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PATTERNED ACTIVITY OF THE BUCCAL GANGLION OF THE NUDIBRANCH MOLLUSC *ARCHIDORIS PSEUDOARGUS*

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INTRODUCTION

In recent years there have been a number of analyses of patterned activity in invertebrate ganglia. Examples include the studies of crayfish swimmeret beat (Hughes & Wiersma, 1960), the flight system of insects (Weis-Fogh, 1964; Wilson, 1966; Wyman, 1966) and crustacean heart-beat control (Maynard, 1966). In the Mollusca, Horridge (1960) described the production of patterned discharges from the cerebral ganglia of the clam *Mya* by electrical stimulation of the cerebral nerves, and Turner (1966) described a similar type of response from the pedal ganglion of *Agriolimax*.

More recently, Dorsett, Willows & Hoyle (1969) have described the production of a patterned sequence of bursts of impulses by a small group of symmetrically placed cells in the pedal ganglion of *Tritonia gilberti* during swimming. These swimming movements were elicited by contact with certain echinoderms, by liquid soap applied to the body surface, or by direct electrical stimulation of certain cells, although the sequence production could also be produced in the isolated brain by electrical stimulation of the cerebral nerves which supply the oral veil. Kandel, Frazier & Wachtel (1969) have also discussed the importance of inhibition in burst production by the abdominal ganglion of *Aplysia californica*.

Until recently the only electrophysiological study of the buccal ganglion was that of Strumwasser (1967), who showed that synchronous bursts were produced in the buccal ganglion of *Aplysia californica* as a result of common synaptic input to symmetrically placed cells. This work has been extended by Gardner (1969), who described both common input and the presence of a single interneurone with input to six cells in the ipselateral ganglion. Levitan, Segundo & Tauc (1970), in another study on the ophistobranch *Navanax inermis*, have described electrical connexions between ten identifiable cells and have given a quantitative description of the coupling coefficients between these cells.

The buccal mass of molluscs has been the subject of many investigations from the point of view of functional morphology (Fretter & Graham, 1962; Nisbet, 1954), but there have been no electrophysiological interpretations of radula movements. In the present study a natural stimulus has been used to induce feeding movements in the isolated buccal mass, and extracellular recording methods have been used to show that the buccal ganglion produces a sequence of bursts during radula movement. An account of the structure and nerve supply of the buccal mass of *A. pseudoargus* has been given elsewhere (Rose, 1970).

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MATERIAL AND METHODS

The induction of feeding movements

The induction procedure involved first starving the animal, then isolating the buccal mass, and finally presenting the sponge (on which the animal normally feeds) to the isolated buccal mass. This frequently had the effect of inducing radula movements similar to those of normal feeding.

Animals were isolated in separate aquarium tanks and starved for 3–4 days prior to the experiment. Isolation was necessary, since animals which were reproducing usually gave poor induction responses. The animals usually fed for periods of 15–20 h, but animals which were feeding also gave poor responses. Starvation for 3 or 4 days seemed to provide optimum conditions. Just before the experiment the animal was presented with sponge as a check, since animals which went to the sponge immediately usually gave the best results.

During the dissection the mantle was first removed from the anterior third of the animal, care being taken to prevent mucus from contacting the buccal mass. The connective tissue covering the brain was then dissected away and the buccal mass, brain, and buccal ganglia were removed from the animal. This was done by cutting through the oesophagus, the paired postero-lateral retractor muscles M 11 (Rose, 1970) and the muscles round the mouth, so that the dissected buccal mass was complete with oral tube and oral tentacles. The branchial, pedal, visceral and rhinophore nerves were cut, but great care was taken not to damage any of the twenty buccal and cerebral nerves supplying the buccal mass, as damage seriously affected the induced responses.

The buccal mass was then pinned out under sea water, with the buccal ganglia uppermost, and a piece of fresh sponge was placed in the bath beside the buccal mass. It was very important that the sponge was fresh, since animals will not normally feed on sponge which is even partially decayed. The breadcrumb sponge, *Halichondria panicea*, was used throughout these experiments. *Archidoris* feeds on other sponges, particularly the red sponge, *Hymeniacidon perleve* (Thompson, 1966), but inferior responses were generally obtained when these were used. In some experiments whole-animal preparations were attempted with the mantle partly removed and the rhinophore nerves left intact. There was little difference between the responses obtained from this nearly intact preparation and those obtained when the buccal mass was completely removed, so the isolated preparation was more frequently used since it was easier to make the recordings in this condition.

Transducer recording of the induced movements

The buccal mass was dissected out in the usual way, and under the experimental conditions described above. It was opened up by a dorsal longitudinal cut along the top of the oral tube and buccal mass as far as the oesophagus, to expose the radula. A needle threaded with cotton was pushed through the radula and underlying odontophore cartilage of one half of the radula, and tied in a loop. The odontophore formed a convenient anchor, as well as being the structure responsible for movement of the radula. The buccal mass was then pinned under sea water with several pins through

e oesophagus posteriorly and one through the ventral wall of the outer constrictor muscle M 5 (Rose, 1969) anteriorly. The other end of the cotton thread was tied to a balanced lever, positioned in line with the radula movements, and radula movements were recorded using a phototransistor arrangement to detect movement of the lever, the resulting voltage change being registered on one beam of a Tektronix 502A oscilloscope. Simultaneous with the transducer measurements, recordings were made either from the ganglion surface or from a buccal nerve, and this activity was fed into the other beam of the oscilloscope. In a number of cases two such recordings were made simultaneously, and the transducer movement and nerve activity from one suction electrode were fed into the same beam of the oscilloscope, using the differential input.

Extracellular recording

Up to eight suction electrodes were used simultaneously to record from the buccal ganglia and nerves. Any combination of four of these electrodes could be selected by a switchboard arrangement. The outputs were led into four a.c. amplifiers, and then into four channels of an ultraviolet recorder (Southern Instruments 10-200). Each suction electrode was mounted on a compact home-made manipulator with a 'universal-joint' construction.

Three general sizes of electrode were used in making extra-cellular recordings from the surface of the ganglion: (*a*) electrodes with the tip diameter the same as the diameter of individual cells were used to record the activity of selected cells; (*b*) slightly larger electrodes were used to record the activity of two to five cells; (*c*) electrodes with a tip diameter of about 300 μ were used to record the activity of most of the cells at the surface of the ganglion.

Intracellular recording

Intracellular recordings were made with glass microelectrodes filled with 3 M-KCl and having a resistance of 5–10 MΩ. A near-unity-gain cathode follower was used. The buccal ganglia were pinned out next to the buccal mass without damaging the nerve supply. The buccal mass was pinned immediately below the buccal ganglia to minimize movements of the ganglia. The ganglia themselves were pulled aside by a pin placed inside the connective on one side. Intracellular recordings were made by making a small hole in the coat of the ganglion with an electrolytically sharpened tungsten needle. In most experiments work was concentrated on the region of the right outer quadrat of the ganglion, and on identifiable units in the nerves.

MATERIAL

Both *Archidoris pseudoargus* and *Aplysia punctata* were available locally, but it was possible to obtain larger numbers of *Archidoris*, and this was also found to be a better preparation from the electrophysiological point of view. Animals were kept in running sea water at room temperature.

RESULTS

Anatomical

Details of the anatomy of the buccal mass and its nerve supply in *Archidoris* have been given by Rose (1970). For the purposes of the present work, the most relevant

points have been summarized in Fig. 1. The paired buccal ganglia are about 1 mm total diameter, and are joined to the cerebral ganglia by the paired cerebro-buccal connectives (C). In addition to a connective, each ganglion gives rise to three buccal nerves, which have been called the radular nerve (B 1), the ventral buccal nerve (B 2) and the dorsal buccal nerve (B 3). The main nerve supply to the buccal muscles is the ventral buccal nerve B 2, which supplies five separate muscles. Both B 1 and B 2 supply the largest muscle, the radular retractor. The dorsal buccal nerve B 3 supplies the constrictor muscles, and a branch supplies the muscles at the opening of the oesophagus, which are concerned with the initiation of peristalsis.

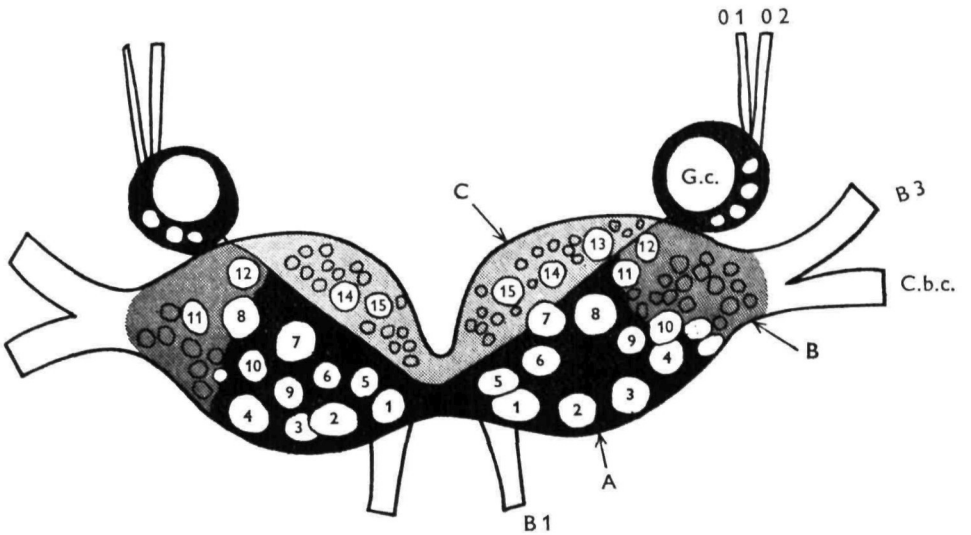

Fig. 1. Diagrammatic representation of the buccal ganglia of *Archidoris pseudoargus* (from Rose, 1970). Each buccal ganglion is divided into three areas: area A contains the largest cells; area B contains medium-sized cells; area C contains the smallest cells. G.c., giant cell of gastro-oesophageal ganglion; O 1 and O 2, oesophageal nerves; B 1, radula nerve; B 2, ventral buccal nerve; B 3, dorsal buccal nerve; C.b.c., cerebro-buccal connective. (Note: in all other figures in this paper the cerebro-buccal connective is labelled as 'C'.) Nerve B 2 is not shown as a separate nerve, because it leaves the cerebro-buccal connective about $\frac{1}{2}$ mm from the ganglion.

The ganglion is made up of three areas of orange-pigmented cells. In area A there are about twelve large cells of 40–60 μ diameter, in area B there are about twenty medium-sized cells of 10–30 μ diameter, and in area C there are large numbers of small cells of 4–8 μ diameter. The work described in this paper concerns the larger cells in area A of Fig. 1.

Attached to each buccal ganglion is a smaller gastro-oesophageal ganglion (200 μ diam.), each ganglion containing a single giant cell together with 3 or 4 medium-sized cells, and giving off two main nerves to the oesophagus, O 1 and O 2, and two smaller nerves to the salivary glands. These giant cells are homologous with the giant cells of the gastro-oesophageal ganglia of *Anisodoris*, described by Gorman & Mirolli (1969).

The induced sequence

Recordings made from the buccal nerves in an isolated buccal-mass preparation, where the animal has not been starved and sponge is not presented, usually show

ly a few active units. Such a recording is shown in Fig. 2A. One unit in the ventral buccal nerve B 2 increases in activity following a short regular burst in the connective. Another unit shows a 1:1 relationship to a 'doublet' of spikes in the connective, and it is later shown to be a branch of this descending fibre in the connective. In Fig. 2B a recording was made from the same nerves, but this time in an animal which was starved for 3 days prior to the experiment and with sponge presented to the isolated buccal mass. This time it is seen that a sequence of bursts occurs in the ventral buccal nerve B 2, having a duration of about 30 s, and being composed of at least five separate units. In addition it should be noted that the 'doublet' of spikes is again recorded in the connective, with a branch in the nerve B 2. The 'doublet' stops as the burst sequence starts with B 2, but again starts firing regularly before the sequence is completed.

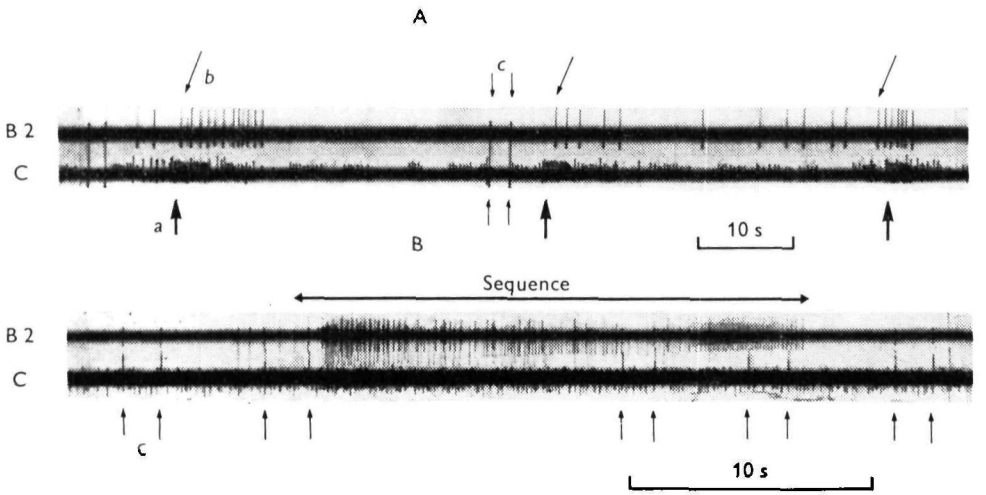

Fig. 2. A, Recordings from C and B 2 from an animal which was previously feeding. *a*, Regularly-firing unit; *b*, unit which closely follows unit *a*; *c*, 'doublet' spike. B, Recordings from C and B 2 from an animal which was starved, and in which the sponge *Halicondria* was presented to the isolated buccal mass. A sequence of activity lasting 25 s occurs in B 2, and the 'doublet' spike (*c*) stops during the sequence.

Symmetry of the ganglia

Two simultaneous recordings were made from a large part of the surface of the right and left buccal ganglia (Fig. 3) and show that a sequence of bursts can be recorded from the right ganglion which closely corresponds to the sequence on the opposite side. Bursts on opposite sides are similar in spike repetition frequency, spike amplitude, and burst duration, and are repeated at regular intervals varying from 1 to 4 min, with very little activity in between. A plot of the change of inter-spike intervals of successive spikes in the first burst on opposite sides (Fig. 4), shows that the onset of the burst in the right ganglion is about 100 ms before that in the left, and the burst in the right ganglion is of higher frequency. The changes in inter-spike interval of the two cells are very similar.

Two simultaneous recordings made from the right and left gastro-oesophageal ganglia (Fig. 5) show that the two giant cells burst nearly synchronously, but again the cell on the right fires a burst of higher frequency than the cell on the left. The branches

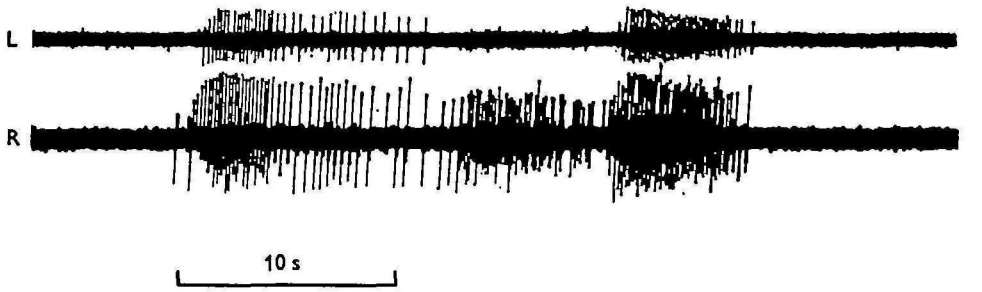

Fig. 3. Symmetry of the left (L) and right (R) buccal ganglia. The recordings were made with a large suction electrode from symmetrically placed regions in each ganglion. The first and last bursts in each sequence are probably from the same cell.

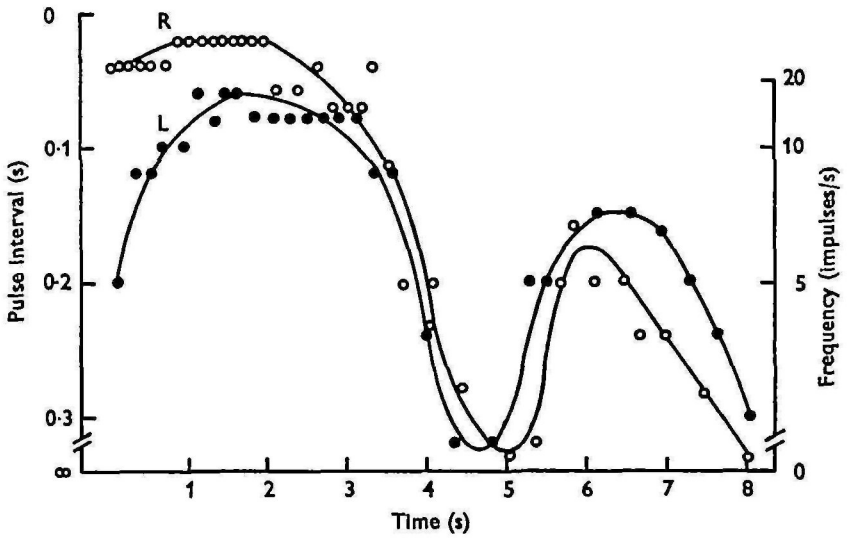

Fig. 4. Plot of inter-spike interval changes for the first pair of bursts in the right (R) and left (L) ganglia shown in Fig. 3.

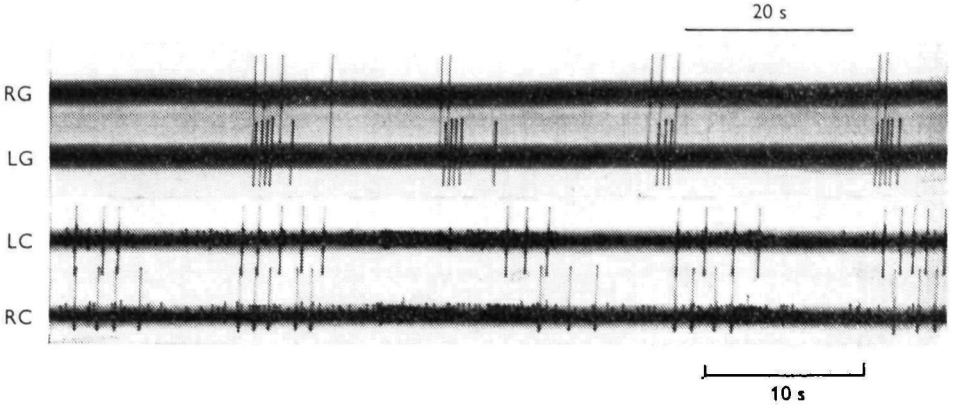

Fig. 5. Paired recordings from the right and left gastro-oesophageal ganglia (RG, LG) and from the right and left connectives (RC, LC) in two different preparations.

the two giant cells have not so far been traced, so that the functional significance of these bursts is not yet clear. Two simultaneous recordings made from the right and left connectives in a different preparation (Fig. 5) show that the 'doublet' of spikes is nearly synchronous (but not 1:1) in the two connectives.

The relationship between the giant cell and this fibre in the connective is not yet known, but preliminary experiments indicate that the axon is not a branch of the gastro-oesophageal giant cell.

Constancy of the sequence

It is of considerable importance to establish whether it is possible to induce a sequence, nearly identical to that shown in Fig. 6, in another individual. Fig. 6A is a recording taken from the same region of the right buccal ganglion as in Fig. 6B, but from a larger animal. The burst sequence is almost identical in the two preparations. One striking visual illustration of the similarity of these two sequences is given by the

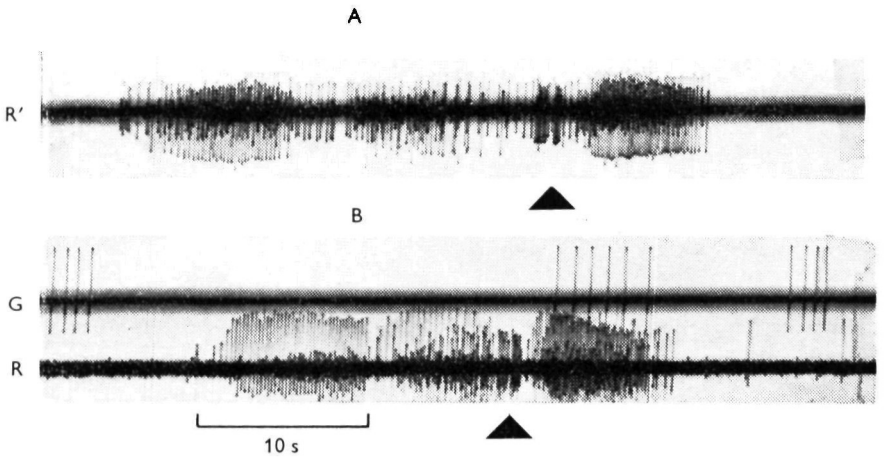

Fig. 6. Recordings made from a large area of the surface of the right buccal ganglion in two different individuals during an induced movement. The two sequences show much similarity (upper trace R' is inverted), which is particularly evident for the two short bursts indicated by the arrow. Recording B was made from the gastro-oesophageal ganglion, and shows the relationship of the burst in the right giant cell to the sequence.

two short bursts at the end of the second long burst, which occur almost exactly at the same time relative to the third long burst in the two animals. This example is useful in showing that the sequence has certain features which are repeatable from one individual to another. About ten preparations were made before this result was obtained, however, so that it can by no means be said that the induction procedure always produces the same sequence of activity in the ganglion. In Fig. 6B the lower trace is a simultaneous recording from the right gastro-oesophageal ganglion and the right buccal ganglion. This shows that the right giant cell of the gastro-oesophageal ganglion fires a burst of impulses in a fixed relationship to the sequence occurring in the buccal ganglion on that side.

Surface recording

It is possible to trace axonal branches from buccal ganglion cells using the method of simultaneous intracellular and extracellular recording (Tauc, 1962; Willows, 1968). The main difficulty with the buccal ganglion of *Archidoris* is that the connective tissue sheath is very tough, and any technique which involves opening the sheath disturbs the cell positions to such an extent that it would not be possible to locate a specific cell in this small ganglion. It is possible to get over this difficulty by replacing the intracellular recording with an extracellular recording using a suction electrode placed over a

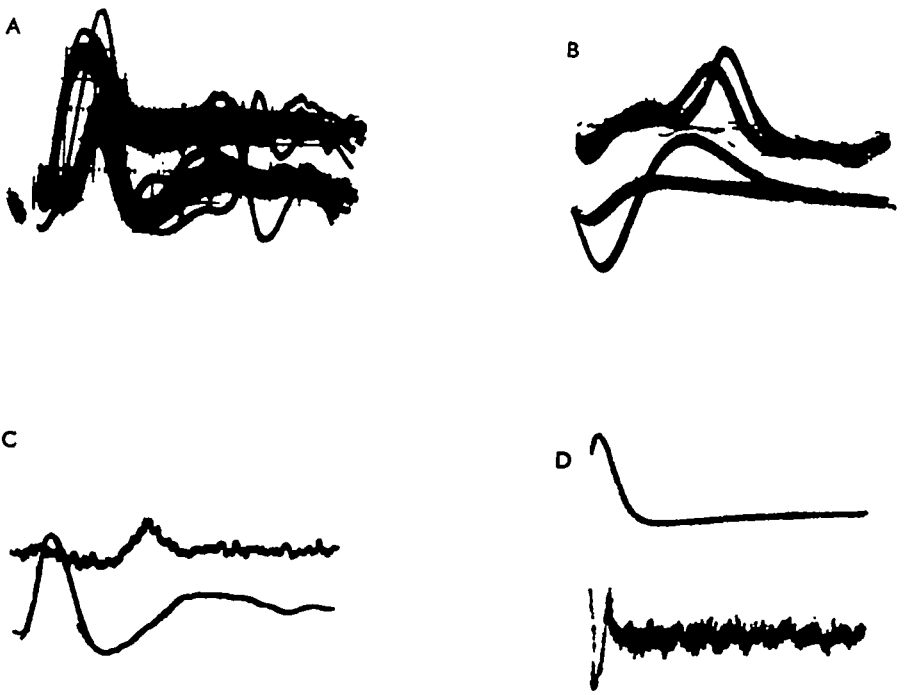

Fig. 7. Comparison of surface and intracellular recording methods in axon mapping. A, Cell recorded with a suction electrode at the surface (upper beam) and suction electrode on B 2 (lower beam); twenty sweeps superimposed. B, Same recording method from a different region of the ganglion (lower beam) and from B 2 (upper beam). C, Extracellular recording of 'doublet' spike action potential in the connective (lower beam) and B 2 (upper beam). D, Intracellular recording from a buccal ganglion cell (upper beam) with a branch in B 2 (lower beam).

specific cell at the ganglion surface. In Fig. 7 recordings made in this way are compared with the intracellular type of recording from a buccal ganglion cell, and there is no significant difference. Using this technique it is possible to locate the cell position at the surface of the intact ganglion and check the position of its axon in all possible nerves.

Transducer recording

Usually few movements are shown by the buccal mass, which is simply isolated from the animal. The induction procedure, however, causes the radula to move in a

manner similar to normal feeding movements (Rose, 1969). Under the best conditions this cycle consists of a forward, then a backward, and finally another forward movement to the resting position. Cinematography of the feeding cycle (Rose, 1970) has shown that the complete feeding cycle lasts 20–25 s. The sequence of overlapping bursts shown earlier (Fig. 3) is of a similar duration, which suggests that the induced movements may be similar to normal feeding movements.

Two types of cell were recorded at the surface of the ganglion: (1) cells whose activity is related to specific movements of the radula, and (2) cells which fire during the radula movement but whose activity is not related to specific radula movements

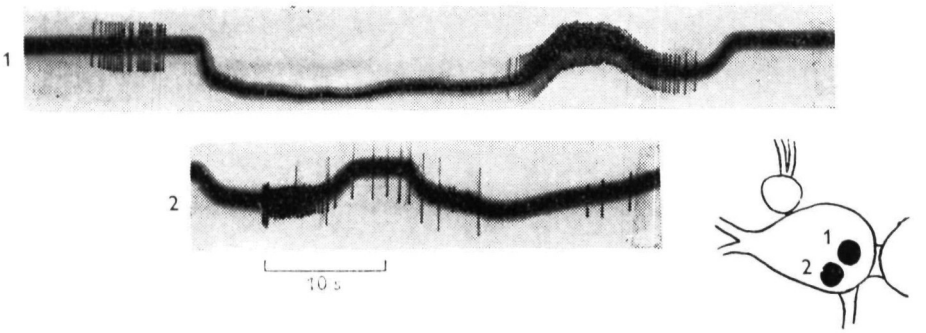

Fig. 8. Recordings from two separate areas (1, 2) of the surface of the left buccal ganglion, stimultaneous with a transducer recording of movements of the left half of the radula. The transducer and extracellular recordings were fed differentially into the same channel of the oscilloscope. Backward movement of the radula is indicated by a downward deflexion of the beam. Diagram shows recording positions on ganglion surface.

recorded by the transducer. Such a division of types is to be expected, since not all of the cells will be involved in the control of the muscles which move the radula. A good example of this distinction is given in Fig. 8, where the upper trace shows a burst which occurs simultaneous with a forward movement of the radula (upward deflexion). With the electrode in a different position on the surface of the ganglion (lower trace) one unit fires during a backward movement and another larger-amplitude spike appears to fire during a forward movement.

The same burst and corresponding movement occurred repeatedly in a given preparation. This is shown clearly in the two examples of Fig. 9. In the upper two traces (A) the same burst always occurs in the same part of the cycle. In the lower traces (B) the burst is always simultaneous with a backward movement of the radula. However, no reliance can be placed on comparisons between individuals, as both strength of the contraction and the loading of the lever varied from one animal to another.

In cases where two simultaneous recordings were made from two regions of the ganglion surface the burst are seen to fire at different times during radula movement. Fig. 10(*a*, *b*) illustrates a case where cells in both regions fire during the first backward movement, and two other larger-amplitude units in the region recorded on the upper trace only fire during a stronger retraction movement. In Fig. 10(*c*, *d*) recordings were made from what were thought to be corresponding regions on opposite sides. In this particular case the bursts on opposite sides were not synchronous, but again showed specific correlations with radula movements.

Detailed analysis of a group of neurones

This particular analysis concentrates on a group of neurones which fire bursts in relation to the cell which was seen to fire first in the sequences shown in Figs. 3 and 6. This particular cell we will call cell A. Cell A always has the highest frequency of the

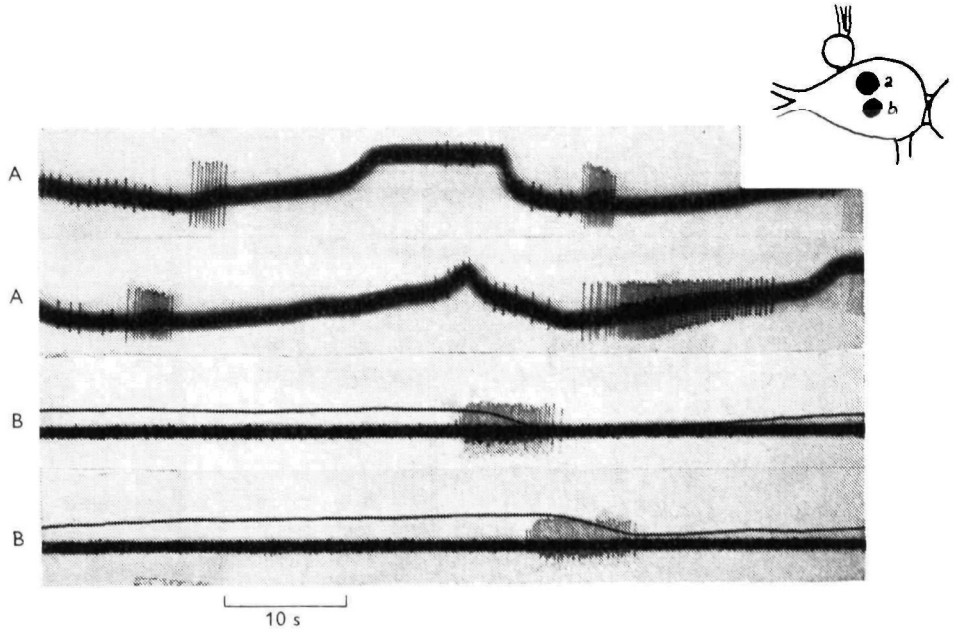

Fig. 9. Further recordings of bursting and radula movement. The upper two recordings (AA) and the lower two recordings (BB) are continuous. A, Four backward movements of the radula (downward deflexion). The burst occurs at approximately the same time in each cycle. B, Two backward movements of the radula simultaneous with another burst recorded at the ganglion surface. Diagram shows recording positions at ganglion surface.

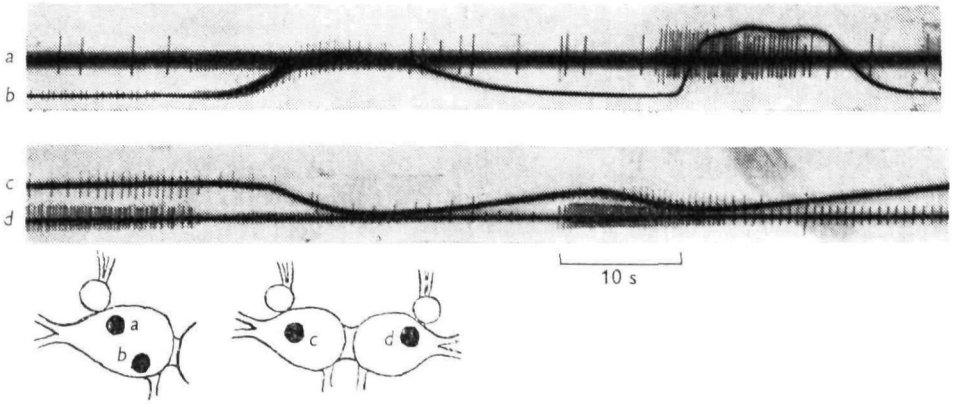

Fig. 10. Paired recordings from the surface of the buccal ganglion simultaneous with a recording of radula movement fed into one beam differentially. (*a*, *b*) Recordings made from two areas of the right buccal ganglion, with different units firing in the two areas during a weak and a stronger backward movement of the radula (upward deflexion of beam *b*). (*c*, *d*) Recordings made from two areas on opposite ganglia. Backward radula movement is a downward deflexion of beam *c*, and the activity of partner cells is not recorded. Diagram shows recording positions at ganglion surface.

[illegible]oup of cells discussed here, and is usually the leading cell in the sequence. Four examples of recordings made from the group of cells associated with cell A are discussed below.

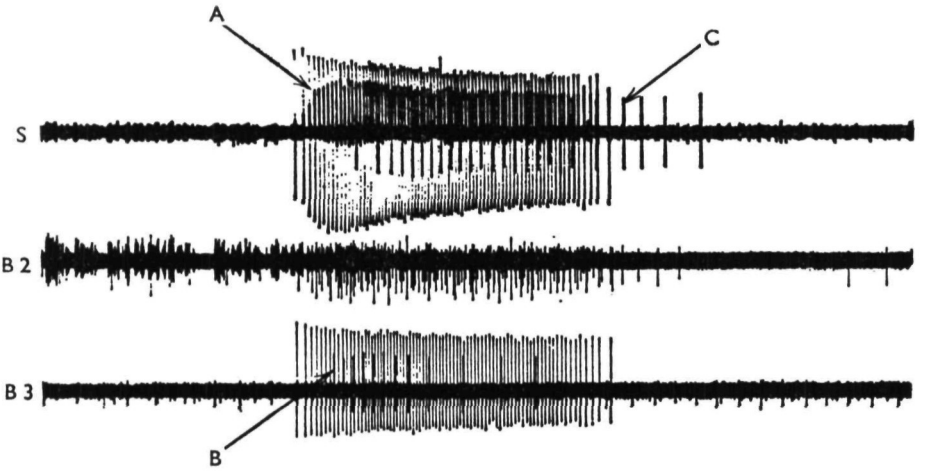

Fig. 11. Three-channel extracellular recording from the ganglion surface (S), B 2, and B 3 (preparation (1)), showing the frequency changes of units A, B and C.

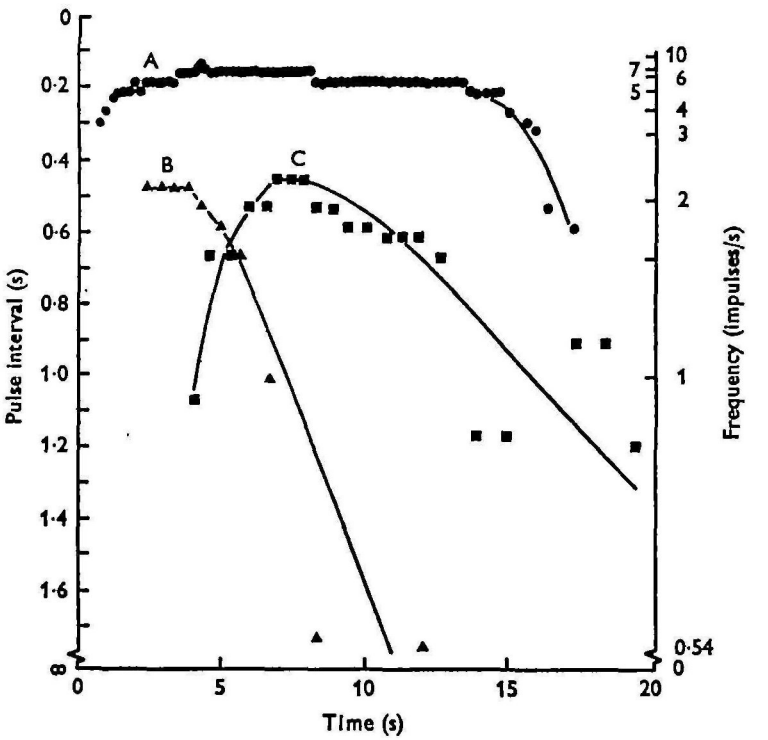

Fig. 12. Inter-spike interval plot of units A, B and C of Fig. 11. There is a reciprocal relationship between the increasing interval (decreasing frequency) of unit B and the decreasing interval (increasing frequency) of unit C.

Preparation (1) (*Figs.* 11, 12).

In this preparation one recording was made from the surface of the ganglion over cells A and E. Cell A is seen to have an axon in the buccal nerve B 3. Two low-frequency cells, B and C, fire in relation to cell A, in addition to a number of less clearly defined units recorded in the ventral buccal nerve B 2. The interval changes of these three units, A, B and C, are plotted in Fig. 12. Cell B, which has an axon in the dorsal buccal nerve B 3 (*a*), fires initially at its highest frequency, but its frequency rapidly falls. The onset of this fall in frequency in cell B is coincident with the start of the burst in cell C. Cell C is situated at the surface of the ganglion close to cell A, since units A and C are recorded by the same electrode; cell C has a higher peak frequency than that of cell B and continues firing for several seconds after cell A has stopped. There appears to be a reciprocal relationship between the rise of frequency (decreasing interval) of cell C and the fall of frequency (rising interval) of cell B.

Preparation (2) (*Figs.* 13, 14)

In the second preparation neurone A was again located in the outer dorsal quadrat of the ganglion, and found to have an axon in the dorsal buccal nerve B 3 (lower trace, Fig. 13 (*a*)). In this example the burst shows a slightly different change of frequency, with a characteristic grouping of impulses in the burst. The recording shown in Fig. 13 (*a*) was made with a suction electrode on the surface of the ganglion over cell A (second trace), and with an electrode on the radular nerve B 1 (upper trace), the ventral buccal nerve B 2 (third trace) and the dorsal buccal nerve B 3 (lower trace). A burst that has been labelled as unit G, occurs in the radular nerve B 1 (upper trace) simultaneously with burst A. The 3-channel recording of Fig. 13 (*b*) was made after removing one electrode from the radular nerve B 1, and moving the suction electrode on the surface to another position near to the position of neurone A. Another unit, neurone E, was recorded at the surface of the ganglion, with an axon in the ventral buccal nerve B 2 (second trace). The interval changes of the two bursts A and E are plotted in Fig. 14, which shows that the changes in interval of one neurone parallel the changes in the other very closely. This will be shown again in preparation (3). It should be noticed that unit E also shows a characteristic grouping of impulses which appear to be coincident with the impulse grouping of neurone A.

Cell B can again be seen in the dorsal buccal nerve B 3 (lower trace), and its onset is coincident with a rapid increase in the frequency of cells A and E, and with the onset of other units recorded in the ventral buccal nerve B 2 (middle trace).

There is an interesting relationship between the remaining units in this recording. Cells A and E fire short bursts of impulses before the long A/B/E bursts, but not after them. Two other cells, X and X′, fire before and after the A/B/E bursts, but their activity is interrupted by these bursts. Unit X (upper trace) has been recorded at the surface with a branch in the dorsal buccal nerve B 3 (lower trace). Unit X′ has a branch in the ventral buccal nerve B 2 (middle trace). These two units give coincident bursts of similar frequency, X′ being of slightly higher frequency than X, and show a distinctive regularity of bursting immediately after the A/B/E bursts, but are more difficult to distinguish than bursts earlier in the recording.

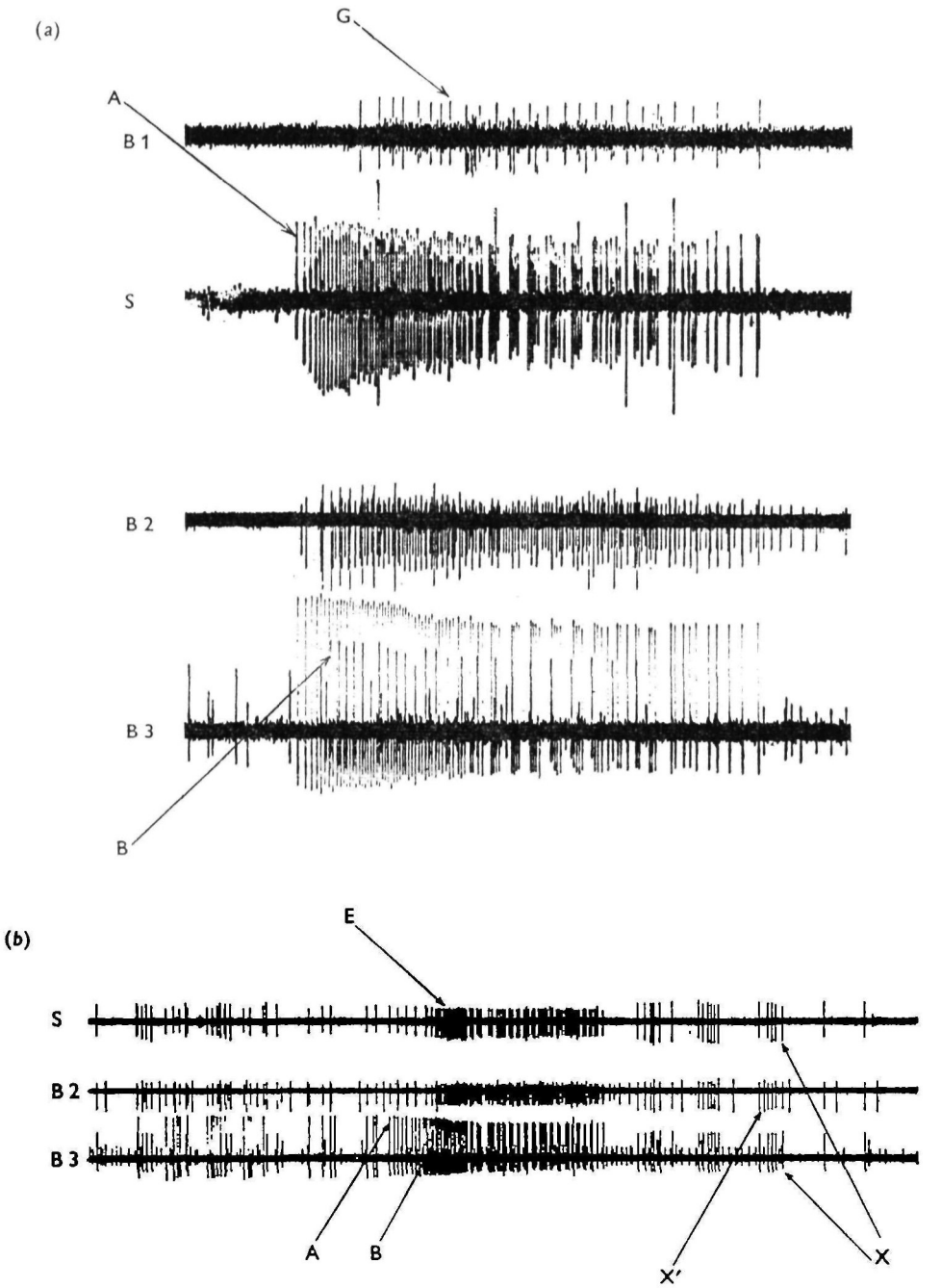

Fig. 13 (*b*). [preparation (2)]. Three-channel extracellular recording from the ganglion surface (S), and the buccal nerves B 2 and B 3. The suction electrode at the surface of the ganglion is over cell E, and units A and B occur in the ventral buccal nerve B 3 (lower trace). In addition units X and X′ fire short-duration bursts. (*a*) Four-channel extracellular recording from the ganglion surface (S), and the buccal nerves B 1, B 2 and B 3. The suction electrode at the ganglion surface is over cell A. The recording shows the relation of unit G (upper trace) to units A and B.

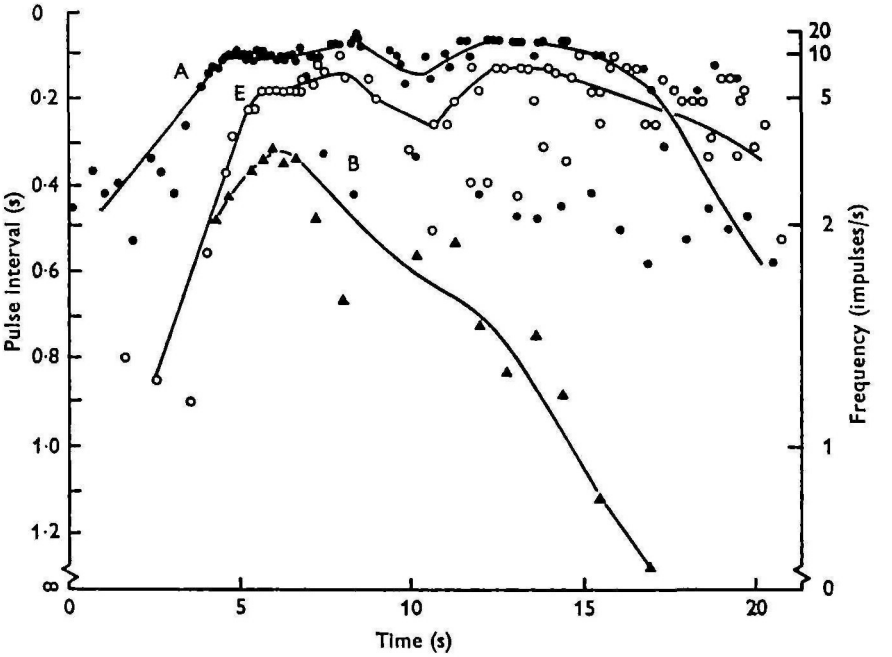

Fig. 14. Inter-spike interval plot of bursts A, B and E, shown in Fig. 13. There is a close relationship between the interval changes of units A and E, and a characteristic fall in frequency of unit B.

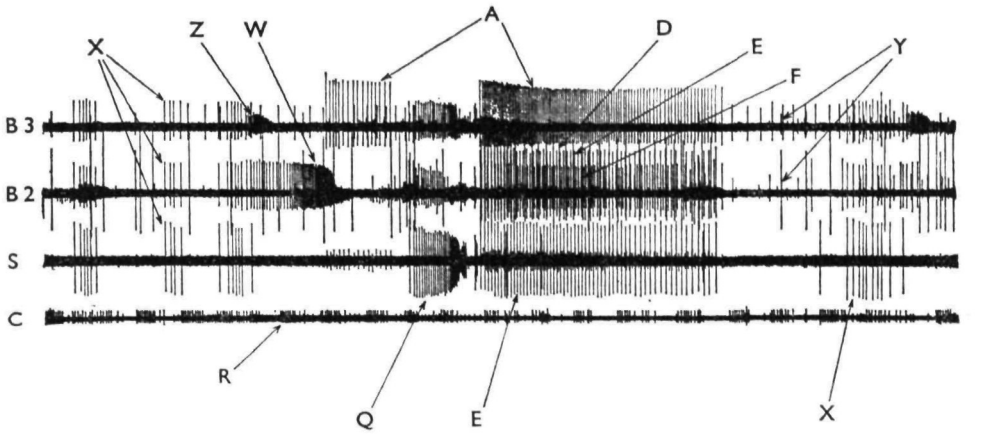

Fig. 15. Preparation (3), 4-channel extracellular recording from ganglion surface (S), C, B 2 and B 3, showing synchronous bursting in units A, E, D and F. The small amplitude spikes recorded at the ganglion surface are from the soma of cell A, with a branch in B 3. Z, Regularly firing burst of increasing frequency. X, Regularly firing burst recorded at the surface, with branches in B 2 and B 3. Q, Burst of increasing frequency, which may be cell X. W, Burst of increasing frequency. Y, Unit producing single spikes, with branch in B 2 and B 3. R, Regularly firing burst in connective.

Preparation (3) (*Figs.* 15, 16)

The most complete example of the group of cells A–H so far recorded is shown in Fig. 15 and analysed in Fig. 16.

A characteristic burst from cell A occurs in the dorsal buccal nerve B 3, whose spikes bear a 1:1 relationship to small-amplitude spikes recorded at the surface (third trace). This provides evidence that the recording area at the surface was very close to cell A. A large-amplitude burst recorded at the surface has a branch in the buccal nerve B 2, and is probably cell E which was recorded in Preparation (2). It can be seen from the graph (Fig. 16) that the interval change of cell E follows that of cell A very closely indeed, as has been remarked previously. In Fig. 20(*a*) the interspike intervals for cell A are plotted against those of cell E, and this shows clearly the linear relationship between them.

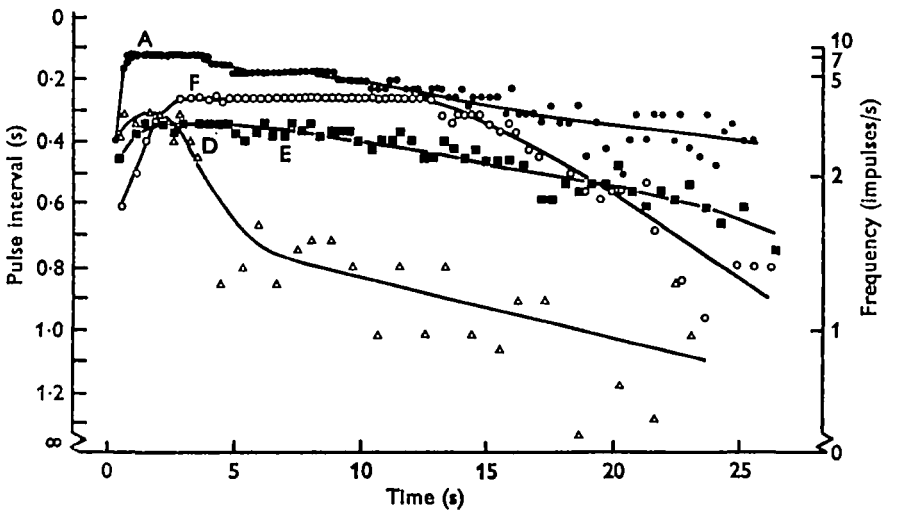

Fig. 16. Inter-spike interval plot of bursts A, E, D and F shown in Fig. 15. There is a close correspondence of interval changes of units A and E.

In this preparation two other units have been recorded in the ventral buccal nerve B 2. One burst, whose spikes are of the largest amplitude (cell D), fires at a lower frequency than the other units, and its frequency falls more rapidly. A fourth unit in this recording which we will call cell F (lowest-amplitude spike in B 2), reaches a maximum frequency which is slightly higher than that of cell E, and maintains this frequency for several seconds and then rapidly falls in frequency. All four bursts in this particular example are seen to start and stop at almost exactly the same time. In addition, a regularly firing unit (R) occurs in the connective, whose activity is unaffected by the A/E/D/F burst complex.

A number of other units can be seen in this recording. Again there is a regularly firing unit which stops as the A/E/D/F burst complex occurs, but this cell appears to have branches in both buccal nerves (B 2 and B 3), so that it is difficult to decide whether it is unit X or X′. In addition, cell Y has branches in the buccal nerves B 2 and B 3, and cell Z fires at regular intervals throughout the recording. Two bursts are of the

'increasing frequency' type (W, Q). It is possible that these are not two additional cells, however, since unit W could be the same as cell F, and the Q burst could be unit X, since it has branches in the buccal nerves B 2 and B 3.

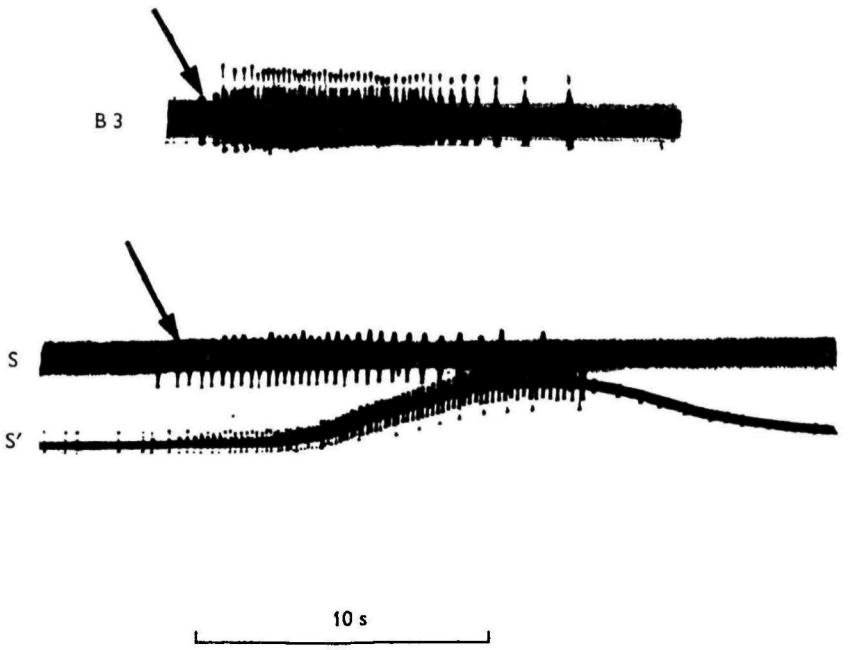

Fig. 17. Preparation (4). Upper recording: single extracellular recording from B 3 showing two units; the larger-amplitude unit is probably cell A, and the unit which is arrowed is cell H. Lower recording: part of recording shown in Fig. 10; unit H (arrowed) is recorded at the surface of the ganglion (S) and two other units are recorded at another part of the surface (S').

Preparation (4) (*Figs.* 17, 18)

Further information on this system comes from a recording made from the dorsal buccal nerve B 3 (Fig. 17). This shows a unit which we call cell H, which fires before a second burst, which is probably cell A. The graph of the interval changes of these two cells (Fig. 17) shows that cell H increases in frequency to a maximum and then its frequency starts to fall at the onset of the burst in cell A. There is a delay of 4–5 s before the frequency of cell A starts to fall rapidly.

It is interesting to compare this record with that shown before in Fig. 10(*a*, *b*) which is shown again in Fig. 17. In this case the unit in the upper trace of the recording (arrowed) is very similar to cell H and another burst, which might be cell E, lags behind the first.

Fig. 20 (*b*) summarizes the positions of the axons of neurons A–H discussed above.

Intracellular recording

It was not possible to obtain simultaneous recordings from two ganglion cells, mainly because of the difficulty of penetration of the sheath in this species. Recordings of intracellular activity simultaneous with extracellular activity were restricted to preparations in which the ganglia were still attached to the buccal mass, and attempts

re made to induce feeding movements while these recordings were being made. Two such recordings are shown in Fig. 19. In Fig. 19(*a*) the cell whose activity was being recorded with the microelectrode was below the threshold for spike generation for several minutes, and little activity was recorded extracellularly in nerve B 2. When sponge was placed near to the preparation a burst of about 10 s duration was recorded

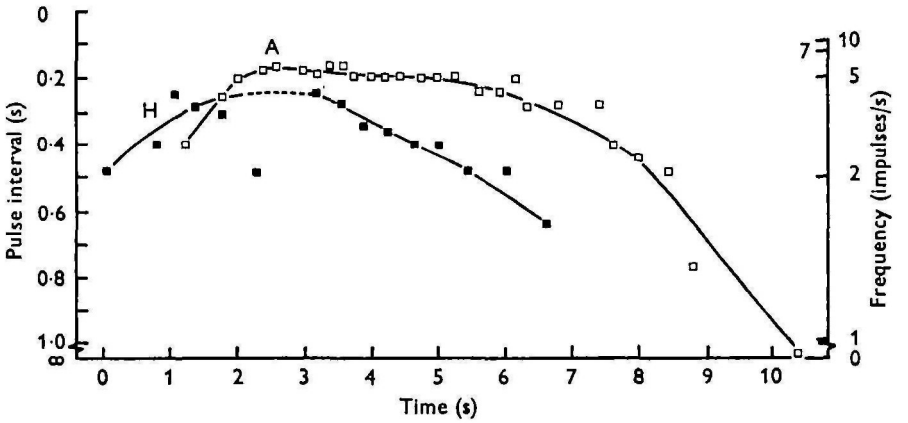

Fig. 18. Interval changes of units A and H (Fig. 17). There is a delay of just over 1 s between the start of cell H and cell A.

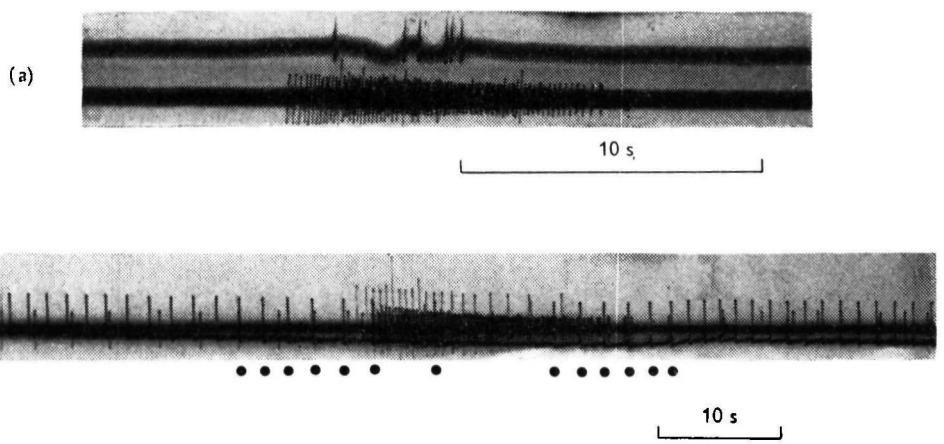

Fig. 20. (*a*) Simultaneous surface extracellular recording (lower beam) and intracellular recording (upper beam) during induction. The recordings were made very close to each other, and there was no activity for several minutes before this recording. The intracellularly recorded cell fires at the same time as the extracellularly recorded bursts, but there is no evidence of a synaptic connection between them. (*b*) Simultaneous extracellular recording from B 3 (upper beam) and intracellular recording from a buccal ganglion cell (lower beam). The intracellularly recorded unit is inhibited as a burst of two units occurs in B 3. Dots placed under intracellularly recorded spikes.

extracellularly, consisting of several units. At the same time the cell whose activity was being recorded intracellularly fired six action potentials, and there was a marked increase in synaptic input to this cell. However, no 1 : 1 correspondence of extracellularly

recorded spikes and postsynaptic potentials was observed. In Fig. 19(*b*) another u showed a marked slowing in spike repetition frequency simultaneous with a burst in nerve B 2, but again the unit responsible for this inhibition could not be distinguished.

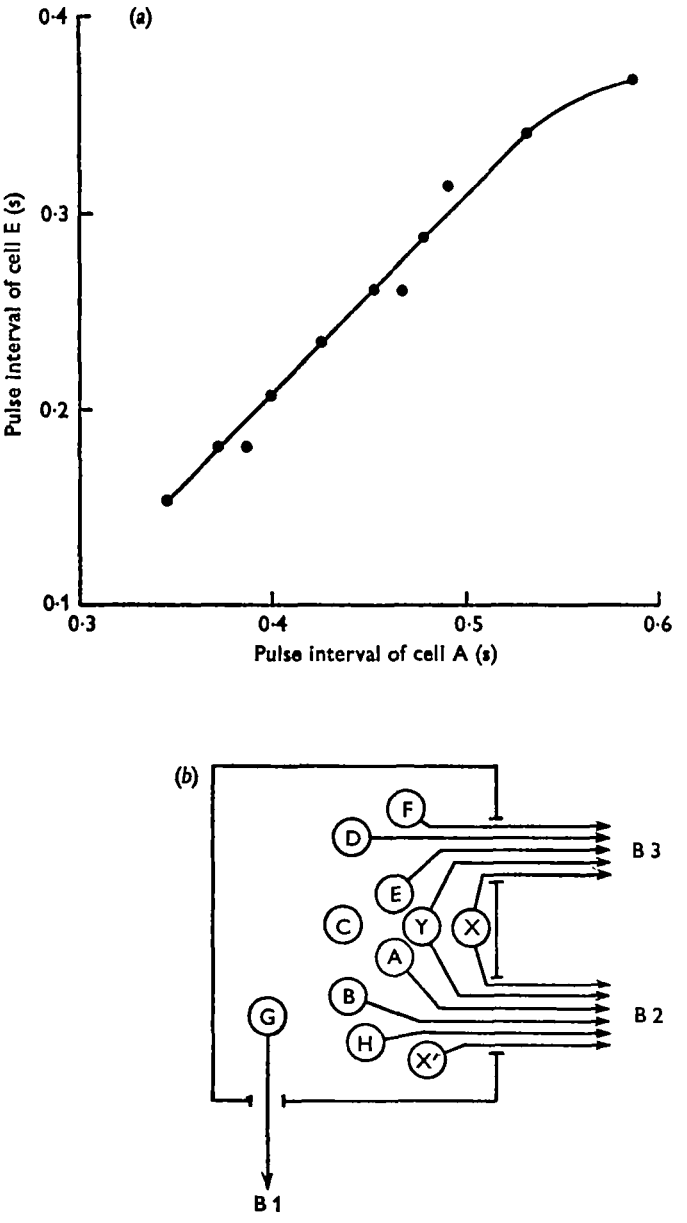

Fig. 19. (*a*) Linear relationship between interval of cell A and of cell E. For any change in the interval of cell A there is an exactly equivalent change in the interval of cell E. (*b*) Diagram shows ipsilateral axon positions found so far for units A–H, X, X′ and Y. B 1, Radular nerve; B 2, ventral buccal nerve; B 3, dorsal buccal nerve.

DISCUSSION

The initial purpose of this work was to activate a network of neurones and relate the activity of individual neurones to the structural connectivity of the network. Although it has been possible to activate the buccal ganglion, the main failing of the work is that there is no intracellular evidence of the connectivity. In an earlier attempt, which was not discussed above, use was made of the fact that recordings can be made from the surface of the ganglion with a suction electrode, and a technique was devised whereby a microelectrode could be introduced inside a suction electrode (unpublished technique). This allowed simultaneous recording of the activity of a group of cells extracellularly, together with the activity of an individual cell recorded intracellularly. Results were obtained with this concentric micro-suction electrode, but the main difficulty was again the penetration of the ganglion sheath with the microelectrode, since in this case it was essential not to damage the sheath, otherwise the cells were immediately sucked into the outer suction electrode.

The results obtained with extracellular recording are interesting in relation to the recent findings of Levitan *et al.* (1970), who gave evidence of linear electrical coupling coefficients between a group of ten identified cells in the buccal ganglion of *Navanax*. The linear relationship between the interspike intervals of neurons A and E in this paper is suggestive of an electrical connexion between these two cells. The similarity of time course of spike repetition frequency of partner cells in opposite buccal ganglia (Fig. 4) also suggests that cells in opposite ganglia could be electrically coupled. Strumwasser (1967) and Gardner (1969) have found that in the buccal ganglia of *Aplysia californica* synchrony between bursts in opposite ganglia is produced as a result of common synaptic input to partner cells. It is quite possible that this is also the case in *Archidoris*. The three main possibilities by which synchrony could occur are mutual excitation (Wilson, 1966), electrical coupling, or common synaptic input, but it is not possible to distinguish between these possibilities on the evidence given in this paper.

The higher frequency and earlier onset of bursts in the right buccal ganglion compared with those in the left ganglion may be significant in relation to normal rasping movements, where the rows of teeth on the right half of the radula appear to be retracted slightly before those on the left (Rose, 1969). This lead of the right half of the radula over the left seems to result in a 'cutting' action of opposite tooth rows. It is possible, however, that the mechanical construction of the radula is such that one side must lead the other during retraction.

Perhaps the most interesting result is that it is possible to induce a sequence of activity in the buccal ganglion with a natural stimulus. Injection of seaweed homogenate into the buccal cavity has more recently been used to induce sequential burst activity in the buccal ganglia of *Aplysia depilans* (in preparation). The sequential pattern of *Aplysia depilans* is very similar to that patterned activity reported by Dorsett, Willows & Hoyle (1969) involved in the co-ordination of swimming in *Tritonia*, which can be elicited by natural stimuli and by electrical stimulation to certain nerves. In relation to full radula movements the sequences reported in this paper are almost certainly partial sequences. There is some evidence that the burst group A/E/F occurs

at the onset of peristalsis in the oesophagus, and it is significant that the axon of cell is found in the nerve which supplies the base of the salivary gland (Rose, 1969).

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