SYNAPTIC RELATIONSHIPS OF THE CEREBRAL GIANT CELLS WITH MOTONEURONES IN THE FEEDING SYSTEM OF *LYMNAEA STAGNALIS*

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SUMMARY

1. The cerebral giant cells (CGCs) of *Lymnaea* have a tonic, modulatory effect on the intensity of output from feeding motoneurones in the buccal ganglia.

2. Short latency, excitatory and probably monosynaptic connexions occur between the CGCs and three identified feeding motoneurones. Unitary excitatory postsynaptic potentials in these motoneurones, following CGC spikes, are of different sizes and durations, and hence have different summation properties.

3. The CGCs make long latency, excitatory polysynaptic connexions with four other feeding motoneurone types.

4. Bursts of spikes in the CGCs, resulting from phasic synaptic input, synchronous with the feeding cycle, amplify their modulatory effect on burst intensity in feeding motoneurones.

5. The different latencies of the CGCs' effects on different motoneurones are appropriate for reinforcing their cyclic burst activity.

INTRODUCTION

The preceding paper (McCrohan & Benjamin, 1980) described the electrophysiology and axonal projections of the cerebral giant cells (CGCs) of *Lymnaea*, a symmetric pair of serotonin-containing neurones (Sakharov & Zs-Nagy, 1968). It showed how the two cells can act as a single unit by virtue of a strong electrotonic junction coupling them, and because they receive apparently common inputs. The CGCs were also shown to receive synaptic inputs synchronous with those received by buccal feeding motoneurones.

This paper describes direct and indirect synaptic connexions made by the CGCs of *Lymnaea* with identified feeding motoneurones of the buccal ganglia. These connexions show similarities with those described in other mollusc species (Berry & Pentreath, 1976a; Cottrell & Macon, 1974; Gillette & Davis, 1977; Paupardin-Tritsch & Gerschenfeld, 1973).

The role of the CGCs is postulated to be a modulatory one, influencing the intensity of motor output from the feeding system. Such a role is discussed in relation to the functioning of the giant cerebral cells in *Planorbis*, *Helisoma*, *Aplysia* and *Pleuro-*

branchaea (Berry & Pentreath, 1976 *a*; Granzow & Kater, 1977; Weiss, Cohen & Kupfermann, 1975; Gillette & Davis, 1977).

MATERIALS AND METHODS

Intracellular recordings were made from neurones in an isolated brain preparation of *Lymnaea*, as described in the previous paper (McCrohan & Benjamin, 1980). The nomenclature and identification of buccal cell types were described by Benjamin & Rose (1979), and the same nomenclature is used in the present paper.

Intracellular injection of tetraethylammonium (TEA) bromide

Microelectrodes were filled with IM TEA bromide (Sigma) solution. A CGC was impaled, and 500 ms, 4–8 nA depolarizing pulses were passed at 1 Hz for 15–20 min. The preparation was then left for up to 5 h before recording, to allow TEA time to diffuse along the axon to the buccal ganglion, where the supposed follower cells of the CGCs are located.

High calcium saline

Most experiments were conducted using a Hepes-buffered saline, as described in the preceding paper (McCrohan & Benjamin, 1980). High Ca^{2+} salines were prepared by increasing the $CaCl_2$ concentration to 40 or 60 mM without altering the concentrations of other ions and substituting tris (BDH) hydrochloride for Hepes as the buffer.

RESULTS

Monitoring the feeding output

The buccal neurones involved in the central generation of feeding in *Lymnaea*, and the inputs they receive, have been described elsewhere (Benjamin & Rose, 1979). The locations in the buccal ganglia of those motoneurones described in this paper, and of the cerebral giant cells (CGCs), are illustrated in Fig. 1. The buccal neurones are numbered 1–7, of which types 1, 2 and 3 occur as a single cell in each ganglion. 4-group consists of one large 4-cell plus approximately six smaller 4-cluster cells; 5-, 6- and 7-cells occur in groups of two or three. Two of these cell types, 4-group cells and 6-cells, have been shown by Rose & Benjamin (1979) to be motoneurones, supplying retractor and protractor muscles respectively, of the buccal mass. 1-cells are probably salivary gland motoneurones as in *Helisoma* (Kater, 1974); 2-, 3-, 5- and 7-cells have all been shown to project to the gut (Benjamin, Rose, Slade & Lacy, 1979). For convenience, all seven buccal cell types are described as motoneurones in the present paper.

The patterns of bursting shown by feeding motoneurones are due to two consecutive phases of synaptic input which occur during radula protraction and the first phase of retraction (Retraction 1) respectively (see Fig. 12*a* for summary). No common inputs to motoneurones occur during Retraction 2, though some motoneurones (e.g. 5 cells) burst on the rebound following an inhibitory input during Retraction 1. The inputs may be excitatory or inhibitory on different types of feeding motoneurone, and each motoneurone receives either one or both of the inputs. The two phases of
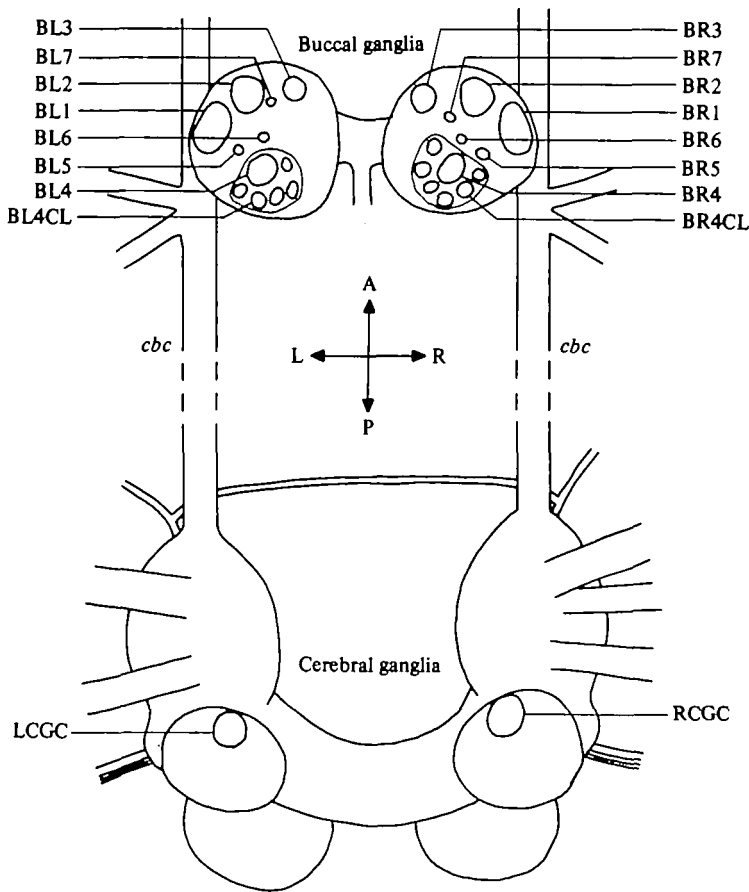
Fig. 1. Dorsal view of cerebral and buccal ganglia of *Lymnaea*, showing the locations of the cerebral giant cells (CGCs) and seven identified buccal cell types. A, anterior; *cbc*, cerebrobuccal connective; L, left; P, posterior; R, right.

input are believed to originate from two coupled networks of interneurones in the buccal ganglia (R. M. Rose, unpublished data). The exact timing of the two input phases of the feeding cycle could be monitored by recording from one or two of the feeding motoneurone types.

Effects of the CGCs on the generation of a feeding output

In Fig. 2 the effect of changes in CGC spike activity on the motor output from the buccal ganglia was examined, using a 4-group cell (retractor motoneurone: Rose & Benjamin, 1979) as a monitor of the feeding cycle. The 4-group cell receives two consecutive phases of inhibitory synaptic input, one during Protraction, and one during Retraction 1, which are followed by a burst of spike activity due to post-inhibitory rebound (Benjamin & Rose, 1979). During the burst electrotonic e.p.s.p.s can be seen, which originate from other 4-group cells which are all electrotonically coupled.

The CGCs have a modulatory effect on the intensity of feeding burst in the buccal motoneurone. Fig. 2(*a*) shows the effect of changes in tonic CGC spike frequency on
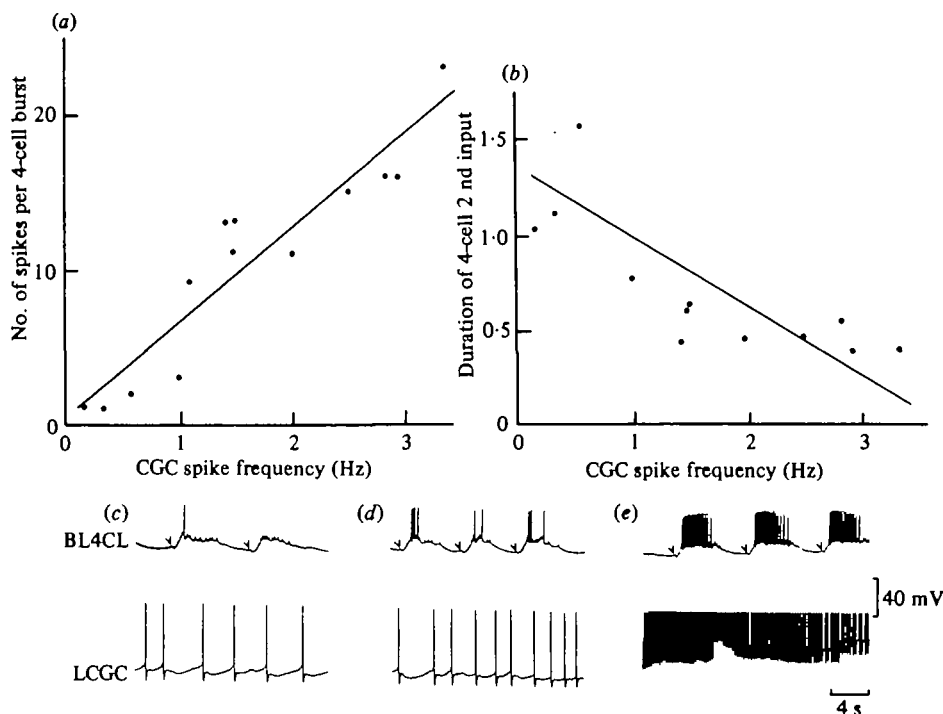
Fig. 2. Effect of tonic CGC spike frequency on strength and timing of 4-group cell bursts. (a) Increased mean CGC spike frequency (maintained by current injection) measured over periods of 20 s, produces increased mean number of spikes per 4-group cell burst, measured over the same periods. Regression line drawn. Correlation coefficient, $r = 0.94$ ($P < 0.001$). (b) Increased mean CGC spike frequency produces a decrease in mean duration of second inhibitory input on a 4-group cell, measured from onset of input to occurrence of first spike. Regression line drawn. $r = 0.89$ ($P < 0.001$). (c), (d) and (e) illustrate the relationships plotted above, in a different preparation. Left CGC spike frequencies were varied by current injection. Onset of second inhibitory input to left buccal 4-cluster cell (BL4CL) marked with arrows.

the number of spikes per burst in a 4-group cell. Tonic CGC spike output was maintained at different levels by current injection, and the mean frequency was measured over periods of 20 s. The mean numbers of spikes per 4-cell burst were taken over the same periods. These two parameters were found to be strongly correlated ($P < 0.001$). Fig. 2(c, d, e) illustrates this relationship using recordings from a different preparation. The CGCs are playing a modulatory role, their tonic spike output determining the intensity of the feeding motor output. In the case of the 4-cell, this would determine the strength of contraction of buccal retractor muscles. Similar effects of CGC spike frequency on burst intensity were observed qualitatively in many preparations on motoneurone types 1, 2 and 3. However, the 4-group cells illustrate the effect most clearly.

4-group cells burst during the second phase of the feeding cycle (Retraction 1) by post-inhibitory rebound (Benjamin & Rose, 1979). The timing of onset of the 4-cell burst varies according to the strength of the rebound from the second inhibitory input. This is reflected by the apparent length of the second inhibitory input, from its onset to the first spike of the burst. Fig. 2(b) shows that the apparent duration of

second phase inhibition on a 4-group cell was dependent on the mean firing frequency of the CGC. Each point on the graph represents the mean of measurements taken over periods of 20 s at different, artificially maintained levels of CGC spike frequency. There is a strong negative correlation between the parameters ($P < $ 0·001). Increased tonic CGC output leads to a decrease in the pre-burst duration of 4-cell second phase inhibition. Fig. 2(c, d, e) illustrates this further. The onset of second phase inhibitory inputs to the 4-group cell are marked with arrows. A possible explanation for the effects of CGC firing frequency on the duration of 4-group second phase inhibition is that the CGCs are providing a background depolarization to the moto-neurone (see next section), so that recovery from inhibition is faster. However, possible direct effects of the CGCs on the interneurones producing inhibition in 4-group cells cannot be entirely discounted.

Artificially evoked changes in CGC spike frequency were not found to alter the frequency of feeding cycles in a preparation showing cyclical activity. For example, in the preparation of Fig. 2(c, d, e), CGC spike frequency was artificially maintained at levels between 0·25 and 4·50 Hz (higher than any observed spontaneous frequency for sustained CGC spike activity). Over this range the frequency of bursts in the 4-group cell (BL4CL), again measured over periods of 20 s, varied only over the range 0·15 to 0·20 Hz, and there was no correlation between CGC spike frequency and burst frequency in the 4-group cell ($P \gg$ 0·1). The observed range of burst frequencies seen in buccal motoneurones can vary between 0·05 and 0·30 Hz (Rose & Benjamin, 1979). On no occasion was cyclical burst activity triggered in feeding motoneurones in a quiescent preparation. Phasic stimulation to mimic the firing pattern often seen in the CGCs during feeding (McCrohan & Benjamin, 1980; Fig. 2c) did not initiate feeding, nor did it reset the phase of feeding cycles when these were already present. Since the CGCs do not act in any way to trigger the onset or determine the period of feeding cycles, it was concluded that they are not command neurones.

The neural mechanisms underlying the CGCs' modulatory effect on the feeding motor output are described in the next section.

Synaptic connexions of the CGCs with identified buccal motoneurones

Seven cell types in the buccal ganglia, previously identified by Benjamin & Rose (1979), were found to respond to stimulation of the CGCs. These responses were all excitatory and could be divided into two types; short latency and probably mono-synaptic, and longer latency polysynaptic effects.

Short latency effects

Three motoneurone types were found in which CGC spikes produced 1:1 excitatory postsynaptic potentials (e.p.s.p.s); 1- and 6-cells, both of which burst during pro-traction, and 4-group cells, described in the previous section.

1-cells. Spikes in a CGC produce 1:1, monosynaptic e.p.s.p.s of approximately 500 ms duration in a 1-cell (Fig. 3). Stimulation of the CGC to cause increased spike frequency led to summating 1:1 e.p.s.p.s (Fig. 3a, b), which could give rise to action potentials (Fig. 3b). Summation of e.p.s.p.s occurred at CGC spike frequencies of
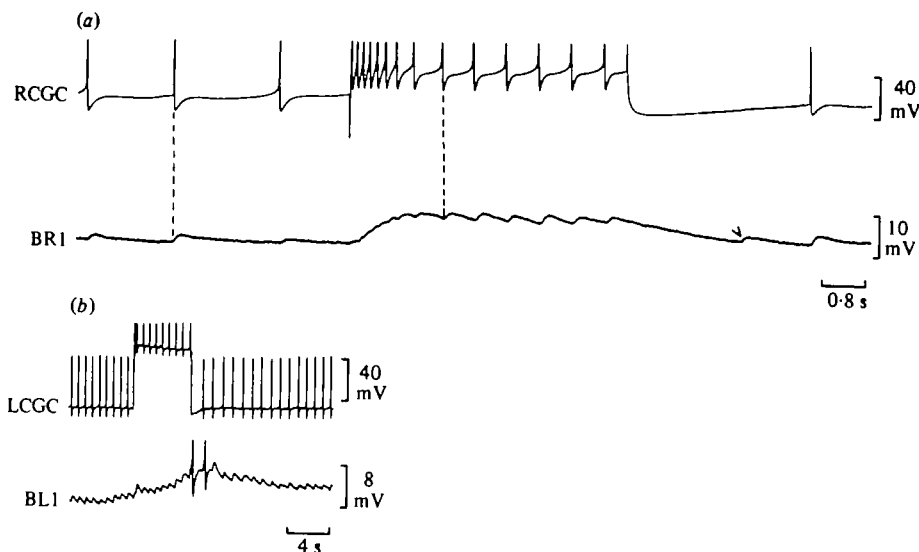
Fig. 3. Short latency, 1:1 e.p.s.p.s seen in 1-cells following CGC spikes. (a) Right buccal 1-cell (BR1) hyperpolarized to prevent spiking. Increased CGC spike frequency, following intracellular stimulation, produces a compound e.p.s.p in BR1. The unitary e.p.s.p (arrowed), occurring in the absence of a RCGC spike, followed a spike in the LCGC. The RCGC soma failed to spike here since it was recovering from after-hyperpolarization. This followed the injection of depolarizing current. (b) Unitary e.p.s.p.s, 1:1 with CGC spikes, in a left 1-cell (BL1) at resting potential, sum to produce firing.

2 Hz and above. The spike frequency seen in CGCs in non-feeding preparations is usually less than 2 Hz, but bursts of spikes up to 5 Hz or more are often seen during the protraction phase of the feeding cycle in a preparation showing rhythmic feeding activity (McCrohan & Benjamin, 1980). Following such CGC burst, summation of 1-cell e.p.s.p.s could occur. The 1-cell e.p.s.p.s follow CGC spikes with constant latency as far as can be measured. Since the CGC spike takes about 8 ms to propagate from cerebral to buccal ganglion, however (McCrohan & Benjamin, 1980), any variation in latency owing to a possible polysynaptic connexion might not be detectable.

The criteria for distinguishing between monosynaptic and polysynaptic transmission have been reviewed by Berry & Pentreath (1976b). Experiments were performed which supported the monosynaptic nature of the CGC-1 cell connexion.

TEA was injected into a CGC and the size of e.p.s.p.s in the postsynaptic cell (1-cell) was monitored. TEA acts on delayed K^+ conductance to increase the duration of the presynaptic action potential, and this in turn leads to an increase in the size of a monosynaptic p.s.p. as a result of increased transmitter release by the presynaptic cell. This technique has been shown to work in *Aplysia* (Kehoe, 1972). Fig. 4 shows the results of injecting a right CGC with TEA. Fig. 4(a) shows 1-cell e.p.s.p.s, 1:1 with CGC spikes before injection. Three hours after injection of TEA, the 1-cell e.p.s.p.s were more than double the amplitude of those recorded before injection (Fig. 4b), indicating a monosynaptic connexion. Because of the long distance involved between the CGC and its follower cell, two problems arose when using this technique on the CGC-1 cell connexion. Firstly, TEA had to be able to diffuse over large
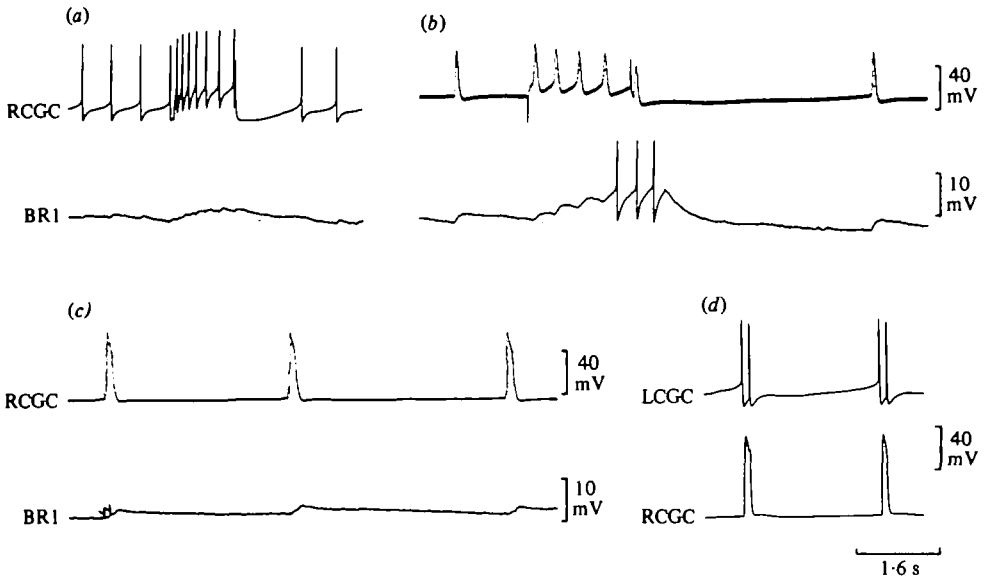
Fig. 4. Effect of injecting TEA into a right CGC on e.p.s.p.s in a right buccal 1-cell (BR1). (a) Before injection. Artificially evoked increase in RCGC spike frequency is followed by 1:1 e.p.s.p.s (approx. 1 mV amplitude) in BR1. (b) 3 h after injection. Long duration RCGC spikes each elicit a single unitary e.p.s.p. (approx 3 mV amplitude) in BR1. (c) 1 h after injection. Each long duration RCGC spike is accompanied by a compound e.p.s.p. in BR1, consisting of two unitary e.p.s.p. components (arrowed). (d) Also 1 h after injection. Each long duration RCGC spike is accompanied by a pair of spikes in left CGC to which it is strongly electrically coupled.

distances. Bryant & Weinreich (1975) have shown in *Aplysia* that TEA can diffuse for several millimetres along an axon, to increase spike duration in the distal terminals, without any detectable amount of TEA leaking out of the cell. A second problem arose because the TEA-lengthened spike in the soma appears to be capable of initiating more than one axon spike. One hour after injecting the right CGC of Fig. 4 with TEA, the CGC spikes were much longer in duration (Fig. 4c), and the 1-cell e.p.s.p.s were larger in amplitude. However, this apparent increase in amplitude was actually an artefact. Each e.p.s.p. was a compound potential resulting from summation of two unitary e.p.s.p.s (the two components are arrowed). The most likely explanation is that long duration CGC soma spikes were accompanied by pairs of normal duration axon spikes, because TEA had not yet had time to diffuse along the axon. The suggestion was supported by Fig. 4(d) in which single long-duration right CGC spikes were accompanied by pairs of spikes in the left CGC. As the cells are strongly coupled in or between the buccal ganglia (McCrohan & Benjamin, 1980), it is likely that the left CGC recording is reflecting the spike activity of the distal axon of the right CGC. However, 3 h after injection of TEA, the 1-cell e.p.s.p.s were again 1:1 with spikes in both the right and the left CGCs. Presumably TEA had now diffused far enough along the axon to prolong spikes in distal terminals so that pairs of spikes no longer occurred.

Another test for a direct synaptic connexion involved immersing the preparation in saline containing 40 mM-Ca^{2+} (10 × normal), for up to 2 h. Austin, Yai & Sato
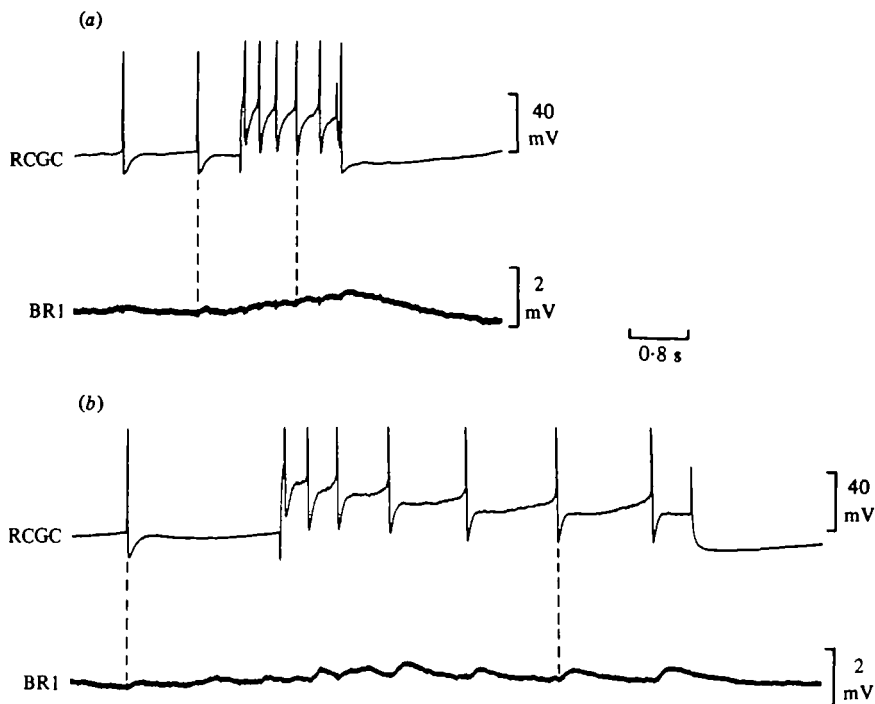
(a)

RCGC

BRI

40 mV

2 mV

0·8 s

(b)

RCGC

BRI

40 mV

2 mV

Fig. 5. Persistence of e.p.s.p.s in right buccal 1-cell (BR1), 1:1 with right CGC spikes, after 2 h immersion in high Ca²⁺ saline. (a) e.p.s.p.s in BR1, 1:1 with spikes in RCGC, following stimulation of RCGC to cause increased spike frequency (normal saline). (b) After 2 h exposure to 40 mM-Ca²⁺ saline. BR1 e.p.s.p.s still 1:1 with RCGC spikes.
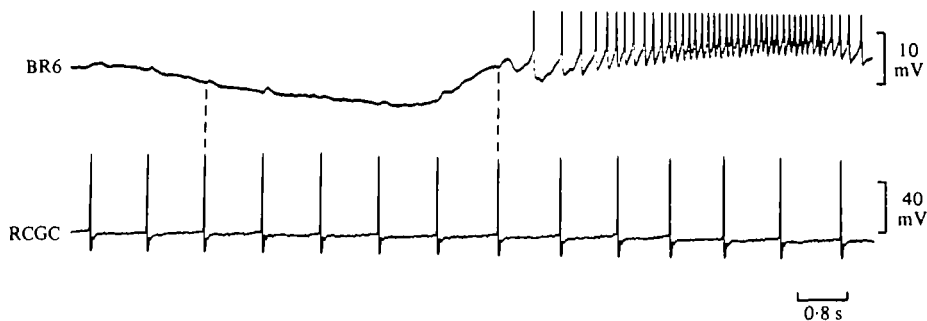
BR6

RCGC

10 mV

40 mV

0·8 s

Fig. 6. Short latency, 1:1 unitary e.p.s.p.s seen in a 6-cell (BR6) following right CGC spikes.

(1967) showed in *Aplysia* that high Ca²⁺ saline leads to increased spike threshold. Any intermediate cell between the CGC and the 1-cell would be likely to fail to fire following some CGC spikes, and the 1:1 relationship between CGC spikes and 1-cell e.p.s.p.s would be lost. Fig. 5 shows that after 2 h of immersion in high Ca²⁺ saline, the 1-cell e.p.s.p.s were still 1:1 with CGC spikes, indicating that this connexion has no intermediate cells, and is therefore monosynaptic. The 1-cell e.p.s.p.s were also larger in high Ca²⁺ (Fig. 5b), presumably because the membrane-stabilizing properties of Ca²⁺ had caused partial hyperpolarization of the 1-cell.
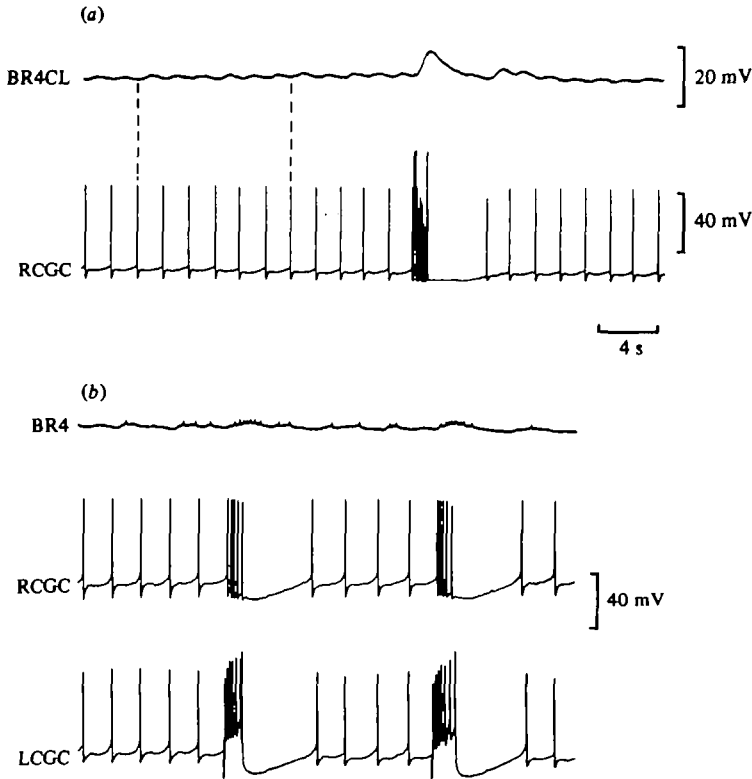
(a)

(b)

Fig. 7. Direct effects of CGCs on 4-group cells. (*a*) Long duration unitary e.p.s.p.s on right 4-cluster cell (BR4CL), 1:1 with spikes in right CGC. Artificially evoked burst in RCGC produces a compound e.p.s.p. in BR4CL. (*b*) Stimulation of left CGC evokes bursts of spikes in both left and right CGCs, and also compound e.p.s.p.s in right buccal 4-cell (BR4). These compound potentials have electrotonic e.p.s.p.s superimposed on them, presumably from other, electrically coupled 4-group neurones, caused to fire following CGC bursts.

6-cells. Small buccal motoneurones, such as 6-cells, were identified by recording from them while recording from other, large, easily identifiable cells such as 1- or 4-cells, and comparing the two phases of synaptic feeding input on the particular motoneurones. 6-cells receive excitatory first phase input. CGC spikes produce constant latency, 1:1 e.p.s.p.s in a 6-cell, of shorter duration than those in the 1-cell (approximately 150 ms) (Fig. 6). These summate at CGC spike frequencies above 6 Hz, to produce a compound e.p.s.p.

6-cells are small and difficult to find. Because of this, further tests for the monosynaptic nature of the connexion were not made. However, the 1:1 occurrence and short latency of e.p.s.p.s support the idea that this connexion is monosynaptic.

4-group cells. CGC spikes produce 1:1 e.p.s.p.s in both 4-cells and 4-cluster cells, which together comprise the 4-group cells (Fig. 7*a*). These e.p.s.p.s were not, however, always seen unless the 4-group cell was artificially hyperpolarized, and they were often masked by electrotonic e.p.s.p.s from other 4-group cells (Benjamin & Rose, 1979) (e.g. Fig. 7*b*). The e.p.s.p.s are of long duration (up to 1 s) and have a slow rise time. Because of the slow rise time, accurate latency measurements were
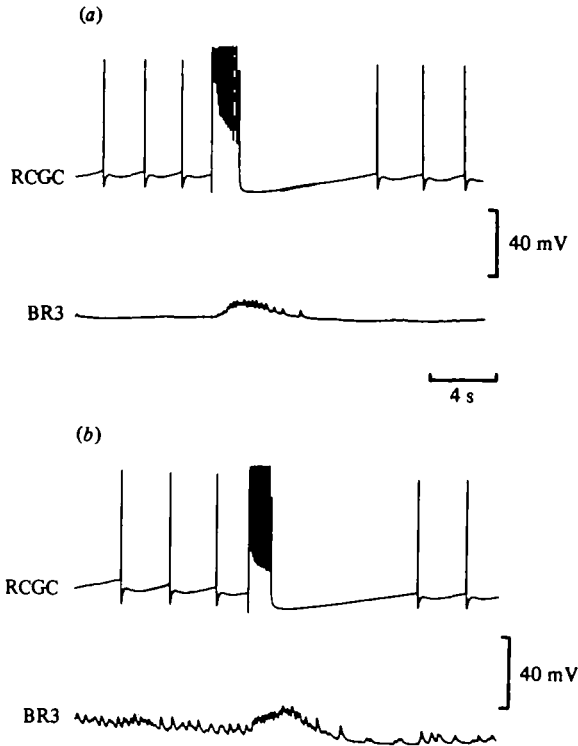
(a)

(b)

Fig. 8. Long latency effect of CGCs on 3-cells. (a) Artificially evoked burst in right CGC is followed, after about 300 ms, by a compound e.p.s.p. in right 3-cell (BR3), superimposed on which are excitatory p.s.p.s. (b) CGC burst causes increased frequency of the same e.p.s.p.s in BR3.

not possible. At increased CGC spike frequency, the 4-group cell e.p.s.p.s summate to produce a compound potential (Fig. 7a, b). In Fig. 7(b) the compound potential has an increased frequency of electrotonic e.p.s.p.s superimposed on it, suggesting that other 4-group cells are also being excited by the increased CGC firing frequency. Because of their long time course, 4-group e.p.s.p.s would sum at low CGC frequencies (1 Hz or above). It is not known whether the CGCs affect all or only some of the 4-group cells directly. However, the direct effects of the CGCs on one 4-group cell can be transmitted to others, owing to the electrotonic coupling within the group.

In summary, the CGCs have 1:1 and presumed monosynaptic connexions with three buccal motoneurone types. However, the effect on these cell types differs according to the particular summating properties of their e.p.s.p.s. A burst of spikes in a CGC produces short latency compound potentials in 1- and 6-cells. The effect of such a burst on a 4-group cell, however, is a compound potential with a slow rise time, and its peak delayed by up to 1 s after the end of the presynaptic burst. The significance of these different summating properties will be discussed later.

Long latency effects

A burst of spikes evoked in a CGC produces long latency excitation on four other identified buccal feeding motoneurones, in which no short latency, unitary 1:1
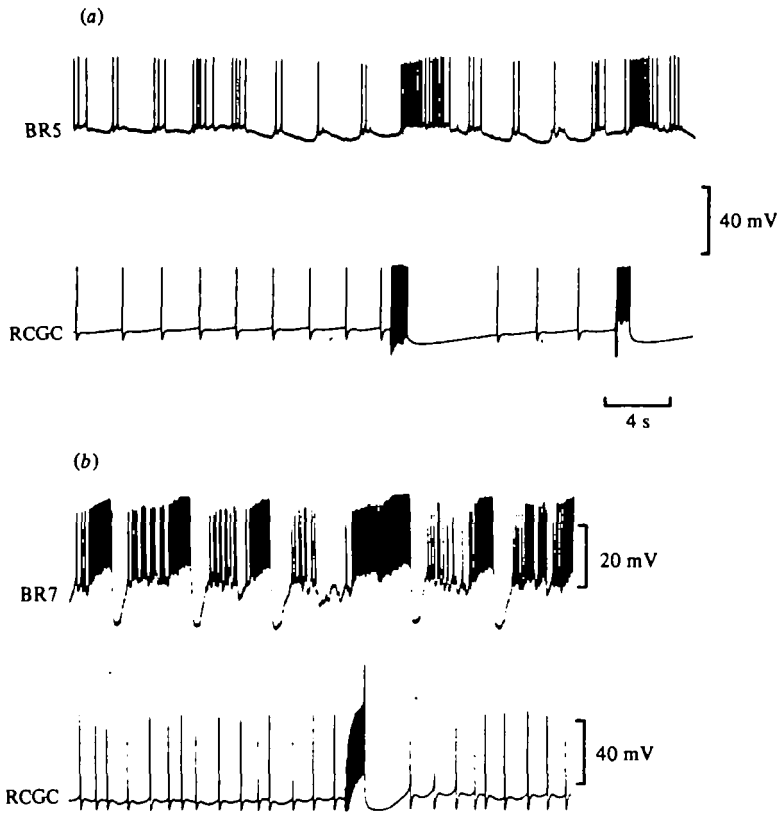
(a)

(b)

Fig. 9. Effects of CGCs on 5- and 7-cells. (a) Bursts in right CGC, following intracellular stimulation, produce increased firing frequency in right 5-cell (BR5). This effect has latency of about 500 ms, and continues for several seconds after the burst. (b) A burst in right CGC is followed by increased spike frequency in right 7-cell (BR7) after latency of about 500 ms.

e.p.s.p.s are seen, following CGC spikes: 3-cells, which burst during the second phase of synaptic input to feeding motoneurones; 5-cells, which fire during the period between feeding cycles; 7-cells, which burst during the first phase of synaptic input; and 2-cells, which burst in both the first and second phases (Benjamin & Rose, 1979; Rose & Benjamin, 1979).

3-*cells*. Fig. 8 shows that a burst of spikes evoked by stimulation of a CGC led to a compound excitatory potential in a 3-cell. This response was delayed for up to 300 ms after the onset of the burst, and persisted for several seconds after the end of the burst. Superimposed on this excitatory wave were small peaks of depolarization (Fig. 8a), identical to those seen following the retraction burst during feeding. These are thought to be caused by input from an interneurone, and are synchronous in 3-cells in both right and left buccal ganglia (Benjamin & Rose, 1979). When they were already present a CGC burst produced an increase in their frequency (Fig. 8b). The CGC must, therefore, influence the 3-cell by activating, directly or indirectly, at least two interneurone types, one leading to the longer depolarizing wave, and one responsible for the brief depolarizing peaks.

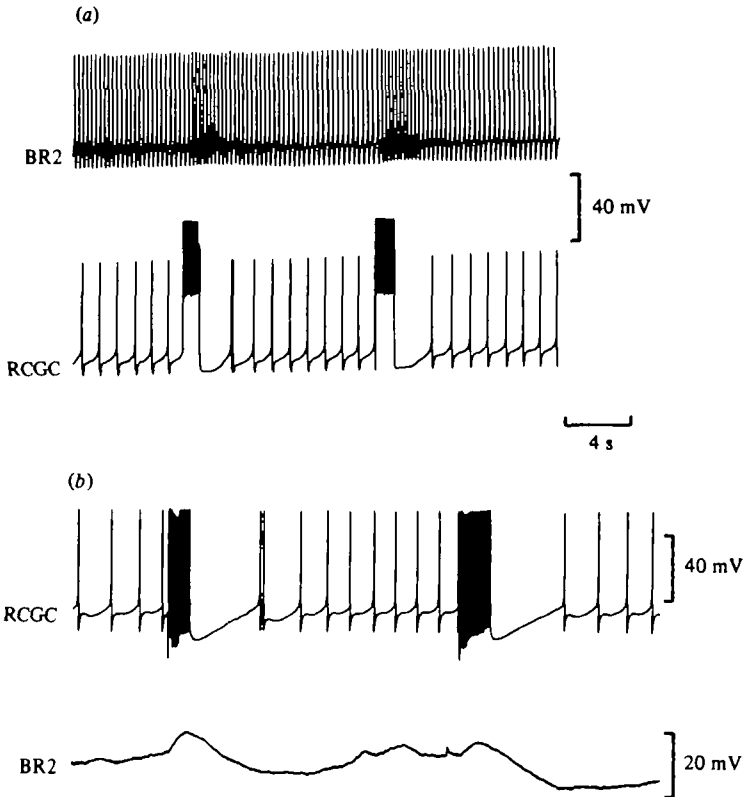Responses in 3-cells to CGC stimulation were reduced or abolished when the

(a)

(b)

Fig. 10. Effect of CGCs on buccal 2-cells. (a) Right CGC bursts following intracellular stimulation, cause increased right 2-cell (BR2) firing frequency after 500 ms delay. (b) BR2, hyperpolarized to prevent spiking, shows underlying compound e.p.s.p.s following RCGC bursts.

preparation was immersed in 60 mM-Ca^{2+} saline, suggesting that this is a poly-synaptic pathway, as previously discussed.

5-*cells*. 5-cells were identified by recording from them while recording from other feeding motoneurones, and comparing the timing and polarity of the two phases of feeding input. 5-cells are not usually silent except during the first and second phases of synaptic input in the feeding cycle, when they receive two consecutive phases of synaptic inhibition. It is difficult to prevent spiking in these cells, even with injection of considerable hyperpolarizing current, so their response to the CGCs was not examined in non-active cells.

Fig. 9(a) shows that a burst of spikes in a CGC caused increased 5-cell spike frequency after a delay of about 500 ms. This response continued for several seconds after CGC stimulation had ceased.

7-*cells*. Like the 5-cells, 7-cells are continuously active except when receiving second phase inhibition following their first phase excitation and burst (Fig. 9b). A burst of spikes in a CGC led to a delayed (500 ms) increase in spike frequency in a 7-cell (Fig. 9b).

2-*cells*. 2-cells are usually active, and are excited by both first and second phase
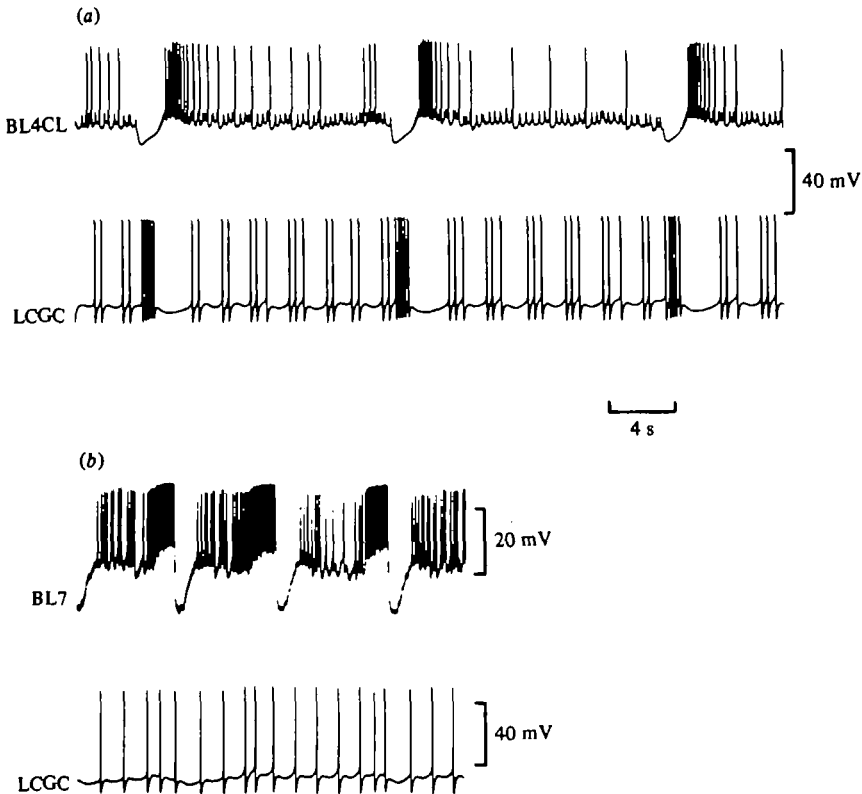
Fig. 11. Two extremes of phasic spike activity seen in CGCs in relation to the feeding cycle, as monitored from buccal motoneurones. (a) Strong bursts seen in left CGC during first input phase (Protraction), which is inhibitory to 4-group cell (BL4CL). (b) Slight increase in LCGC spike frequency during first input phase, which is excitatory to 7-cell (BL7).

inputs. Fig. 10(a) shows a CGC burst followed by increased firing rate in a non-patterning 2-cell. This response was delayed for about 500 ms after the onset of the CGC burst and continued for several seconds after it had finished. Fig. 10(b) shows that the response in a 2-cell (hyperpolarized to prevent spike activity) is a compound e.p.s.p. formed by summation of e.p.s.p.s which are not 1:1 with the CGC spikes. The 2-cell response was not always seen, suggesting that it depends on one or more intermediate cells, whose activity is not wholly determined by the CGCs. It was abolished by immersion of the preparation in 60 mM-Ca^{2+} saline.

Summary: tonic and phasic effect of the CGCs

The presence of excitatory inputs in the seven cell types (described above), following CGC stimulation, is probably sufficient to explain the excitatory effects of tonic increase in CGC firing frequency on the feeding output (monitored from 4-cells, and described in a previous section, Fig. 2). Increased tonic CGC spike output would excite all seven motoneurone types to some extent, and so increase the strength of their output, by increasing their tendency to fire. However, evidence presented in the previous paper (McCrohan & Benjamin, 1980) shows that CGCs do not always have
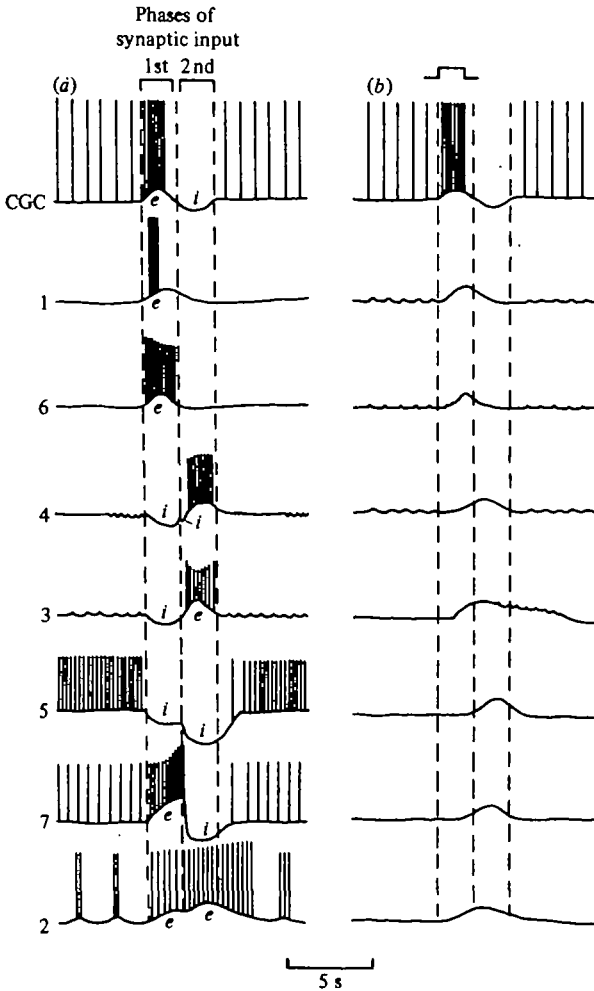
Fig. 12. Phasic modulatory effect of CGCs on feeding motoneurones. (a) Patterned spike activities of CGC and buccal cell types 1–7 during first (Protraction) and second (Retraction 1) phases of synaptic input in the feeding cycle. E.p.s.p.s in motoneurones from CGCs not shown. e, i, Excitatory and inhibitory input respectively. (Adapted from Benjamin & Rose, 1979.) (b) Compound potentials produced in motoneurones by an artificially evoked CGC burst (similar to that seen during the feeding cycle) alone. The timing of onset and cessation of these effects is shown. (E.p.s.p.s not drawn to scale.) For more details, see text.

a regular pattern of spike activity. Their spike frequency is often phasic, especially during feeding. This was shown to be due to two consecutive phases of synaptic input to the CGCs, excitation followed by inhibition. These inputs are synchronous with the two phases of synaptic input received by feeding motoneurones. Since this input often evokes cyclical CGC spike activity, it is reasonable to postulate that the CGCs could also be having a phasic modulatory effect on the output of buccal motonuerones.

Fig. 11 shows two extremes of phasic firing activity seen in a CGC in relation to the feeding cycle as monitored in a 4-group cell and a 7-cell. In Fig. 11(a) the CGC bursts strongly for 1 s at 5 Hz during the first phase of input to feeding motoneurones

(Protraction), and does not fire at all during the second phase (Retraction 1). In Fig. 11(b) the CGC's spike frequency increases only very slightly during the first phase. The activity of CGCs seen in most feeding preparations varies somewhere between these two extremes, and therefore the phasic effects of the CGCs would vary. What causes this variation in the response of a CGC to these phasic inputs is not known.

Fig. 12 illustrates how the different timing of the effects of a CGC burst (such as that seen in Fig. 11a) on different feeding motoneurones, could serve to strengthen bursts in motoneurones in both the first and second phases of the feeding cycle. Fig. 12(a) relates the spike activity of a CGC to those of seven different buccal motoneurone types during the course of a single feeding cycle. Excitatory and inhibitory synaptic inputs are denoted 'e' and 'i' respectively. Fig 12(b) summarizes the effect of an apparently identical, but artificially evoked, CGC burst alone on the membrane potentials of the seven cells.

Both 1- and 6-cells burst during the first (Protraction) phase, and their bursts are strengthened by short latency summating e.p.s.p.s in the presence of a first phase CGC burst.

4-group cells burst in the second (Retraction 1) phase, on the rebound from a large first phase inhibitory input plus a smaller second phase inhibition. The compound potential resulting from the CGC burst has slow rise time and a delayed peak. This would promote the 4-group cell's rebound from inhibition during the second half of the first phase, and thus advance the onset of its retraction burst. This advanced timing, together with a strengthening of 4-cell burst, is equivalent to the effect of a tonic increase in CGC firing frequency (Fig. 2).

3-cells receive a delayed excitatory input from the CGC, which does not start until the second half of the first phase. At this time the 3-cell is recovering from a first phase inhibitory feeding input. The CGC's effect is to promote this recovery and also to enhance the effect of the second phase excitatory input, thus producing a stronger 3-cell burst.

5- and 7-cells are each receiving an inhibitory feeding input during the second phase when the full effect of the CGC's long latency excitation is present. This could serve to lessen the degree of hyperpolarization so that post-inhibitory recovery would occur sooner.

The 2-cell burst spans both input phases of the feeding cycle. The frequency of spikes in this burst is increased following a CGC burst.

The CGC burst can therefore strengthen feeding bursts in motoneurones that fire during either protraction (cells 1 and 6) or retraction (cells 4 and 3), owing to the different characteristics of postsynaptic responses in the various motoneurone types. Such phasic effects of the CGCs are permitted because they receive inputs synchronous with those on the feeding motoneurones themselves.

In the 4-group cells, both phasic and tonic increases in CGC spike frequency can produce an advanced onset of the second phase retraction burst, together with a strengthening of this burst. Replacing a continuous high frequency output from the CGCs, by accurately timed high frequency bursts during the first phase of the cycle, may represent the more controlled way of achieving this end. This suggestion may equally well apply to the other motoneurones.

DISCUSSION

Function of the cerebral giant cells

The results presented here show how the CGCs in *Lymnaea* modulate the strength of the feeding motor output, but do not initiate feeding cycles. Such a modulatory function for giant serotonin neurones has been demonstrated in *Planorbis* and *Aplysia* (Berry & Pentreath, 1976a; Weiss *et al.* 1975). In these species, like *Lymnaea*, the effect of the giant cerebral cells on buccal motoneurones is to produce alterations in the intensity of motoneurone output, rather than to initiate rhythmic feeding activity. An additional finding in *Lymnaea* is that the CGCs' modulatory effect is precisely timed, owing to phasic input synchronous with the feeding cycle. Bursts of spikes in the CGCs during feeding produce excitatory effects with different latencies on different motoneurone types. These latencies are appropriate for reinforcing the feeding synaptic input already present on the motoneurones, thus strengthening motoneurone bursts.

The giant cerebral cells of *Helisoma* and *Pleurobranchaea* have been postulated to have a command function (Granzow & Kater, 1977; Gillette & Davis, 1977). In both these species, increased spike activity in the giant cerebral cells initiated cyclical bursting, which was monitored from buccal motoneurones in *Helisoma*, and extra-cellularly from buccal nerves in *Pleurobranchaea*. Increased giant cerebral cell spike activity was also shown to influence the frequency of feeding cycles. This suggested that, in these species, the giant cerebral cells act on the interneurones which are presumed to impose rhythmic activity on the feeding motoneurones.

The CGCs of *Lymnaea* may, in some situations, appear to have a 'command' function, owing to their ability to increase the intensity of output from feeding motoneurones. Feeding motoneurones receive two phases of synaptic input, which are responsible for producing rhythmic feeding bursts (Benjamin & Rose, 1979). In some preparations these inputs occur in motoneurones whose activity is at a very low level. This means that bursts of spikes are either not generated, or are very small. An increase in CGC spike activity would, as has been shown, increase the strength of motoneurone activity so that stronger bursts would occur in response to the phasic feeding inputs. In this way a rhythmic burst pattern of motor output to the buccal muscles would have been switched on. This would not, however, involve a true switching of the central pattern generator.

Feedback to the CGCs from the feeding pattern generator

Phasic inputs to giant cerebral cells, synchronous with feeding cycles, have been shown in several mollusc species. As in *Lymnaea* (McCrohan & Benjamin, 1980), there is a dual phasic input to the metacerebral giant cells of *Pleurobranchaea* (Gillette & Davis, 1977). This consists of an excitatory input during eversion of the buccal mass, and inhibition during withdrawal. The source of these inputs in *Pleurobranchaea* was shown to be neurones of the feeding system, which discharge rhythmically. This feedback from the feeding system to the metacerebral giant cells has been suggested to be a positive feedback mechanism which sustains, amplifies and phase-locks the feeding motor output (Gillette & Davis, 1977).

Since the two phasic inputs to the CGCs of *Lymnaea*, described in the previous

paper (McCrohan & Benjamin, 1980), are synchronous with the two phases of synaptic input received by buccal motoneurones during feeding (Benjamin & Rose, 1979), it is possible that they come, directly or indirectly, from the two networks of buccal interneurones that are thought to provide these inputs (R. M. Rose, unpublished data). If this is so, then a system of positive feedback similar to that described for *Pleurobranchaea* (Gillette & Davis, 1977) may be working in *Lymnaea*. The strength of phasic inputs to the CGCs would be correlated with the strength of input to motoneurones from the feeding pattern generator, thus amplifying the CGCs' modulatory effect on the motor output.

In *Helisoma*, the giant cerebral interneurones receive an inhibitory input synchronous with the inhibitory input to protractor motoneurones (Granzow & Kater, 1977). A phasic excitatory input to giant cerebral cells has been found in *Helix* and *Aplysia* (Weiss & Kupfermann, 1976; Kandel & Tauc, 1966) though, in the former, it has not yet been correlated with feeding motor activity.

Synaptic connexions of the CGCs

Both direct and indirect connexions between giant serotonin-containing cerebral cells and identified buccal neurones, similar to those described here for *Lymnaea*, have been shown in several mollusc species (Cottrell & Macon, 1974; Gillette & Davis, 1977; Paupardin-Tritsch & Gerschenfeld, 1973; Berry & Pentreath, 1976a). In *Lymnaea* they all appear to be excitatory though inhibitory effects have been demonstrated in other species.

The giant cerebral cell system is already being used to study the mechanism of action of the neurotransmitter, serotonin (Cottrell & Macon, 1974; Paupardin-Tritsch & Gerschenfeld, 1973; Gerschenfeld & Paupardin-Tritsch, 1974). The identification of a number of follower cells in which postsynaptic responses differ from each other in amplitude and time course, as has been described here in *Lymnaea*, additionally provides a useful system for the study of the variety of membrane properties underlying these responses.

The identification of homologous or equivalent buccal motoneurones in different species could yield comparative information regarding the possible evolution of particular connexions.

Isolated versus whole-animal preparations

The work described in this paper was performed on isolated brain preparations. This was a satisfactory method for studying the effect of the CGC interneurones on a group of motoneurones. However, when studying more fully the higher-order role of these interneurones, it may prove necessary to consider the use of whole animal or semi-isolated preparations, especially when sensory input to the cells is being examined. It has been shown in the giant cerebral neurones of *Archidoris*, for example, that the shadow response in whole animal preparations differs from that in isolated brains (Blackshaw & Dorsett, 1976). In *Pleurobranchaea* the phasic input to the metacerebral giant cells during feeding was shown to have a greater effect on spike frequency in whole animal than in isolated brain preparations. In *Lymnaea*, preliminary studies on semi-isolated preparations suggest that contraction of buccal

muscles following artificial stimulation may lead to changes in spike activity in the CGCs. Such effects could not be investigated in an isolated preparation.

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