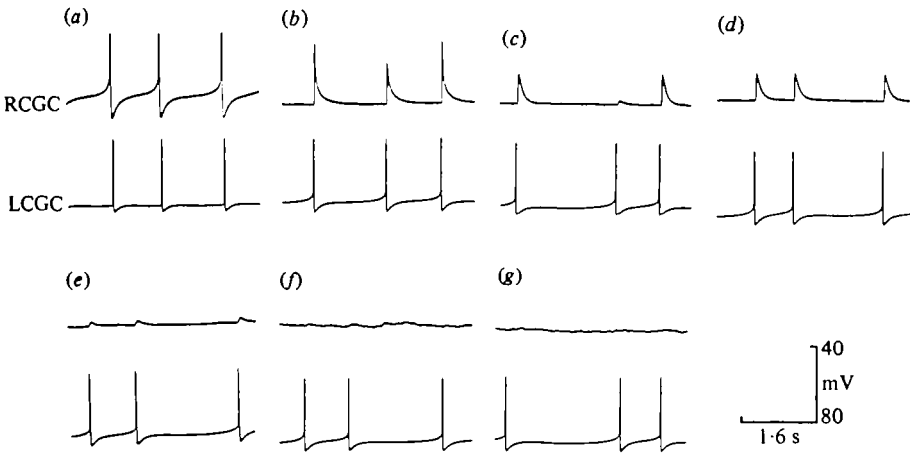

Fig. 6. Effect of progressive hyperpolarization of a right CGC on its response to a presynaptic spike in a left CGC. Level of hyperpolarization of the right CGC: (*a*) −50 mV (resting potential). Shows 1:1 spike transmission. (*b*) −90 mV. 1:1 spikes in right CGC are axon spikes, revealed after failure of soma spikes. (*c*) −130 mV. Axon spikes smaller in amplitude. (*d*) −150 mV. One spike disappears to reveal a smaller response. (*e*) −170 mV, and (*f*) −210 mV. Responses become progressively smaller in amplitude owing to failure of spike propagation further from the cell body. (*g*) −250 mV. Responses disappear, indicating electrotonic transmission.

a response in the other cell of the same form and polarity as the stimulus, but greatly attenuated (Fig. 4*a*). To ensure that the response in the left CGC of Fig. 4(*a*) was not a signal artifact, the electrode was withdrawn from the cell. No response was now seen following injection of current into the right CGC. The apparently weak coupling ratio is not, however, a good indication of the efficacy of the junction. A better indication is given by the 1:1 spike activity of the two cells, even at high firing frequencies (Fig. 4*c*). The apparent anomaly between this 1:1 firing and the low coupling ratio was explained by the location of the junction at a considerable distance from the cell bodies, in or between the buccal ganglia (described later). A square current pulse applied to one cell body would be greatly attenuated before being recorded in the other cell body, owing to the distance travelled. An indication of this distance involved in spike transmission from one cell body to the other is given by the long delay of 20 ms between the onset of spikes in the pre- and postsynaptic cells (Fig. 4*b*).

Loss of soma-spikes in the postsynaptic cell occurs if the presynaptic cell is made to burst at high frequencies by injecting it with pulses of depolarizing current over a period of several minutes (Fig. 5). However, a postsynaptic response of some kind is always seen following a presynaptic spike (Fig. 5*b*, *c*, *d*). The absence of response fatigue is further evidence for electrical coupling between the CGCs. The low amplitude postsynaptic cell response seen in the absence of a full soma spike (Fig. 5) is probably a distant axon spike, initiated in the buccal ganglia, that has failed to invade the more proximal parts of the axon.

Fig. 6 shows the effect on spike transmission of progressive hyperpolarization of one CGC (the postsynaptic cell) by injection of d.c. The postsynaptic response

decreases in amplitude and disappears when the postsynaptic membrane potential is set at −250 mV (Fig. 6*g*) again indicating an electrotonic postsynaptic response rather than a chemical one (which would increase in amplitude with progressive hyperpolarization of the cell membrane). The changes in postsynaptic response amplitude with progressive hyperpolarization are sudden rather than gradual. Fig. 6(*b*) shows postsynaptic axon spikes, longer in duration and smaller in amplitude than the soma spikes of Fig. 6(*a*). The axon spike amplitude drops to a lower level at a membrane potential of −130 mV (Fig. 6*c*), and at −170 mV these spikes fail and reveal much smaller underlying responses (Fig. 6*e*). These results may be explained by postulating points of low excitability along the length of the CGC axon. Then increased hyperpolarization of the cell body would be expected to lead to successive blocking of spike propagation past more distal low excitability zones.

None of the postsynaptic CGC responses described above are affected by high Mg^{2+} (50 mM), low Ca^{2+} saline, or by high Mn^{2+} (10 mM) saline, both of which have been found to block chemical synapses in several species (Meiri & Rahmimoff, 1972; Nicholls & Baylor, 1968) including *Lymnaea* (Janse, 1976). Immersion of preparations in either of these salines for several hours led to increased CGC membrane potential and cessation of spike activity, but had no effect on 1:1 spike transmission between the cells.

The consequence of the strong coupling between the CGCs is to make the two cells act as a single functional unit. As described in previous sections, this neural unit receives symmetrical synaptic input from right and left sides of the brain, and anatomical studies suggest that the output from the two CGCs is symmetrical also (see later).

Location of electrotonic junction

To locate the site of the electrotonic junction between the CGCs, different connectives between left and right halves of the brain were cut and the effects on the coupling of the neurones were observed. Cutting the main cerebral commissure did not abolish coupling (Fig. 7*a*), nor did bisection of the whole brain down its midline, leaving the buccal commissure intact (Fig. 7*b*). This located the electrotonic junction in the buccal ganglia, and this conclusion was confirmed by cutting the buccal commissure and totally abolishing coupling in nearly all preparations (Fig. 7*c*). Occasionally a small degree of coupling persisted after section of the buccal commissure (Fig. 7*d*). This may be due to a second connexion between the CGCs in the subcerebral commissure (intact only in a few preparations) or it could be explained by postulating a mono- or polysynaptic pathway connecting the two cells within the brain.

Axonal projections of the CGCs: electrophysiology

Extracellularly recorded spikes in some nerve roots of the central nervous system occurred 1:1 with intracellularly recorded spikes in the CGCs (Fig. 8). This suggests that axons of the CGCs occurred in these nerves, although the nerve spikes could also have come from cells which fire 1:1 with the CGCs. The evidence given in the next section shows that it is unnecessary to consider this second possibility.

Fig. 8 shows that the left CGC has an axon projecting to the left laterobucca

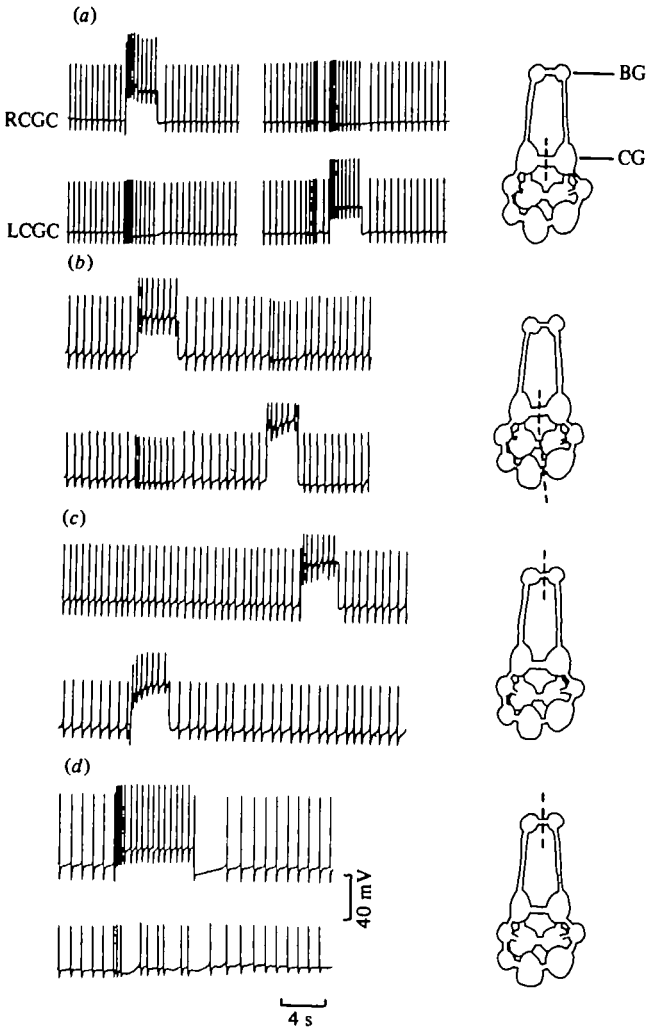

Fig. 7. Location of the site of the electrotonic junction between CGCs, in or between the buccal ganglia. The connectives sectioned are indicated in the drawing of the brain accompanying each recording. (*a*) 1:1 spike transmission remains after cutting the main cerebral commissure. (*b*) 1:1 spike transmission remains after bisecting the whole brain, leaving the buccal commissure intact. (*c*) 1:1 spiking abolished after cutting the buccal commissure. (*d*) A small degree of coupling may remain after cutting the buccal commissure. BG, buccal ganglia; CG, cerebral ganglia.

nerve (Fig. 8*a*, *b*) and the left ventrobuccal nerve (Fig. 8*c*). Similar experiments showed that this cell also had an axon in the single postbuccal nerve, and in the left dorsobuccal nerve. Other experiments showed that the right CGC had symmetrical projections, i.e. to the right (ipsilateral) laterobuccal, ventrobuccal and dorsobuccal nerves, and to the postbuccal nerve. No contralateral projections to nerves in the buccal ganglia have been found by this method, nor have projections been found leaving the cerebral ganglia on either side except in the ipsilateral cerebrobuccal connective. When two cells normally fire 1:1 it is difficult to know which

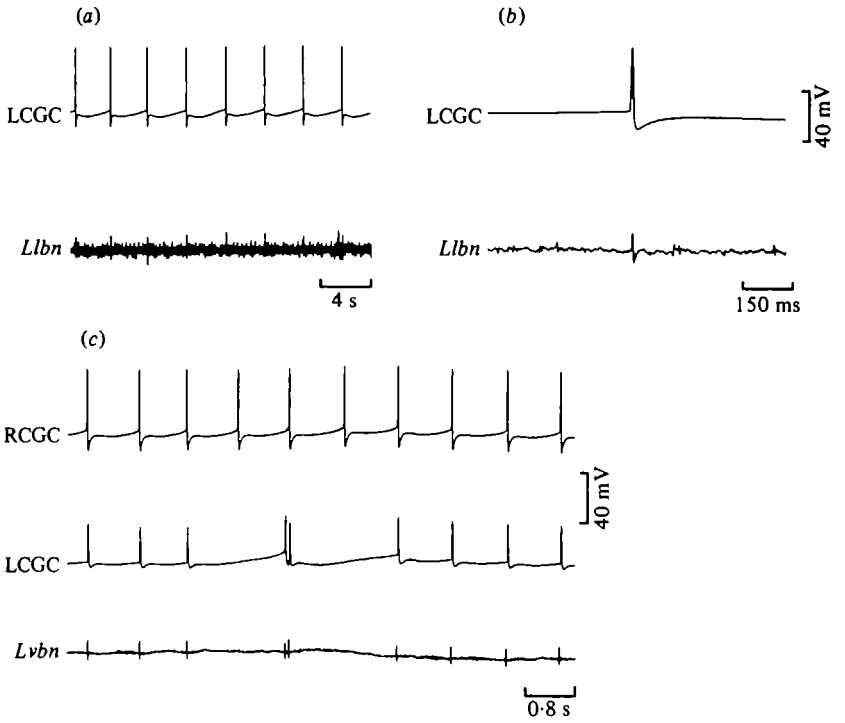

Fig. 8. Extracellular recordings from buccal nerves. (*a*) Left CGC recorded with left latero-buccal nerve (*Llbn*) showing 1:1 spikes. (*b*) The same, but on an extended time base to show the latency between spikes. (*c*) Left ventrobuccal nerve (*Lvbn*) recorded with left and right CGCs firing slightly asynchronously. Lvbn spikes follow those in the left CGC only, indicating that only the left CGC has a projection in this nerve.

cell is responsible for an extracellularly recorded action potential. This problem was partly resolved by the type of experiment shown in Fig. 8(*c*). In this preparation the left and right CGCs did not always fire 1:1, and the left ventrobuccal nerve spikes followed those of the left CGC rather than the right. This suggests that an axon of the left CGC, and not of the right CGC occurred in this nerve. That the two CGCs have only ipsilateral projections to nerves of the buccal ganglia was confirmed by the anatomical methods that are described in the next section.

Morphology of the CGCs

Three staining techniques were used to examine the morphology of the CGCs. Procion Yellow injected into a cell gave information about the dendritic processes adjacent to the cell body, but did not provide information about distant axonal projections, since it apparently fills axons by passive diffusion. Horseradish peroxidase (HRP) is actively transported along axons and revealed more distant axonal projections. Cobalt back-fills confirmed the presence of axonal projections in buccal nerves, but was not useful for examination of fine dendritic processes in unintensified whole mount preparations. Morphological studies of left and right CGCs showed them to have symmetrical anatomy. The overall axonal projections of the CGCs are summarized in Fig. 1.

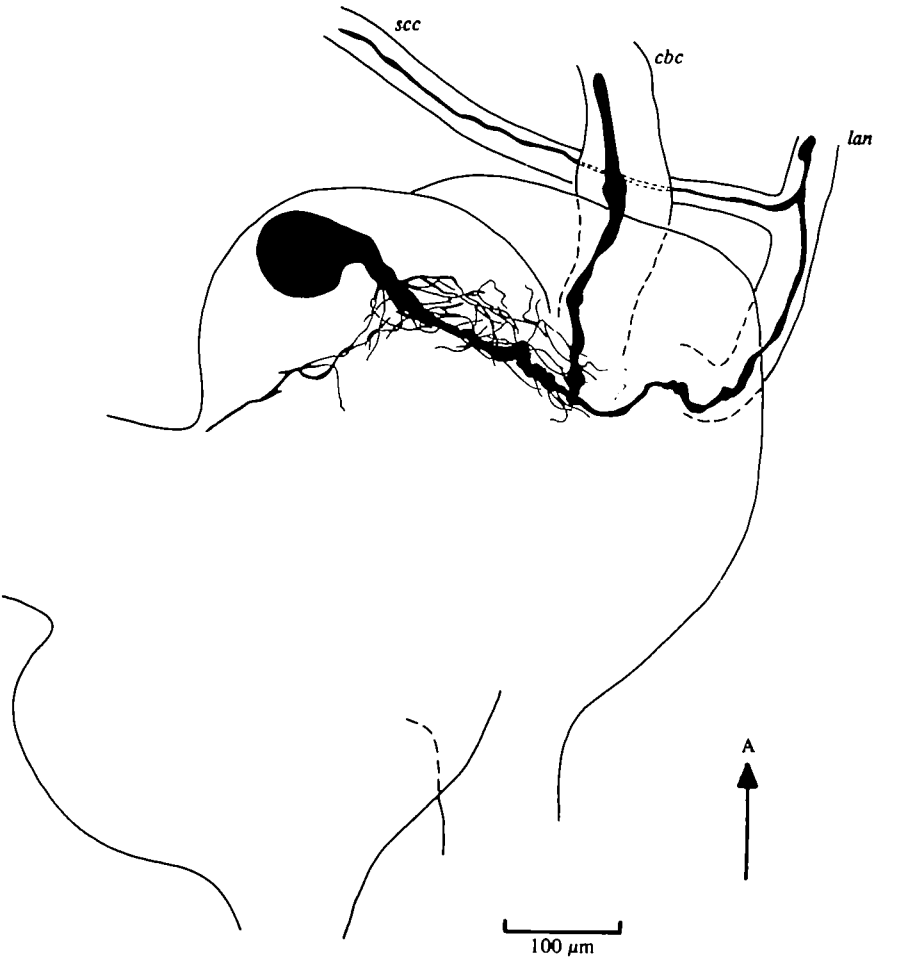

Fig. 9. Drawing of Procion Yellow filled right CGC, showing extensive fine branching in the neuropile of the cerebral ganglion. Axon branches project to the right cerebrobuccal connective (*cbc*), labial artery nerve (*lan*) and subcerebral commissure (*scc*).

Procion Yellow injection

Fig. 9 shows a reconstruction of a right CGC injected with Procion Yellow dye. There is extensive fine branching in the cerebral ganglion's neuropile (Fig. 9, Fig. 12*c*) which could be the site of integration of sensory input from the cerebral nerves. One branch (found in several CGCs injected) approaches the cerebral commissure but does not enter it. The axon passes ventrally in the cerebral ganglion and branches once in the ventral lobe, before leaving via the labial artery nerve and the cerebrobuccal connective. The former branch divides again almost immediately, one half entering the subcerebral commissure which connects with the labial artery nerve on the opposite side. This left to right connexion may provide a means for explaining the small amount of CGC coupling sometimes seen after sectioning the buccal commissure (Fig. 7*d*). The labial artery nerves are a pair of very fine nerves leaving the ventral lobes

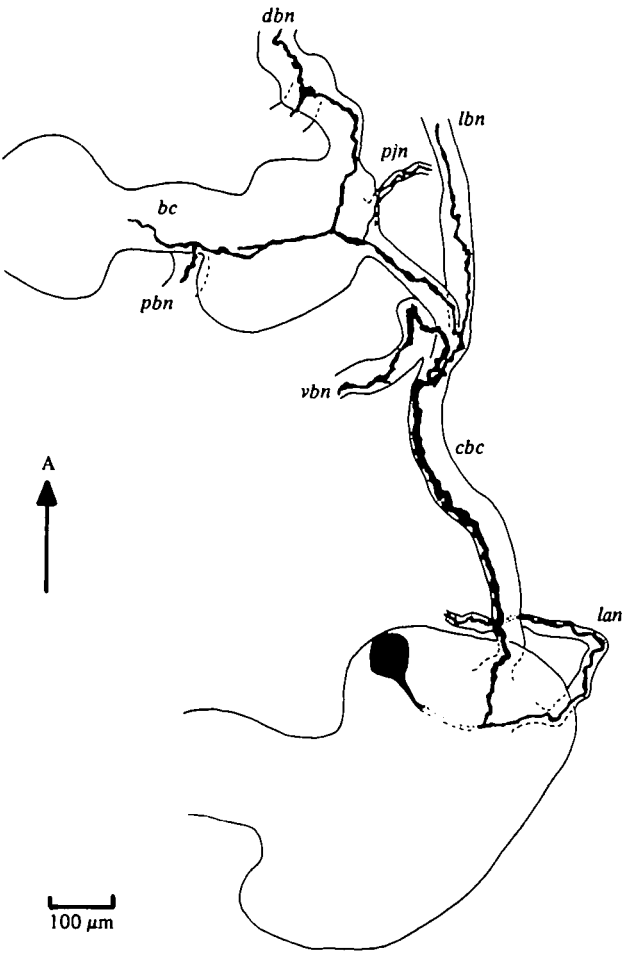

Fig. 10. Drawing of horseradish peroxidase filled right CGC showing axon projections in the following nerves: right cerebrobuccal connective (*cbc*), labial artery nerve (*lan*), ventrobuccal nerve (*vbn*), laterobuccal nerve (*lbn*), posterior jugalis nerve (*pjn*), dorsobuccal nerve (*dbn*), postbuccal nerve (*pbn*) and buccal commissure (*bc*). No contralateral projections were seen.

of the cerebral ganglia (Lacaze-Duthiers, 1872). The subcerebral commissure is an anastamosis of these nerves. The labial artery nerves run alongside the labial arteries to the lip region, as described by Joosse (1964) and therefore constitute one of the lip nerves. The CGC axon forms a large portion of the thickness of this nerve, certainly at its cerebral origin.

Procion Yellow filled axons were followed along the cerebrobuccal connective almost as far as the buccal ganglion, and in one preparation a branch entered the ventrobuccal nerve.

Horseradish peroxidase injection

The HRP injection technique had certain limitations. Diaminobenzidine, used to visualize the enzyme, did not efficiently penetrate the deep neuropile of the large cerebral ganglia, so the fine processes in the cerebral ganglion appeared faint. How-

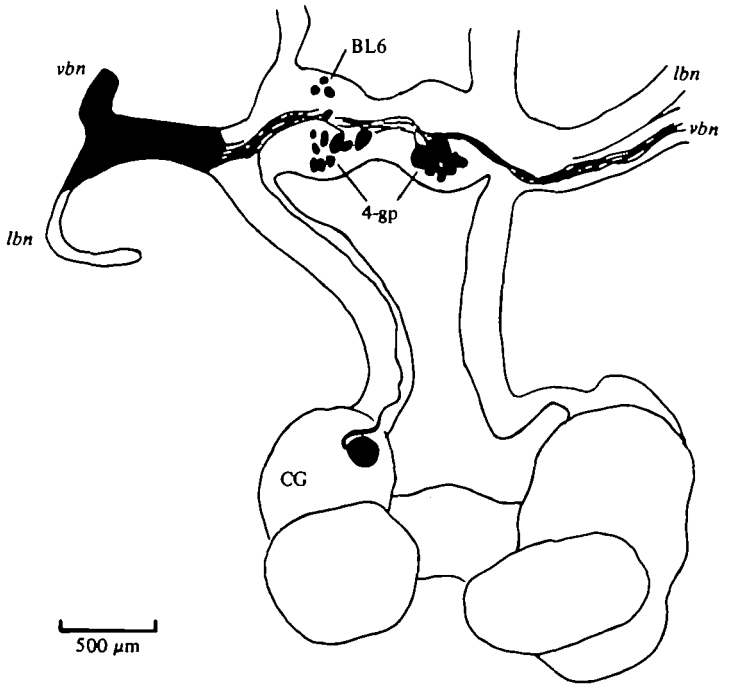

Fig. 11. Drawing of cells filled by retrograde injection of cobalt into the left ventrobuccal nerve root. Cells filled include the right and left 4-group cells (4-gp), possible left 6 cells (BL6) and the right CGC. *cbc*, Cerebrobuccal connective; CG, cerebral ganglion; *lbn*, laterobuccal nerve; *vbn*, ventrobuccal nerve.

ever, the axon could be followed from the cell body to where it emerged from the ventral lobe. In the buccal ganglia a considerable amount of background staining was present, so that fine axon branches were not easily distinguished (Fig. 12*d*). In the reconstruction (Fig. 10) only intensely stained processes were drawn. Fig. 10 shows a reconstruction of a HRP-injected right CGC. All ipsilateral buccal nerves contain axon projections which could be followed to the cut ends of the nerves. Only one branch was found in each nerve. No contralateral axon projections were found.

Cobalt back fills

Back fills of dorsobuccal, ventrobuccal (Fig. 11) and laterobuccal nerves, and of the cerebrobuccal connective (Fig. 12*a*, *b*) led to filling of the ipsilateral CGC with cobaltous chloride, but not of the contralateral CGC. These results confirm the projections described in the previous sections.

Cobaltous chloride, Procion Yellow and HRP have all been shown to cross electrotonic junctions under certain conditions. However, since no contralateral projections of the CGCs were found using these techniques it was concluded that this did not occur in the preparations described above.

Conduction time of CGC axons

Fig. 13 summarizes an experiment which can account for the delay of approximately 20 ms between onset of spikes in the two CGCs, which were previously shown to be connected by a presumed zero-delay electrotonic junction. The right CGC's soma-spike was recorded intracellularly (using electrode *S*), and its axon spike recorded at various points along the route connecting the two CGCs. After sectioning the left cerebrobuccal connective at point A, the soma spike-axon spike delays measured at point *A* (R_1); the base of the left ventrobuccal nerve (R_2); and the base of the right ventrobuccal nerve (R_3) were measured as 16, 12 and 8 ms respectively. The right cerebrobuccal connective was then sectioned at point *B* and a delay of 0 ms was recorded between spikes in the soma and in the right cerebrobuccal connective at this point (R_4). This zero-delay suggests that the spike initiation zone of the right CGC was approximately midway between the cell body and R_4.

It was concluded from these measurements of soma spike-axon spike latencies that the 20 ms delay between right and left CGC spikes can be accounted for by conduction time in the connectives, without having to postulate any synaptic delay due to a possible chemical synapse. The axon spikes recorded from the left cerebrobuccal connective at R_1 and in the left ventrobuccal nerve at R_2 could be due to axon projections of the right CGC entering these nerves from the buccal ganglia. However, since no other techniques revealed such contralateral CGC axon projections, it seems more likely that they are antidromic spikes initiated in the axon of the left CGC (now cut off from its cell body) by electrotonic transmission from the right cell. Such isolated axons have previously been shown to be capable of spike activity (Tauc, 1962).

DISCUSSION

The CGCs: a single neural unit

The electrophysiological and anatomical evidence presented here shows that the two CGCs act as a single, bilaterally-symmetrical, functional unit, potentially capable of producing identical effects on follower cells on both sides of the brain. The occurrence of 1:1 spike activity in the two cells is mainly due to their strong electrotonic coupling, but is reinforced by their apparently common synaptic input.

The very strong electrotonic junction suggests that there is a functional requirement for 1:1 firing of the CGCs. Electrotonic coupling occurs between the giant cerebral neurones of *Planorbis* and *Ariolimax* (Berry & Pentreath, 1976; Senseman & Gelperin, 1973), but it is not always present in the former and does not always lead to 1:1 spike activity in the latter. The cerebrobuccal interneurones of *Helisoma* show

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Fig. 12. (*a*) Photograph of cells filled by retrograde injection of cobalt into the left cerebrobuccal connective. (*b*) Drawing of the same, showing small cells in the left cerebral ganglion (CG) and the left CGC on the ventral surface of the anterior lobe (*al*). (*c*) Photograph of section through the proximal axon of the Procion Yellow filled CGC illustrated in Fig. 10, showing fine branching. (*d*) Photograph of section through axon branches of the HRP filled CGC illustrated in Fig. 11, showing stained material (arrowed) in the right cerebrobuccal connective (*cbc*) and dorsobuccal nerve (*dbn*). BG, Buccal ganglion.

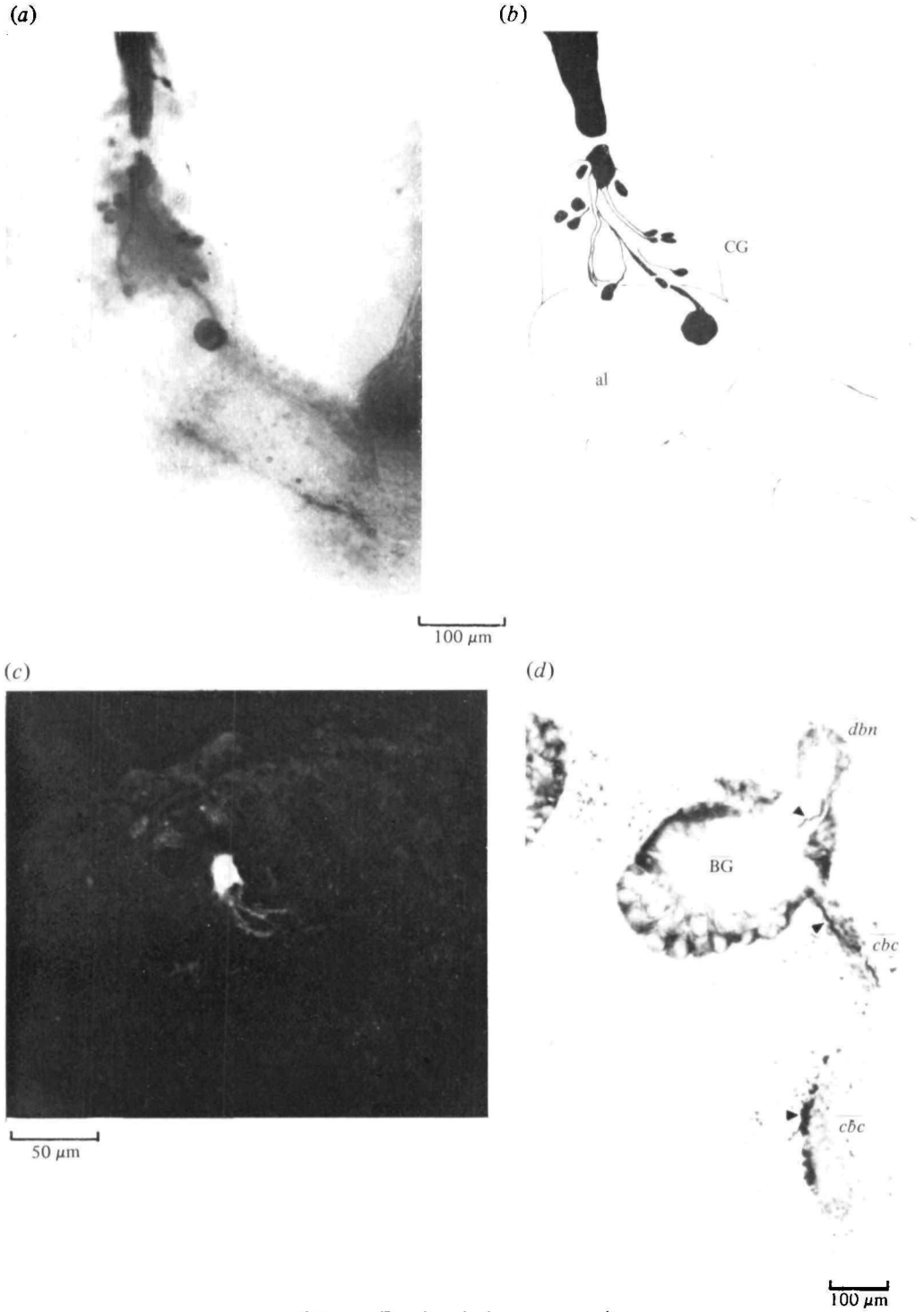

FIG. 12. For description see opposite.

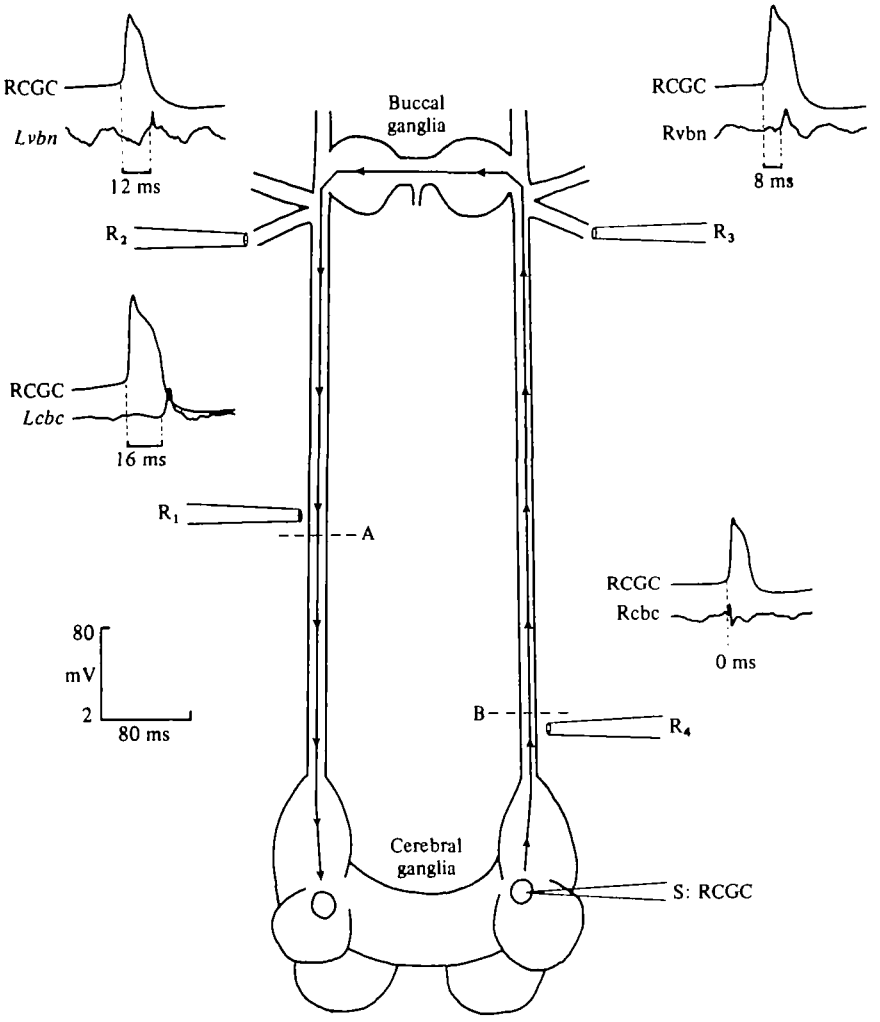

Fig. 13. Axonal conduction times of right CGC, to account for 20 ms delay between right and left CGC soma spikes. S, intracellular recording electrode in right CGC body. R_1 to R_4, extracellular recording electrodes at different points on the assumed conduction pathway between right and left CGCs (indicated by arrows). Inset are recordings showing delays between onset of right CGC soma spikes and axon spikes recorded at R_1 to R_4, all shown on the same scale, as indicated. See text for more details. *Lcbc* and *Rcbc*, left and right cerebrobuccal connectives; *Lvbn* and *Rvbn*, left and right ventrobuccal nerves.

1:1 spike activity in about one-third of preparations (Granzow & Kater, 1977). This was supposed to be due to a labile chemical synapse connecting the cells, since no transmission of d.c. current between somata was observed. However, as the presynaptic to postsynaptic spike latency was 25 ms (long even for a chemical synapse), the 1:1 firing in *Helisoma* may be found to be due to a distally situated electrotonic junction. This could explain both the long latency (compare 20 ms in *Lymnaea*) and the apparent absence of d.c. transmission. This illustrates the problems which may arise in studying a pair of neurones whose follower cells and synaptic connexions (in this case an electrotonic junction) are up to 2·5 mm distal to their cell bodies. Recording

from the soma may not always give a complete picture of the activity of distant processes.

The location of the CGCs' electrotonic junction in the buccal ganglia may be functionally important. The following paper (McCrohan & Benjamin, 1980) gives evidence that the CGCs have modulating effects on the output of buccal motoneurones concerned with feeding, and an electrotonic junction situated in the buccal ganglia would ensure exact synchrony of CGC spiking at their site of action. A junction located in the cerebral commissure, where it is thought to be in *Planorbis* (Berry & Pentreath, 1976) could lead to a delay between spikes in the two CGCs of several milliseconds (the time taken for a spike to cross the cerebral commissure from one cell body to the other) at the level of the buccal ganglia. Benjamin & Rose (1979) have demonstrated that synchronized activity in equivalent left and right buccal motoneurones is important, and synchrony of CGC activity on both sides would help to maintain this.

Spontaneously occurring synaptic inputs to the CGC neural unit on both sides of the brain appear to originate from a common source. Such inputs have also been found on the metacerebral giant cells of *Helix* (Kandel & Tauc, 1966), which are supposed homologues of the CGCs. Giant cerebral cells in other species appear to receive independent inputs, in addition to the common ones (Berry & Pentreath, 1976; Weiss & Kupfermann, 1974).

Anatomical studies support the notion of the CGCs acting as a single unit. Contralateral axonal projections of giant cerebral cells have been shown in *Helix*, *Planorbis* and *Pleurobranchaea* (Pentreath & Cottrell, 1974; Berry & Pentreath, 1976; Gillette & Davis, 1977), but appear to be absent in *Lymnaea*. Precise symmetry of spike activity would make such projections unnecessary. The anatomy of the CGCs of *Lymnaea* appears to be less complex overall than that of giant cerebral neurones in other species. Giant cerebral cells in all species examined have axonal projections in the ipsilateral cerebrobuccal connective and buccal nerves, and in one or more ipsilateral lip nerves. However, the CGCs in *Lymnaea* differ in having no contralateral projections. Contralateral buccal nerve projections are found in most other species (Weiss & Kupfermann, 1976; Berry & Pentreath, 1976; Cottrell, 1977; Gillette & Davis, 1977) and projections from the contralateral cerebral ganglion are found in some (Cottrell, 1977; Senseman & Gelperin, 1973). Anatomical studies of the giant serotonin neurone of *Helix* have demonstrated the presence of two or more axon processes running parallel along some nerves (Pentreath & Cottrell, 1974). This phenomenon was not observed in *Lymnaea*. The relatively simple anatomy worked out for the CGCs in *Lymnaea* is unlikely to be due to bad filling of the cells with dye since Procion Yellow and especially HRP were both seen to travel long distances (several millimetres).

Possible functions of the CGCs

The likely destinations of the CGCs' axonal projections suggest possible effector sites which could be studied; namely the lips, the buccal musculature and the buccal ganglia. Modulatory effects of giant cerebral neurones on muscle contraction have already been shown in the lip muscle of *Planorbis* (Pentreath, 1973) and the buccal

musculature of *Aplysia* (Weiss, Cohen & Kupfermann, 1975). Such direct effects on muscles may also be present in *Lymnaea*. The following paper (McCrohan & Benjamin, 1980) will describe the effects of the CGCs on buccal motoneurones. Similar connexions have been found with buccal neurones in *Helix*, *Planorbis*, *Aplysia* and *Pleurobranchaea* (Cottrell & Macon, 1974; Berry & Pentreath, 1976; Weiss & Kupfermann, 1976; Gillette & Davis, 1977).

On the basis of the results presented here, it is concluded that the CGCs of *Lymnaea* are homologous to the giant cerebral neurones described in other molluscs, although there are anatomical differences between the CGCs of *Lymnaea* and those of other species. A comparison of the role of these cells in different species should give some insight into the evolution of higher control elements in behavioural networks.

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