J. exp. Biol. **122**, 237–256 (1986)
Printed in Great Britain

THE MORPHOLOGY AND PHYSIOLOGICAL PROPERTIES OF THE SMALL WHITE NEURONES IN THE BUCCAL GANGLIA OF *TRITONIA HOMBERGI*

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Accepted 2 December 1985

SUMMARY

The two small white (W) cells in the buccal ganglia of *Tritonia hombergi* can initiate and modulate cyclic activity in the pattern generating neurones which drive feeding activity in the buccal mass.

They also make extensive monosynaptic connections with the buccal motoneurones, generating EPSPs on protractor (P) cells, IPSPs on retractor (R) cells, and EIPSPs on the small radula flattener (F) cells. Two F motoneurones receive a chemically mediated, facilitating EPSP from the W cells.

Inactive W cells receive weak excitatory feedback from the pattern generating network interneurones (FPG) in phase I of the feeding cycle and also from some F cells. Prolonged depolarization of one W cell recruits the other. When both are active they adopt a patterned burst mode with a common inhibitory input in phase I.

INTRODUCTION

The neural mechanisms which control the rhythmic cycle of activity shown by the buccal mass and structures associated with the mouth during the capture, ingestion and swallowing of food have been studied in a variety of pulmonate and opisthobranch molluscs (Kater, 1974; Siegler, Mpitsos & Davis, 1974; Woollacott, 1974; Bulloch & Dorsett, 1979*a*,*b*; Rose & Benjamin, 1979).

In *Tritonia hombergi* the buccal mass undergoes three phases of activity during the feeding cycle. The first phase, protraction, is followed by two phases of retraction – radula retraction and flattening – each phase being driven by groups of identified motoneurones in the buccal ganglia called the P, R and F cells (Bulloch & Dorsett, 1979*a*). Essentially similar sequences have been reported in the closely related species *T. diomedea* (Willows, 1978) and in the pulmonate *Lymnaea* (Goldschmeding & DeVlieger, 1975; Rose & Benjamin, 1979), the individual differences between these genera probably reflecting adaptations for dealing with the different physical nature of the food.

In *Tritonia hombergi* the patterned output of the buccal motoneurones is primarily determined by common inputs from three multi-action interneurones whose activity can be recognized by their characteristic and synchronized synaptic input to the

Key words: *Tritonia*, feeding interneurones, modulation.

motoneurone groups (Bulloch & Dorsett, 1979*b*). In *Lymnaea* similar pattern generating neurones have been identified by direct recording (Rose & Benjamin, 1981). Other sources have been found to influence the buccal motor network either by direct synaptic input or by their action on the pattern generating interneurones (FPGs). These include both mechanoreceptors and chemoreceptors in the anterior gut (Goldschmeding & Jager, 1973; Kater & Rowell, 1973; Audesirk, 1979; Audesirk & Audesirk, 1980; Dorsett & Sigger, 1981). In *T. diomedea* a presumed buccal motoneurone may also initiate and modulate activity in the pattern generating network (Willows, 1980).

In the search for the pattern generating interneurones in *T. hombergi*, a number of interneurones were found whose properties could not be fully characterized as part of the pattern generating network yet whose action reliably initiated feeding activity from the motoneurones (Bulloch & Dorsett, 1979*b*). Among these were two small white cells which are often visible on the dorsal surface of the buccal ganglia. These interact with the motoneurones either by direct synaptic connections, indirectly through the pattern generating interneurones, or both. White cells with some comparable properties have also been reported from the buccal ganglia of *T. diomedea* (Willows, 1980; Masinovsky, Lloyd & Willows, 1985) and *Pleurobranchaea* (Gillette & Davis, 1977; Gillette, Gillette & Davis, 1980, 1982). Other interneurones such as the paired cerebral giant cells also influence the feeding output pattern in *T. hombergi* and other molluscs (*Pleurobranchaea*, Gillette & Davis, 1977; *Lymnaea*, McCrohan & Benjamin, 1980) but are not always active during spontaneous bouts of feeding activity. In *Aplysia* these cells have been implicated in the arousal associated with feeding behaviour (Kupfermann & Weiss, 1982).

MATERIALS AND METHODS

Details of the head–buccal mass preparation used in these experiments were as in a previous paper (Bulloch & Dorsett, 1979*a*). The buccal mass and peri-oral region were dissected out and supported on a wax-covered platform inserted down the oesophagus. The buccal ganglia were stabilized by pins pushed through the oesophageal wall into the wax. Apart from cutting the peripheral pleural and pedal nerves, the circumoesophageal ganglionic complex was not disturbed. The white neurones are normally located in the area F3–4 on the topographical map given previously (Bulloch & Dorsett, 1979*a*). Details of cell morphology were obtained by dye-iontophoresis using microelectrodes filled to the tips with a 4% solution of Lucifer Yellow CH layered with a 1% solution of lithium chloride. The initial resistance of electrodes used was in the range of 40–50 MΩ. Dye was injected using currents of 10 nA until the cell and axon could be distinguished when viewed through a dark blue filter. Some preparations were left overnight at 5°C to allow the dye to diffuse. For drawing and photography the ganglia were mounted in 120% sea water and viewed by u.v. light under a Leitz Dialux 20 microscope fitted with a B12 excitation filter and a K515 barrier filter. The hypertonic saline helped to flatten the ganglia by partially dehydrating the living cells and considerably improved the

resolution of fine processes arising from the axons. Identification of the white cells was confirmed physiologically in each case by simultaneous recording with a second electrode from the neurones LB8 or RB8 which receive powerful excitatory input from the white cells.

For most experiments intracellular recordings were made from three buccal neurones simultaneously in a combination chosen from the two white cells and a pair of identified motoneurones. Sequences of activity from selected combinations were stored on separate tracks of a Racal Store 14DS tape recorder. Paired interactions between W cells and motoneurones were routinely checked for electrotonic coupling by the application of hyperpolarizing and depolarizing currents to the soma of each of the pair. Other interactions were considered to be monosynaptic when an EPSP followed the presynaptic impulse 1:1 at frequencies up to $10\,s^{-1}$, with short but constant latencies of 6–10 ms for ipsilateral combinations and in the region of 12–20 ms for contralateral ganglionic pairings (Fig. 3). These criteria have been considered as strong evidence for monosynaptic connections (Kandel, 1976). Further tests such as the introduction of high Mg^{2+}–low Ca^{2+} in the bathing solution were not possible in the through-flow sea water bath used in these experiments.

RESULTS

White cell morphology

The white cells are between 20 and 30 μm in diameter. The colouration is variable, ranging from an obvious white to an indeterminate grey tone. The cell lies below the surface and can be difficult to penetrate as the electrode pressure may displace it to one side. Eight white neurones were filled with Lucifer Yellow CH and showed extensive diffusion into the axons. The white cells are bipolar neurones, the axons arising separately but close together on the medial surface of the soma (Fig. 1A,B). The two axons are consistently seen to be of unequal diameter, the larger following the central axon tracts to enter the ipsilateral gastro-oesophageal nerve in which it continues without branching until just below the gastro-oesophageal ganglion. Here it divides, the larger branch entering a nerve which passes to the oesophageal wall and the finer branch continuing through the ganglion to enter the most medial nerve on its distal border (Fig. 1A). The structures innervated by these nerves are not known. Other small branches arise from the main axon just before it enters the gastro-oesophageal nerve, and terminate in an arborization in the neuropile where axons enter and leave the ganglion through the trunk formed by the fused bases of the buccal nerves 2, 3 and the cerebrobuccal connective (CBC).

The smaller diameter axon passes towards the buccal commissure; in three preparations the axon underwent a characteristic flexion in the neuropile some 100 μm from the soma. A number of fine processes arise from this segment of the axon, generally directed posteriorly and towards the areas of the neuropile occupied by the dense dendritic arborization of the motoneurones, (Fig. 2A–C), but extensive branching in this region was seen in only two preparations. On entering the

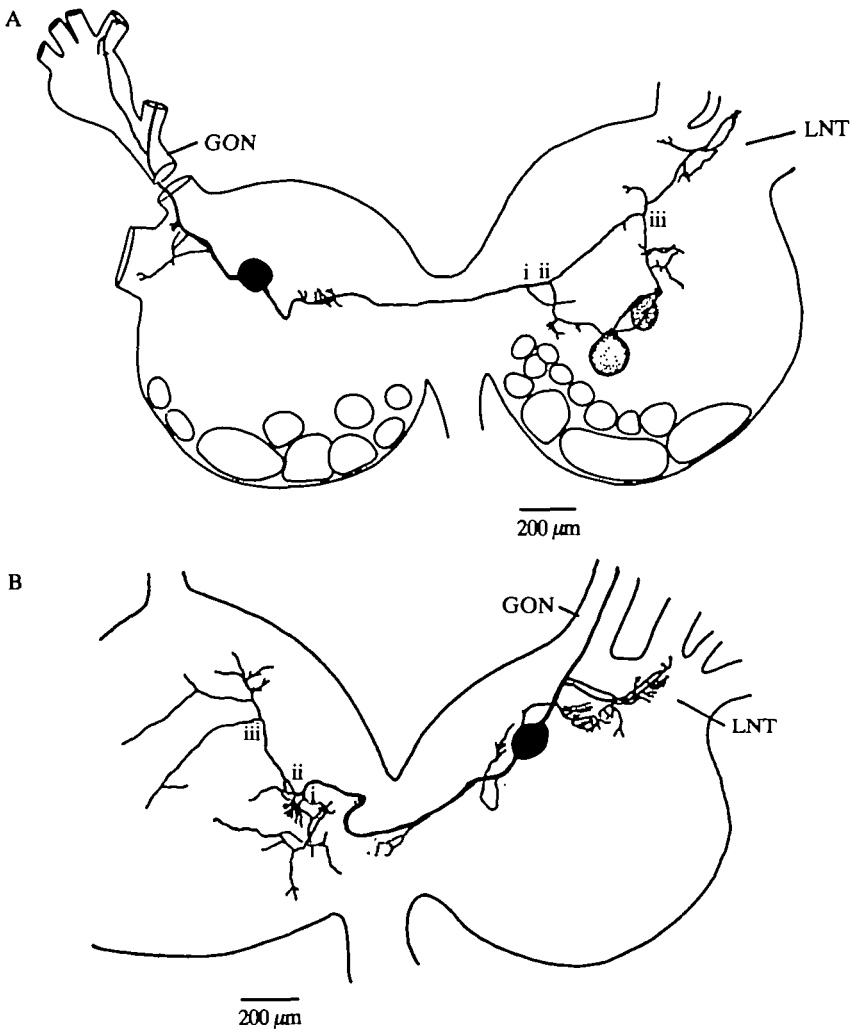

Fig. 1. Drawings of Lucifer Yellow preparations of W cells. (A) Cell in left buccal ganglion. The broad axon in the left gastro-oesophageal nerve (GON) enters small branches, one just below and the other just distal to the ganglion. Both axons branch in the region of the lateral nerve trunk (LNT) and three prominent branches arise from the axon in the opposite ganglion (i, ii, iii). The dotted outlines represent two neurones which are dye-coupled to the W cell in the opposite ganglion. (B) The W cell of the right side. Numbers refer to previously identified cells.

contralateral ganglion the axon passes towards the lateral nerve trunk where it terminates in a small arborization comparable to that of the other side. In all preparations three major branches were seen arising from this axon as it crossed the contralateral neuropile, directed towards and overlapping the areas occupied by the dense dendritic arborizations of the motoneurones grouped along the posterior border of the ganglion (Fig. 2; and see Bulloch, 1977). These processes show both secondary and tertiary branching but the fine arborizations characteristic of the postsynaptic motoneurones were never observed. Two preparations of W cells

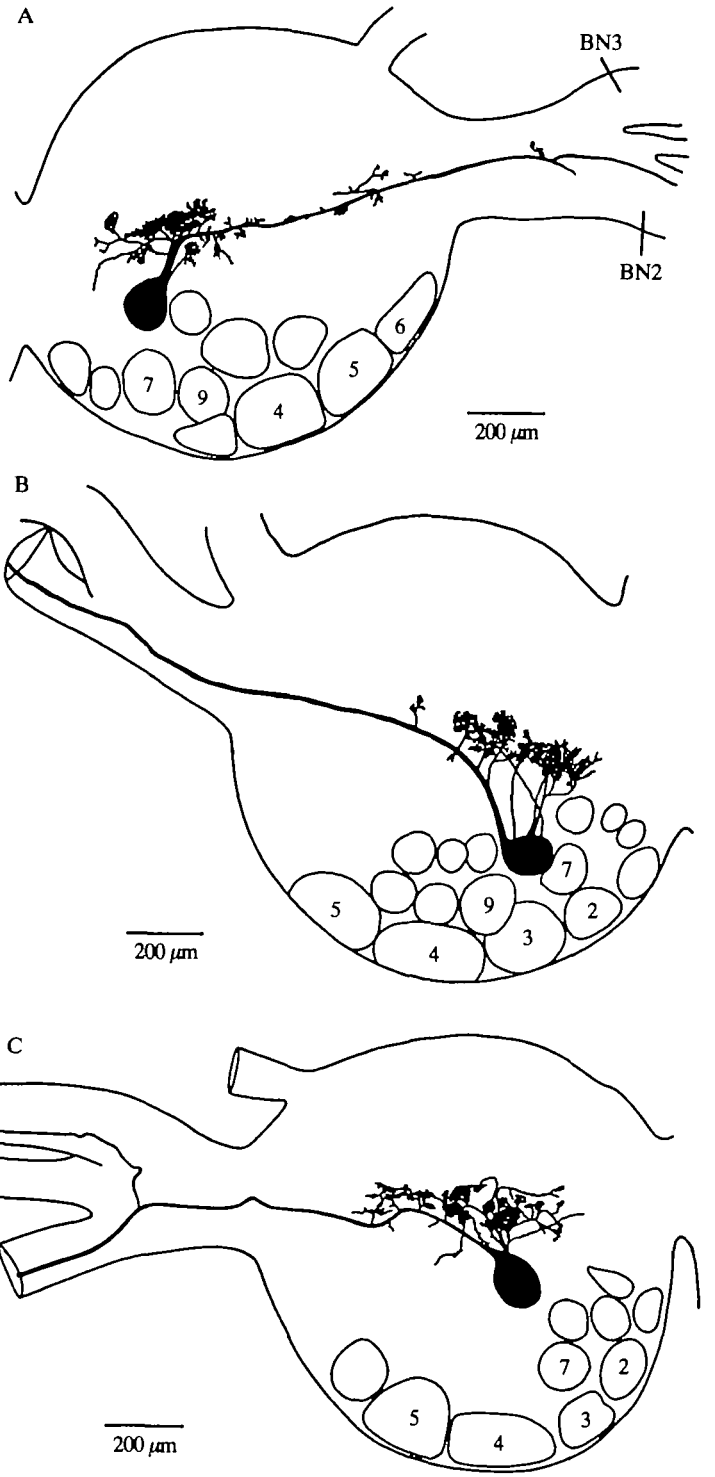

Fig. 2. Drawings of the F cells B8 and B81. (A) Lucifer preparation of RB8 showing branched dendritic processes arising from the soma and the proximal axon. These divide and become finely varicose so that the neurone appears to have a luminous cloud in a specific area of the neuropile. (B) LB8. In this preparation four neurites arise from the soma and together with small branches from the proximal axon form a dendritic field in the neuropile in an area corresponding to that of RB8. (C) Lucifer preparation of LB81. The morphology is generally similar to the previous neurones but the dendritic tree arises mainly from branches of a stem process alongside the main axon. The axon divides to enter BN2 and BN3. Numbers refer to previously identified cells.

examined after allowing some 12 h for dye diffusion to occur revealed faint outlines of one or two cell bodies in the opposite ganglion which were apparently dye-coupled to the white cell (Fig. 1A). No corresponding cells were seen on the same side. Dye-coupling has frequently been associated with electrotonic coupling between cells, but numerous recordings from all the accessible surface neurones in the area failed to reveal any that were electrically coupled to the contralateral white neurone.

Morphology of neurones B8, B81

Two F-type neurones in each buccal ganglion respond monosynaptically to impulses in the W cells with large, facilitating EPSPs (Fig. 3A–F). One pair of these neurones corresponds topographically to the identified cells B8, while the other pair B81, located in area D-6 (Bulloch & Dorsett, 1979*a*, fig. 3), were not previously specified. LB8 and RB8 are monopolar neurones around 100 μm in diameter (Fig. 2A,B). The single axon penetrates the neuropile and turns laterally to enter buccal nerve 2 (BN2) on the same side. Between two and four fine neurites 2–300 μm long arise directly from the soma, passing centrally into the neuropile where they undergo terminal branching to form a three-dimensional array of fine varicose processes whose exact configuration is difficult to resolve (Fig. 2A,B). A number of similar processes arise from the initial 2–300 μm segment of the axon to occupy adjacent areas of the neuropile. It is assumed that these structures represent the principal synaptic areas of the neurones, as little or no branching occurs in more distal regions of the axon within the ganglion. The morphology of LB81 and RB81 generally corresponds to that of the B8 neurones, except the axon divides in the lateral nerve trunk to enter both BN2 and BN3. In this pair the finely divided varicose branches tend to arise from a single thicker stem process adjacent to the main axon (Fig. 2C), and the fluorescent cloud they present in Lucifer Yellow preparations occupies a more central area of the neuropile.

W cell interactions with protractor motoneurones (P cells)

It has been reported that the left white cell (LBW) generates a facilitating EPSP on the ipsilateral P motoneurone LB2 (Bulloch & Dorsett, 1979*b*). Subsequent recordings from this and other members of the P cell group have confirmed that the P cells 2, 6 and 13 receive 1:1 EPSPs with a short and constant latency from both white cells (Fig. 3H). In LB2 and LB6 the amplitude of the EPSP from the ipsilateral white cell is 0·5–1 mV which summates at frequencies above 3 s^{-1} but does not markedly facilitate (Fig. 4A,B). LB13 receives a very small EPSP from LBW but at impulse frequencies above 3 s^{-1} a second interneurone input appears which closely follows the firing pattern in the W cell, but has a latency of 700 ms (Fig. 4C,D). This is too long for a monosynaptic connection. A prolonged depolarization of the W cell (Fig. 4E) can lead to patterned bursting in P cells which continues after the W cell activity has terminated, resulting from activation of the synaptic inputs associated with the feeding pattern generating interneurones 1 and 1A described previously (Bulloch & Dorsett, 1979*a*).

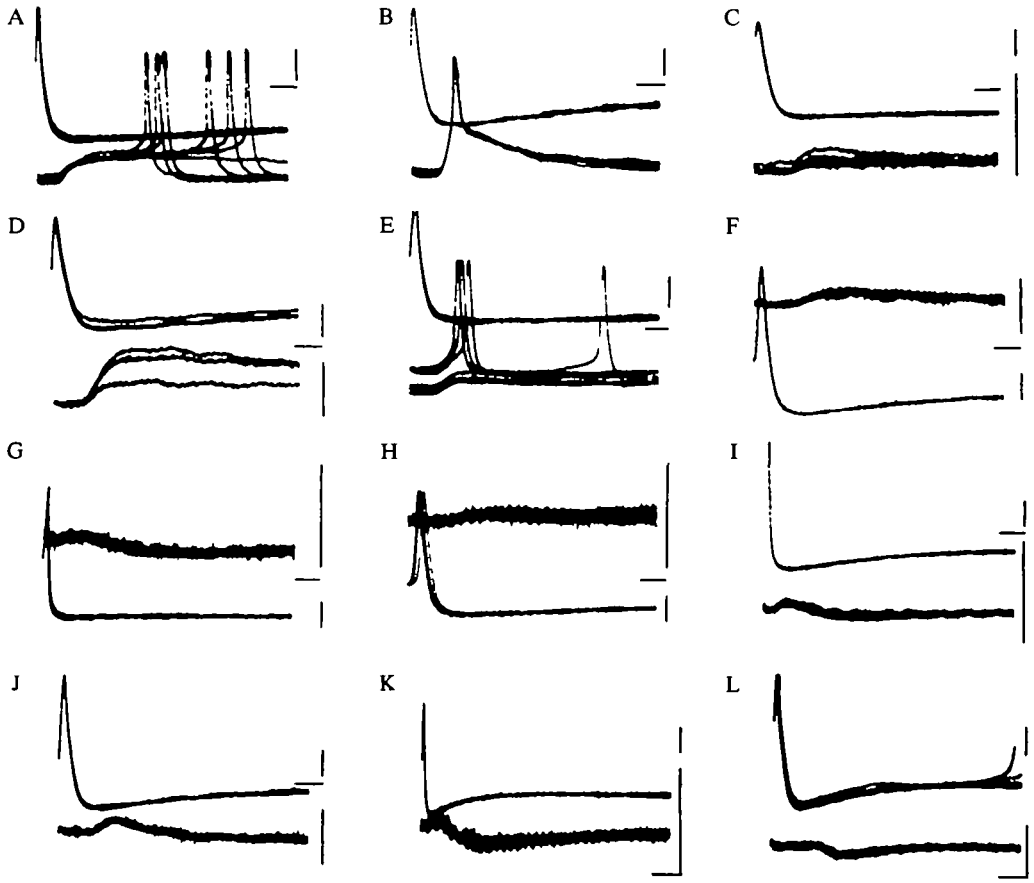

Fig. 3. Monosynaptic connections between buccal motoneurones and the W cells. The sweeps are triggered on the W cell spike. Each record consists of not less than seven successive sweeps superimposed to show constant latency of the PSP. (A) RW and R8 showing 6 ms short latency ipsilateral EPSP. (B) LW and L8. (C) LW and R8 10 ms contralateral EPSP. (D) RW and R81 showing facilitation of the EPSP. (E) LW and L81. Lowest trace shows EPSPs while that above shows EPSP and spikes. (F) LW and R81. (G) LW and L15 showing an EIPSP in an F motoneurone. (H) RW and R2 (P motoneurone) showing an EPSP with an 8 ms latency. (I) RW and R1 (an R motoneurone) showing an EIPSP with a 5 ms latency. (J) RW and the F cell L10 showing an EIPSP with latency of 11 ms. (K) RW and F cell L19 showing an EIPSP with latency of 10 ms. (L) RW and L4 (R motoneurone) showing a simple IPSP. Scales: vertical, 20 mV; horizontal, 10 mV except K, 50 ms and L, 20 ms.

Interactions with retractor motoneurones (R cells)

The action of the W cells on members of the R group motoneurones is more complex than that on the P cells. In quiescent preparations individual impulses in the W cells result in monosynaptic IPSPs in B7 and B9 (Figs 3I–L, 5A,B), but in B4 and B5 at spike frequencies above $3\,s^{-1}$ these summate to produce a 3–5 mV hyperpolarization of the cell membrane and often involve the recruitment of a second inhibitory interneurone (Fig. 5C,E). In each case the short latency inhibition is followed by a depolarization of variable amplitude with a latency of around 2 s,

probably due to the activation of interneurone 2 (see below). The R cell LB3 receives a monosynaptic EIPSP from the W cells, repetitive stimulation causing summation of the inhibitory component and a progressive hyperpolarization of 2–3 mV with an enhancement of the EPSP amplitude (Fig. 5D). Increasing levels of hyperpolarizing current applied to LB3 lead to reversal of the slow inhibitory wave (Fig. 5Fi–iii).

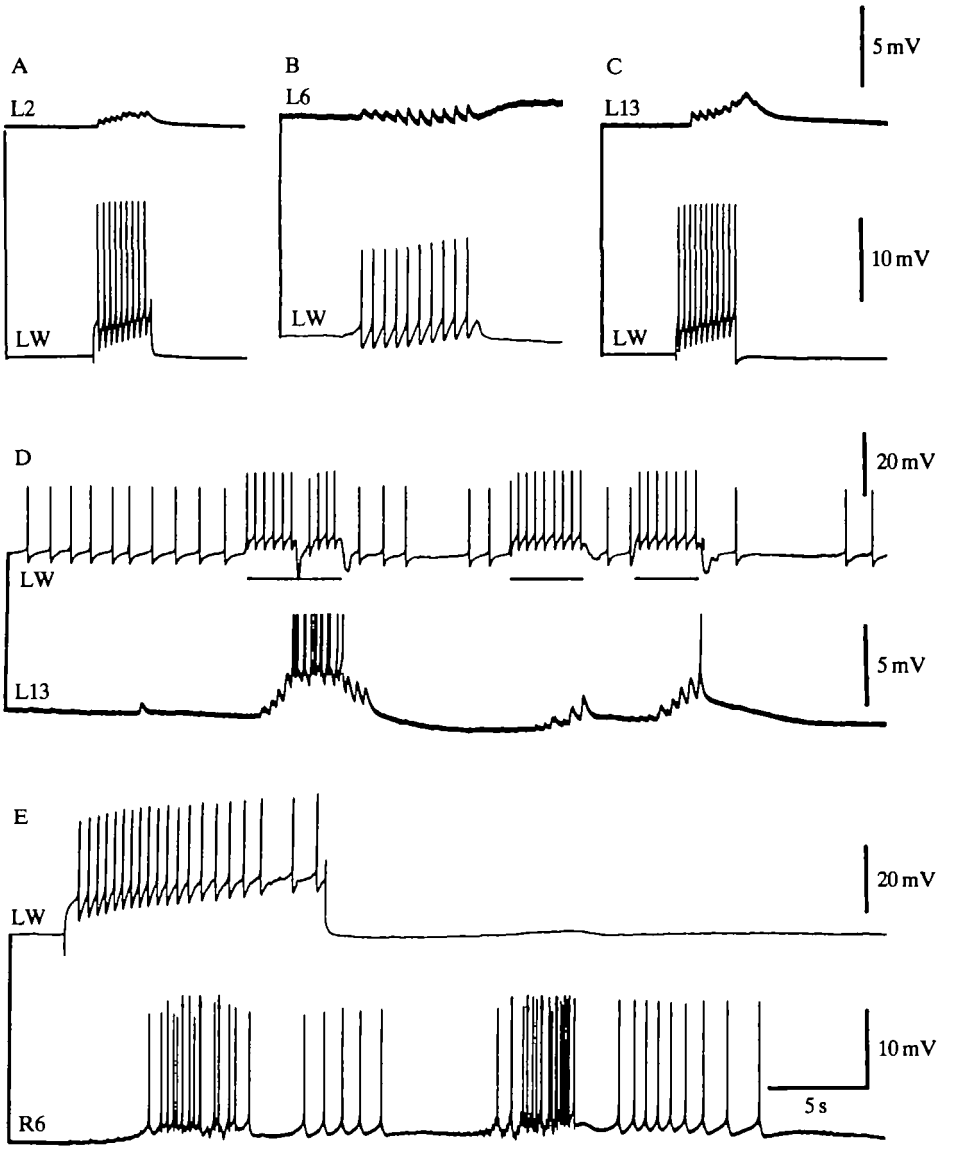

Fig. 4. W cell interactions with P group neurones. (A),(B) Monosynaptic EPSPs with L2 and L6. (C) Indirect excitation of L13. (D) Low frequency spiking reveals a small, possibly monosynaptic, 1:1 EPSP with L13. Higher frequencies evoke the larger EPSP (bar) which brings the cell to spike threshold and induces an IPSP in the W cell. This second indirectly evoked EPSP is from interneurone 1A of the FPG network. (E) A prolonged burst in a W cell induces a series of feeding cycle bursts in the P cell R6.

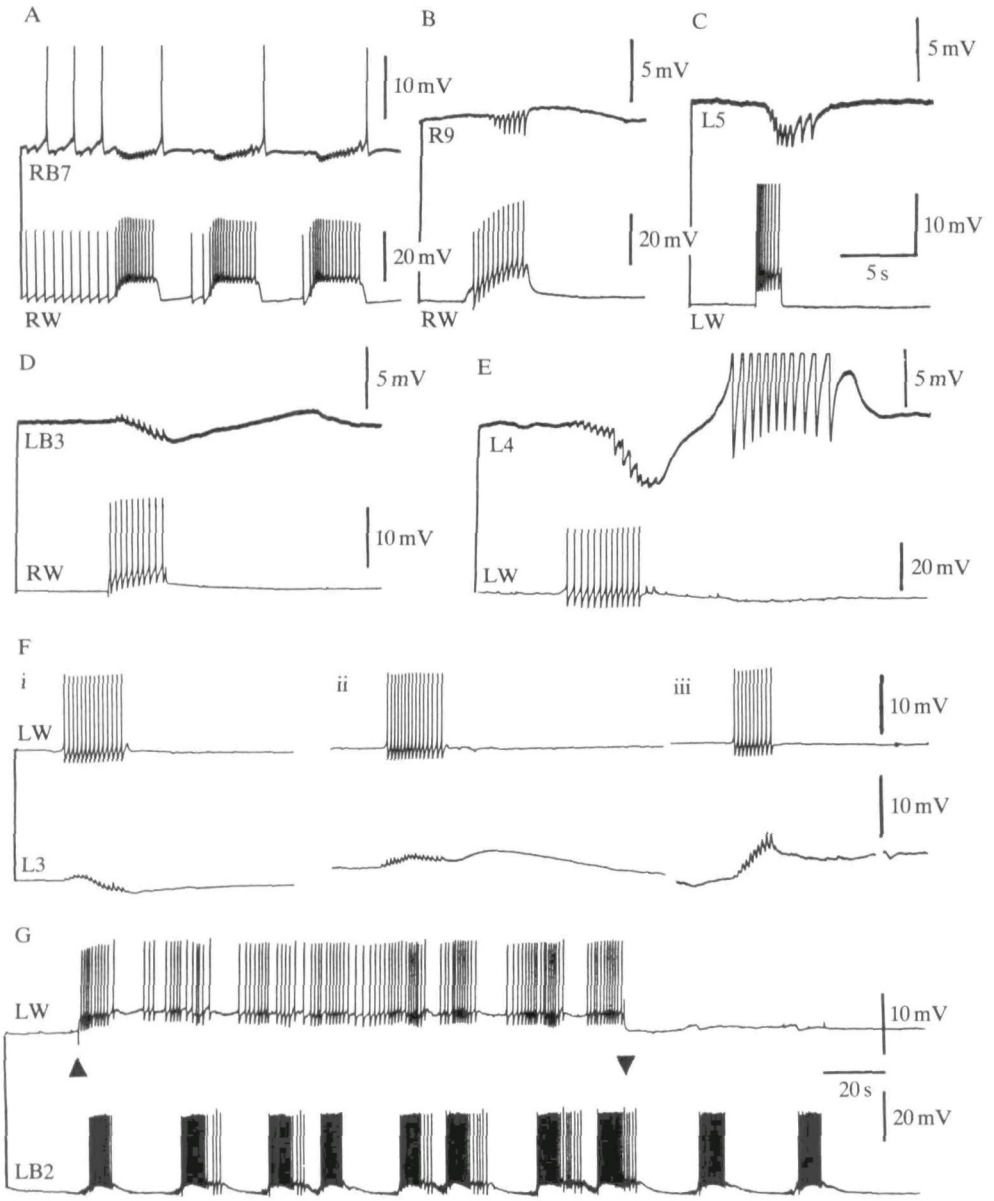

Fig. 5. W cell interactions with R cells. (A) Monosynaptic IPSP with R7. (B) Facilitating IPSP with R9. (C) A rapid burst evokes a possibly disynaptic IPSP in B5, which as a similar amplitude to the interneurone 1A input in this cell. (D) An EIPSP in L3. (E) A series of spikes produce small monosynaptic IPSPs in L4, but ultimately recruit a second larger IPSP followed by a large depolarizing wave and spiking. These long latency inputs are from interneurone 1A and interneurone 2 of the FPG. (F)(i) At normal membrane potential the inhibitory component of the L3 EIPSP gradually hyperpolarizes the membrane. (ii) Moderate hyperpolarizing current (2 nA) produces the equilibrium potential for the IPSP. (iii) Larger currents (5 nA) reverse the IPSP and enhance the EPSP. (G) Prolonged W cell depolarization generates typical feeding cycles in L2 by activating the FPG network. Note the development of patterned bursting in the W cell with inhibition during phase I of the cycle. A–F upper time scale.

The inhibition in R group neurones that follows a short burst of impulses in the W cells is frequently followed by a wave of depolarization that can lead to spiking (Fig. 5D,E). This is probably due to the activation of interneurone 2 in the FPG network, which drives R group neurones during phase II of the feeding cycle. Prolonged firing of a W cell leads to patterned bursting in the R group neurones in which the three types of synaptic input from the FPG network can be resolved (Fig. 5G). Termination of W cell activity is followed by a further two cycles in the pattern generating network which also has synaptic feedback to the W cell.

Interactions with the F group motoneurones

There is now some evidence that the F group motoneurones that fire during phase III of the buccal cycle can be divided into two physiological types. The majority of this group, including neurones B10–19 respond to impulses in the white cells with a small monosynaptic EIPSP having an amplitude of 1 mV (Fig. 3J,K). The inhibitory component summates at frequencies above $2\,s^{-1}$ to hyperpolarize the cell (Fig. 6A–C). As in the R group, a burst of impulses in the W cells often leads to delayed excitation of the F cells through the FPG network; the synaptic inputs from interneurones 1, 1A and the characteristic long inhibitory wave from interneurone 2 following in their proper sequence after a latency of several seconds (Fig. 6C). Prolonged depolarization of the W cells leads to the development of cycles of patterned bursting in the F group characteristic of the feeding sequence (Fig. 6D,E). At frequencies around $1\,s^{-1}$ one sometimes observes progressive activation of the FPG interneurone inputs, with the intensity of the interneurone 2 discharge increasing until its full amplitude is expressed, causing the F cell to fire by post-inhibitory rebound and overcoming the inhibitory influence of the W cell input. The effects of the FPG network on the W cell can also be observed as an inhibition in phase I and a vigorous burst extending from the onset of phase II into phase III (Fig. 6D,E). Normally no evidence is obtained of any feedback between the motoneurones and the W cells. On one occasion a long latency excitation feedback was found between LW and LB15. Bursts of impulses in the F cell evoked by depolarization, postinhibitory rebound or following interneurone 2 inhibition resulted in 1–2 impulses in the W cell after a delay of 2–4 s (Fig. 6F).

Interactions with F cells R8, R81

The F cells may be separated into two groups on the basis of their interactions with the W neurones. Two symmetrical pairs of F cells L-RB8, L-RB81 respond uniquely to single W cell impulses with large facilitating EPSPs having amplitudes of 1–2 mV (Fig. 7A,C). L8 and R8 are normally so responsive to W cell spikes that it is difficult to obtain isolated EPSPs even when the cells are strongly hyperpolarized; this property is particularly noticeable in the contralateral connections. The neurones L81 and R81 respond to W cell impulses with an EPSP which rapidly facilitates so that 1–2 impulses in either W cell are sufficient to bring both L81 and R81 to spike threshold. Under moderate hyperpolarization, and at a spike frequency of $3\,s^{-1}$ in

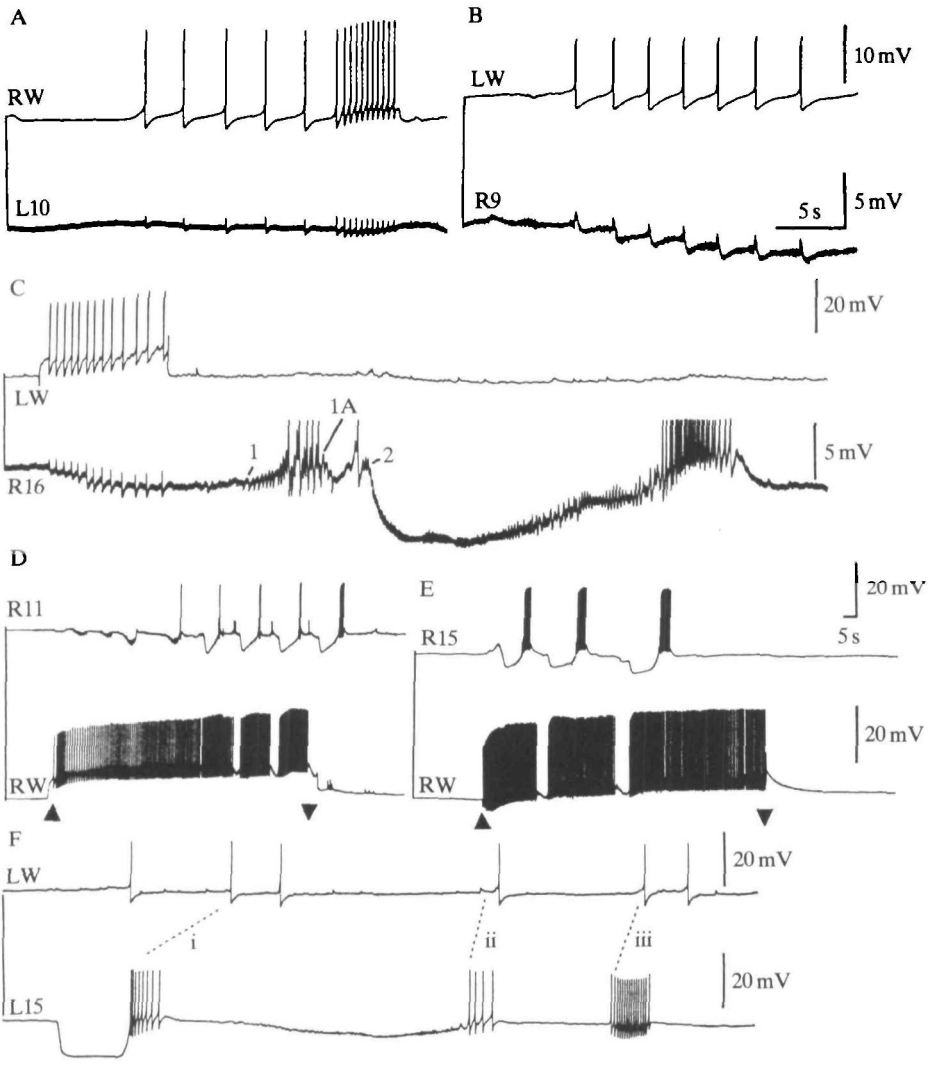

Fig. 6. Interactions with F cells. (A),(B) Biphasic PSP in contralateral L10 and R9. (C) Impulse train in W cell generates a BPSP in R16 followed by one cycle of inputs from the FPG interneurones (1, 1A, 2). The spikes in R16 are truncated. (D) Prolonged depolarization of RW leads to feeding pattern activity in R11. Note the increasing amplitude of the inhibitory interneurone 2 input during the first few cycles and the adoption of the patterned burst mode in the W cell with inhibition in phase I. (E) Similar action of RW on R15. (F) Feedback from an F cell to the W cell. L15 is fired by (i) postinhibitory rebound, (ii) weak interneurone 2 input, (iii) depolarization. In each case F cell activity leads to a few spikes in the W cell after a delay. A,C,F, upper time scale; B, upper scale 1 s; D,E, lower scale.

the contralateral W cell, the EPSP in R81 facilitates from 1 mV to a peak of 6 mV after seven impulses (Fig. 7A–B). The latency for the W cell EPSP in both pairs of F cells is approximately 7 s for the ipsilateral connections and between 15 and 17 ms for the contralateral pairing. The synaptic inputs from the W cells are 2–3 times larger than those from other unidentified sources. Unlike other pairs of F cells reported

previously (Bulloch & Dorsett, 1979*b*) no evidence was obtained of electrotonic coupling between these two cell pairs in any combination. Despite the dye-coupling that was observed between the W cells and a pair of neurones in the opposite buccal ganglion in positions approximately corresponding to neurones B8 and B81, no electrotonic junctions were found between these neurones and the opposite W cell. An extensive search for coupling to other F cells also proved negative, as did attempts to initiate feeding cycles by depolarizing neurones 8 and 81.

Both B8 and B81 tend to fire spike doublets or triplets resulting from depolarizing afterpotentials (DAPs) in both pairs of cells (Thompson & Smith, 1976; Bulloch & Willows, 1981). In B8 the peak amplitude of the DAP occurs 100 ms after the spike

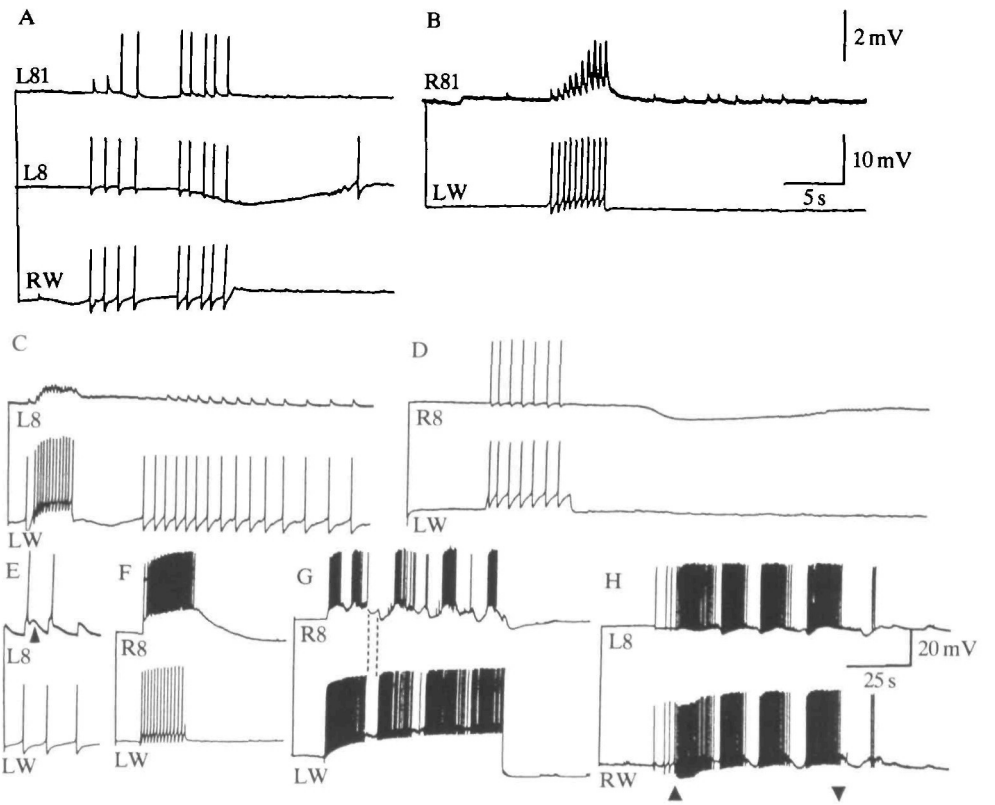

Fig. 7. Responses of the F cells B8 and B81 to W cell stimulation. (A) Simultaneous records from L8 and L81 to spikes in RW (aligned from multichannel tape records). (B) Facilitating EPSPs in R81. (C),(D) Interactions of LW with B8 in both ganglia. Occasionally, as here, stronger connections were found contralaterally. (E) A depolarizing after-potential in L8 (arrowhead) follows the spike. (F) High frequency burst in R8 in response to depolarizing LW. Spike ratio was 4:1. (G) Patterned bursting in R8 following depolarization of LW. Inhibition of W cell coincides with phase I inhibition in the F cell. The W cell EPSP does not overcome the phase I and II inhibition from the FPG interneurones. (H) Depolarization of RW drives L8. Firing interrupted in both cells late in phase I, but RW burst overcomes weak phase II inhibition in this preparation. Arrowheads, depolarizing current. A–E, calibration as in lower trace B. F–H, as in H.

and decays exponentially over 2–300 ms (Fig. 7E). A train of impulses in the W cell at 3–4 s^{-1} can lead to a high frequency burst in B8 with spike ratios of about 4:1 (Fig. 7F).

Prolonged depolarization of a W cell for periods between 15 and 160 ms has two principal actions on the B8 and B81 neurones. The initial effect, due to the direct synaptic pathway, is to generate a high frequency discharge in both cells. After a period of 5–15 s activity, an inhibition occurs in the motoneurone coincident with a slight increase in the firing rate of the W cell (Fig. 7G). The motoneurone then fires a second burst which continues until a further inhibition occurs, this time common to both neurones. The W cell recovers from this inhibition within 1–2 s to resume firing at an increased rate, but the B8 neurone experiences a second large inhibitory wave which has the characteristics of the interneurone 2 input to these neurones. As the inhibitory influence of interneurone 2 declines, B8 fires its characteristic phase III burst resulting from postinhibitory rebound and the excitatory drive from the W cell (Fig. 7G,H). Interneurone 2 inhibition in the F group following W cell activation often shows a progressive build up in intensity during the first few cycles of FPG activity (Fig. 6D) and occasionally B8 continues to fire in phase II as a result of the strong excitation from the W cells.

When stimulated individually both the B8 and B81 neurones consistently produce movements associated with the contraction of muscle group 4 (inner lip retraction) and muscle groups 5 and 6 (radula retraction and flattening) which occur during phase III of the feeding cycle. Their axons leave the ganglia in nerves which serve these muscles but direct innervation has not been established. As they do not drive any other motoneurones so far recorded, it is provisionally assumed that they are motoneurones serving these muscles.

Interactions between W cells

Simultaneous recordings from both W cells provide no evidence of any monosynaptic pathway between these cells, but they receive common inputs from a number of unidentified sources (Fig. 9C).

Prolonged depolarization of one W cell will activate its partner after a latency of 2–10 s (Fig. 8A–C). The spiking in both cells is periodically interrupted by a simultaneous inhibitory input lasting about 5 s, although this is shorter in the cell being depolarized. The reciprocal excitatory nature of the pathway between the W cells is also emphasized by the observation that the patterned bursting continues for one or two cycles after the depolarizing current in one neurone ceases.

The synchronized burst patterning seen in the W cells depends upon spike activity in both partners (Fig. 10). If one neurone of an active pair is hyperpolarized to prevent spiking or reduce it to frequencies below 2 s^{-1}, patterned bursting in the partner is replaced by slow iterative spiking. Upon release from hyperpolarization, burst patterning is resumed by both cells. This mode of activity in the W cells is accompanied by feeding movements of the buccal mass which are more vigorous than those seen during spontaneous buccal cycles when the W cells are silent.

Inputs to the white cells

Evidence has been presented showing that prolonged depolarization of the W cells causes indirect excitation of the buccal motoneurones by activating the FPG network (Figs 5F, 6D,E, 7G,H). Simultaneous recording from a motoneurone and a W cell provides information relative to the timing of two synaptic inputs to the W cells that seem to be derived from the FPG network. In preparations where both W cells are active, there is a periodic inhibition of both neurones in phase I, followed by an acceleration of the firing rate coincident with the onset of phase II. Neither of these events can be elicited by stimulating single motoneurones from any group. W cells do show slight postinhibitory rebound but this does not produce spike frequencies equivalent to those seen during phase II firing.

Concurrent recording from a W cell and a motoneurone during spontaneous FPG cycles where the W cell is inactive reveals that the W cells undergo a 1–2 mV depolarization in phase I at the time of the increase in the firing rate of interneurone 1

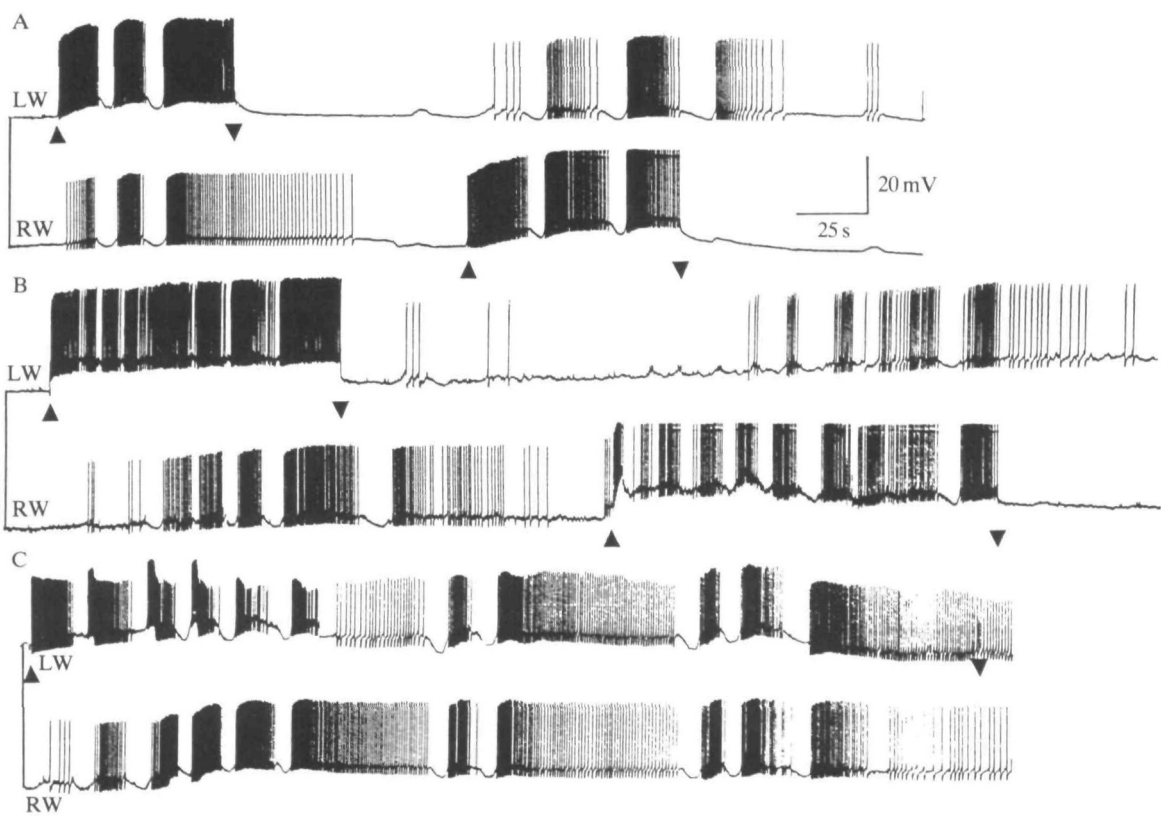

Fig. 8. Interaction between W cells. (A) Depolarization of LW (arrowheads) excites RW and is followed by patterned bursting. In the same preparation depolarization of RW excites LW. Note the depolarizing waves in RW accompanying the terminal bursts in LW. (B) A similar experiment from another preparation. (C) Patterned activity in both cells following prolonged (360 s) depolarization of LW. Note the almost identical pattern.

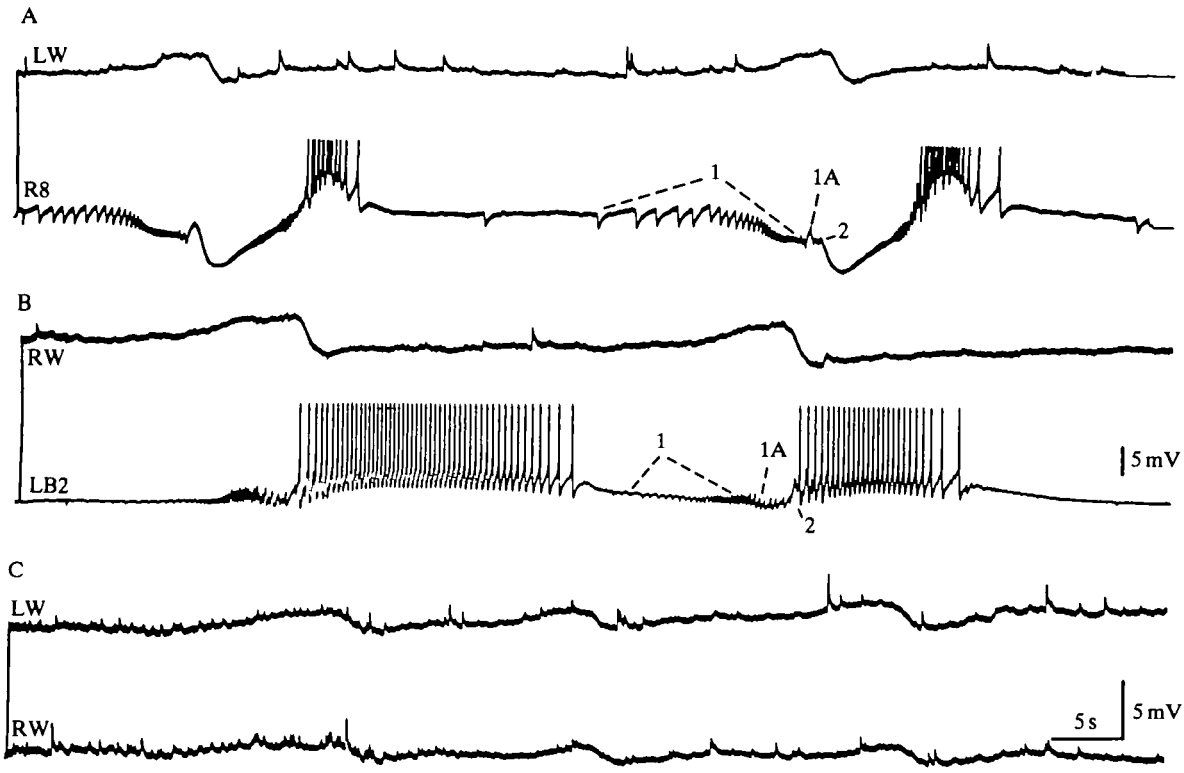

Fig. 9. Inputs to W cells. (A) Recording from an inactive W cell and an F cell being driven by spontaneous activity in the FPG interneurone network. The W cell is depolarized during phase I in parallel with the increased frequency of the interneurone 1 PSP (1, 1A). There is a rapid repolarization at the time of the interneurone 2 input (I2). (B) Similar recording from a W cell and an R cell, showing the phase I depolarization and repolarization in phase II. (C) Simultaneous recordings from the two W cells during spontaneous FPG cycles. Both neurones receive the same inputs. Spikes in A and B are truncated.

and the activation of interneurone 1A (Fig. 9A,B). At the onset of interneurone 2 activity the W cell membrane repolarizes and may temporarily become 1 mV hyperpolarized with respect to its earlier resting level. These fluctuations in membrane potential are common to the W cells and it is improbable that they represent movement artefacts as no disturbance to the recording on the other trace is observed, but no unitary PSPs can be resolved which indicate direct inputs from the multi-action interneurones of the FPG network (Bulloch & Dorsett, 1979*b*).

DISCUSSION

The white cells in the buccal ganglia of *T. hombergi* both initiate and enhance the protraction–retraction cycles in the isolated buccal mass preparation. They do this by exciting the premotor interneurone network which determines the patterned motor output during feeding activity.

In this action they resemble other white buccal neurones present in *Pleurobranchaea* and *T. diomedea* (Gillette & Gillette, 1983; Willows, 1980). Although analogous in their function, considerable variation is found in the morphological and physiological characteristics of the white cells in the three species. In *T. diomedea* the B11 and B12 neurones are unipolar with an axon in the ipsilateral gastro-oesophageal nerve, only the B12 cell having a contralateral branch which makes the synapse with the B5 motoneurone. The white cells of *Pleurobranchaea* are also unipolar with a dendritic field close to the soma, but the axon crosses to the gastro-oesophageal nerve on the opposite side. In *T. hombergi* the neurone is bipolar with a well developed system of branches in both ganglia.

The synaptic properties of the white neurones in the three species are equally divergent. In *Pleurobranchaea* the two cells are strongly coupled electrotonically, yet do not synapse with the motoneurones. In neither species of *Tritonia* are the cells coupled, but in *T. hombergi* they make extensive chemical synaptic contacts with the

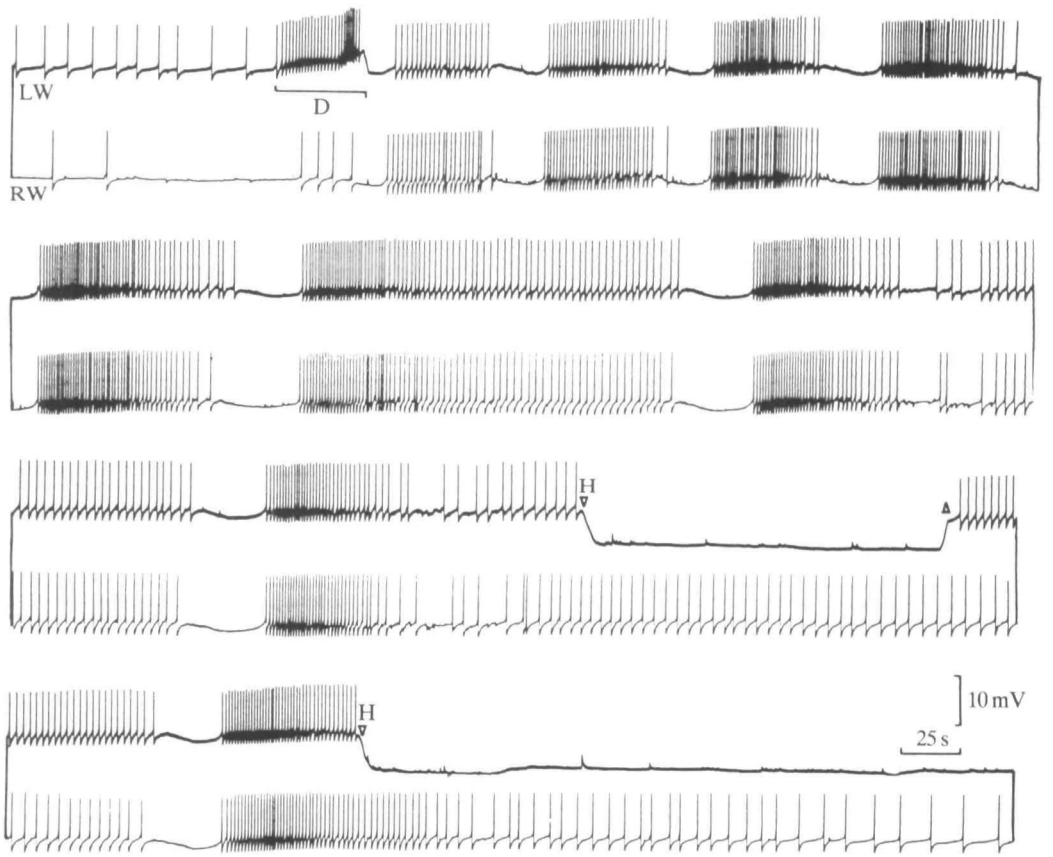

Fig. 10. Patterned bursting depends on activity of both W cells. A short depolarization (D) of LW initiates 3 min continuous burst activity in both neurones. Hyperpolarization of LW (H) causes RW to spike slowly and continuously. Release leads to resumption of patterned bursting.

motoneurones, whereas only electrical coupling has been detected with unspecified motoneurones at this level in *T. diomedea*. The large facilitating excitatory synapses made with the F cells B8 and B81 are not reported from *T. diomedea* but there is a biphasic synapse on the motoneurone B5, an F cell which has the ability to initiate cyclic feeding activity in this species.

Relationships with the FPG network

The nature of the synaptic contacts of the W cells on the buccal motoneurones of *T. hombergi* is at first sight inconsistent with their observed firing during phase II–III in some spontaneous buccal cycles. At this time they are co-active with the R cells (which they inhibit) and out of phase with the P cells (which they excite). However their spike frequency during these cycles does not exceed $2\,s^{-1}$, which might be expected to only marginally influence the excitability of the motoneurones other than B8 and B81. From these observations one might conclude that the principal action of the W cells is on the interneurones of the FPG network.

Unitary synaptic inputs from the FPG interneurones cannot be resolved in the W cells, but the simultaneous ramp depolarizations that are seen in non-spiking W cells during phase I of spontaneous feeding cycles (Fig. 9) are not seen at any other time, and appear to be derived from phase I interneurone activity, indicating some form of positive feedback from the FPG network. This input can give rise to occasional spikes in the W cell during phase I and may be followed by similar activity in phase III as a result of feedback from some F cells.

In some preparations the W cells are more active than in others and exhibit a high level of synaptic activity and spontaneous spiking associated with continuous feeding movements in the buccal mass. In other quiescent preparations the W cells are silent but depolarizing currents of around 1 nA injected into the soma lead to spiking at around $2\,s^{-1}$ which eventually recruits the partner in the opposite ganglion, followed by activation of the common inhibitory input. This sequence is associated with the excitation of the FPG network monitored by the characteristic synaptic inputs of these interneurones in the motoneurones. A similar transition from quiescence to synaptically driven activity is also seen in *Pleurobranchaea* white cells following the stimulation of the oral veil with appetitive substances (Gillette & Gillette, 1983).

A model summarizing the main features of the W cell interactions is given in Fig. 11. The reciprocal excitatory connection between the W cells is provided by the centres '*e*', two of which are provided as both W cells must be active to excite the common inhibitory input '*i*'. The activity of the inhibitory input to the W cells ('*i*') is phase-linked to the FPG interneurone 1A, but is separated by the finding that spontaneous FPG cycles are not accompanied by phase I inhibitory input in silent W cells. It is envisaged that the centres '*e*' would gate the action of '*i*'.

The W cells may excite the FPG interneurones through two routes. Excitation of interneurone 1A could result from the activation of '*i*', but a more probable route, suggested by the fact that the FPG inputs are normally elicited in their proper temporal sequence, is by the excitation of interneurone 1. If this connection was

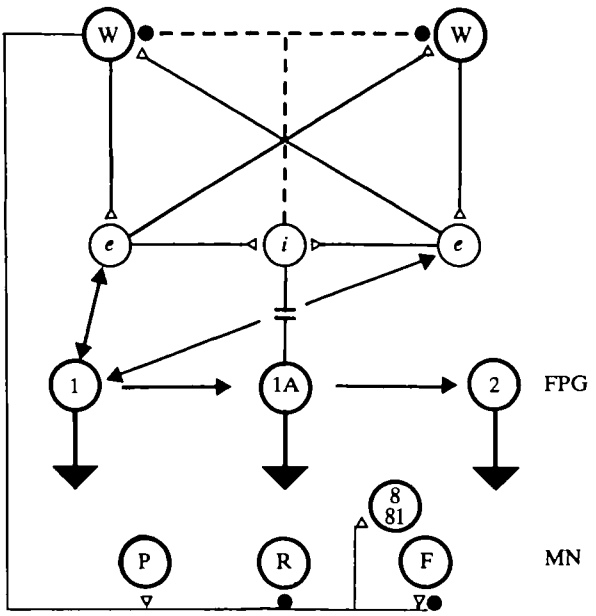

Fig. 11. Hypothetical model accounting for the interactions between the W cells and the FPG and motoneurone network described in the text. Excitatory pathways between the W cells, and the W cells and motoneurones terminate in open triangles, inhibitory pathways terminate in closed circles. The W cells excite each other indirectly through '*e*', and both need to be active to excite the common inhibitory input '*i*' (dotted line). The arrowed lines suggest excitatory pathways with the FPG interneurones and motoneurones (MN) based on observed interactions monitored at motoneurone level.

reciprocal it would also provide the source of the early phase I depolarization seen in inactive W cells (see Fig. 9).

One pair of white cells in *T. diomedea* (B11) contain large amounts of a small cardioactive peptide (SCPB) together with acetylcholine (Masinovsky *et al.* 1985). The other pair (B12) only contain the peptide. It may be anticipated that the W cells in *T. hombergi* are also peptidergic, but the anatomy suggests a closer homology to the B12 neurones which have a branch to the contralateral ganglion. No release sites for the peptide have been identified in the ganglionic sheath or along the gastro-oesophageal nerve and their action outside the ganglion is not known.

In *Pleurobranchaea*, appetitive substances applied to the oral veil result in depolarization and prolonged burst episodes in the ventral white cells (VWC) which drive the buccal motor network (Gillette & Gillette, 1983). Direct stimulation of the VWC also produces a transient contraction of the longitudinal muscle of the oesophagus. Gillette & Gillette suggest that the VWCs have a command role in the feeding behaviour of *Pleurobranchaea*, the criteria for which are not met in the present observations on *T. hombergi*.

There is considerable functional resemblance between the W cells of *T. hombergi* and the slow oscillator (SO) neurone in the buccal ganglion of *Lymnaea* (Rose & Benjamin, 1981). Direct synaptic input from the SO to the N1 neurones excites the FPG interneurones in their proper sequence, while the SO activity becomes

phase-locked to the buccal cycle by inhibitory feedback from the interneurones. In *Lymnaea* the inhibition of SO occurs during the retraction phases of the cycle. The SO also makes monosynaptic connections to buccal motoneurones.

The feeding movements of the buccal mass of *Tritonia* are more vigorous, and the period between individual cycles is least when the W neurones are co-active with the FPG network. Thus the W neurones can both initiate and modulate feeding activity, but their activity is not necessary for its expression in the isolated buccal mass. At present, no special function, apart from their motor activity, can be assigned to the buccal neurones B8 and B81. Their unique synaptic relationship to the W cells argues for some special role in the neural circuit associated with the behaviour, and will be the subject of further investigation.

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