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THE INTEGRATION OF THE PATTERNED OUTPUT OF BUCCAL MOTONEURONES DURING FEEDING IN *TRITONIA HOMBERGI*

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SUMMARY

Three phases of activity may be recognized in the buccal mass of *Tritonia hombergi* during the feeding cycle. These have been termed Protraction, Retraction and Flattening. Each phase is driven by a group of motoneurones along the posterior border of the buccal ganglia. The patterned bursting observed in the motoneurone groups during feeding activity is phased by synaptic inputs which are common to two or more groups. Evidence is presented which indicates these inputs are derived from three unidentified multi-action interneurone sources within each buccal ganglion, and whose action primarily determines the patterned output of the motoneurones. Electrical coupling between between synergistic motoneurones and, in one case, post-inhibitory rebound, contribute to the synchronization of group activity. Proprioceptive input to the motoneurones was not identified, but may project to the interneurones.

Some small neurones having synaptic inputs on the motoneurones appropriate to two of the interneurones were found, but require confirmation in this role. The cerebral giant cells synapse on representatives of three motoneurone groups, and also activate the buccal interneurones driving the feeding cycle. The patterned activity of the motoneurones can occur in the absence of cerebral cell activity.

INTRODUCTION

The molluscan buccal mass is a convenient system for the study of the neural organization underlying simple behaviour patterns. It is one of the few structures in gastropods where the muscles are anatomically distinct, and is innervated by the paired buccal ganglia which contain relatively few neurones, often large enough to permit intracellular recording from identifiable cells. The feeding habits of species studied in this way range from rasping with the radula (Kater, 1974), biting and swallowing (Davis & Mpitsos, 1971; Willows, 1977) or a powerful suck (Woollacott, 1974). These activities are regularly occurring or repetitive events, driven by the patterned discharge of groups of motoneurones in the buccal ganglia (Rose, 1971, 1972; Berry, 1972*a*, *b*; Siegler, Mpitsos & Davis, 1974).

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Several mechanisms have been recognized as contributing to the generation of synchronized bursts in synergistic groups of motoneurones, including such properties as electrical coupling, post-inhibitory rebound, an inherent 'burstiness' developing from sustained depolarizations, and common synaptic inputs. The latter may come from proprioceptors with cell bodies in the buccal mass or central ganglia (Kater & Rowell, 1973; Siegler, 1977), or from interneurones in the buccal or cerebro-pleural ganglia (Gardner, 1971, 1977). In *Helisoma* a network of electrically coupled cells with intrinsic rhythmic properties are thought to provide the drive and timing for antagonistic groups of motoneurones (Kater, 1974), but in other preparations the interneurones themselves have not been identified (Seigler, 1977).

In a previous paper (Bulloch & Dorsett, 1979*a*) we have described the rhythmic feeding movements of the buccal mass of *Tritonia hombergi*, and specified the function and patterned activity of some 40 motoneurones along the posterior dorsal border of the buccal ganglia. Here we show how the motoneurone activity of each cycle is divided into three phases, the sequence being primarily determined by synaptic inputs from three unidentified interneurone sources within each buccal ganglion.

MATERIALS AND METHODS

Details of the preparations used in these experiments are given in an earlier paper (Bulloch & Dorsett, 1979). Briefly they consisted of the buccal mass cut out with the mouth and surrounding structures on the anterior region of the head. The buccal tube was often opened ventrally to permit viewing of jaw and radula movements from beneath by means of a small mirror. The buccal ganglia were supported from beneath by a wax-covered platform inserted down the oesophagus. Apart from cutting the pedal and pleural nerves, the cerebro-pleuro-pedal complex was not disturbed, the cerebral nerve supply to the head region remaining intact. When recording simultaneously from cerebral and buccal neurones, the cerebral nerves were cut to allow the ganglia to be drawn back to lie alongside the buccals with the connective intact.

The preparations were maintained in Perspex chambers in running sea water, where they remained in good condition for 24–36 h.

Neural correlates of buccal mass activity

Tritonia hombergi feeds by biting large pieces from the surface of the colonial coelenterate *Alcyonium digitatum*. It achieves this by movements of its powerful scissor-like jaws, assisted by the radula within the buccal mass.

Details of this process are given in the previous paper (Bulloch & Dorsett, 1979). The individual buccal cycles are made up of a sequence of movements which can be divided conveniently into three phases termed (1) Protraction, (2) Retraction and (3) Flattening. These terms refer to the action of the radula, but they are accompanied by opening and closing movements of the jaws and inner and outer lips, which form an integral part of the feeding process.

The jaw and radula movements in each phase of the cycle have been correlated with patterned bursting activity in corresponding groups of P, R and F motoneurones in the buccal ganglia (Bulloch & Dorsett, 1979). A fourth group, called the M cells,

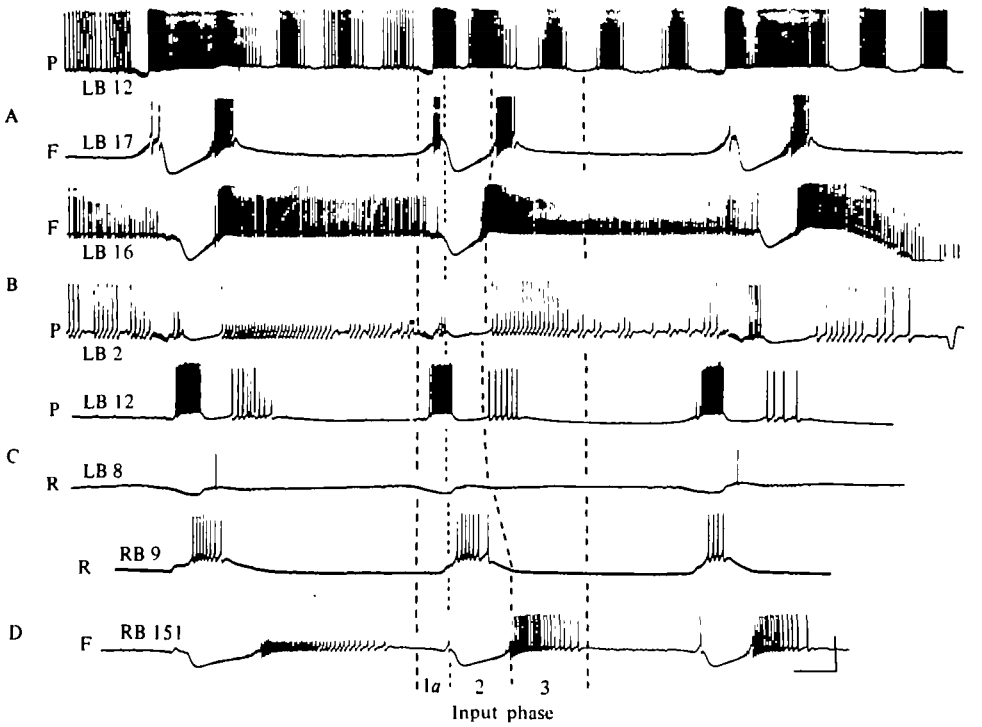

Fig. 1. A composite recording from pairs of cells in spontaneously active preparations, aligned to illustrate their activity during the three phases of buccal mass activity. Calibration: 50 mV, 5 s.

retract the outer lips as the mouth and jaws open. In spontaneously active preparations recordings from pairs of motoneurones can subsequently be aligned to represent the activity of many cells during a typical buccal cycle (Fig. 1).

Synaptic inputs to the buccal motoneurones

The neural mechanisms responsible for integrating the motoneurone activity have two principal requirements. In order to produce co-ordinated movements they must (*a*) ensure that each group fires in the correct phase of the cycle and (*b*) bring about the contemporaneous activation of members within a group.

Recordings from pairs of buccal motoneurones indicate that they receive chemically mediated synaptic inputs from three sources, each of which is common to two or more of the motoneurone groups. The p.s.p.s have been designated Inputs 1, 1*a* and 2, to indicate the phase of the buccal cycle with which they coincide. Our evidence suggests that the inputs come from three unidentified interneurones whose activity is the principal factor in timing the output of the motoneurone groups.

Synaptic activity in Phase 1

The Phase 1 movements of the buccal mass are those associated with mouth opening, including retraction of the inner and outer lips, radula protraction and flattening and jaw opening. These movements result from activity of the P and M cells, and those members of the F group which fire during Phase 1.

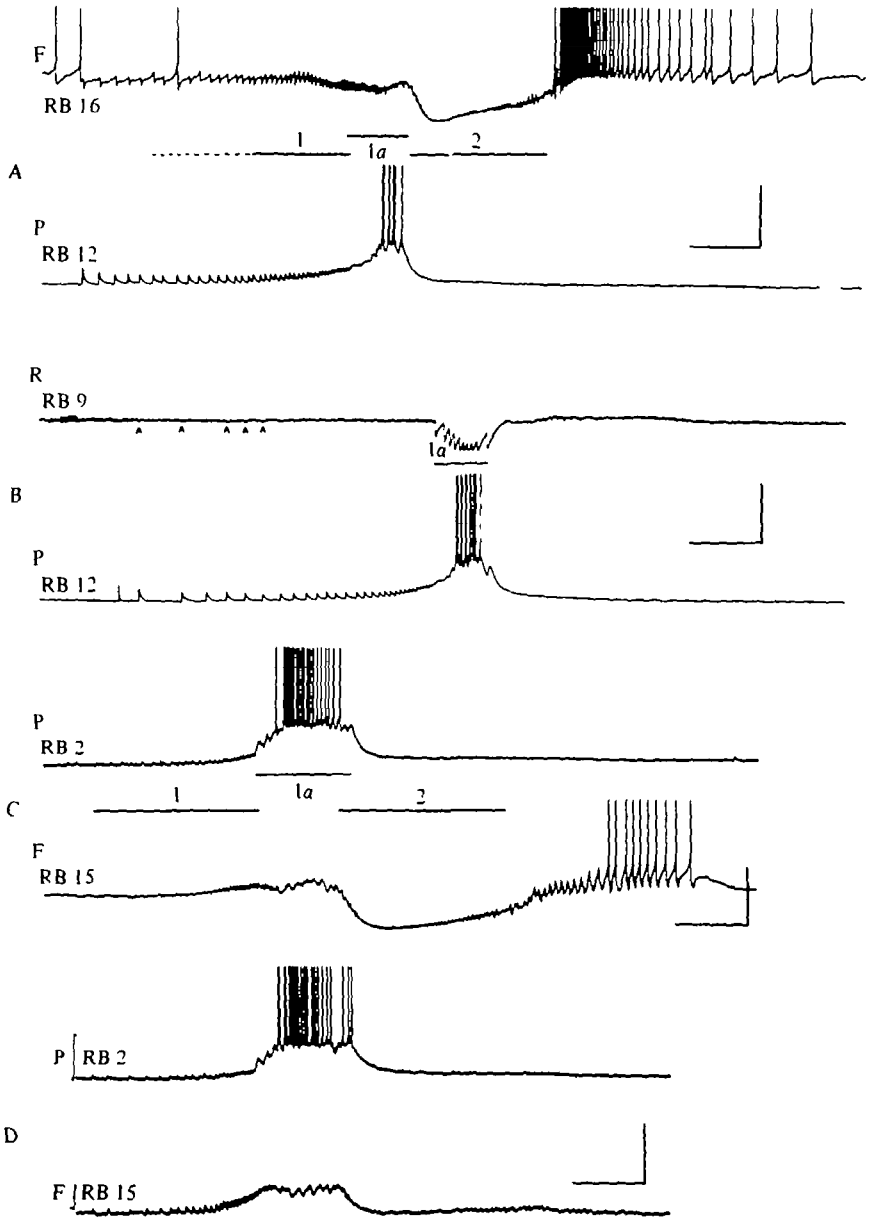

Fig. 2. Interneurone inputs to the motoneurones. A, An F cell (upper trace) and a P cell (lower). The synaptic input from Interneuron 1 is expressed as an EIPSP in the F cell and an EPSP in the P cell. Interneurone 1*a* produces a large EPSP and a burst in the P cell, but individual p.s.p.s from 1*a* are difficult to resolve in the F cell, although there is a depolarization of the membrane. The large inhibitory wave caused by Interneurone 2 is only received in the F cell. B, An R cell (upper) and a P cell (lower). The inhibitory inputs from Interneurone 1 are very small in the R cell, but occur 1 : 1 with the EPSP in the P cell. The 1*a* inhibitory input is is clearly seen in the R cell. C, A P (upper) and an F (lower) cell showing synaptic activity in the F cell during Input 1*a*, and the LIW associated with Input 2. D, Same pair of cells with the F cell membrane hyperpolarized to the equilibrium potential for Input 2. Input 1 now appears as an EPSP while 1*a* is still inhibitory in sign. Calibrations: 50 mV, 5 s.

The P cell burst is preceded by a slowly accelerating EPSP which appears between 5 and 30 s before the onset of Phase 1. This p.s.p. is received simultaneously by all neurones in the P group, and has been called Input 1 (Fig. 2A–D). Input 1 has an initial frequency of about 1 s^{-1}, increasing to 5 s^{-1} just prior to the Phase 1 burst. Its amplitude is 1–2 mV and may summate at the higher frequencies, but it is not directly responsible for the P cell burst.

The burst follows the onset of a second, larger EPSP which is superimposed upon Input 1, and rapidly depolarizes the P cells beyond spike threshold (Fig. 2A–D). This second EPSP, termed Input 1*a*, may attain amplitudes of up to 5 mV, but its frequency rarely exceeds 2–3 s^{-1}. Input 1*a* is common to all three identified P cells (B 2, 12, 13) but is difficult to resolve during spike activity.

At the end of Phase 1 the synaptic inputs from these two sources terminate and the P cells return rapidly to their resting potential. In some preparations P cells fire intermittently in Phases 2 and 3 (Fig. 1), but such activity is without observable effect on the radula and jaw movements in the latter part of the cycle, due perhaps to the lower impulse frequency in the neurones.

Although the principal activity of the F cells is seen in Phase 3, some members of this group fire a short burst during Phase 1, driving the radula flattening and inner lip retraction that occur at this time. The synaptic inputs causing the Phase 1 burst in these F cells are often difficult to resolve, but Input 1 can be recognized as an inhibitory or an EIPSP which occurs 1 : 1 with the EPSP in the P cells, and is presumably derived from the same source (Fig. 2A, C). The overall tendency of the Input 1 p.s.p. at the lower frequency is inhibitory, and may serve to terminate the Phase 3 bursts of the previous cycle, but occasional spikes may be generated by the excitatory component of the p.s.p.

Strong hyperpolarization of the F cell through the recording microelectrode converts Input 1 into an e.p.s.p. which summates at higher frequencies (Fig. 2D).

During Phase 1 considerable variation is observed in F cell activity. Many neurones show a depolarizing shift in the membrane potential while continuing to show small amplitude inhibitory potentials similar to those of Input 1 (Fig. 1A, D). The overall depolarization often leads to a short burst of impulses, although the number of spikes generated in any one cell may vary in successive cycles. In other F cells large amplitude IPSPs or EIPSPs are superimposed upon the smaller IPSPs, and can frequently be correlated with the 1*a* input in P group motoneurones (Fig. 2A, D). Other members of the F group do not receive 1*a* inputs directly, but are electrically coupled to members that do.

The R cells are not active during Phase 1, but dual recordings with P and F cells show they also receive Inputs 1 and 1*a*. In R cells these are manifest as inhibitory rather than biphasic or excitatory potentials (Figs. 1C, 2B, 3B, C). The Input 1 IPSP is often difficult to resolve, having an amplitude of less than 1 mV, with little effect on the level of the membrane potential. It also occurs 1 : 1 with the EPSP in the P cells and is derived from the same source. During Phase 1 the R cells are hyperpolarized by up to 10 mV by large amplitude IPSPs that correspond to Input 1*a* as seen in the P cells (Figs. 2B, 3B, D).

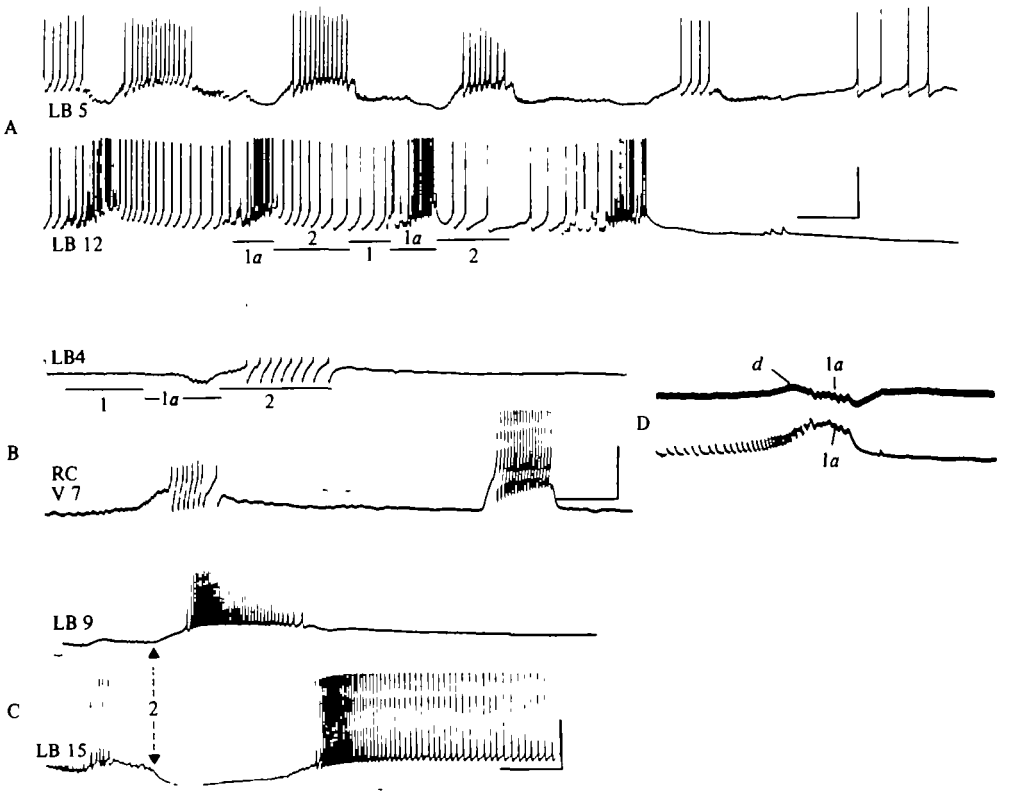

Fig. 3. Interneurone inputs to the R cells. A, Spontaneous cycles in the giant R cell (LB 5 upper) and a P cell (lower). The R cells are depolarized by Input 2, following the P cell burst. The R cell burst may be partially terminated by an inhibitory input which may be derived from Interneurone 1 or the Phase 3 burst in the F cells. B, The 1 and 1*a* interneurone inputs to the R cell (upper) are common to a P-type cell in the cerebral ganglia (lower). A driven burst in the P cell does not activate the 1*a* p.s.p. in the R cell. C, A single cycle in an R cell (upper) and an F cell (lower) showing the simultaneous onset and opposite sign of Interneurone 2 in these motoneurones. D, Common p.s.p.'s from Interneurone 1*a* seen in an R cell (upper) and a P cell (lower). The P cell was hyperpolarized to prevent spiking. *Calibration*: 50 mV, 5 s.

Synaptic activity in Phase 2

Phase 2 activity in R cells is seen as a depolarizing wave on which individual EPSPs cannot always be resolved (Figs. 1 C, D, 3 A). This leads to a burst of impulses in these neurones at frequencies between 1 and 3 s^{-1}, driving the jaw closure and radula retraction movements associated with this phase.

The depolarization of the R cells coincides with the onset of a Long Inhibitory Wave (LIW) in the F cells (Fig. 3C), hyperpolarizing the membrane to around −75 mV. Small amplitude IPSPs are visible on the falling and ascending sections of the waveform which suggest that LIW is the product of summating individual potentials. These attain maximum frequencies around 10 s^{-1}, at which point their amplitude is small and close to their reversal potential (Fig. 2C, D). The depolarizing wave in the R cells and the LIW in the F cells appear simultaneously, have a similar configuration, but are of opposite sign. The one is never recorded in the absence of th

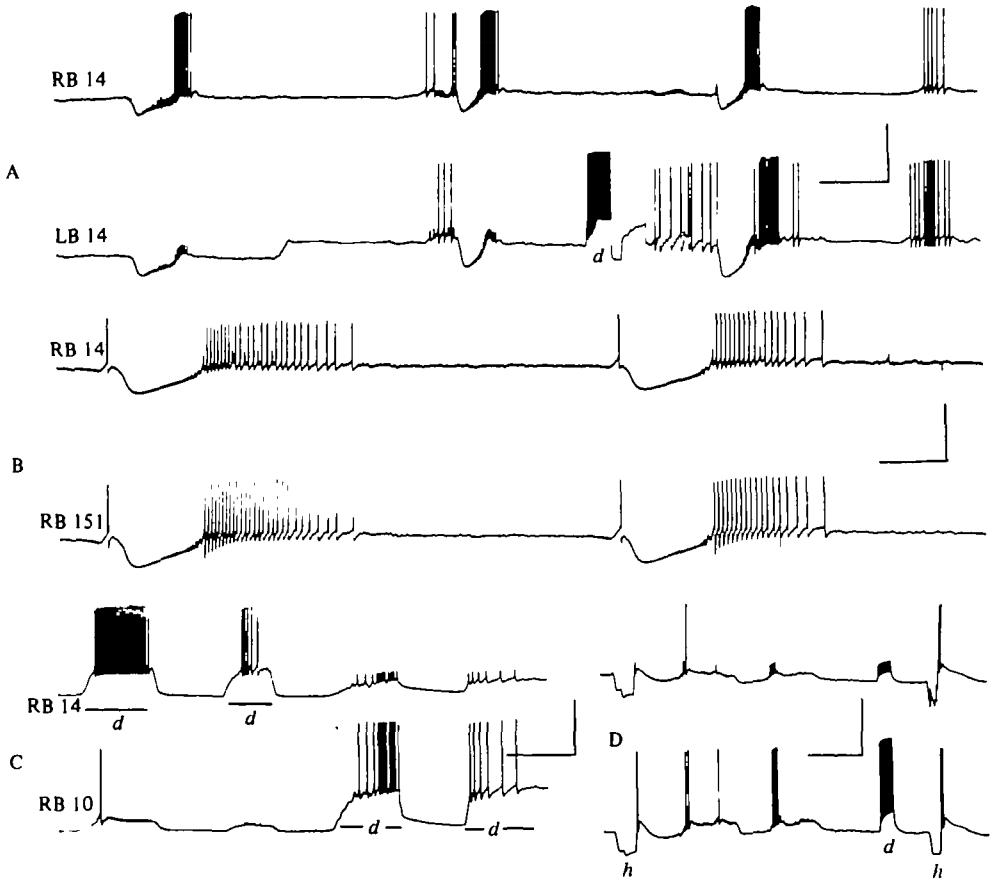

Fig. 4. Inter-relationship of F cells. A, A symmetrical pair of F cells which are not themselves coupled, but receive common inputs from one or more members of the group. B, A pair of F cells which are coupled electrically. C, A second pair of electrically coupled F cells. Spike burst induced by depolarization (*d*). D, The same pair showing that post-inhibitory rebound in one cell (*h*) can lead to spikes in both. Calibration: 50 mV, 5 s.

other. It is therefore suggested that these are synaptically mediated events of opposite sign having a common origin. This source has been termed Input 2.

Phase 3 activity in F cells

Examination of the Phase 3 burst in the F cells, causing the two halves of the radula to separate and flatten, has failed to reveal any dependence on an underlying synaptic input. A property of F motoneurones, not shared by P or R cells, is that of post-inhibitory rebound excitation. The number of spikes generated depends upon the duration and the amplitude of the previous hyperpolarization (Fig. 4D). Thus the immediate consequence of the release of the F cell population from Phase 2 inhibition by Input 2 is the generation of an intense burst of impulses in all members of the group. This effect is reinforced by positive feedback supplied by electrical coupling between individuals in the F cell population (Fig. 4B–D), which generates spike

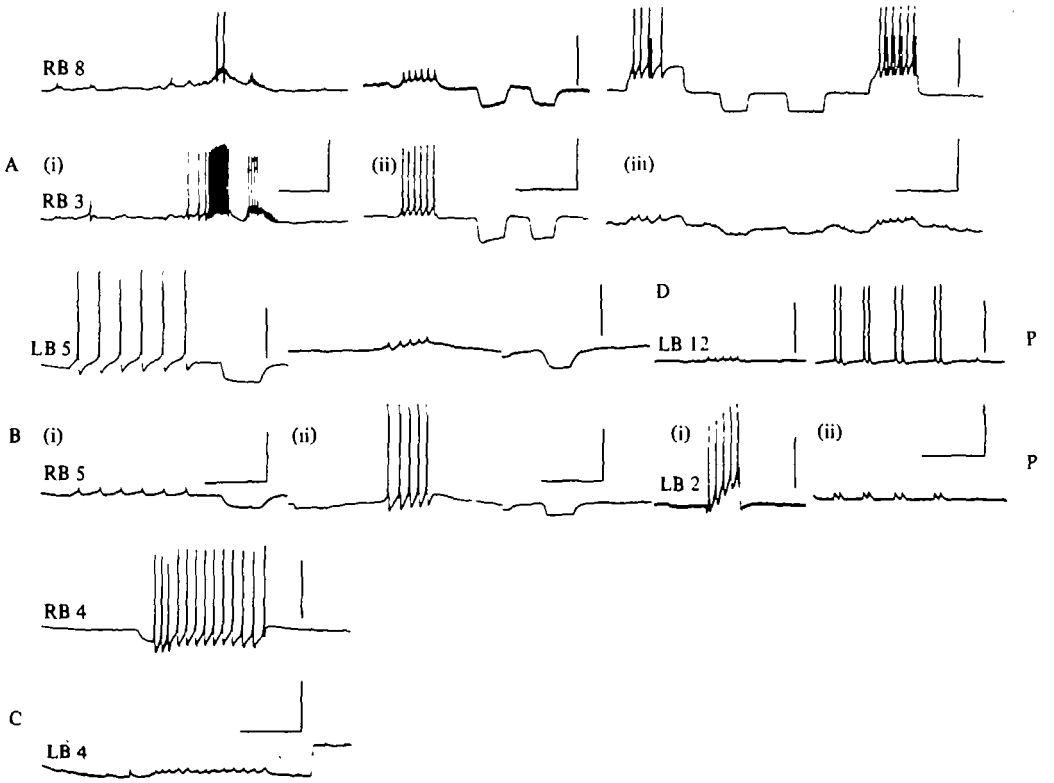

Fig. 5. Electrical coupling in R and P motoneurones. A, i–iii. Coupling in the R cells RB 3 and 8. B, i–ii. Coupling in the giant R pair B 5. C, Coupling in the pair B 4. D, Coupling in the P cells LB 12 and 2. Calibration: presynaptic cells, 50 mV; postsynaptic cells, 5 mV, 5 s.

frequencies up to 15 impulses/s^{-1} in individual neurones. The bursts are terminated by the decay in the rebound effect and by the onset of Input 1 inhibition of the next cycle.

Electronic coupling in motoneurones

Burst synchronization within the P, R and F groups is also aided by electronic junctions between members of the same group. Among the P cells coupling has been recorded between LB 2 and LB 12 and pair B 12 of each side (Fig. 5 D). Of the R cells, the giant cells 4 and 5 are both coupled to their contralateral partners, and similar junctions are found between RB 3–8, RB 4–9, and LB 3–6 (Fig. 5 A–C). Similar junctions are found between pairs of F cells (Fig. 4 B–D). The degree of coupling is normally insufficient for a spike train of 5 impulses/s^{-1} in one cell to cause a spike in its partners. However, intense depolarization in some F cells which normally fire at high frequencies in Phase 3 will often induce one or two spikes in a partner to which it is coupled. The function of coupling would seem to be that of equalizing the membrane potential within the group rather than to synchronize individual impulses.

Interactions between P, R and F motoneurones

In the many recordings made from motoneurone pairs during the present investigation, no direct synaptic contacts were found between neurones generally confirming

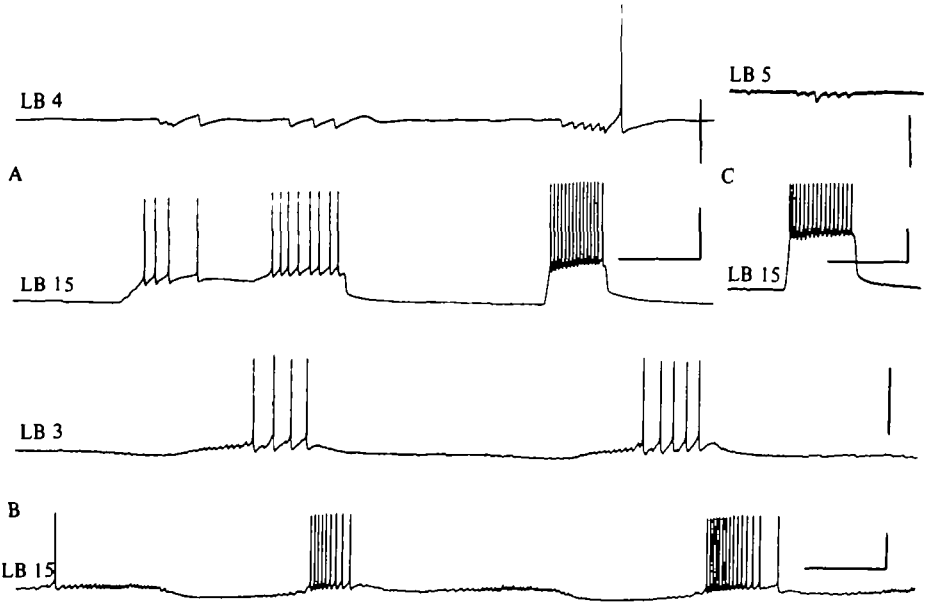

Fig. 6. Interactions between motoneurone groups. A, F cell excites a polysynaptic inhibitory input to the R cell. The inhibitory potential is complex having a late excitatory component. B, The same F cell (lower) has a similar relationship to another R cell. C, The same F cell also inhibits the R cell LB 5 polysynaptically. Calibrations: 50 mV, 5 s.

results of other workers, and only a few instances of indirect polysynaptic interactions were encountered. Examples of P–R and reciprocal excitatory pathways were both obtained, but only by stimulating the presynaptic neurone for a prolonged period at frequencies higher than those observed during normal activity. At this stage no particular significance is attached to these results. On one occasion an F cell identified as LB 15 was bound to activate a short latency, indirect inhibitory pathway to the R cells LB 4 and 5. The IPSP in LB 4 had a late excitatory component which at higher frequencies was sufficient to cause a spike in the R cell (Fig. 6A). Pathways such as this are of interest in view of the Phase 2 inhibitory potentials often seen in R cells, and may be involved in terminating their activity (Fig. 6B).

In other preparations excitatory pathways were found between the F cells LB 9 and 10 and an R cell, LB 6. There was no correspondence between spikes and EPSPs so the pathway is presumably indirect.

The cerebral giant cells and buccal motoneurones

The cerebral giant cells located on the anterior border of the cerebral ganglia send axons to the buccal ganglia and nerves through one or both cerebro-buccal connectives (Dorsett 1967). Monosynaptic connexions have been identified with representatives of all three motoneurone groups, but such interactions are not common. Impulses in the cerebral giant cell LC 1 are followed by 1 : 1 EPSPs in the identified P cells LB 2 and 12 and other unidentified members of this group (Fig. 7C, G, H, I) over a wide range of frequencies, and are considered to be monosynaptic.

The R cells LB 1 and 3 receive a monosynaptic IPSP following the LC 1 spike, after a long but constant latency. In both cases the inhibitory potential has a late excitatory

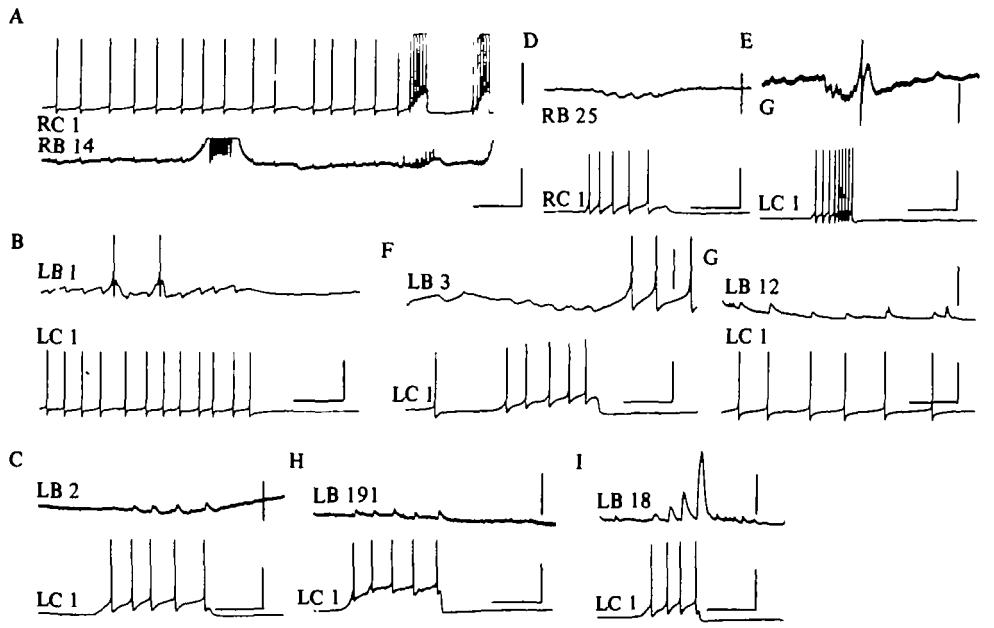

Fig. 7. Monosynaptic connexions of cerebral giant cells. A, Cerebral giant cell has an EIPSP on an ipsilateral F cell. B, LC 1 has an IEPSP on the R cell (upper). C, LC 1 has a facilitating EPSP with the P cell LB 2 (upper). D, RC 1 inhibits an unidentified cell (area J-3). E, Inhibitory synapse formed with the large G cell in the area H-3. G, EPSPs from LC 1 in the P cell LB 12. F, IEPSP with an R cell. H and I, EPSPs in two small neurones from an area close to the M cells. LB 18 has a facilitating response. Calibrations: intracellular records of presynaptic neurone, 50 mV; post-synaptic cells, 5 mV. Horizontal 5 s.

component that may generate a spike during or following the cerebral cell activity. Occasionally a small excitatory potential precedes this p.s.p. forming an EIEPSP (Bulloch, 1977). The large 'G' cell close to the origin of the gastro-oesophageal nerve is also inhibited by LC 1 (Fig. 7E).

Among the F cells, LB 14 displayed a 1 : 1 correspondence between an EIPSP and the cerebral cell spike after a latency of 20 ms. Similar inputs were observed to LB 10 and 17. The configuration of the p.s.p. is similar to Input 1 in these cells, but they do not follow its characteristic temporal pattern (Fig. 7A).

Indirect interactions with the cerebral giants

Although the cerebral giant cells synapse with relatively few buccal motoneurones, they are effective in evoking the patterned feeding bursts and associated buccal mass movements. This action derives from their excitatory effect on the buccal interneurones that phase the feeding cycle (Fig. 8C).

A short burst of impulses in either cerebral giant cell frequently results in excitation of P and F cells, and inhibition of R cells, through activation of Input 1*a* (Fig. 8A, B, D). Such bursts are typically followed by Input 2 without further impulses in the cerebral giant, and may be followed by a further spontaneous burst sequence, introduced by properly phased activity of Input 1 (Fig. 8E, F, H).

Although impulses in the cerebral giants can initiate activity in a previously quiescent preparation, the evidence suggests that it is not a required preliminary to

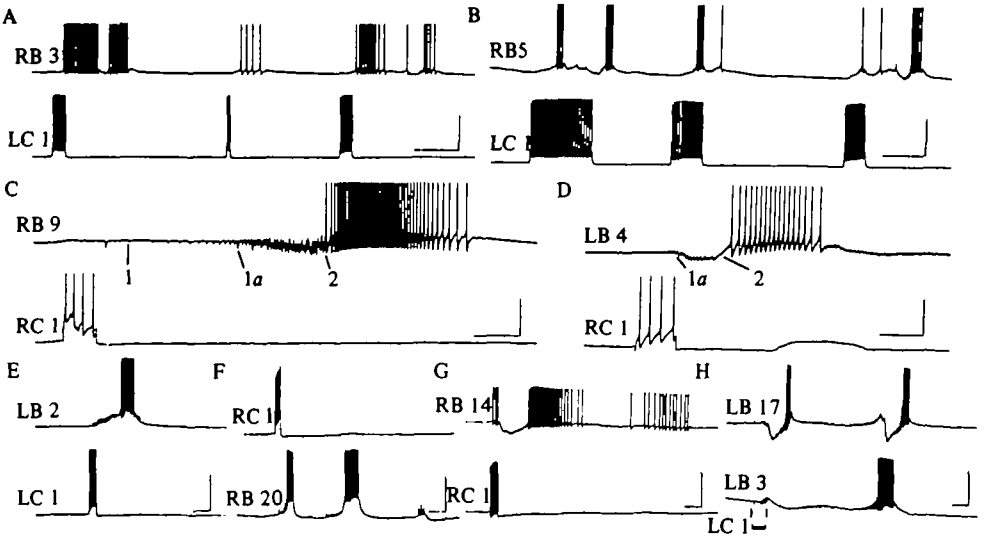

Fig. 8. Motoneurone responses resulting from cerebral giant action on interneurones. A and B, Delayed bursts in two R cells following cerebral cell activation of Interneurone 2. Response is broadly proportional to number of cerebral cell spikes. C, A correctly phased sequence of interneurone action resulting from four spikes in the cerebral giant cell produces a burst in the R cell. D, Four spikes in the cerebral cell produce a short latency burst in which the Interneuron 1 input is reduced or missing. Interneuron 1 *a* is followed by 2. E, Activation of a P cell following Interneurone 1 discharge. F, Activation of a P cell is followed by a second, spontaneous cycle. G, The burst in RC 1 activates the F cell through the Interneurone 1 *a* input. This is followed by Interneuron 2 inhibition and the Phase 3 burst. H, Responses in an F (upper) and R (lower) cell following a cerebral cell burst. The first short latency cycle is followed by a complete spontaneous sequence. Calibration: 50 mV, 5 s.

Table 1. *Summary of properties of proposed interneurones*

	P cells	R cells	F cells	Phase in cycle
Interneuron 1	EPSP	IPSP or IEPSP	EIPSP or IEPSP	5–30 s before, to end of Phase 1
Interneuron 1 *a*	Large EPSP	Large IPSP	EIPSP/IPSP	During Phase 1
Interneuron 2	?	EPSP	IPSP	During Phase 2

feeding behaviour. Spontaneous cyclic movements occur in the absence of activity in the cerebral giants, and their precise role is at present uncertain.

The buccal interneurones

The correct sequencing of buccal mass activity has been shown to depend on three sources of synaptic input to the principal groups of motoneurones. The common inputs ensure contemporaneous activation of individuals within each group, which is assisted by electrical coupling between synergists. Experiments with isolated buccal ganglia indicate that the sources of this synaptic input are unidentified interneurones within the buccal ganglia. These have been designated Interneurones 1, 1 *a* and 2. Comparison of recordings from symmetrical pairs of motoneurones has indicated some common and some separate inputs, and it is therefore proposed that each ganglion has its own set of interneurones which synapse on ipsilateral and contralateral motoneurones. Whereas Inputs 1 and 1 *a* may represent the output of single pairs of interneurones,

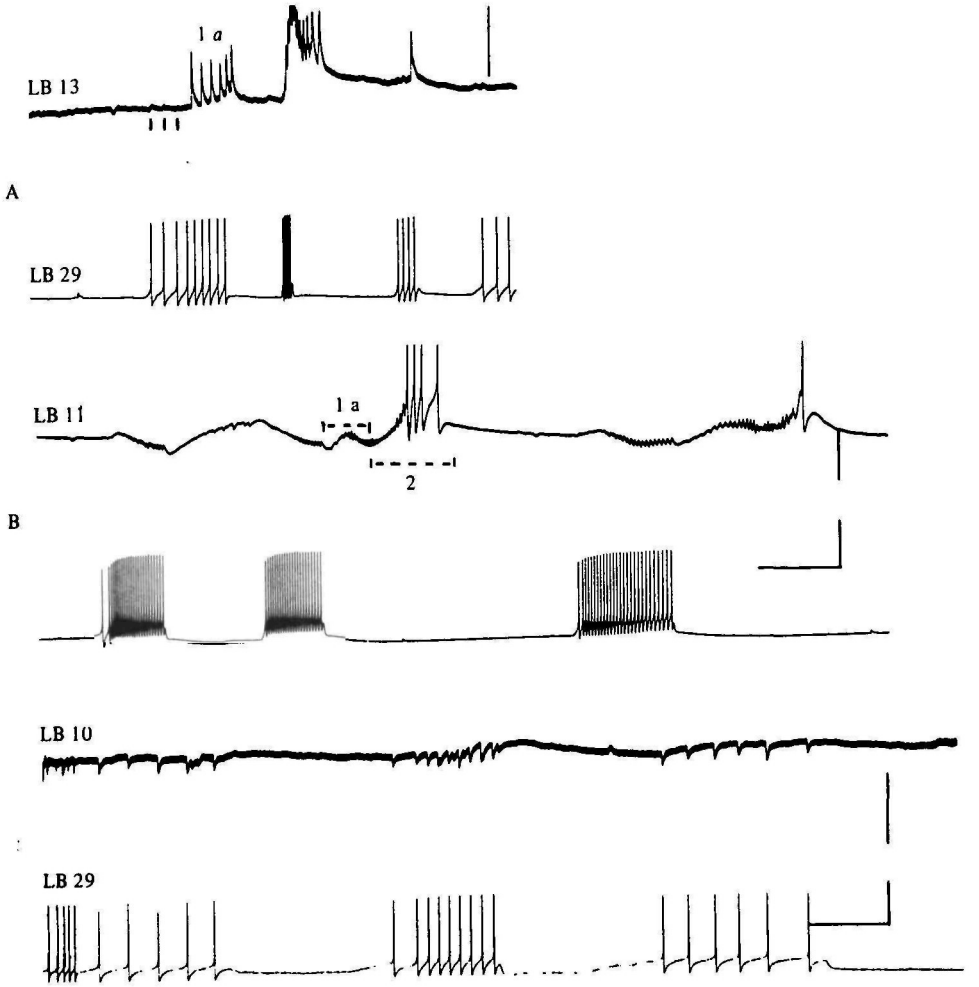

Fig. 9. Interneurone 1, candidate LB 29. A, Sequence showing how LB 29 (lower) has a small amplitude EPSP in the P cell (upper), but activates a large EPSP polysynaptically. The large p.s.p. may be Interneuron 1 *a*. B, LB 29 produces a monosynaptic IPSP in the R cell (upper), but is followed by what appears to be a typical 1 *a* inhibitory input. C, LB 29 has a monosynaptic inhibitory input to another R cell. Calibration: (A) upper 10 mV, lower 20 mV; (B) 20 mV; (C) upper 10 mV, lower 20 mV. Horizontal, 5 s.

there is some evidence to suggest that Input 2 may be derived from two or more closely coupled sources with each ganglion (Bulloch, 1977), which synapse independently with the motoneurone groups. Such an interneurone–motoneurone model is in general agreement with those suggested by Kater (1974) and Berry (1972*b*). Extensive searches for the interneurones have been made both in the isolated ganglia and the semi-intact preparation. Candidate interneurones were required to have multi-action, chemically mediated synapses on the P, R and F motoneurones, in a manner consistent with Table 1. They should also generate the patterned activity seen in spontaneous and induced feeding cycles. Although no neurones were found which satisfied the criteria entirely, a few showed some properties required of the prospective interneurones.

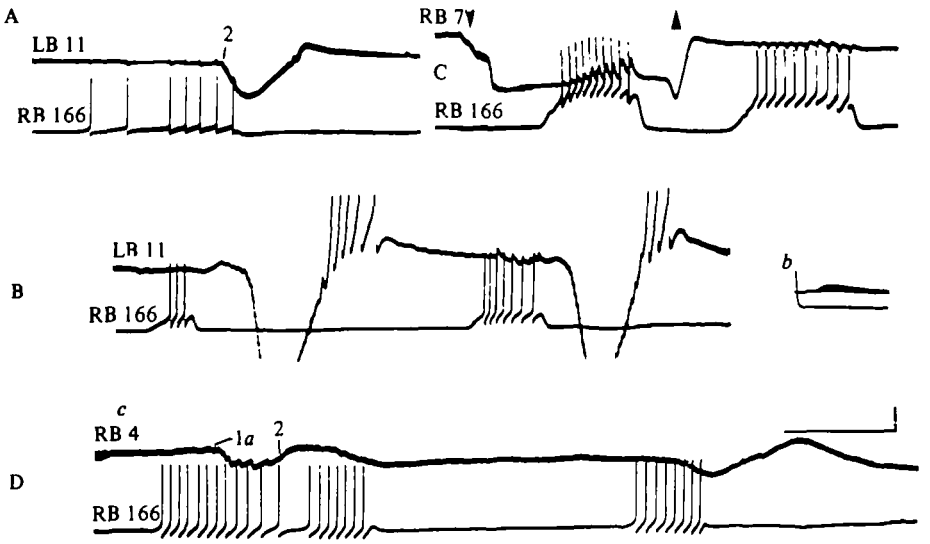

Fig. 10. Interneurone 1, candidate RB 166. A, EIPSP in an F cell. B, Complete buccal cycles following RB 166 stimulation. The inhibitory component of the p.s.p. is not obvious at this time (*b*). Sweep triggered on spike showing facilitating p.s.p. with latency of 20 ms. C, IEPSP in an R cell. When hyperpolarized (arrows) the p.s.p. converts to a facilitating EPSP. D, Interaction with the R cell, RB 4. Although the p.s.p. is not resolved, 166 activates both inputs 1*a* and 2. Calibration: 50 mV, 5 s.

Interneurone 1, *candidates LB* 29, *RB* 166

This neurone is a small unidentified cell in the region LB G-4, which made monosynaptic connexions with P and R cells. An impulse train in LB 29 generated a typical Phase 1 burst in the P cell LB 13, accompanied by jaw opening movements (Fig. 9A). Hyperpolarization of the LB 13 membrane revealed a small EPSP having 1 : 1 relationship with the spike in LB 29, which is rapidly obscured by a larger polysynaptic p.s.p. which, at normal levels of membrane potential, generates the Phase 1 burst. LB 29 also formed inhibitory synapses with the R cells LB 10 and 11 (Fig. 9B, C). Termination of an impulse train in LB 29 is followed by a second inhibitory input and a depolarizing wave, characteristic of Inputs 1*a* and 2. Further observations on this neuron were curtailed before its behaviour during spontaneous cycles could be established.

Another small neurone RB 166, located in area F-4 of the buccal ganglion, synapsed with both ipsilateral and contralateral motoneurones. Upon initial penetration, it produced an EIPSP in an F cell, which subsequently converted to an EPSP, probably due to the escape of Cl^- from the electrode. It also formed synaptic contacts with R cells, producing an IEPSP with a facilitating excitatory component in RB 7 and a small amplitude IPSP in RB 4. A few spikes in RB 166 consistently activated Interneurones 1*a* and 2, and the cell itself was inhibited slightly by Interneurone 2 (Fig. 10).

Interneurone candidate, L–RB-W

These neurones are a symmetrical pair of small cells, 30 μm in diameter, located in the area F-3 on the dorsal aspect of the ganglion. They are distinguished from their

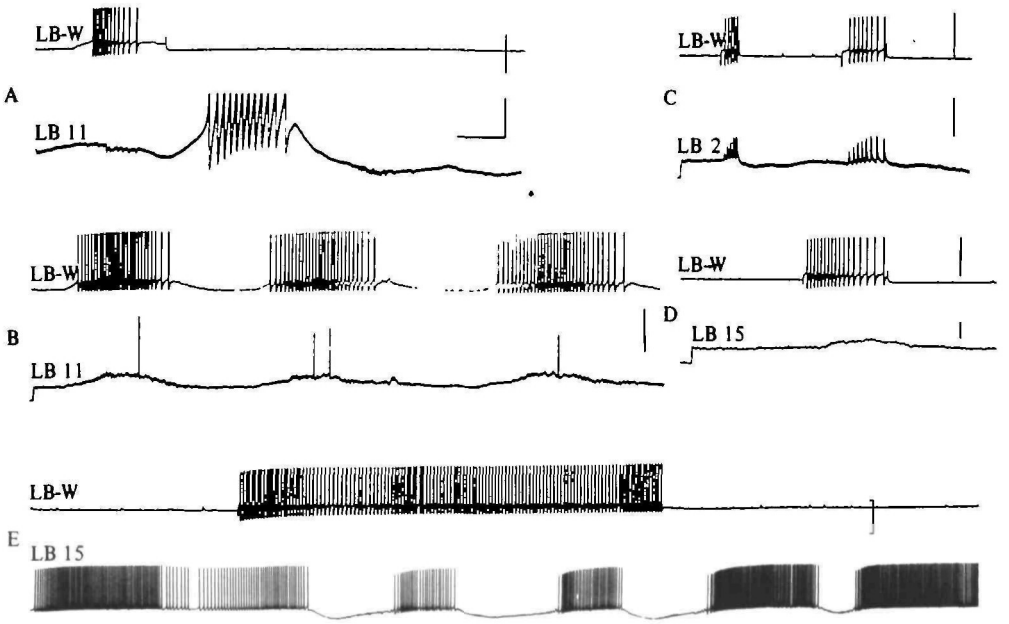

Fig. 11. Interneurone 1*a*, candidate LB-W. A, LB-W inhibits the R cell (lower) and generates and Input 2 burst. B, A prolonged discharge induced in the W cell generates an e.p.s.p. discharge with occasional spikes in the same R cell. This may be from Interneuron 2. C, The W cell produces a large facilitating EPSP in the P cell, LB 2. D, the W cell burst produces a polysynaptic depolarizing wave in the F cell. E, A prolonged burst in the W cell imposed a patterned discharge in the F cell by activating an inhibitory input (Input 2?). The F cell resumed continuous firing after the last inhibitory period. Calibration: (A) upper 50 mV, lower 10 mV; (B) 50 mV; (C, D) upper 50 mV, lower 10 mV; (E) 50 mV. Horizontal, 5 s.

neighbours by their greyish-white colouration. Successful recordings have been obtained on relatively few occasions, but these indicate the W cells are potent activators of co-ordinated buccal mass activity.

The W neurones establish monosynaptic connexions with all three principal motoneurone groups. The P cell, LB 2 responds with a large EPSP which strongly facilitates with repetition (Fig. 11 C), and the effect being still noticeable some 12 s later. However, the EPSPs do not appear to generate the typical Phase 1 burst at the frequencies characteristic of Interneurone 1*a* discharge.

LBW also made a large EPSP and A spike with LB 17, which in this instance showed the impulse pattern associated with an M cell. Monosynaptic connexions have not been demonstrated with F cells, although a pulse train in the W neurone produces a depolarizing wave after a latency of 1–2 s (Fig. 11 D).

The response in R cells is more complex (Fig. 11 A, B), single impulses in LBW resulting in a monosynaptic IPSP which may have a second, slower inhibitory component. The latter is not conspicuous with single impulses, but a spike train hyperpolarizes the R cell by 10–12 mV, perhaps by summation of the slower inhibitory potential. The inhibition is followed by a spontaneous depolarization and spike burst in the R cells, presumably resulting from the activation of Interneurone 2. On two occasions the W cell produced a depolarizing p.s.p. in an R cell, once when the membrane potential of the postsynaptic cell was unusually high (reversed i.p.s.p.?)

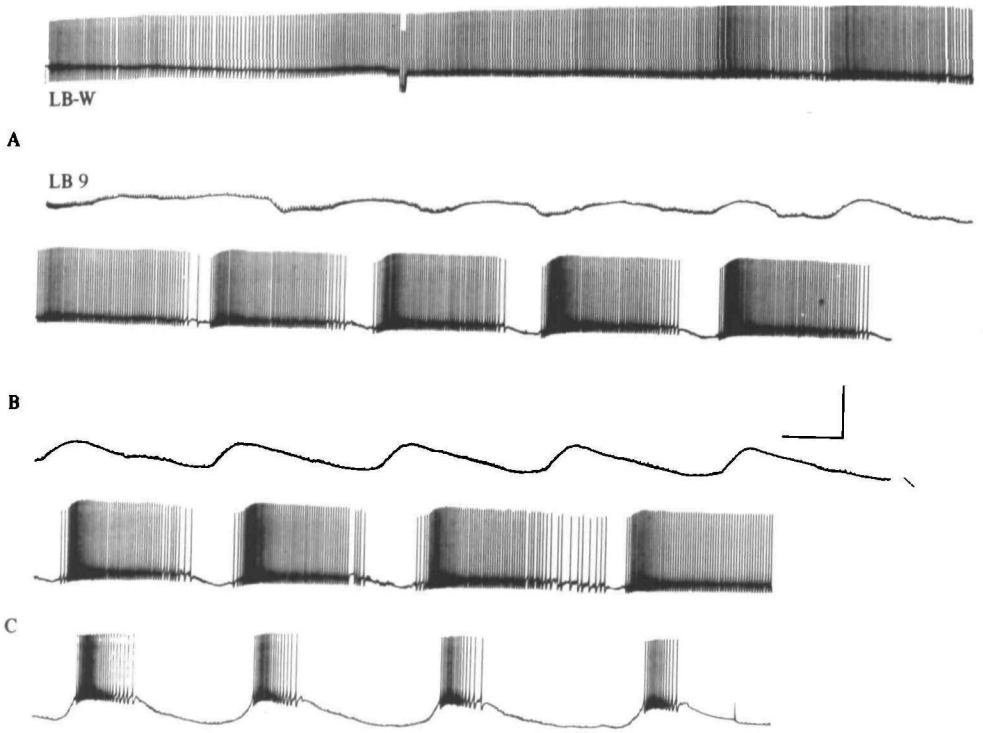

Fig. 12. Continuous discharge in the W cell leads to the build up of depolarizing waves in an R cell (A). These increase in amplitude (B) to become a typical Phase 2 discharge (C). At the same time the W cell is periodically inhibited to produce patterned bursting. The W cell burst leads the depolarization in the R cell. Calibration: 50 mV, 5 s.

and once when the R cell appeared to be weakly coupled to the W cell. A prolonged discharge in the W cell, following the initial penetration, gives way after some 40 s to a phase of patterned bursting, which corresponds to the development of depolarizing waves in an R cell monitored simultaneously with a second electrode. These waves ultimately developed into spike bursts in the R cell, following the peak impulse activity in the W cell (Fig. 12).

An impulse train in LB-W also led to patterned bursting in an F cell, LB 15, which had previously been firing continuously (Fig. 11 E). The F cell pattern resulted from a periodic inhibitory input activated by the W cell discharge (Interneurone 2 ?), although less prolonged stimulation of the W cell at a time when the F cell was inactive revealed a second weak excitatory pathway (Fig. 11 D).

Thus the white neurones inhibit the R cells, excite P cells and activate other neurones which resemble Interneurone 2 in their action. Preliminary results from more recent experiments indicate they also activate Interneurone 1 *a*, are themselves inhibited by Interneurone 2, and are weakly excited by the post-inhibitory rebound of some F cells.

DISCUSSION

The most obvious feature distinguishing the feeding mechanism of *Tritonia* from that of gastropods such as *Helisoma* or *Pleurobranchea* is the division of the buccal

cycle into three phases rather than two. Thus the patterned output is not derived from two alternating sets of protraction/retraction (eversion/withdrawal) motoneurones, but from the sequential activation of the P, R and F motoneurone groups.

Several lines of evidence suggest that the motor output to the buccal mass is derived mainly from the connexions and properties of the buccal motoneurones rather than from proprioceptive inputs. The most important of these comes from the study of the synaptic inputs to the motoneurones, which shows their activity is principally fashioned by three multi-action interneurone sources within the buccal ganglia. In contrast to the findings of other workers (Kater & Rowell, 1973; Seigler, 1977), synaptic inputs, such as those derived from proprioceptors, were not found during the present study, nor was afferent traffic detected in any centrally isolated buccal nerves during spontaneous activity cycles or manipulated movements of the jaws (Bulloch, 1977). On the other hand, mechanical stimulation of the oesophageal wall was a most effective way of stimulating the buccal cycle.

The present experiments have shown that Interneurone 1 normally precedes and overlaps Interneurone 1*a*, the simplest interpretation being that the former excites the latter after attaining a threshold frequency. The mechanism involved in the transition from Phase 1 to Phase 2 activity is less certain. The 1*a* input to the P cells normally terminates abruptly, coinciding with the onset of the LIW in F cells and the depolarization of R cells caused by Interneurone 2, suggesting that the Phase 1 interneurones are inhibited by Interneurone 2. However, occasionally Interneurone 1*a* activity may overlap Interneurone 2 by 1–2 s.

The sequential activation of Interneurones 1, 1*a* and 2 could be governed by reciprocal inhibitory connexions, combined with properties such as post-inhibitory rebound in Interneurone 2, which would result in its excitation following the discharge of the Phase 1 interneurones. There is the additional possibility of feedback from the motoneurones, corollary discharge neurones or proprioceptors to the interneurones themselves, which could also contribute to the phase mechanism timing the buccal cycle (Spira & Bennett, 1972; Gardner, 1977; Gillette & Davis, 1977).

Despite extensive searches no cells were found which could be unequivocally identified as having all the required properties of the interneurones, but several cells were found which showed some of their characteristics.

The two small unidentified cells RB 166 and LB 29, are multi-action neurones having many features of Interneurone 1, including the ability to activate Interneurones 1*a* and 2 in their proper sequence. LB 29 had a burst pattern similar to an F cell, and both were strongly inhibited by Interneurone 2. The opportunistic nature of recording from such small cells, prevented the acquisition of all the information required to establish their role as interneurones.

The role of the W cells is not fully understood. Their synaptic contacts with the P and R cells may be identified with Interneurone 1*a*, but their most interesting property is the ability to impose a patterned output on the F and R group through the apparent activation of Interneurone 2. In doing so, the W cells develop a patterned discharge themselves with a maximum spike frequency in advance of the peak depolarization of the R cells, suggesting that their activity leads Interneurone 2 rather than forming part of it. Further studies of these cells are in progress.

The role of the cerebral giant cells

The cerebral giant cells of *Tritonia hombergi* appear to be homologues of similarly placed neurones in a number of other gastropods, in that they synapse with neurones in the buccal ganglia, have axons in the buccal nerves and contain serotonin (Weinreich *et al.* 1973; Gerschenfeld & Paupardin-Tritsch, 1974; Cottrell & Macon, 1974; Gillette & Davis, 1977; Berry & Pentreath, 1976; Gelperin, 1975).

They interact with the buccal motoneurones at two levels; by direct synaptic contacts with certain P, R and F cells, and indirectly by activating the three interneurones that generate the motor output. Their involvement in the feeding behaviour is slightly ambiguous, for while a few spikes or a slow iteration in the giant cells is capable of generating rhythmic activity in a previously inactive buccal mass, vigorous buccal activity may also occur when the cerebral cells are silent. No evidence has been found of phasic feedback to the cerebral giants from the motor network or during movements of the buccal mass, as reported for *Aplysia* (Kupfermann & Weiss, 1974) and *Pleurobranchaea* (Gillette & Davis, 1977). The direct effects of the cerebral giant cells on the buccal musculature (Pentreath, 1973; Weiss, Cohen & Kupfermann, 1975) has not been studied here, although the axon passes to the periphery through branches in the buccal nerves and in cerebral nerve 4.

In view of their effect on the buccal mass as a whole, part of their function appears to be to raise the level of excitability in the interneurone–motoneurone network controlling the feeding behaviour, and influencing its position in the behavioural hierarchy by their action. Further work is needed on the long term activity of these neurones and the nature of their synaptic contacts in the cerebral neuropil.

Functional aspects of the synaptic inputs

Several authors have recorded complex synaptic input potentials in molluscan neurones, but their functional significance has yet to be established (Gardner & Kandel, 1972; Fiore & Meunier, 1975; Shimahara & Tauc, 1975). Observations on the configuration of Input 1 in F cells has suggested a possible function of the EIPSP. At its onset the inhibitory component predominates and may serve to terminate the Phase 3 burst from the previous cycle, ensuring relaxation of muscles 4 and 5 before the next one. As the EIPSP accelerates towards Phase 1 the F cell if often slightly depolarized, due either to facilitation of the excitatory component, desensitization of the inhibitory one, or both. In either case it may prepare the way for the short Phase 1 burst. The low amplitude inhibitory potentials recorded from R cells from Interneurone 1 and the IEPSPs from interneurone candidate RB 166 and the cerebral giant cells have no obvious function other than to ensure relaxation of the jaw closer muscles and the radula retractors during Phase 1.

The Phase 2 burst in R cells is often terminated by a series of IPSPs during Phase 3 (Figs. 4A, B, 12A), which ensure relaxation of the jaw closer and radula retractor muscles. The source of this inhibitory input might be Interneurone 1 *a* or from contacts with F cells such as LB 15 (Fig. 7). The significance of the contacts on the large R cells 4 and 5 might be that these cells could provide more current to repolarize smaller members of the group through electronic junctions.

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