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MOTOR NEURONE COORDINATION AND SENSORY MODULATION IN THE FEEDING SYSTEM OF THE MOLLUSC *PLEUROBRANCHAEA CALIFORNICA*

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SUMMARY

This study on the marine mollusc, *Pleurobranchaea californica*, examined the electrophysiological basis of coordinated motor neurone activity associated with the intrinsic feeding rhythm of the buccal ganglion, and the sensory modulation of this rhythm.

Paired intracellular microelectrode recordings from antagonist motor neurones showed that each received alternating barrages of EPSPs and IPSPs; as one of the pair was depolarized, its antagonist was hyperpolarized. PSPs of opposite polarity in antagonists were one-for-one with each other, suggesting that common presynaptic interneurones were responsible. There was no evidence for direct synaptic interaction between antagonist motor neurones. Paired recordings from synergist motor neurones showed that they received similar barrages of PSPs which alternately depolarized and hyperpolarized them. Underlying these parallel changes in potential were one-to-one PSPs of the same polarity, again suggesting driving by common presynaptic interneurones.

The feeding rhythm that was recorded from the buccal ganglion with the buccal mass attached was compared quantitatively with that recorded from the same ganglion after deafferentation. There were significant differences in parameters of the rhythm measured under the two conditions. Rhythmic afferent activity was recorded from the distal stumps of buccal roots, during rhythmic feeding movements of the buccal mass. The spiking of different units was associated with different phases of the movement cycle. Sensory cells with central somata were stimulated individually in the isolated ganglion, and two effects were recorded. Some sensory neurones excited withdrawal motor neurones and inhibited eversion motor neurones; others inhibited withdrawal motor neurones.

INTRODUCTION

The marine mollusc *Pleurobranchaea californica* feeds by the rhythmical eversion and withdrawal of its proboscis, which consists of an oral tube and underlying muscular buccal mass. The rhythmic movements are produced by the coordinated activity of several groups of muscles, including those of the buccal mass, the mouth and oral veil region, and the body wall (Davis & Mpitsos, 1971; Davis, Siegler & Mpitsos, 1973;

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Lee & Liegeois, 1974). Within the central nervous system there are at least two separate pattern-generating 'centres'; the buccal ganglion and the brain (cerebro-pleural ganglion). In the absence of sensory input, both of these ganglia can produce a cyclically patterned motor output, in response to tonic electrical stimulation of certain peripheral nerves. This output corresponds in essential features to rhythmic feeding behaviour; namely, alternating bursts of spikes occur in motor neurones that innervate antagonistic muscles of the buccal mass (buccal ganglion) or mouth and oral veil region (brain), and the frequency of this alternation can be varied over a range similar to that seen during feeding in the intact animal (Davis & Mpitsos, 1971; Davis *et al.* 1973).

The present study will examine two different aspects of the buccal ganglion feeding rhythm. The first section considers the central mechanisms that coordinate the activity of synergistic and antagonistic buccal ganglion motor neurones. Intracellular recordings from single eversion or withdrawal motor neurones showed that they received cyclic excitatory post-synaptic potentials (EPSPs) and inhibitory post-synaptic potentials (IPSPs) during the rhythmic motor output of the isolated nervous system (Siegler, Mpitsos & Davis, 1974). These synaptic events were the primary cause of the rhythmic patterns of spikes in motor neurones, though intrinsic properties of the motor neurones, such as post-inhibitory rebound, also contributed to the spiking patterns of motor neurones (Siegler *et al.* 1974). Previous indirect evidence suggested that one source of such synaptic inputs might be other motor neurones, for, in some, IPSPs corresponded with action potentials recorded extracellularly in a buccal ganglion root (Siegler *et al.* 1974). The possibility of direct interactions between motor neurones was tested in the present investigation by making intracellular recordings from pairs of motor neurones in the isolated buccal ganglion.

So far, the centrally generated elements of the feeding rhythm have been emphasized. In the intact animal, however, sensory feedback from movements of the buccal mass and associated structures, must normally act in combination with the intrinsic, central rhythm to effect an appropriate motor output. *Pleurobranchaea* is carnivorous, and in the laboratory can be induced to feed on items of widely different bulk and consistency, including macerated or small strips of squid or other meat, and comparatively large irregularly shaped items, such as other *Pleurobranchaea*. Solid food is swallowed whole, rather than being torn off in pieces, and several successive cycles of buccal mass contractions may be required to ingest a single large item. Consequently, even within a single meal, there might be considerable variation in the material that is accommodated within the buccal mass. The second section of this study is thus concerned with the way in which the central rhythm is modulated by sensory input. The feeding rhythm of the buccal ganglion with the buccal mass attached was compared with that of the isolated buccal ganglion, to determine the effects of sensory feedback upon the central rhythm. The nature of this sensory feedback was examined by recording afferent activity in buccal roots during naturally occurring contractions and imposed movements of the buccal mass. Somata of single sensory cells were stimulated in the isolated ganglion while recording the activity in the motor neurones.

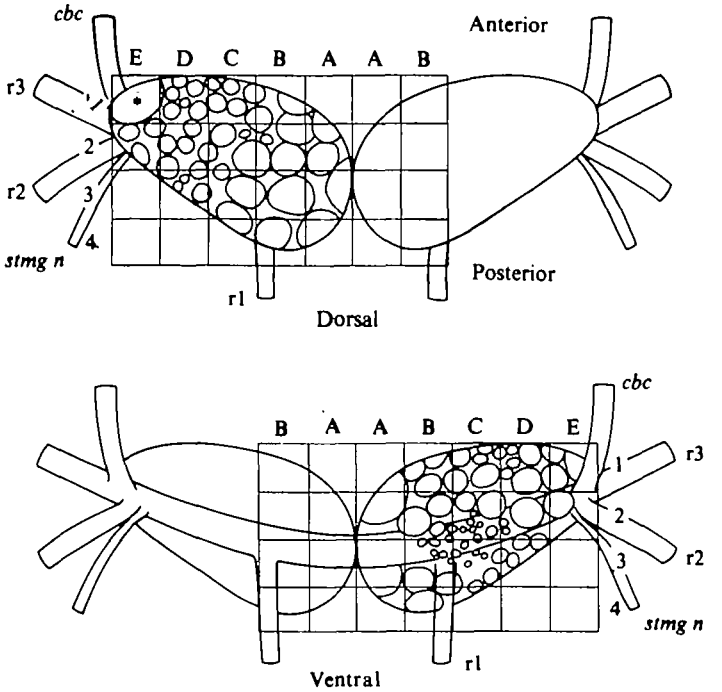

Fig. 1. Drawings of a typical buccal ganglion and its major nerves. Somata are shown for the left half only; the position of cells on the opposite half is typically similar. Positions of the somata are noted according to the superimposed grid, giving column, row, side of the ganglion, and aspect. Somata that occupy more than one quadrant are recorded with reference to their centrepoint. Positions of axons are given according to the side of the ganglion and of the root. For example, the starred cell, which has an axon in the right cerebrobuccal connective, is recorded as (E1 left dorsal; Rcbc). r1, r2 and r3 are sensorimotor roots 1, 2 and 3, which innervate the buccal mass, *stmg n* is the stomatogastric nerve, which innervates the oesophagus, and *cbc* is the cerebrobuccal connective.

MATERIALS AND METHODS

Experiments were performed on buccal ganglia and/or buccal masses isolated from *Pleurobranchaea californica*. The animals ranged from 50 to 250 g in weight and their buccal ganglia measured 2–4 mm across. In the first part of the study 40 animals were used; in the second part, 45 animals. They were collected locally or obtained from Pacific Bio-marine Co., Venice, California, and maintained in running sea water.

Central mechanisms of motor coordination

Experimental procedure

All experiments were performed on the isolated buccal ganglion. The ganglion was pinned to the Sylgard-coated surface of the recording chamber, and bathed in Millipore filtered seawater (0·45 μm pore) containing 0·5% glucose (w/v), adjusted to pH 7·4. The temperature of the bath was maintained between 13–16 °C. The connective tissue was dissected from the ends of the cut nerve roots, and all but the inner transparent layer was removed from the surface of the ganglion.

Extracellular recordings were made with glass suction electrodes attached end-on to nerve roots. Intracellular recordings were made from neurone somata, using glass

capillary microelectrodes filled with 0·6 M-K_2SO_4. The microelectrodes had a resistance of 5–20 MΩ. The positions of motor neurone somata were recorded using the grid shown in Fig. 1. The feeding rhythm was elicited by tonic stimulation to the paired stomatogastric nerves (both in the same suction electrode), with electrical shocks of 1–20 ms duration at a frequency of 0·5–20 Hz. Standard electrophysiological equipment was used throughout. Permanent records were made with a continuous recording oscilloscope camera or a chart pen recorder (Gould-Brush 200).

Identification of motor neurones

The majority of motor neurones penetrated in the isolated buccal ganglion could be readily designated as 'eversion' or 'withdrawal'. These terms are used with reference to the feeding behaviour of the intact animal. During feeding, the alternate eversion and withdrawal of the buccal mass is coordinated with the rhythmic movements of the intrinsic musculature (Davis & Mpitsos, 1971). 'Withdrawal (phase)' motor neurones innervate intrinsic muscles of the buccal mass whose contractions are coordinated with withdrawal, and 'eversion (phase)' motor neurones innervate intrinsic muscles whose contractions are coordinated with eversion. Eversion and withdrawal motor neurones burst alternately during rhythmic feeding activity in the isolated buccal ganglion (Davis *et al.* 1973). Somata of the two groups of motor neurones are distinctive in size and position (Siegler *et al.* 1974). Thus motor neurones could be classified by their firing pattern in the feeding cycle, and by the positions of their axons and somata (see also Siegler *et al.* 1974 for detailed physiological criteria).

A small proportion of motor neurones could not be readily classified. The timing of rhythmic bursts of spikes in these cells varied, relative to the major withdrawal and eversion bursts, as the frequency of the feeding rhythm changed. Since the functional significance of such changing patterns is unknown, the present report is limited to those motor neurones that could be easily classified. It must be recognized, however, that not all aspects of the pattern can be explained in terms of the simple scheme used here.

Sensory modulation of the feeding rhythm

General experimental and recording techniques were as above. In preparations which included the buccal mass, glucose was omitted from the bathing solution.

Experimental preparations

The three following preparations of the buccal mass and/or buccal ganglion were used in this second group of experiments. (1) *Buccal mass with buccal ganglion attached.* After opening the animal by a dorsal midline incision, the cerebrobuccal connectives were cut and the buccal mass-buccal ganglion complex was removed by cutting through the outer covering of the oral tube anterior to muscle 6 (see Davis *et al.* 1973 for numbering of muscles), and through the oesophagus, close to the stomach. After the stomatogastric nerves were dissected free from the oesophagus, the oesophagus was cut away near its juncture with the buccal mass. All but the inner transparent layer of connective tissue was removed from lengths of intact nerves. The buccal mass was held dorsal surface up by a single dissecting pin through its ventral anterior edge. Cyclic contractions of the buccal mass were elicited by tonic electrical stimulation to the paired stomatogastric nerves, and resembled contractions that can be seen through

the translucent body wall of small (1–2 cm) intact feeding animals. The frequency of feeding contractions could be varied by changing the parameters of stimulation (i.e. voltage, duration, and frequency of shocks) to the stomatogastric nerves. Unless so stimulated, the buccal mass was quiescent. (2) *Buccal mass.* The buccal mass was removed from the animal as described above and the buccal ganglion was cut away, leaving the buccal roots, which were attached to the buccal mass, as long as possible. (3) *Isolated buccal ganglion.* This was prepared in the same way as described above.

Experimental procedure

Effects of deafferentation upon the motor rhythm. In each preparation the feeding rhythm was compared for the following three conditions. (1) *With sensory feedback.* Using the buccal mass with the buccal ganglion attached, a single root was cut (either one of the paired root 1's, which contain eversion motor neurones, or one of the paired root 3's, which contain withdrawal motor neurones), and efferent activity was recorded from the central stump. Activity in intact roots was recorded *en passant.* Parameters of stimulation of the stomatogastric nerves were adjusted to obtain regular rhythmic feeding contractions of the buccal mass. (2) *Without sensory feedback but with the same stimulation parameters.* The buccal ganglion was then deafferented by cutting all remaining intact roots peripheral to the recording sites, without removing the electrodes. The feeding rhythm was elicited by stimulating at the same parameters as before deafferentation. (3) *Without sensory feedback but with more intense stimulation.* The parameters of stimulation were increased (e.g. higher voltage, longer pulses, etc.) to elicit the feeding rhythm at the same frequency as that recorded before deafferentation of the buccal ganglion ((1) this para.). For each of these three experimental conditions, records of 10–20 cycles of feeding were analysed. Interburst interval was measured from the midpoint of successive withdrawal or eversion bursts. The number of spikes per burst was counted, then divided by the burst duration to give the average frequency of spikes within a burst. Within each preparation the appropriate measures (see Results) were compared using a one-tailed *t*-test. Differences were significant to at least the 0·01 level.

Characterization of sensory feedback from the buccal mass. To record sensory firing resulting from buccal mass movements, the following two preparations were used. (1) *Buccal mass with buccal ganglion attached.* A single root (one r1 or r3) was cut and afferent activity was recorded from the peripheral stump. Feeding contractions of the buccal mass were elicited by the stimulation of the stomatogastric nerves. In some of these experiments the buccal mass was threaded from the mouth opening to the oesophagus opening with $\frac{1}{8}$ in. O.D. plastic tubing, to simulate the presence of food in the buccal cavity. (Presence of the tubing did not alone stimulate feeding movements; as with the empty buccal mass, these did not begin until the stomatogastric nerves were stimulated.) (2) *Isolated buccal mass.* Afferent spikes were recorded from the peripheral stumps of buccal roots, in response to contraction of the buccal mass elicited by electrical stimulation of other roots, or mechanically imposed movements of buccal mass parts.

Effects of single sensory cells on motor output. An isolated buccal ganglion was used. Activity in buccal roots was recorded extracellularly, and single motor neurones and

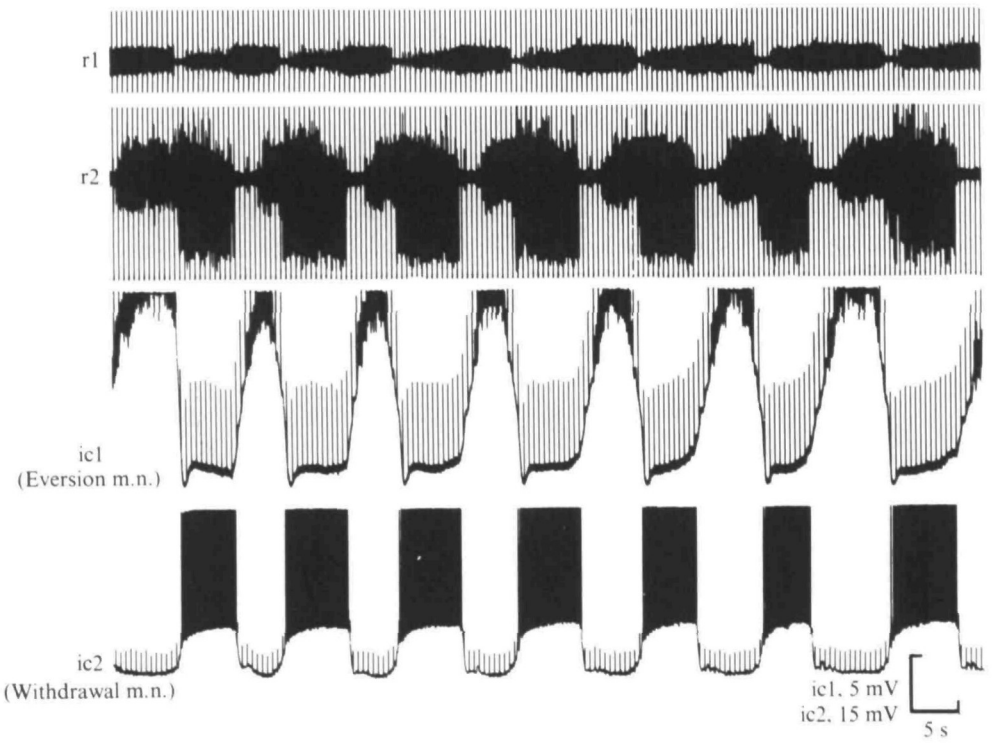

Fig. 2. Rapid alternation of activity in an eversion motor neurone (ic1) and a withdrawal motor neurone (ic2) during feeding output elicited by tonic stimulation to the stomatogastric nerves (note stimulus artifact on all traces). Spikes in ic1 are off scale. Cell position: ic1 (C–D rt. dorsal; Rr1); ic2 (A_3 rt. dorsal; Rr2). r1 and r2 are recorded ipsilateral to ic1 and ic2.

sensory neurones were recorded intracellularly. Motor neurones were identified as described above. Sensory neurones were identified as described in Results.

RESULTS

Central mechanisms of motor coordination

Coordination of antagonists

Two patterns of rhythmic activity could be recorded intracellularly from pairs of withdrawal and eversion motor neurones: (1) depolarization and hyperpolarization alternated, out of phase in the two (Fig. 2) or (2) there was a period of relative quiescence between depolarization of an eversion motor neurone and the ensuing depolarization of a withdrawal motor neurone; eversion depolarization then followed withdrawal depolarization with a short latency (i.e. withdrawal-eversion-pause-withdrawal and so forth) (Fig. 3). In every preparation the frequency of the feeding rhythm could be varied several-fold by changing the parameters of stimulation to the stomatogastric nerves. The first pattern was typical of the fastest feeding frequencies recorded from a preparation, whereas the second was typical of the slowest frequencies. In effect, the phase position of the eversion depolarization in the withdrawal cycle decreased as the interburst interval increased, a relationship described previously in measurement of motor activity that was recorded extracellularly (Davis *et al.*, 1973).

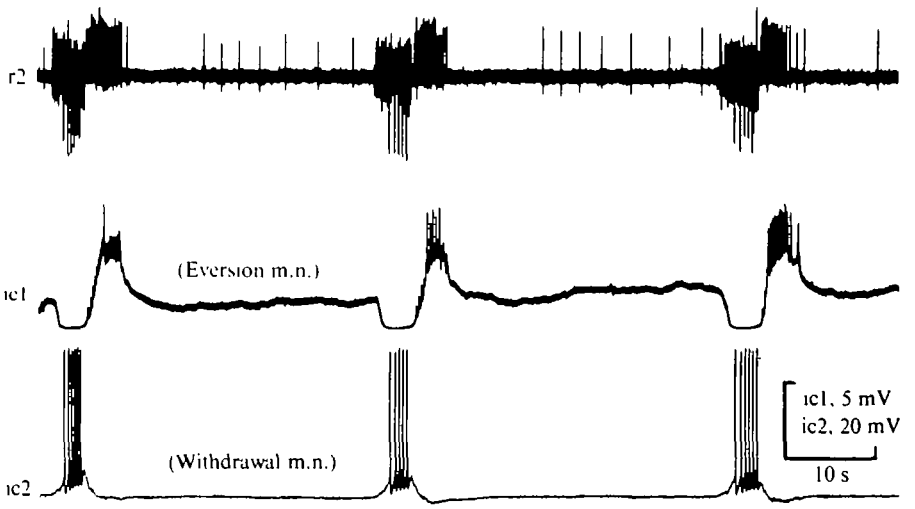

Fig. 3. Latency locking of synaptic activity in a withdrawal (ic2) and eversion (ic1) motor neurone during spontaneous rhythmic buccal ganglion output. Cell position: ic1 (C–D 2 rt. dorsal; Rr1); ic2 (A3 rt. dorsal; Rr2). r2 is recorded ipsilateral to ic1 and ic2

During rhythmic motor output a small proportion of PSPs of opposite polarity in antagonist motor neurones are 'one-to-one' or 'common' (Fig. 4*A*). By this it is meant that individual PSPs in one cell can be matched with individual PSPs in the other cell, for long periods, and that these pairs of PSPs always occur at a fixed latency relative to each other. Such one-to-one correspondence of IPSPs and EPSPs suggests that antagonist motor neurones share inputs from neurones with opposite post-synaptic effects in the two. Although the change of the membrane potential in one neurone can be almost a mirror image of that in the other (Fig. 4*B*, *C*), the majority of PSPs recorded in antagonist motor neurones do not correspond one-to-one. The depolarization of one motor neurone thus appears to be driven by a population of presynaptic cells that are distinct from those that drive the coinciding hyperpolarization of an antagonist.

No evidence was found for direct synaptic interactions between antagonist motor neurones. Spikes in one motor neurone, evoked by injection of current, did not result in one-to-one PSPs in an antagonist. Some cells were penetrated, however, whose spikes did evoke one-to-one PSPs in a motor neurone; these cells were identified as sensory. The failure to find direct connexions between antagonist motor neurones might simply reflect the fact that not all possible pairings were recorded. But, extracellular recordings from roots (where spikes of many motor neurones could be measured) showed no motor neurones whose spikes corresponded one-to-one with PSPs in an antagonist. The rhythmic alternation of inputs to eversion and withdrawal motor neurones therefore probably arises from interneurones and not from direct, and reciprocal, synaptic interaction between the motor neurones.

Coordination of synergists

Intracellular recordings from pairs of withdrawal motor neurones revealed similar changes of their membrane potentials during rapid rhythmic motor output (Fig. 5*A*).

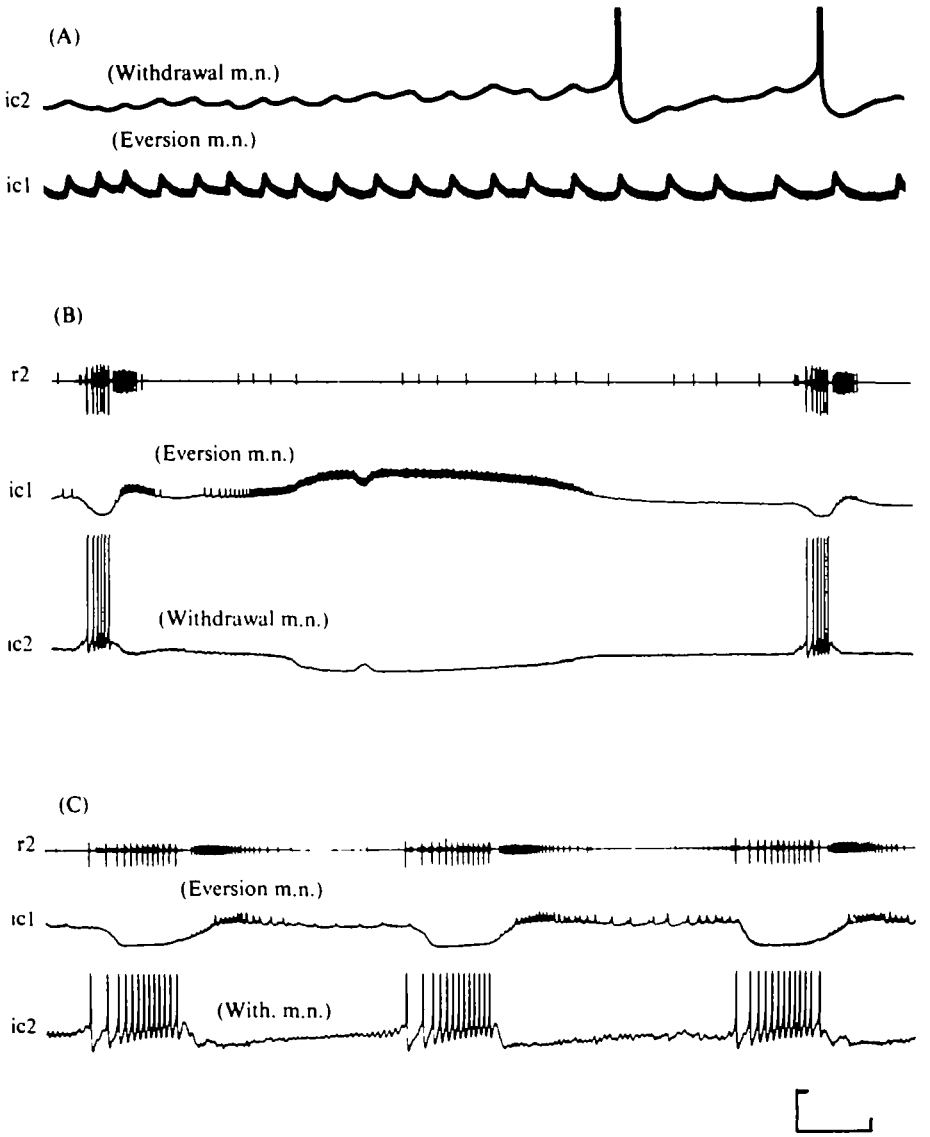

Fig. 4. Synaptic inputs to antagonist motor neurones. (*A*) EPSPs in an eversion motor neurone (ic1) correspond one to one with IPSPs in a withdrawal motor neurone (ic2). This sequence shows the first few spikes of an ensuing burst in the withdrawal motor neurones. (*B*, *C*) Opposite changes in membrane potential in the same two cells. Cell position: ic1 (C2 rt. dorsal; Rr1); ic2 (A3 rt. dorsal; Rr2). r2 is recorded ipsilateral to ic1 and ic2. Calibration mark. Vertical: *A*, ic1, 4 mV; ic2, 7 mV; *B*, ic1, 15 mV; ic2, 10 mV; *C*, ic1 and 2, 20 mV. Horizontal: *A*, 1·2 s; *B*, 3 s; *C*, 4 s.

The most conspicuous feature of these parallel potential changes was the occurrence of one-to-one PSPs of the same polarity in the two cells (Fig. 5*B–E*). In some pairs of withdrawal motor neurones, up to five different pairs of PSPs could be distinguished, on the basis of their change in amplitude with changes in membrane potential and their pattern of occurrence. This was true in both ipsilateral (Fig. 5*A–C*, *E*) and contralateral (Fig. 5*D*) motor neurones. The occurrence of one-to-one PSPs in pairs

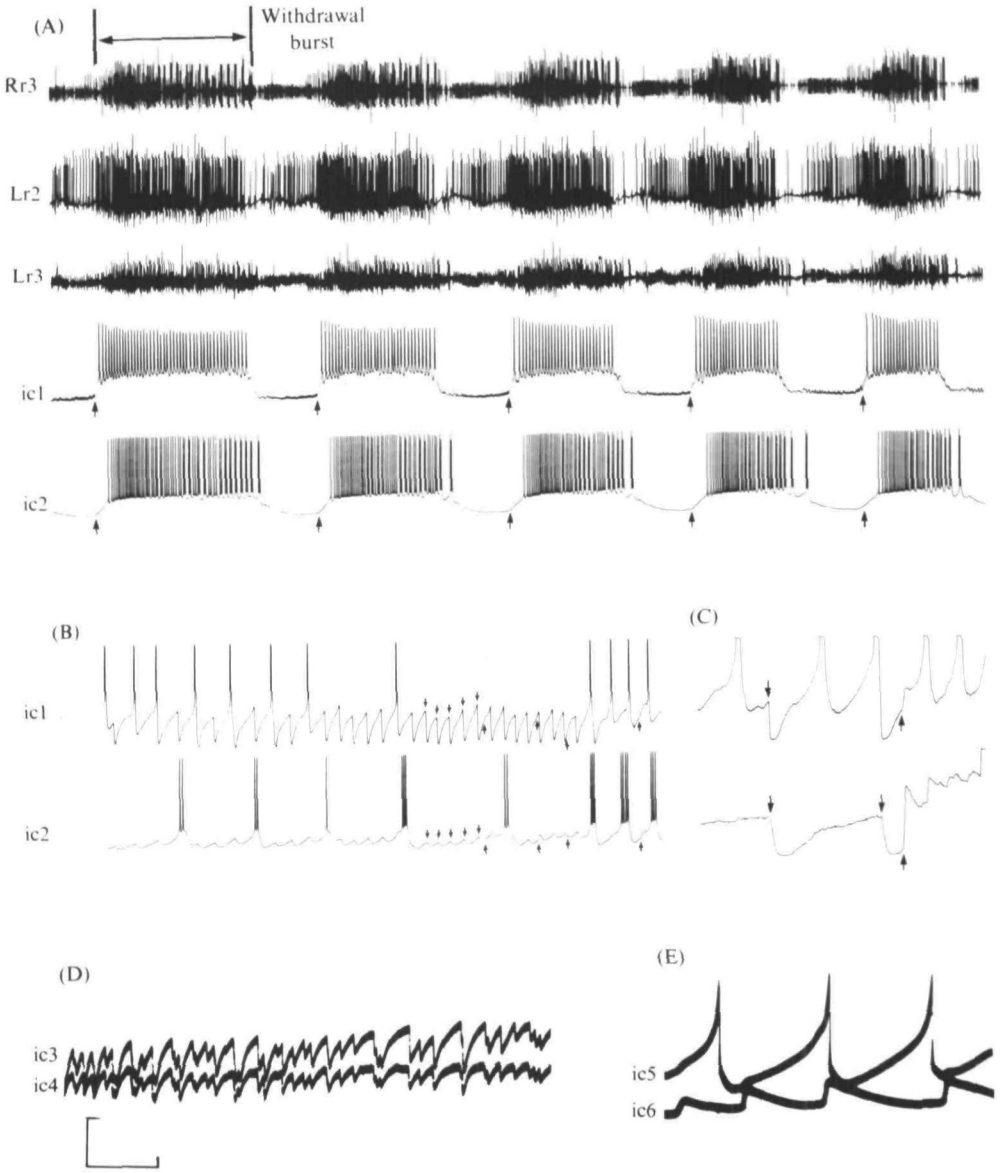

Fig. 5. Coordinated firing and common synaptic inputs to withdrawal motor neurones. (*A*) Patterned spikes during rhythmic feeding, driven by tonic stimulation to the stomatogastric nerves. A barrage of IPSPs ends simultaneously in the two cells (upward arrows) though spikes in ic1 typically precede those in ic2 by almost 1 s. (*B, C*) Underlying common IPSPs are marked by downward arrows, EPSPs by upward arrows. (*D, E*) Records from two other pairs of withdrawal motor neurones. Cell position ic1 (C3 left dorsal; Lr2, Lr3); ic2 (C2 left dorsal; Lr3); ic3 (A2 left dorsal; Lr3), ic4 (A1 rt. dorsal; Rr2); ic5 (B2 rt. dorsal; Rr2); ic6 (A3 rt. dorsal; Rr3). Abbreviations: The prefix *R* indicates right root of pair; *L* indicates left root of pair. Calibration mark. Vertical: *A*, ic1, 4 mV, ic2, 30 mV; *B*, ic1 and 2, 15 mV; *C*, ic1, 4 mV, ic2, 3 mV; *D*, ic3 and 4, 2 mV; *E*, ic5 and 6, 1 mV. Horizontal: *A*, 3 s; *B* and *C*, 1·5 s; *D*, 1·25 s; *E*, 250 ms.

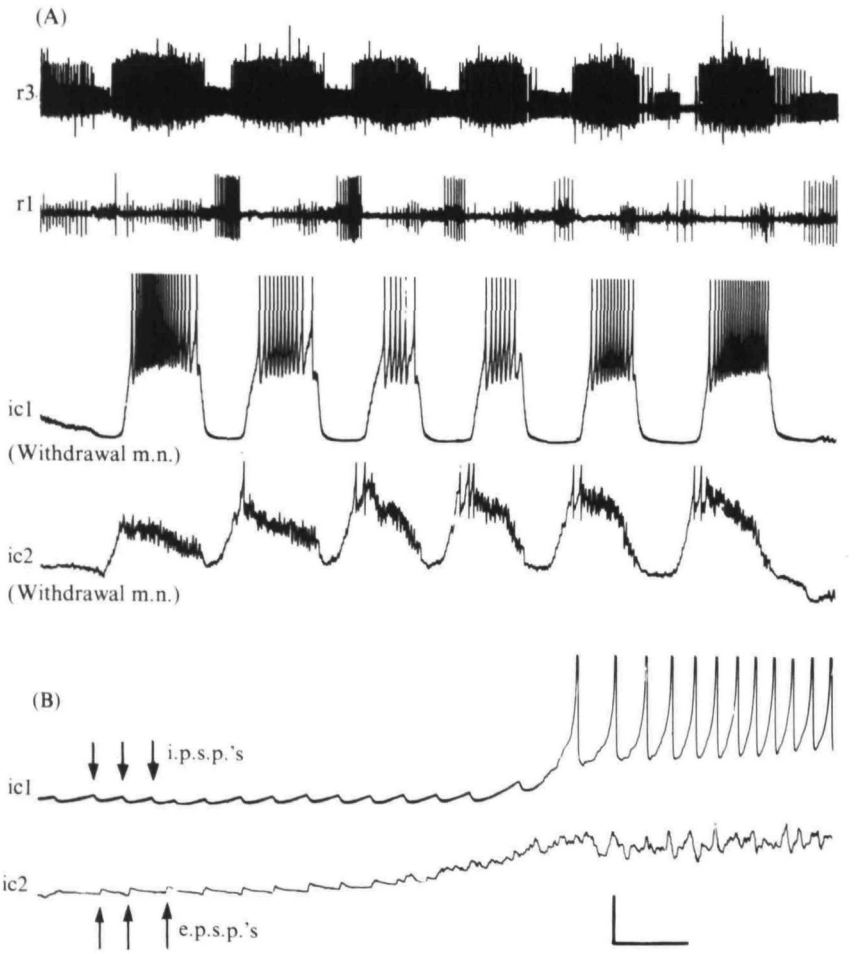

Fig. 6. Latency between cyclic inputs to two withdrawal motor neurones. (*A*) Depolarizing wave and spikes in one (ic2) leads that in the other by 3–4 s. Spikes in ic2 are partially off scale. (*B*) Higher gain records reveal that the earlier depolarization of ic2 is due to a train of ESPSs, which occur while ic1 is still inhibited by another independent train of IPSPs. Cell position: ic1 (B2 rt. dorsal; Rr3); ic2 (B2 rt. dorsal; Rr3) r1 and r3 are recorded ipsilateral to ic1 and ic2. Calibration. Vertical: *A*, ic1, 9 mV, ic2, 5 mV; *B*, ic1 and 2, 3 mV. Horizontal: *A*, 10 s; *B*, 1 s.

of withdrawal motor neurones suggests that they have common premotor neurones. Nevertheless some pairs of withdrawal neurones, which had many common synaptic inputs, showed differences in the onset of depolarization, and initiation of spiking, or onset of hyperpolarization and termination of spiking (e.g. Fig. 5*A*). In some pairs of cells independent PSPs could be distinguished that could account for these differences. For example, the onset of depolarization, and firing in one neurone preceded that in the other by 3–4 s (Fig. 6*A*). The leading cell received a train of EPSPs, which occurred while the other cell was hyperpolarized by an independent train of IPSPs (Fig. 6*B*).

In none of the pairs of withdrawal motor neurones recorded did intracellular depolarization or hyperpolarization of one result in similar changes in the membrane potential of the other. In less than 5% of over 250 recordings from withdrawal motor neurones, an intracellularly stimulated depolarization, which did not evoke spikes,

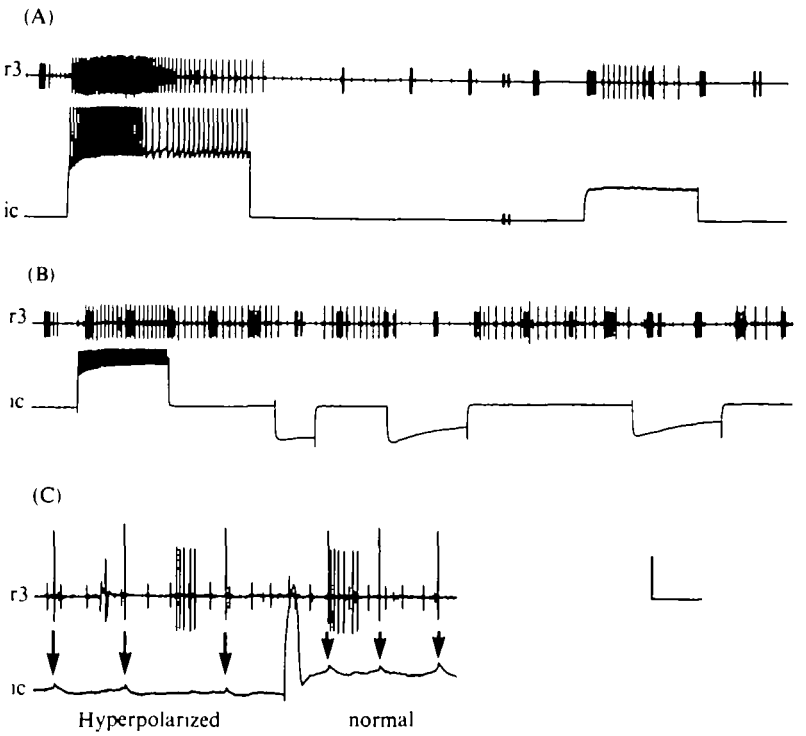

Fig. 7. Interaction between withdrawal motor neurones. (*A*) When a withdrawal motor neurone is depolarized and made to spike, a number of other withdrawal units in r3 are recruited; some of the same units (the largest, and the smallest distinguishable) fire when the motor neurone is depolarized to a level which is below its spike threshold. The bars denote a 5 s gap in the record. (*B*) The same motor neurone is again depolarized and at least two units (again the largest and the smallest) begin to spike in r3. During three subsequent imposed hyperpolarizations, spiking in these units stops or is slowed. (*C*) Spikes in the largest of these extracellularly recorded withdrawal motor neurones corresponds one to one with a small biphasic potential (arrows) in the penetrated cell. Its amplitude did not change when the cell was hyperpolarized (first half of record). The DC balance of the intracellular trace was changed at the offset of hyperpolarization (second half of record) to maintain the position of the record. Cell position is (C_3 rt. dorsal; Rr2, Rr3). Its spike in r3 is small and not distinguishable in the records shown here. r3 is recorded ipsilateral to ic. Calibration. Vertical: *A*, 9 mV; *B*, 40 mV; *C*, 4 mV. Horizontal: *A* and *B*, 4 s; *C*, 1 s.

increased the frequency of extracellularly recorded spikes in other withdrawal motor neurones (Fig. 7*A*). Intracellularly stimulated hyperpolarization lowered the frequency of spikes of the same motor neurones (Fig. 7*B*). In either case some of these spikes corresponded one-to-one with small biphasic PSPs in the stimulated motor neurone, which did not change size with hyperpolarization (Fig. 7*C*). These interactions would result if the motor neurones are electrotonically coupled by a non-rectifying synapse.

Sensory modulation of the rhythm

Influences of sensory feedback on the central rhythm

Deafferentation of the buccal ganglion resulted in changes in the burst period and duration, and in the frequency of spikes within individual bursts of the feeding rhythm.

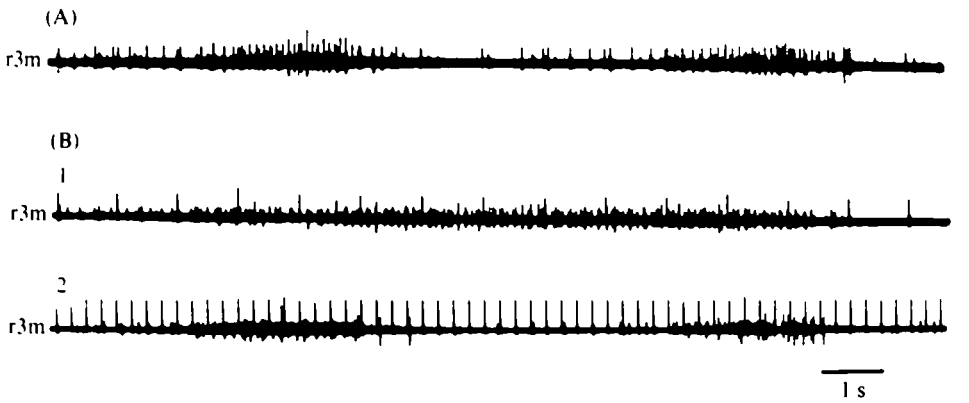

Fig. 8. The effect of deafferentation upon rhythmic feeding output in withdrawal motor neurones. (*A*) Recordings from a buccal ganglion-buccal mass preparation shortly before deafferentation of the buccal ganglion. A single r3 was cut peripheral to the recording site to insure that only efferent activity was recorded; all other major sensorimotor roots were intact. The feeding rhythm was elicited by tonic stomatogastric nerve stimulation (note stimulus artifacts). Two withdrawal bursts are shown. (*B*) Recordings from the same preparation 10 min after deafferentation of the buccal ganglion. Injury discharge had subsided within 5 min of cutting roots. In (1) stimulus parameters were identical to *A*. One withdrawal burst is shown. In (2) stimulus parameters were increased to elicit feeding at a rate comparable to that before deafferentation. Two withdrawal bursts are shown. Note weaker bursts. *A* and *B* are part of a longer record (consisting of 15 cycles of feeding activity for each condition), from which measures presented in text were made. Abbreviations: the suffix *m* indicates that root is cut and only motor activity is recorded.

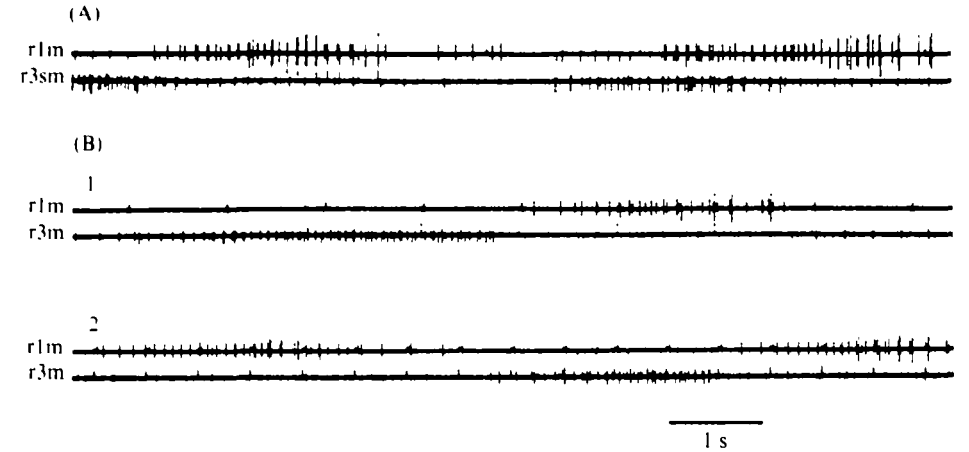

Fig. 9. The effect of deafferentation upon rhythmic activity of eversion motor neurones. (*A*) Recordings from buccal ganglion-buccal mass preparation shortly before deafferentation of the buccal ganglion. A single r1 (upper trace) had been cut peripheral to the recording site to insure that only efferent activity was recorded; the ipsilateral r3 (lower trace) and all other roots were intact. Two eversion bursts are shown. (*B*) Recordings from the same preparation, shortly after cutting the remaining roots. In (1) the stimulus parameters were identical to those in (*A*). One eversion burst is shown. In (2) the stimulus parameters were increased to elicit the maximum feeding rate for the preparation. Two eversion bursts are shown. *A* and *B* are part of a longer record, (consisting of 17 cycles of feeding activity for each condition) from which measures in text were made. Abbreviations: the suffix *sm* indicates that root is intact and both sensory and motor activity are recorded; *m* indicates that root is cut and only motor activity is recorded.

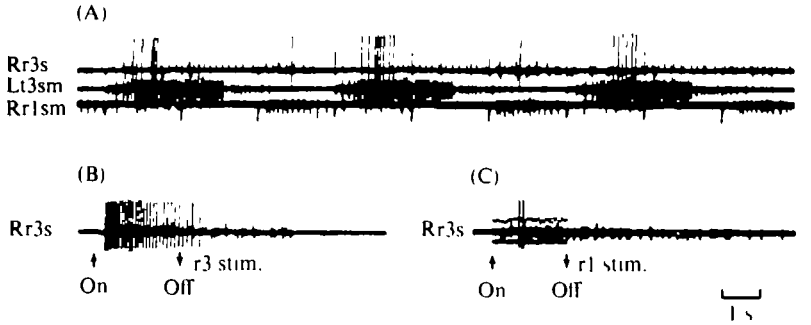

Fig. 10. Sensory discharge recorded in r3 during movements of the buccal mass. The buccal cavity was threaded with plastic tubing. (*A*) Rhythmic discharge was recorded from the distal stump of Rr3, in an otherwise intact buccal ganglion-buccal mass preparation, during cyclic feeding contractions. (*B*) Sensory discharge in response to a prolonged and strong withdrawal movement, confined to stimulated side, elicited by stimulation of the contralateral r3. 20 Hz shocks begin at upward arrow and end at downward arrow. (*C*) Sensory discharge in the same root in response to a prolonged strong eversion movement, elicited by stimulation of the ipsilateral r1. Stimulus as in (*B*). In both (*B*) and (*C*) the observed movement and the sensory discharge outlasted the stimulus for more than 2 s. The largest unit in (*B*) is the same one that fired during the withdrawal phase of the cycle in (*A*), and the largest unit in (*C*) is the same one that fired during the eversion phase in (*A*). Abbreviations: *sm* indicates that root is intact and both sensory and motor activity are recorded; *s* indicates root is cut and only sensory activity is recorded; otherwise as in Fig. 5.

Figs. 8 and 9 show short stretches of rhythmic activity recorded from buccal ganglia during two such deafferentation experiments.

Burst period. There was a marked increase in the burst period of the feeding rhythm, in response to identical stimuli to the stomatogastric nerves. The average time between the midpoint of successive bursts (whether measured in eversion or withdrawal bursts) was typically two to three times greater after the buccal ganglion was deafferented. For example, in the preparation shown in Fig. 8, the mean interburst interval was $7{\cdot}2 \pm 0{\cdot}9$ s before, and $17{\cdot}2 \pm 2{\cdot}2$ s after deafferentation; in Fig. 9 it was $5{\cdot}9 \pm 0{\cdot}3$ s before, and $17{\cdot}2 \pm 1{\cdot}9$ s after deafferentation.

Burst duration. In the withdrawal phase deafferentation caused an increase in the mean duration of bursts (e.g. Fig. 8, $2{\cdot}9 \pm 0{\cdot}1$ s before, and $10{\cdot}0 \pm 2{\cdot}4$ s after, deafferentation). This increase, however, was related to the overall increase in burst period with deafferentation. When the deafferented (isolated) buccal ganglion was stimulated at an intensity sufficient to return the feeding rhythm to near its previous higher frequency, withdrawal phase bursts again became comparably shorter (e.g. Fig. 8, $2{\cdot}9 \pm 0{\cdot}1$ s before deafferentation, and $3{\cdot}2 \pm 0{\cdot}3$ s after deafferentation, increased stimulus intensity). In the eversion phase there was no change in the duration of the burst with deafferentation (e.g. Fig. 9, $3{\cdot}2 \pm 0{\cdot}3$ s before and $3{\cdot}4 \pm 0{\cdot}4$ s after deafferentation).

Frequency of spikes. With deafferentation the mean frequency of spikes (spikes s^{-1}) within both withdrawal and eversion bursts decreased (e.g. Fig. 8, $65{\cdot}7 \pm 1{\cdot}7$ before, and $31{\cdot}5 \pm 2{\cdot}4$ after, deafferentation; Fig. 9, $16{\cdot}8 \pm 1{\cdot}5$ before, and $10{\cdot}5 \pm 1{\cdot}4$ after deafferentation). As with burst duration, this change was in part related to the decrease in feeding frequency, for when the isolated buccal ganglion was stimulated at an intensity sufficient to return the feeding rhythm to near its predeafferentation frequency, the frequency of spikes was also increased (e.g. Fig. 8, $31{\cdot}5 \pm 2{\cdot}4$ after deafferentation

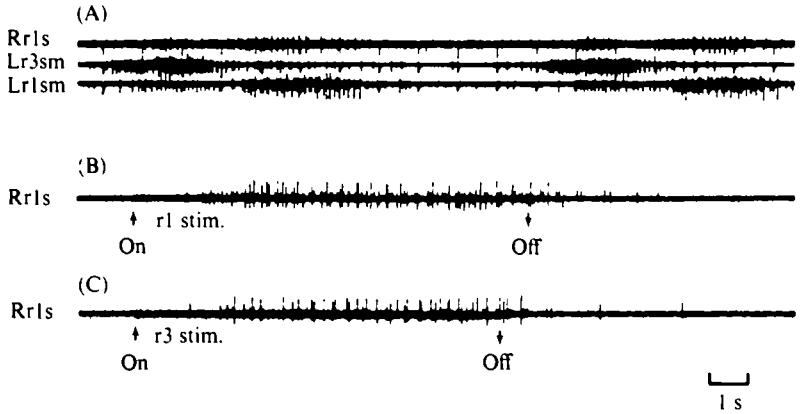

Fig. 11. Sensory discharge recorded in r1 during movements of the buccal mass. The buccal cavity was empty. (*A*) Rhythmic discharge was recorded from the distal stump of Rr1, in an otherwise intact buccal ganglion-buccal mass preparation, during rhythmic feeding contractions. (*B*) Sensory discharge in response to a prolonged eversion movement elicited by stimulation of contralateral r1. (*C*) Sensory discharge in response to a prolonged withdrawal movement confined to the stimulated side, elicited by stimulation to ipsilateral r3. *B* and *C* are records from an isolated buccal mass. The stimulus is as in Fig. 10*B* and *C*. Abbreviations as in Figs. 5 and 10

and 52·5 ± 1·5 after, increased stimulus intensity, Fig. 9, 10·5 ± 1·4 after, and 12·3 ± 1·3 after, increased stimulus parameters). In addition, some larger units fired less frequently in the isolated buccal ganglion, particularly those in root 1 (Fig. 9).

Sensory feedback from movements of the buccal mass

Sensory feedback was recorded from the peripheral stumps of single cut buccal ganglion roots in otherwise intact buccal ganglion-buccal mass preparations. Afferent discharge recorded from root 3 (withdrawal) was rhythmic during rhythmic contractions elicited by tonic stimulation to the stomatogastric nerves. A few units could be reidentified in successive cycles of activity. For example (Fig. 10*A*), one or two small units were recruited, and continued to fire during the transition from eversion to withdrawal. One large unit fired during the withdrawal burst and another fired at the midpoint of the eversion burst. These large units spiked only when the buccal mass was at the most extreme positions of the movement cycle. Prolonged contractions of the buccal mass, which were stronger than those usually seen during cyclic feeding, could be elicited by direct stimulation to the appropriate nerve roots. This resulted in more prolonged and stronger afferent activity in root 3 (Fig. 10*B*, *C*); for example, one of the large units spiked more frequently (Fig. 10*B*). In general, in all preparations studied, the stronger the eversion or withdrawal contractions, the stronger the afferent discharge recorded (i.e. more units of larger size, firing more frequently). Rhythmic afferent discharge corresponding to eversion and withdrawal movements was also recorded from root 1 (eversion). The largest units were recorded during the eversion phase of the contraction cycle (Fig. 11*A*). As for root 3, prolonged stronger contractions of the buccal mass elicited by direct stimulation of appropriate roots, resulted in prolonged sensory discharge in root 1 (Fig. 11*B*, *C*). Afferent activity during contractions of the buccal mass also could be recorded from the peripheral stump of root 2. Cutting this root interfered with cyclic movements of the buccal mass more than did

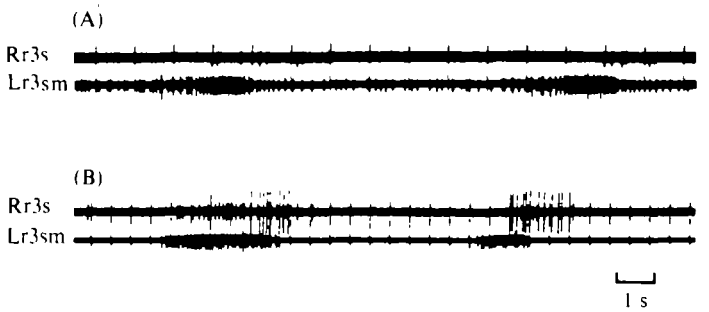

Fig. 12. Effect of buccal mass distension on sensory activity during cyclic feeding contractions. A single r3 has been cut and recordings are made from the distal stump; other roots of the buccal mass are intact. Feeding movements are elicited by tonic stimulation of the stomatogastric nerves. (*A*) The buccal mass is empty and afferent discharge is weak. (*B*) In the same preparations, the buccal mass cavity is threaded with plastic tubing. Afferent discharge is increased. Abbreviations as in Figs. 5 and 10;

cutting other roots. After cutting the movements were not well defined, and rhythmic discharge was not apparent.

The strength of afferent discharge during cyclic contractions was related to the degree of distension of the buccal mass. Only a few units fired in root 3 when the buccal cavity was empty (Fig. 12*A*). Additional, larger, units were recruited when, in the same preparation, the buccal mass was threaded with plastic tubing to simulate the presence of food (Fig. 12*B*). Discharge in root 3 was similarly altered by distension of the buccal mass.

Mechanically impressed movements of the buccal mass similar to those that occur during the withdrawal (eversion) phase of contraction readily elicited afferent discharge (probably from different units). Such movements include: (1) forced retraction (protraction) of the radular sac, (2) bending the lateral teeth convex (concave) with respect to the anterior-posterior axis of the buccal mass, (3) forcing the lateral teeth toward (away from) the midline, and (4) moving the radula backward (forward) relative to its attachment with the lateral teeth.*

Effects of single sensory neurones

There are sensory neurones with somata located in the buccal ganglion (Siegler *et al.*, 1974). These neurones have axons in buccal roots 1 or 3, and their somata are located in the anterior lateral region of the buccal ganglion. Although the cells spiked cyclically with cyclic buccal mass contractions, they were judged to be sensory, rather than motor neurones on the following grounds: (1) an absence of synaptic input; (2) spikes in the soma, caused by natural or imposed movements, occurred at a constant latency after the corresponding spikes in buccal roots; and (3) soma spikes were sharply rising with no synaptic prepotential (Siegler *et al.*, 1974). The position of the somata, the distribution of their axons in peripheral roots, and their characteristic lack of cyclic firing in the isolated ganglion (even during vigorous rhythmic motor output) allowed the identification of these sensory cells in the isolated ganglion.

In the present experiments, the effect of spikes in sensory cells, on the feeding output

* Terminology is that used by Davis & Mpitsos (1971). Observation of the buccal mass, in the present experiments, confirmed their description of buccal mass movements, as shown in their Figs. 14 and 18, except, unlike Fig. 18*B*, the lateral teeth do not maintain the same shape throughout the feeding cycle, but during withdrawal, curve inward toward the midline of the buccal mass.

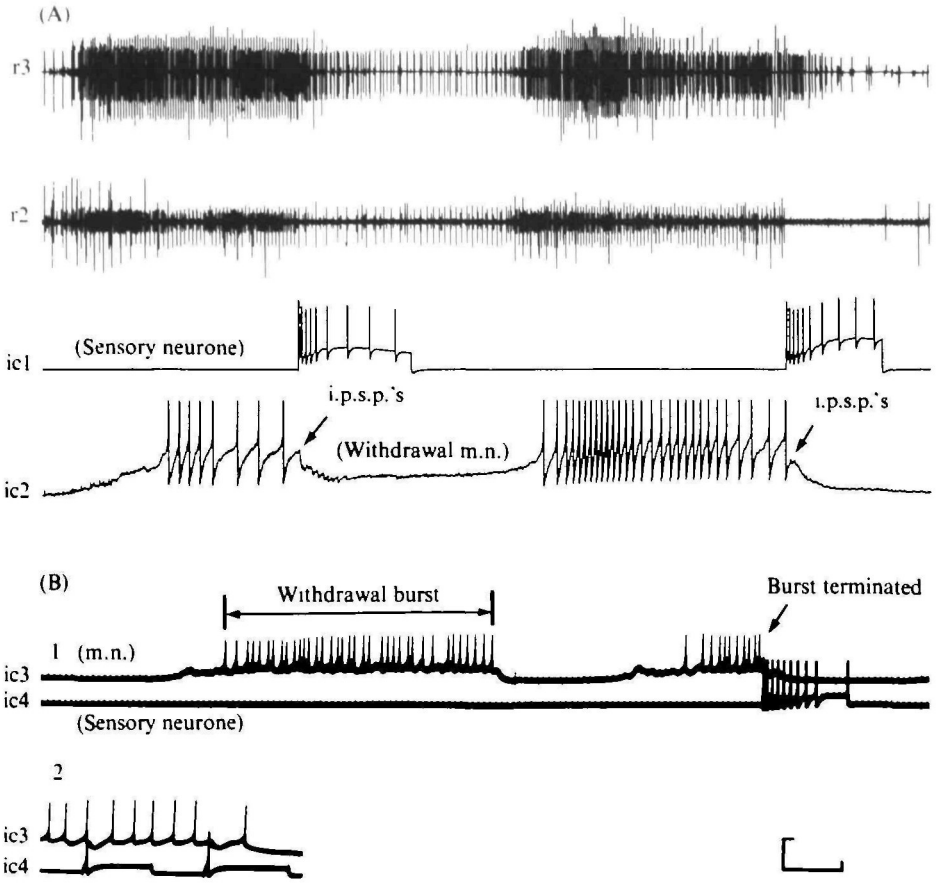

Fig. 13. Effect of spikes in a central sensory neurone upon withdrawal motor neurones, during cyclic feeding in an isolated ganglion. (*A*) Intracellular depolarization of the sensory neurone (ic1) evokes spikes, and initiates a barrage of IPSPs in a contralateral withdrawal motor neurone (ic2) which stops firing. Stimulation of ic1 also slows or stops spikes in several ipsilateral withdrawal motor neurones, recorded extracellularly in r2 and r3. (*B*) (1) Spikes in another sensory cell (ic4) result in premature termination of a burst in a withdrawal motor neurone (ic3). (2) IPSPs in the motor neurone are correlated one for one with firing in sensory cell. Cell ic1 (D1 rt. ventral; Rr3), ic2 (A3 left ventral; Lr3), ic3 (A3 rt. dorsal; Rr3), ic4 (D2 rt. dorsal; Rr1-r3). r3 and r2 are recorded ipsilateral to ic1 and ic2. Calibration. Vertical: *A*, ic1, 15 mV; ic2, 4·5 mV; *B*(1), ic3, 37 mV; ic4, 42 mV; *B*(2), ic3, 41 mV; ic4, 48 mV. Horizontal: *A*, 4·5 s; *B*(1), 1125 ms; *B*(2), 520 ms.

of the buccal ganglion, was assessed in the isolated preparation. These sensory neurones could be driven at only relatively low frequencies in response to imposed depolarization, (typically 4–8 Hz, compared to > 25 Hz for motor neurones), yet could affect the activity of the motor neurones. Firing in single different sensory cells either terminated (Fig. 13), or initiated (Fig. 14) firing in withdrawal motor neurones. In the latter, ongoing spikes in eversion motor neurones were simultaneously terminated (Fig. 14). Intracellular recordings from eversion or withdrawal motor neurones revealed that their spiking was terminated by a barrage of IPSPs, which far outlasted the occurrence of spikes in the sensory cell (Fig. 13*A*, 13*B*(1), Fig. 14*B*). In some motor neurones, single IPSPs could be correlated one to one with spikes in the sensory cell (Fig. 13*B*(2), Fig. 14*C*). The IPSPs followed at constant latencies, which, though

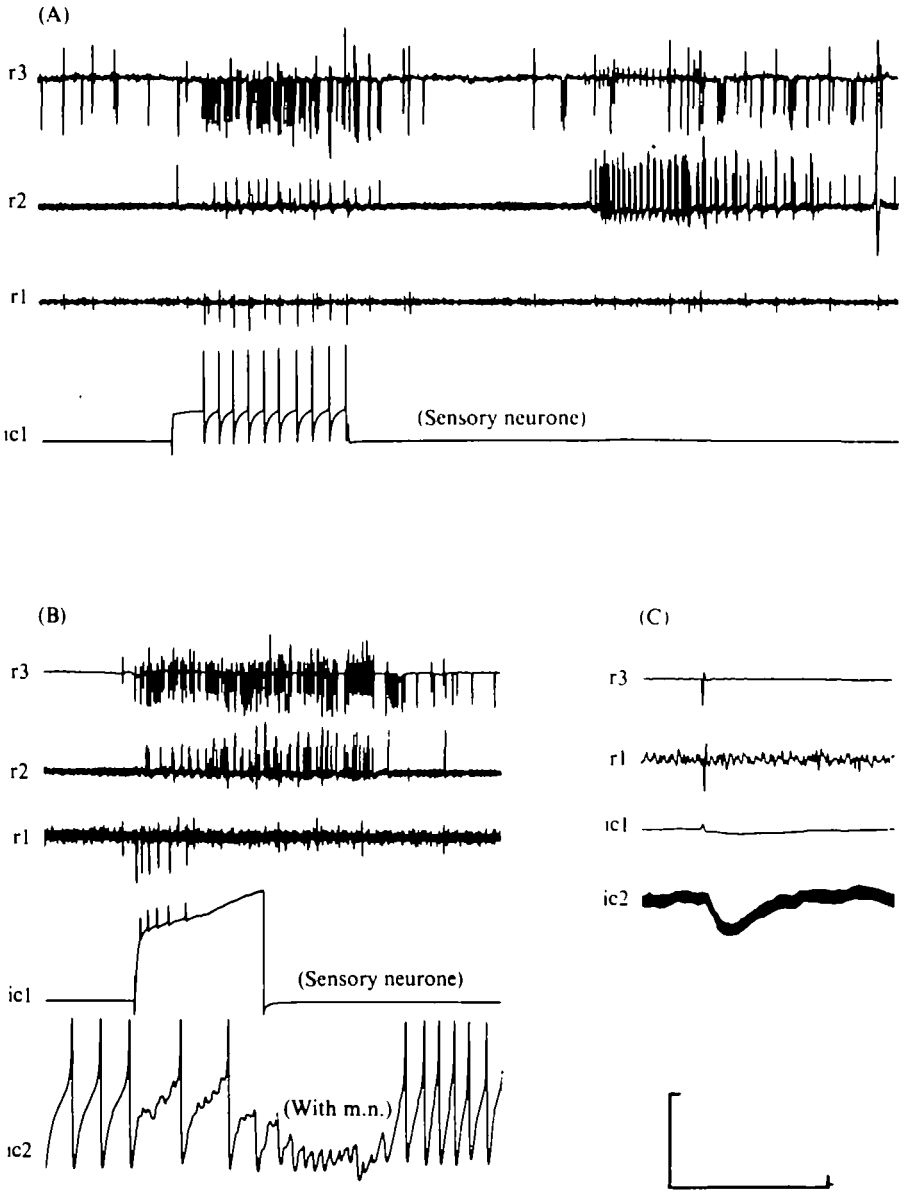

Fig. 14. Effect of central sensory neurone spikes on motor neurones in an isolated buccal ganglion. (*A*) Intracellular depolarization of a sensory neurone (ic1) in a quiescent preparation causes it to spike and initiates a burst of spikes in withdrawal motor neurones, recorded in ipsilateral r3. A burst of spikes in r2 eversion motor neurones often follows withdrawal activity with a latency of 2–3 s. (*B*) Spiking in eversion motor neurone (ic2) is inhibited during withdrawal burst by a barrage of IPSPs, one for one with action potentials. Cell position: ic1 (E2 left dorsal; Lr1-r3), ic2 (C2, left dorsal). r1, r2 and r3 are recorded ipsilateral to ic1 and ic2. Calibration. Vertical: *A*, ic1, 90 mV. *B*, ic1, 90 mV; ic2, 15 mV. *C*, ic1, 90 mV; ic2, 18 mV. Horizontal: *A* and *B*, 2 s; *C*, 400 ms.

long, (20 ms), are of the same order as those observed for known monosynaptic connexions in other molluscan nervous systems (see Berry & Pentreath, 1976, for a review).†

DISCUSSION

The rhythmic alternation of antagonist motor neurones appears to be coordinated in part by interneurones that have opposite post-synaptic effects on the two populations. Such dual-effect neurones can be inferred from the occurrence of one-to-one PSPs, of opposite polarity, in antagonist pairs of motor neurones. Only direct recordings from these supposed interneurones could rigorously exclude the possibility that the one-to-one PSPs arise, instead, from different but coupled interneurones. Dual-effect interneurones have been identified in other molluscs (e.g. Kandel, Frazier & Coggeshall, 1967; Gardner, 1971; Berry & Cottrell, 1973) and have been inferred to exist in insects (Burrows & Horridge, 1974) and crustaceans (Selverston & Mulloney, 1974). Such organization may represent one general mechanism for antagonist reciprocity, as noted by Kennedy & Davis (1976). The PSPs that are independent, but occur at approximately the same time in antagonists, must come from other coordinated interneurones which each have outputs to only one member of an antagonist pair.

The alternating pattern of activity recorded from antagonists apparently does not arise as a result of direct interactions between these motor neurones. It is likewise thought that the motor neurones do not interact directly, or that any direct interactions are not by themselves sufficient to generate the rhythmic motor output, for a variety of other rhythmic motor outputs. These include flight in the locust (Burrows, 1973), ventilatory movements in *Limulus* (Wyse, 1972), tail movements during backward walking in the crayfish (Kovac, 1974) and feeding in the snail *Helisoma* (Kater, 1974). The stomatogastric ganglion of the spiny lobster is the only well-documented example where direct reciprocal connexions between motor neurones appear to be primarily responsible for the alternation of firing in antagonists (Mulloney & Selverston, 1974).

In withdrawal motor neurones, both ipsilateral and contralateral activity is coordinated by synchronous barrages of PSPs of the same polarity. Again, common driver neurones can be inferred from the presence of many different one-to-one synaptic events in pairs of withdrawal motor neurones. Such one-to-one PSPs in synergist motor neurones have also been found to contribute to their coordinated activity in other molluscan, (e.g. Kater, 1974; Mayeri *et al.*, 1974) as well as arthropod (Sandemann, 1970; Burrows & Rowell, 1973) nervous systems. Conversely, in *Pleurobranchaea*, slight phase differences between some synergist pairs of motor neurones can, in part, be attributed to independent synaptic inputs. Intrinsic properties of the motor neurones may also contribute to the patterning of the motor output (Siegler *et al.*, 1974). This contrasts with the organization of the protractor motor neurones in the buccal ganglion of *Helisoma*; these apparently received identical synaptic inputs, and motor neurone properties alone are thought to determine their sequence of firing (Kater, 1974).

† Recordings were made from relatively few pairs (< 10) of sensory and motor neurones, so the present description is not intended to imply that other possible interactions do not occur. For example, it is not known whether or not sensory neurones which inhibit withdrawal motor neurones also simultaneously excite eversion motor neurones, or if withdrawal motor neurones excited by sensory firing (Fig. 14) receive EPSPs one to one with sensory spikes (as for the IPSPs).

Electrotonic coupling between synergist motor neurones is a feature of other molluscan feeding systems (e.g. Levitan, Tauc & Segundo, 1970; Spira & Bennett, 1972; Berry, 1972; Kater, 1974). In the present experiments strong non-rectifying electrotonic coupling was seen between the 'efference copy' neurones (Davis *et al.*, 1974) of the buccal ganglion (M. Siegler, unpublished observations), but no direct electrophysiological evidence for electrotonic coupling between motor neurones was obtained. Indirect evidence suggested, however, the presence of one or a few pairs of withdrawal motor neurones that were electrotonically coupled. This has been confirmed by R. Gillette (personal communication), who has recorded simultaneously from electrotonically coupled pairs of withdrawal, and eversion motor neurones. These were found in a region of the buccal ganglion not studied here.

The neurones and interconnexions responsible for the generation of rhythmic feeding motor output in the buccal ganglion of *Pleurobranchaea* have not been identified in the present experiments. But, as in most other systems studied (e.g. locust flight, Burrows, 1973; snail feeding, Kater, 1974; crayfish tail movements, Kovac, 1974; *Limulus* ventilation, Wyse, 1972), the immediate cause of cyclic activity in motor neurones appears to be rhythmic synaptic inputs from interneurones. In *Helisoma* the premotor interneurones are part of an electrotonically coupled network of cells; it is thought that this network has intrinsic rhythmic properties, which determine the timing of the motor output of the buccal ganglia (Kater, 1974). In other systems, it is not known whether the cells that are responsible for rhythmic synaptic inputs to the motor neurones are themselves part of the 'central pattern generator', or if they are, instead, driven by yet 'higher order' cells. Indeed, it may be impossible to make such distinctions in networks that have a high degree of reciprocal interaction within and between organizational 'levels' (Davis, 1976). In *Pleurobranchaea*, experiments must now be addressed to the identification of the interneurones, and the unravelling of their interconnexions. It seems unlikely that further analysis, directed solely at the motor neurone level, will be useful in revealing the source and cause of the intrinsic rhythmic activity of the buccal ganglion.

After eliminating sensory feedback, there were several changes in the feeding rhythm, even though other stimulus parameters were unchanged. The frequency of the rhythm was significantly lower, the duration of withdrawal burst increased, and the frequency of spikes within both withdrawal and eversion bursts decreased. Changes qualitatively similar to these are observed in the isolated buccal ganglion, when the frequency of the feeding rhythm is changed; for example, at lower frequencies of the rhythm, withdrawal bursts are longer and the frequency of spikes within them is lower (Davis *et al.*, 1973). This raises the possibility that the different changes observed with deafferentation were interrelated by an effect upon a central mechanism, rather than each representing an independent effect of removing sensory input. For example, comparison of withdrawal or eversion bursts from similar feeding frequencies shows that there is no difference in their duration in isolated and intact ganglia. It is therefore likely that the relationship between burst duration and feeding frequency is determined wholly by the central connexions of interneurones and motor neurones, as studied in the isolated ganglion. In contrast, the frequency of spikes within both eversion and withdrawal bursts is greater with sensory feedback, than in the isolated ganglion, when compared at similar feeding frequencies. Thus there is an excitatory effect of sensory

input that is independent of the change in burst duration and feeding frequency. This would explain the observations (M. Siegler, unpublished data) that in the isolated ganglion, some motor neurones (particularly the largest) are only occasionally depolarized to spike threshold, even during the fastest rhythmic frequencies. Presumably the recruitment of these motor neurones partially explains the more intense spiking within bursts with the addition of sensory input.

There is no direct evidence as to the precise connexions by which sensory feedback alters the motor output. Because there is a change in the frequency of the feeding rhythm with deafferentation it can be assumed that sensory feedback affects the central network that produces the rhythm. This necessarily implies, further, that sensory effects are mediated by the activity of interneurones (as argued in previous sections, interneurones must be involved in the pattern generation). Also consistent with this idea are the effects obtained when single sensory cells are stimulated in the isolated ganglion. The resulting barrage of synaptic and spike activity recorded in the motor neurones far outlasts the duration of spiking in the sensory cell itself, and antagonist motor neurones are, simultaneously, oppositely affected. Interneurones are implicated in these two effects because: (1) there are apparently no direct (i.e. monosynaptic) excitatory connexions between synergist motor neurones sufficient to sustain a burst, and (2) there are no direct inhibitory connexions between antagonist motor neurones, whereby firing in one group of motor neurones would directly inhibit the antagonist group. One way that this evidence for interneurone effects can be reconciled with that for monosynaptic sensory-motor connexions is by supposing that the effect upon interneurones is mediated through the changing activity of the motor neurones themselves. This possibility is suggested by the finding that some buccal motor neurones also make central connexions upon interneurones (W. J. Davis and R. Gillette, personal communication). Another possibility, not necessarily exclusive of the first, is that there are also direct sensory-interneurone connexions.

The present results on the effects of sensory feedback upon the central rhythm provide an interesting comparison with those obtained in investigations of the feeding system in the pond snail, *Helisoma*. There, in both the buccal ganglion-buccal mass and in the isolated buccal ganglia, motor neurones are phasically activated as part of a central programme (Kater & Rowell, 1973; Kater, 1974). But, in *Helisoma*, unlike *Pleurobranchaea*, the frequency of the feeding rhythm does not change when the buccal ganglion is deafferented (Kater & Rowell, 1973). The only reported effects of sensory feedback are to alter the duration of motor neurone bursts and the frequency of spiking within them. No interneurones were found that receive sensory input (Kater & Rowell, 1973). The interneurones thought to generate the rhythm (the 'cyberchron' network of Kater, 1974) do not appear to be affected, and any differences between the isolated the intact rhythm can be explained by monosynaptic connexions between stretch receptor neurones and motor neurones. One way these differences between *Pleurobranchaea* and *Helisoma* can be expressed is by saying that in *Helisoma* feedback from movements 'reinforces' the central rhythm, whereas in *Pleurobranchaea* sensory feedback can also 'regulate' the frequency of the central rhythm.

In *Pleurobranchaea* observation of the buccal feeding movements of partially dissected preparations (whole animal with buccal mass exposed), and consideration of the buccal mass anatomy, suggest that both phases of the feeding cycle are subject to

variations in load. During withdrawal, the lateral teeth are pressed toward the midline and drawn backward, and at the same time the radula is pulled backward by the radular sac (see Davis & Mpitsos, 1971). The greater the bulk in the buccal mass, the stronger the contractions of its muscles must be to complete these movements. Likewise, contractions that occur during eversion, such as those responsible for the forward movement of the radula and radular sac, must be stronger to compensate any additional load. A two-phase variation in load is reflected in recordings of afferent activity in response to both active muscle contractions, and externally imposed movements similar to those which occur during feeding. Within individual preparations, spikes in different afferent units can be associated with different movements, either eversion or withdrawal. Some of these large units are recruited only when the buccal mass is distended by bulk (internal load), and their firing is more intense during stronger, more prolonged movements. In the intact animal, such sensory feedback would allow adjustment of the central motor program to compensate for load variations throughout the feeding cycle.

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