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A SYSTEM OF ELECTRICALLY COUPLED SMALL CELLS IN THE BUCCAL GANGLIA OF THE POND SNAIL *PLANORBIS CORNEUS*

BY MICHAEL S. BERRY†
Department of Zoology, University of Bristol

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INTRODUCTION

Much of the work in molluscan neurophysiology has been centred on the large identifiable cells rather than on the small cells which constitute a far greater proportion of the total neurone population. The main reason for this is that experiments on identifiable cells can be repeated exactly whereas this is not possible if the same cells cannot be recorded from preparation to preparation. However, in some ganglia (see below) small, electrically coupled cells have been found whose electrophysiological properties and behaviour are so similar that the behaviour of any such cell may be largely predictable even though the specific cell itself cannot be identified. In some respects this has simplified analysis because large numbers of small, visually unidentifiable cells can be readily distinguished from other cells in the ganglia by their properties.

Among such groups are the 'bag' cells in the abdominal ganglion of *Aplysia* (Frazier *et al.* 1967; Kandel, 1969; Kupfermann, 1967) and the 'trigger' cells in the pleural ganglia of *Tritonia* (Dorsett, Willows & Hoyle, 1969; Willows & Hoyle, 1969). The former have neurosecretory properties and the latter can elicit locomotor activity (evasive swimming).

The experiments reported here describe a similar population of electrically coupled small cells in the buccal ganglia of *Planorbis*, studied in preparations of isolated ganglia. There are two buccal ganglia, joined by a commissure, and each communicates with the rest of the central nervous system via a cerebro-buccal (c.b.) connective. They innervate the buccal mass symmetrically and control its feeding movements. They also supply the oesophagus, over which they have less direct control. Several large somata have been identified and will be described in future publications. Each has a symmetrical partner in the opposite buccal ganglion.

In the population of coupled small cells the behaviour of an individual resembles that of its neighbours in the group and is to a large extent predictable. Activity spreads throughout the groups in both buccal ganglia and elicits sequences of patterned impulse activity in other neurones. These are similar to the patterns recorded from the same neurones during feeding movements in preparations where the buccal ganglia remain connected to the buccal mass. In this respect the buccal group of cells resembles the trigger neurones of *Tritonia* and may fulfil an analogous function. Despite

† Present address: Wellcome Laboratories Pharmacology, Gatty Marine Laboratory, University of St Andrews, Scotland.

the lack of data, derived from intact preparations, which might yield information about their exact behavioural role, the population of cells described here will likewise be referred to as a trigger group or system because of its widespread and long-lasting excitatory effects.

METHODS

Specimens of *Planorbis corneus* were maintained in aquaria at room temperature (18–22 °C). They were supplied with pond weed and survived for several months. Experiments were performed at room temperature with the preparation in saline that was frequently renewed. The saline, based on the results of an analysis of *Planorbis* haemolymph by Kostyuk (1968), had the following composition in mM/l: Na, 46·2; K, 1·3; Mg, 1·5; Ca, 4·5; HCO_3, 6·9; Cl, 46·6.

In order to isolate the buccal ganglia the shell was first cut off and the snail was immersed in saline. A median dorsal longitudinal incision exposed the buccal mass and oesophagus, and the latter was cut and pulled through the nerve ring to reveal the buccal ganglia at its anterior end. After removal of the salivary glands the buccal nerves and connectives were cut, leaving as great a length as possible. The isolated buccal ganglia were transferred to a wax dish containing 100 ml of saline and pinned, dorsal surface uppermost, to dental wax embedded in the bottom of the dish. Six to twelve micro-pins were used; these were cut very short to allow the electrodes to pass over rather than between them. The pins were placed through the connective tissue between the nerves so that the sheath over the ganglia was stretched slightly; the sheath itself was not pinned and was therefore not damaged. The cells, which showed very clearly through the sheath, could then be penetrated easily with microelectrodes without its removal. It was most important to pin the ganglia out so that the sheath was adequately stretched; too little or too much stretch meant that microelectrode penetration was impossible or the cells were damaged.

The microelectrodes were pulled from thick-walled Pyrex capillary tubing (diSPo micro pipettes) with a modified Nastuk electrode puller which allowed a cooling jet of air to be directed on to the electrode tip during the final pull. This resulted in electrodes which for the same tip size and shape had a much shorter and stouter shank (Weevers, 1971). They were filled with 2 M potassium acetate and their resistance ranged from 10 to 60 MΩ. The electrodes were inserted into the cells under visual control by gentle tapping of the micro-manipulator. An alternative method of penetration was to mount the electrode on a modified loudspeaker (Weevers, 1971) and apply a short-duration current pulse to drive the electrode through the sheath. This method was often used for insertion of a second electrode when recording from two cells simultaneously; it produced less vibration than tapping the manipulator, and was less likely to dislodge the first electrode.

The two microelectrodes led to cathode followers and each could be switched to a bridge circuit for simultaneous recording and passage of current into the cell with the same electrode. The resistance of one bridge arm in series with the microelectrode was high (500 MΩ) in order to avoid variations in the current applied to the cell. Records, displayed on a Tektronix 502 oscilloscope, were photographed direct. Extracellular records from nerves were made with three suction electrodes which led first to a switch box where they could be switched for stimulating via RF isolation

units. Amplifying and stimulating equipment was of the conventional type. For recording on three or more channels an ultraviolet recorder (Southern Instruments Ltd.) was used.

RESULTS

(1) *Size and distribution of trigger cell somata, and their identification*

Unlike the coupled small cells of *Tritonia* and *Aplysia*, those in the buccal ganglia of *Planorbis* do not form a discrete anatomical population, but they do occur in large numbers among the very small cells in the region of the commissure. They are also found in smaller numbers, apparently randomly distributed, in the rest of the ganglia. Those in *Tritonia* also occur near the commissure (pleural), and in *Aplysia* where the connectives emerge. Their wide distribution in *Planorbis* makes it difficult to determine their numbers. Up to ten have been recorded in a single ganglion and there are probably many more; only superficial cells were impaled, and near the commissure there are several layers of cells overlying the neuropil. The size of recorded trigger-cell somata ranged from 10 to 50 μm.

In order to locate a trigger cell repeated penetrations were made of different small somata near the commissure. Once penetrated a cell could be identified as belonging to the trigger system by a lack of spike or synaptic activity and by its effect, when stimulated intracellularly, on identified large neurones and on units recorded from the nerves. The presence of strong electrotonic coupling with similar small (trigger) cells provided final confirmation. The detailed nature of the effects of trigger-cell stimulation on other neurones is discussed later but can be summarized briefly here: (1) There is an initial hyperpolarization in four identified motoneurones (cells 3–6) in each buccal ganglion, and in the majority of unidentified large cells. This is followed by complex synaptic activity lasting for several seconds or even minutes after the trigger-cell discharge, and usually resulting in a burst of spikes. (2) There is a similar effect on each giant cell (cell 1) which supplies the oesophagus. (3) There is an excitatory effect on the second largest soma (cell 2) in each ganglion. In no case could similar effects be produced by stimulating a small cell which lacked the electrotonic coupling with others having the same actions.

(2) *Initiation of activity and its conduction through the system*

The trigger cells are usually silent but activity may be elicited either by intracellular stimulation of any neurone in the system or by stimulation of any buccal nerve or connective. Once initiated it spreads throughout the coupled cells of both ganglia, which all tend to fire together (Fig. 1 A, B, C). The cells produce single spikes or a short burst, and several later bursts may follow the initial discharge for several seconds. A proportion of the cells commonly fails to reach threshold (Fig. 1 D), but unless they are in sufficient numbers this does not prevent the spread of activity. Electrotonic coupling can be demonstrated by hyperpolarizing one cell and recording attenuated hyperpolarizations in other trigger cells including those in the opposite ganglion (Fig. 1 E, F). Some or all of the trigger cells must send axons across the commissure.

The strength of the coupling between individual cells, measured by the transmission of intracellular pulses, varies considerably and is presumably determined by the

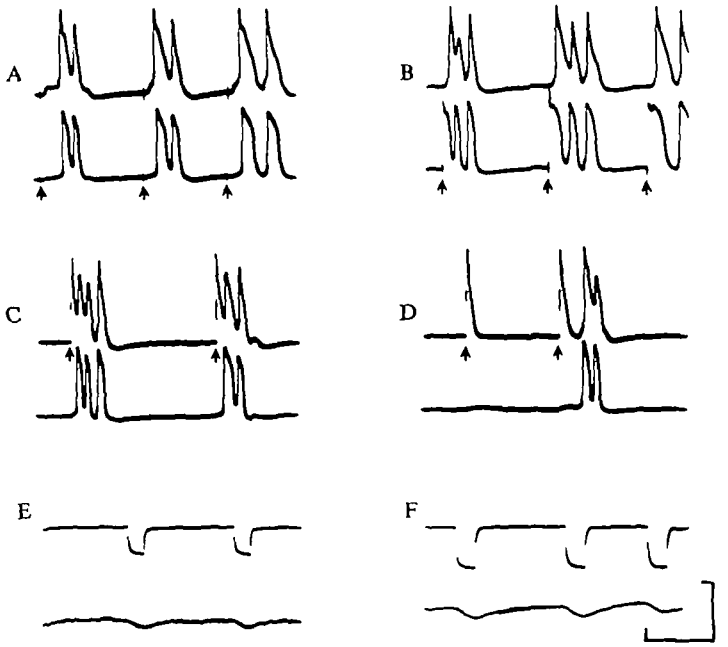

Fig. 1. The initiation and spread of excitation throughout cells of the trigger system. A–D, Simultaneous records from two trigger cells in opposite ganglia showing synchronous short bursts of spikes produced (A) by single stimuli to the left c.b. connective, (B) by intracellular stimulation of the right cell (lower trace), and (C) by intracellular stimulation of the left cell (upper trace). D shows a failure of spread of excitation after the first stimulus to the left cell. Stimuli are arrowed; intracellular stimuli were 10 msec duration. E and F illustrate the electrotonic coupling between these two cells; 100 msec hyperpolarizing pulses (higher intensity in F) applied to the left cell are recorded attenuated in the right cell. Calibration: (A–D) upper, 60 mV; lower, 50 mV; (E and F) upper, 20 mV; lower, 10 mV. Time calibration: 300 msec.

geometry of the cells and proximity of synaptic contacts. Weak coupling may indicate that transmission is not direct but via other trigger cells. Coupling is not always weaker between cells in opposite ganglia than between those in the same ganglion in spite of the greater distance of conduction. This is more evident when transmission of spikes rather than applied pulses is considered because there is then apparently active rather than decremental conduction in the commissure. An understanding of the nature of the spread of excitation requires knowledge of the type and extent of interconnections.

Fatigue or failure of spread of excitation eventually occurred if a stimulus to a nerve or trigger cell was repeated immediately after the end of each elicited response. Preparations varied quite widely in the rate at which the trigger cells became fatigued; sometimes 1 or 2 sec and at other times as many as 30 sec had to be allowed between the end of one conducted response and the application of a stimulus to elicit another response. Where further bursts followed the initial response these would stop first, and then later the initial burst would stop. The fatigue, which appeared to be caused partly by a rise in threshold, could to some extent be overcome either by increasing the intensity of stimulation to a nerve, which increased the number of trigger cells firing, or by stimulating at high frequency. In both cases summation of EPSP's to threshold occurred, as a result of spatial and temporal summation respectively. Fig. 2

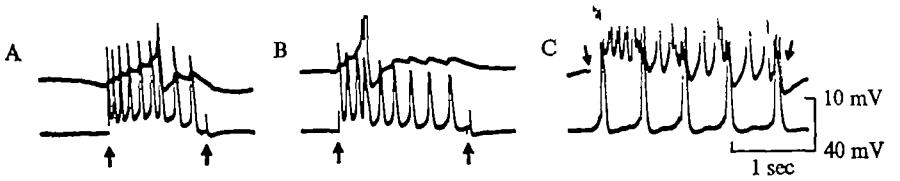

Fig. 2. Summation and facilitation of electrotonic EPSP's in fatigued trigger cells. Simultaneous records from two closely coupled cells in opposite ganglia which had been fatigued by repeated connective stimulation until a single EPSP no longer reached threshold. A, Intracellular stimulation of the left cell (lower trace; arrows indicate on and off of stimulus) causes spikes which gradually become longer and produce larger EPSP's (facilitation) in the right cell. B, Further fatigue causes even the first few spikes to be of long duration, and threshold is reached more rapidly than in A even though the spikes are at lower frequency. C, Stimulation of the right cell produces summating EPSP's in the left cell.

illustrates summation of EPSP's to threshold in two closely coupled cells in opposite ganglia which had been fatigued by prior high-frequency stimulation. It is noticeable that the spike duration is not constant, and the longer the duration of the spike the larger the electrotonic EPSP it produced (see Waziri, 1969); the possible significance of this in offsetting fatigue will be discussed later.

(3) *Configuration of action potentials*

The time course of action potentials in trigger cells is extremely variable. In any one cell enormous changes may take place both in the duration and in the shape of the spikes during repetitive firing. Typically the spike has a rapid rise time, and it is in the decay that the variations occur, either in the form of a smooth, gradual increase in duration during repetitive discharge, or sudden depolarizing deflexions (Fig. 3).

The deflexions appear to result from electrotonic transmission of action potentials from closely coupled neighbours. They do not usually occur if few of the neighbouring cells are excited, for example during stimulation of a c.b. connective at low intensity and low frequency (Fig. 3A); the spikes then have a 'normal' time course for molluscan central neurones. At higher stimulus intensities more trigger cells are stimulated and a burst of spikes results, with the characteristic shapes shown in Fig. 3B. Deflexions occur simultaneously with discharges in certain other trigger neurones (Fig. 3C), but if one such neurone alone is stimulated intracellularly then the electrotonic EPSP's, or coupling potentials, that it produces are small (up to 10 mV). The deflexions, however, may be much larger than this, and may therefore represent summed activity in a number of neighbouring neurones. An alternative explanation is that the deflexions are caused by spikes initiated in a process of the cell which then invade the soma electrotonically. The very rapid rise of some spikes further suggest that they originate some distance from the soma; they resemble antidromic spikes produced by stimulation of a nerve.

The smooth increase in duration of action potentials is presumably a property of the cell itself; similar smaller changes occur in many cells if they are driven at high frequency, or even during a natural spontaneous burst (Strumwasser, 1967). In trigger-cell spikes the main changes take place in the duration of either the initial or final part of the falling phase. The changes described were characteristic of trigger cells but of no other neurones that were recorded from the buccal ganglia.

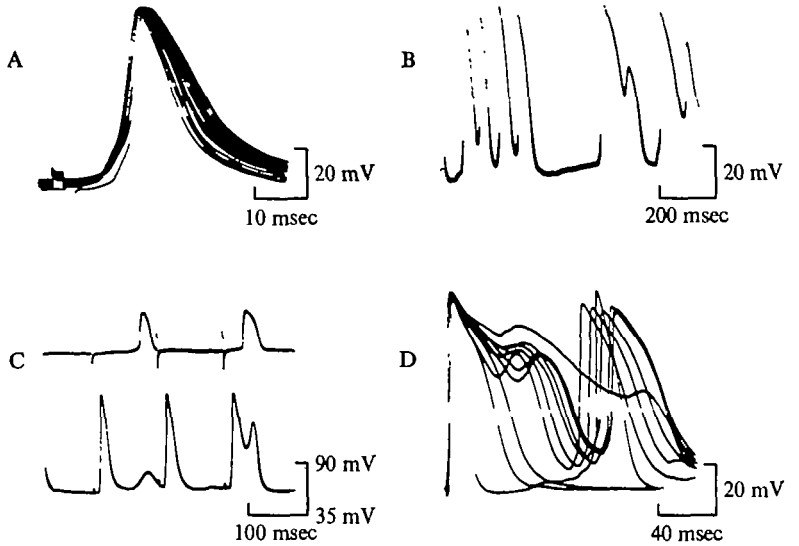

Fig. 3. Changes in the configuration of the action potential in trigger neurones. A, B, and the lower trace of C are from the same trigger cell. All stimuli were applied to the ipsilateral c.b. connective. A, Superimposed sweeps showing spikes produced by low-intensity stimulation (1 per 10 sec). Few neighbouring cells discharged and the time course of the action potential is 'normal'. B, Burst of spikes elicited by a single high-intensity stimulus. C, Repeated low-intensity stimulation. A trigger neurone from the opposite ganglion is recorded simultaneously. D, Trigger cell from a different preparation showing a very marked change in configuration during repeated stimulation (1/sec). In B–D note the smooth increases in duration and the depolarizations during the falling phase. The latter are associated with other trigger cell spikes (C), and are believed to result from summation of input from coupled cells, or possibly the electrotonic invasion of the soma by spikes initiated distally in a cell process (see text).

(4) *Integration in the trigger system by increase in spike duration during repetitive firing*

The increase in action-potential duration when discharging at high frequency is very marked in some molluscan neurones (e.g. Strumwasser, 1967). In some cells of the buccal ganglion trigger system changes take place which are as large as or larger than any hitherto described. Since it is relatively easy to find closely coupled cells in the trigger system it was possible to study the effect of increase in duration of the action potential on the post-synaptic cells. For this purpose recordings were made from trigger cells which were close together in the same ganglion. Passive electrotonic conduction between such cells tends to be strong, so any changes in duration of soma spike in one cell would be likely to affect the soma of the other. It would not then matter if the spike in the process linking the cells did not also increase in duration.

Fig. 4 illustrates the interactions between two trigger cells, about 50 μm apart, one of which showed the typical increase in duration of its action potentials. Passive conduction was very much greater than between cells in opposite ganglia. Although the differences were not measured quantitatively, the more rapid rise and decay of transmitted pulses are evident (Fig. 4A; compare Fig. 1E, F). Spikes were elicited in one of the cells by repeatedly stimulating it antidromically (see next section) via the ipsilateral c.b. connective. These spikes gradually increased in duration from about 20 to 60 msec. In its coupled neighbour, which lacked an axon in the connective, there was a concurrent increase in height and duration of electrotonic EPSP's which

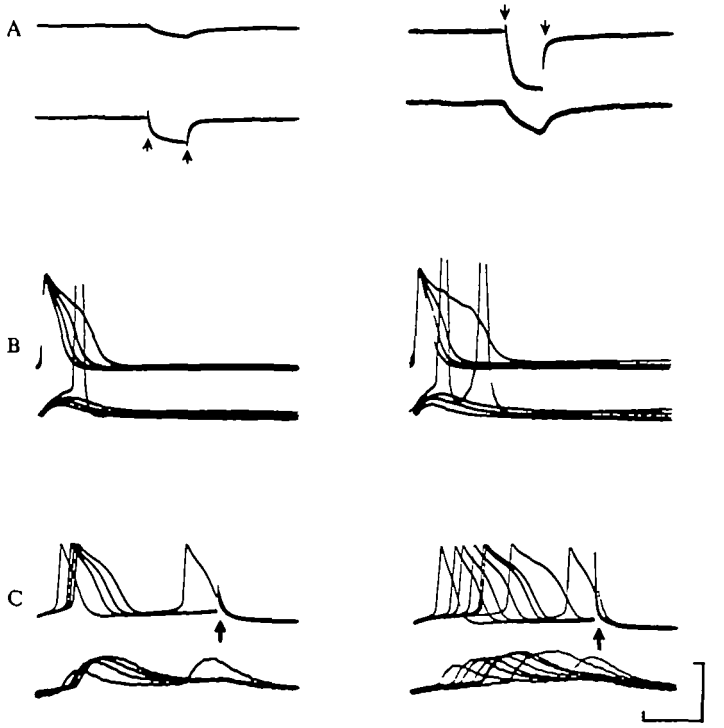

Fig. 4. Increase in size of electrotonic EPSP's produced by spikes which increase in duration with fatigue in closely coupled trigger cells. Simultaneous records from two cells 50 μm apart; the action potentials of the cell recorded on the upper trace in each case increased in duration on repetitive stimulation. A, Demonstration of strong electrotonic coupling by transmission of applied hyperpolarizing pulses. B, Repetitive stimulation of the ipsilateral c.b. connective at 1/sec antidromically elicits spikes (upper trace) which progressively increase in duration. Concurrently, EPSP's in the other cell increase in size and eventually reach threshold. The EPSP associated with the very long spike triggers two discharges (four sweeps superimposed). C, Repetitive intracellular stimulation (upper trace; arrow indicates 'off') causes progressively lengthening spikes which produce larger EPSP's (lower trace) although they do not reach threshold. Several sweeps are superimposed. In B and C a period of stimulation to cause fatigue had been given prior to the recording. Calibration: (A) upper, 20 mV; lower, 10 mV; (B) upper, 35 mV; lower, 20 mV; (C) upper, 50 mV; lower, 10 mV. Time calibration: (A) 100 msec; (B and C) 40 msec.

eventually reached threshold with little or no summation (Fig. 4B). To test whether the increased spike duration was directly responsible for the increased EPSP size, rather than both being a consequence of increase in common input, the pre-synaptic cell was repeatedly stimulated intracellularly (Fig. 4C). Its action potentials again increased in duration and, although the spike threshold of the post-synaptic cell was not reached, the size and duration of the EPSP's were also considerably increased. This might be expected for two electrotonically coupled cells, the increase in the size of the EPSP's resulting from an increased duration of action current which can charge the capacity of the post-synaptic cell membrane more fully before decaying. However, the correlation of long-duration spike and large EPSP may be the result of some third process as yet unknown.

Fig. 2 shows a similar action between neurones in opposite ganglia, in which summation as well as increase in size of EPSP's (facilitation) occurred. The figure clearly

illustrates how fatigue can be offset by the concurrent increase in spike duration. The cells were fatigued (i.e. threshold raised and spike duration increased) by repetitive stimulation of a c.b. connective. One of the cells was depolarized by the same amount after a short (Fig. 2 A) and long (Fig. 2 B) period of connective stimulation. The effects of its spikes on the post-synaptic cell were studied in the two cases. In Fig. 2 B, although fatigue was greater than in Fig. 2 A and the spikes produced EPSP's at lower frequency, threshold was reached more quickly because the fatigue had caused even the initial spikes to be of long duration. These long spikes produced large EPSP's, fewer of which were necessary for summation to threshold than the small EPSP's produced by short-duration spikes. These results show that the effects are large enough to be of significance, but the importance for the system as a whole is unknown because only a proportion of trigger cells showed the large increase in spike duration. The possible integrative significance of these properties will be discussed in detail.

(5) *Input and output of the trigger system*

Before discussing the output of the trigger system to other cells of the buccal ganglia, its peripheral axon distribution will be considered. The evidence for an axon branch was (*a*) production of an antidromic response and (*b*) the production on intracellular depolarization of intracellular and axon spikes with a 1:1 correspondence and with constant latency. It was occasionally difficult to be certain that the responses were not due to the close coupling between cells because often one cell would follow another on a 1:1 basis for long periods of time. To overcome this, stimuli were applied for relatively long periods to reduce the effective strength of coupling by fatigue.

The majority of trigger cells appear to have no peripheral axons, but a few have processes in almost any of the buccal nerves or c.b. connectives; some have single and others multibranched axons. A number of cells have axons which cross the commissure and enter contralateral nerves. Apart from trigger cells few others were found which had axons in the connectives.

Analysis of synaptic input was also made difficult by the close coupling because it was often impossible to determine whether an EPSP elicited by stimulation of a nerve or of a connective was the result of input from outside the system or whether it resulted from the antidromic stimulation of another trigger cell. Therefore large numbers of different neurones in the buccal ganglia were stimulated intracellularly, but no synaptic input to the system was revealed; neurones in the rest of the central nervous system were not tested. All the synaptic activity was excitatory and appeared to be mediated electrically.

(6) *Influence of the trigger system on other neurones in the buccal ganglia*

Activity in the trigger system, elicited by intracellular stimulation of one of its cells, produces long-lasting effects on large numbers of neurones in the buccal ganglia. The effects are especially large if long periods of rest (*c.* 30 sec) are allowed between stimuli so that the trigger system is not fatigued. Activity then spreads more readily between the electrically coupled members and the initial burst is often followed by several later bursts, consequently with greater effect. In addition to fatigue within the system when stimuli are repeated at too short intervals there also appears to be adaptation in

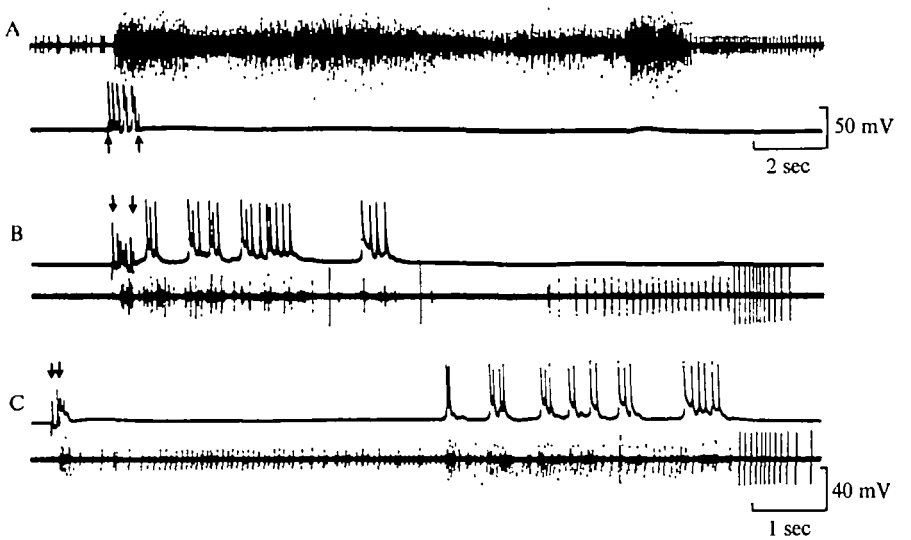

Fig. 5. Influence of the trigger system on units recorded from a c.b. connective (A) and dorsal buccal nerve (B and C). In each record activity in the system is elicited by intracellular stimulation (arrowed) of one of its cells. In B and C the large unit at the end of the records is cell 4. Note that there are 'spontaneous' trigger-cell bursts.

the cells that are directly or indirectly influenced by the system, i.e. failure of a response may occur at two levels.

Fig. 5 shows the effect of trigger-cell output on activity recorded extracellularly from a c.b. connective and a dorsal buccal nerve respectively. In Fig. 5A the stimulus produced a burst of spikes which elicited a train of impulses in the connective. If stimuli were repeated at short intervals the connective response eventually became a short burst of spikes, and then failed completely. In this preparation the full response could be elicited again after about 20 sec.

The response of the dorsal nerves is illustrated in Fig. 5B, C. There is one dorsal nerve on each side, emerging from the c.b. connective close to the buccal ganglia. In intact preparations the nerves show a sequence of impulse activity during each rasping movement of the radula, and one unit in particular is readily identifiable. This unit, numbered cell 4, fires a burst at the end of the sequence. In isolated preparations it fires a burst following connective stimulation or trigger-cell stimulation (Fig. 5B, C), and activity in many other neurones can be related temporally to its firing. In Fig. 5 the stimulated trigger cell produced further bursts after the initial response; this is not necessary for triggering activity in cell 4 but it does tend to modify preceding activity. The ease with which the response could be elicited varied considerably from preparation to preparation. On repetitive stimulation cell 4 eventually ceased to fire or produced only one or two spikes and preceding units dropped out. The variations in the rate of failure of the response in different preparations will be discussed later.

Intracellular recordings were made from many of the large somata in the buccal ganglia to determine the effect on them of trigger-cell stimulation. Six large cells have been identified in each buccal ganglion, each with a symmetrical partner in the other ganglion: (1) a 'giant' cell (cell 1) which supplies the oesophagus, (2) a large neurone of unknown function (cell 2) which is coupled electrotonically to its partner, (3) three

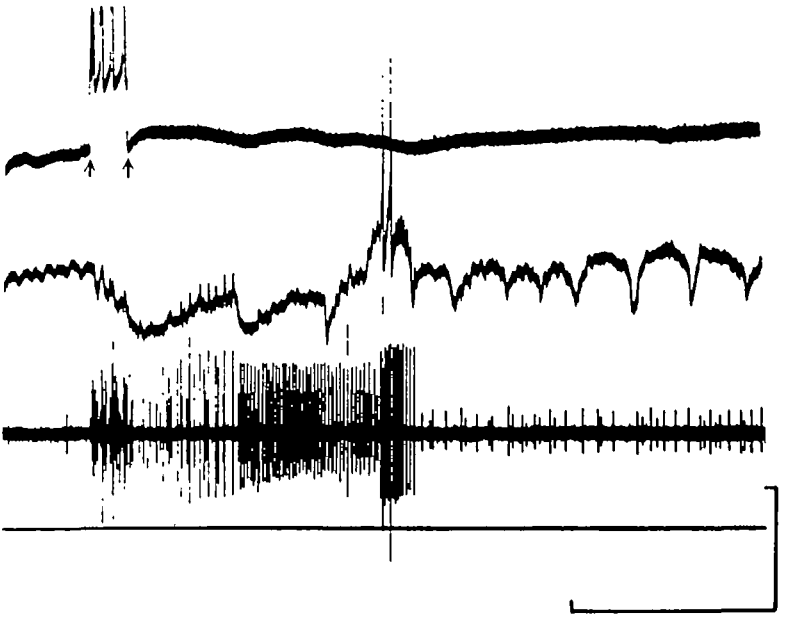

Fig. 6. Input to cell 3 initiated by stimulation of the trigger system. A trigger cell, stimulated intracellularly (upper trace; stimulus arrowed), is recorded simultaneously with the contralateral cell 3 (middle trace) and dorsal nerve. An initial hyperpolarization in cell 3 is followed by two bursts of short-duration EPSP's from cell 4 (cell 4 spikes in the dorsal nerve are synchronous with the EPSP's). The response is similar both to that produced by stimulation of a c.b. connective and to a single cycle of rhythmic input in intact preparations. Calibration: upper and middle, 10 mV. Time calibration: 3 sec.

motoneurones to the buccal mass (cells 3, 5 and 6) plus cell 4 which has already been mentioned and is presumed to be a motoneurone because it produces a burst during each rasping movement but whose function has not yet been confirmed. When recorded in intact preparations many of the unidentified large neurones behaved like cells 3, 5 and 6 in producing muscle twitches in the buccal mass on a 1:1 basis with spikes elicited by intracellular depolarization. They are therefore regarded as motoneurones. Cells 3 and 4 in the same ganglion are coupled electrotonically – note the 1:1 correspondence between cell 4 spikes in the dorsal nerve and EPSP's in cell 3 (Figs. 6, 10).

The effect of trigger-cell stimulation on most of the large cells is an initial large hyperpolarization. This is followed by a period of complex synaptic activity which often produces spikes. The influence on cell 3 is shown in Fig. 6 where its activity is compared with that in the ipsilateral dorsal nerve. Fig. 7 shows typical effects of the trigger system on a number of unidentified large cells. In Fig. 7C a cell in which the post-synaptic effect was excitatory fired at very high frequency rather than being hyperpolarized. These and many other of the effects are identical to those produced by stimulation of a nerve or connective. Such stimulation almost certainly acts indirectly (see section 5 above) via the trigger system which has axons in most of the nerves. Threshold for a response in a large cell, produced by stimulation of a c.b. connective, was nearly always the same as threshold for activity or spread of activity in the trigger system.

The system has opposite effects on the two largest neurones in each ganglion; it

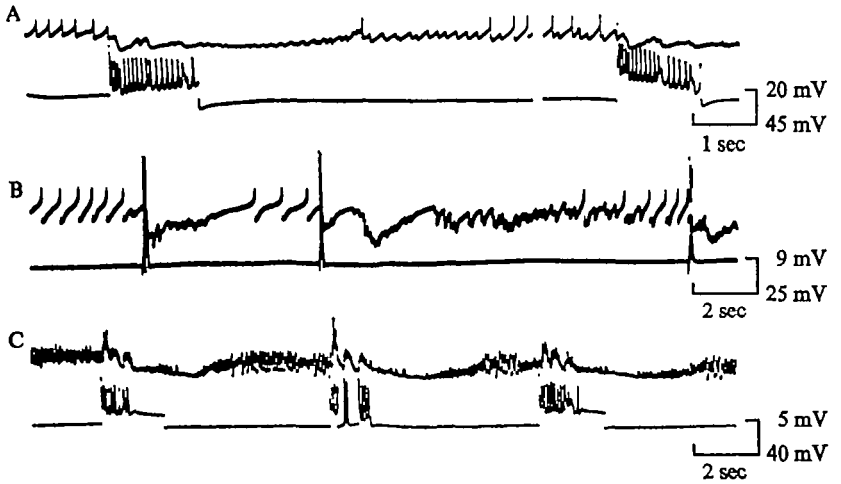

Fig. 7. Influence of the trigger system on some unidentified large cells. Each record shows a large cell (upper trace) recorded simultaneously with a contralateral trigger cell stimulated intracellularly. A and B show the usual inhibition which outlasts the trigger-cell activity. C shows a neurone receiving EPSP's which is made to fire bursts of high-frequency spikes.

inhibits the giant cell and excites cell 2. Fig. 8B shows the hyperpolarization of a giant cell produced by intracellular stimulation of a trigger cell. The first response is usually a very small depolarization. The biphasic nature can be seen more clearly in Fig. 8A, where intracellular stimuli of shorter duration and lower intensity were given in order to produce a smaller response. In the neighbourhood of the giant cells are some small neurones whose electrophysiological properties are similar to those of the giant cells themselves, and Fig. 8C shows the inhibition of one such neurone by trigger-system activity. By contrast, cell 2 is strongly excited. Each trigger burst produces a depolarization (Fig. 8D). Very strong depolarization is often followed by a short period of inactivity.

The trigger system was found to have an effect on every soma impaled, but no cell was found to have any effect on the system. This might be expected if it does in fact function as a trigger for feeding; no feedback from cells in the ganglia would be necessary, although there might be feedback from the buccal mass. If this is the function, however, the relative rapidity with which the system usually becomes fatigued is difficult to explain because feeding proceeds continuously for very long periods. The answer to this problem might be found in the few preparations in which the system did not readily become fatigued. In these preparations the later trigger bursts, which follow the initial directly elicited burst, did not quickly die out but continued for several minutes, often at very regular short intervals. In addition, instead of several such bursts occurring during a single long sequence of dorsal nerve activity (Fig. 5B, C), usually a single burst was followed by one or several sequences (Fig. 9). The increased rate at which the cycles were repeated meant that in the identified motoneurones (and other unidentified large cells) the depolarization, which follows the initial hyperpolarization, had to occur much more rapidly than usual. Cell 4, for example, then reached threshold for a burst soon after hyperpolarization. Any further trigger-cell bursts during the depolarization phase prolonged the delay of onset of cell 4 firing and increased the activity preceding it in the dorsal nerve (Fig. 10).

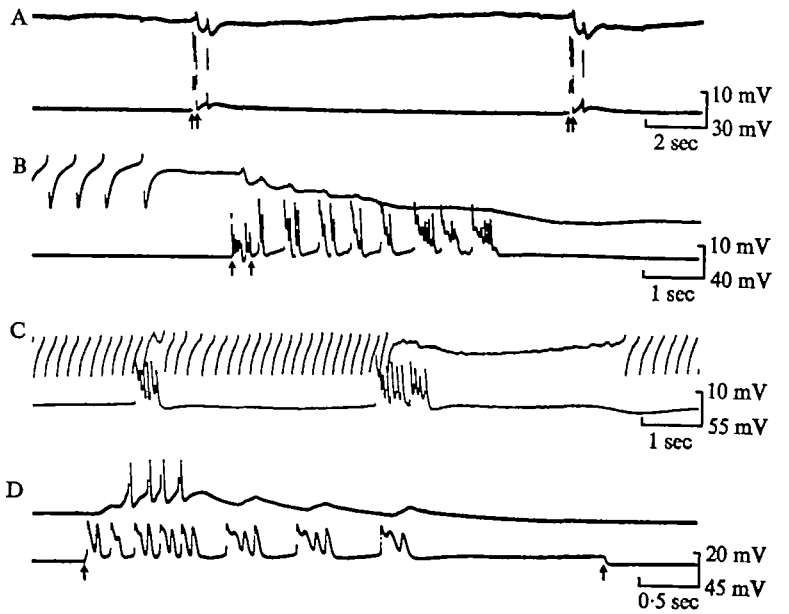

Fig. 8. Opposite synaptic actions of the trigger system on the two largest neurones of each buccal ganglion – cell 1 (giant cell) and cell 2. The cells are recorded simultaneously with a trigger neurone stimulated intracellularly (stimuli arrowed). A, Short bursts of trigger cell spikes produce biphasic deflexions of possibly compound origin in the giant cell. B, Prolonged activity produces long-lasting inhibition. C, Small neurones close to the giant cells and with similar properties are also inhibited. D, Cell 2 is strongly excited, and each trigger-cell burst produces a depolarization. Cells were contralateral in B, C and D and ipsilateral in A.

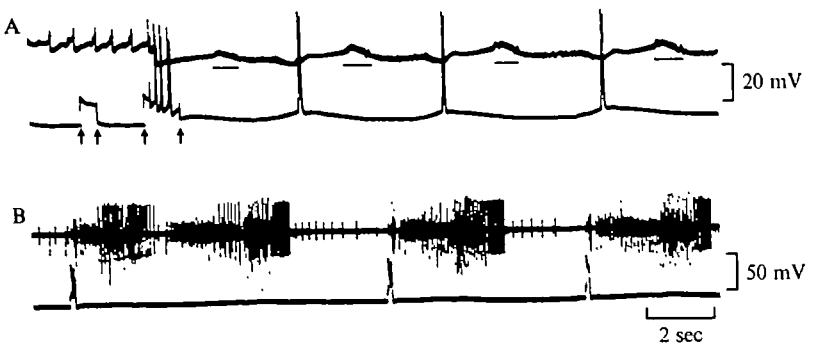

Fig. 9. Spontaneous rhythmic behaviour in the trigger system. A, The second, threshold stimulus to a trigger cell (lower trace) initiates spontaneous bursting. Each discharge is followed by a burst of EPSP's in cell 3 (upper trace, bursts underlined), produced by spikes in the ipsilateral coupled cell 4. B is from the same preparation after spontaneous activity had stopped. Sequences in the dorsal buccal nerve (upper trace) are readily elicited by trigger-cell stimulation (lower trace). This is characteristic of preparations showing spontaneous bursting. Each sequence following stimulation is similar to that occurring spontaneously. The burst of spikes at the end of each sequence is from cell 4.

In most of the usual preparations cell 4 became depolarized well after these bursts had ceased so that they had no such delaying effects. In all of the few preparations which showed this tendency for the trigger system to produce regular spontaneous bursts, and for the identified motoneurones to become depolarized rapidly after hyperpolarization, the sequence in the dorsal nerve and activity in other nerves could be

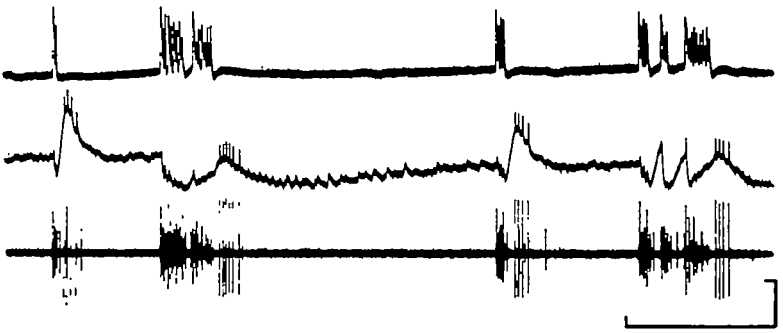

Fig. 10. Example of an 'excitable' preparation in which cell 3 (middle trace) depolarizes rapidly following inhibitory input initiated by trigger-cell stimulation (upper trace). The lower trace is from the ipsilateral dorsal nerve. Usually the inhibitory input, which is common to cells 3 and 4, lasts much longer and the burst in cell 4 (large unit in the dorsal nerve record) is delayed. Calibration: upper, 50 mV; middle, 10 mV. Time calibration: 2 sec.

initiated very readily. This suggests that not only the trigger cells but the ganglia as a whole were in a more active or excitable state than was usually found.

Results from these 'excitable' preparations show that the system can, in certain circumstances, produce bursts at short, regular intervals for quite long periods, and can elicit burst activity in other cells. It is not known what changes take place in the system (or in these other cells) to enable it to do this, or whether the activity actually originates in the system or is triggered from elsewhere. Nevertheless, the evidence, and comparison with the trigger cells in *Tritonia*, strongly suggest that this system in the buccal ganglia does in fact function as a trigger for feeding, but recordings in intact preparations must be made for confirmation.

DISCUSSION

Electrotonic coupling between neurones in molluscs

Electrotonic coupling between nerve cells has been demonstrated in a variety of vertebrate and invertebrate species (Bennett *et al.* 1967). In molluscs numerous cases have been discovered recently (Dorsett *et al.* 1969; Frazier *et al.* 1967; Kandel, 1969; Tauc, 1969; Waziri, 1969; Willows & Hoyle, 1969). Electrotonic coupling has been found in the few buccal ganglia that have been studied – for example, in *Aplysia* (Gardner, 1969), *Navanax* (Levitan, Tauc & Segundo, 1970) and *Anisodoris* (Gorman & Mirolli, 1969; coupled cells are in the oesophageal plexus on to which buccal ganglion cells synapse). In *Planorbis*, besides the trigger system, several large cells were found to be electrotonically coupled, including one pair in opposite ganglia.

Electrical connexions have often been regarded as a mechanism for rapid transmission and synchronous mass activity (see Bennett, 1968). However, this cannot occur in many of the cases mentioned because transmission of spikes from cell to cell is poor. A different function for the electrotonic coupling of numbers of cells is discussed separately by Levitan *et al.* (1970) and by Willows & Hoyle (1969) for large coupled cells in the buccal ganglia of *Navanax*, and for the pleural network of *Tritonia* respectively. Briefly, synaptic input to *one* cell of the group is less effective in causing a polarization change than it would be if the neurone were electrically isolated. This is because there is a larger membrane area to be charged. Only when a sufficient number

of inputs to the different cells of the group occur simultaneously can the cells fire, and the coupling can then aid synchrony. In this way only particular, large stimuli can excite the group which triggers a behaviour pattern. The cells of the group select inputs that affect many cells in the network simultaneously and reject those that excite only a few components.

The trigger system in *Planorbis* would also seem to function in this way. Although activity in a single cell can spread through the system there is rapid fatigue, and for further conduction to occur large numbers of cells must be stimulated via a nerve or a connective. This will be discussed in more detail later when considering the behavioural role of the system.

Coupling between trigger cells, and the conduction of spikes through the system in Planorbis

By recording from the trigger cells two at a time it has been possible to determine certain features of their connexions and interactions. They are coupled electrotonically and spikes are conducted to the cells of both ganglia which tend to fire together. Activity is followed by a rise in threshold, which may be overcome by summation and facilitation of EPSP's. Usually activity must be elicited by stimulation, but it does occur spontaneously.

This leaves many basic questions about the system unanswered. (1) What are the nature and extent of cell processes and connexions? When intracellular current pulses applied to one cell are recorded in the other trigger cells does this indicate direct connexions or are the pulses conducted via other cells? (2) Are there any specializations within the system? For example, are there particular pathways of conduction so that activity need not invade the whole network? (3) Where does spontaneous activity originate? Does it occur at fixed or fluctuating points in the system or is it initiated by input from elsewhere? (4) How many cells are there in the system?

To obtain answers to these types of questions requires two main approaches. (1) A detailed histological study, including dye injection, to examine cell processes. This would be made rather difficult by the fact that the somata do not form a distinct anatomical population, though neuropil processes might. The cell bodies may have characteristic properties which allow them to be identified histologically, like, for example, the bag cells of *Aplysia* (Frazier *et al.* 1967). (2) A multi-electrode study. By recording from more than two cells at a time it should be possible to study the conduction of activity (both spontaneous and elicited) more fully. Technically this should not be too difficult because, although the cells are small, they are relatively easy to penetrate and are very stable. Willows & Hoyle (1969) have recorded with up to four microelectrodes from the pleural network in *Tritonia*. A comparison with similar systems such as this, which are more amenable for study, may provide useful information about the system in *Planorbis*.

Input and output of the trigger system

No input could be demonstrated, but there are widespread peripheral and central cell processes including those to the rest of the CNS. It is not known whether the peripheral axons are motor, sensory or neurosecretory (the trigger cells in *Tritonia* have peripheral axons which are motor). Central processes appear to synapse directly

with large numbers of cells in the buccal ganglia; stimulation of a trigger cell usually caused an immediate response as well as prolonged synaptic activity. Information about such synaptic contacts awaits histological analysis.

Increase in spike duration and its possible integrative significance

Increase in spike duration with repetitive firing, especially at high frequency, is a common phenomenon, although a decrease in spike duration occurs in some cases (e.g. tadpole skin cells; A. M. Roberts, personal communication). The increase can vary considerably in extent. Strumwasser (1967), for example, shows the gradual large increase in spike duration in an identified cell in the abdominal ganglion of *Aplysia* during a spontaneous burst. In some of the trigger cells in *Planorbis*, spikes, which were repeatedly elicited either by intracellular depolarization or antidromic stimulation of an axon, increase up to 15-fold in duration. Some of the increase could be explained by electrotonic invasion from other trigger cells, but in general it appeared to be a property of the cell itself, because of its gradual smooth nature.

Since increases in spike duration often occur in cells that are damaged by electrode penetration, it was necessary first to be sure that this was not the cause. The following points suggest that the cells were not damaged. (1) Penetration was quite easy, requiring very little movement of the electrode, and it resulted in a sharp change in potential. It was much easier than for many of the large cells. (2) There was never a 'penetration burst' or the continuous firing shown by many damaged cells. (3) A large stable resting potential (40–60 mV) could be recorded for several hours, occasionally more than 12 h. (4) Action potentials were large (45–80 mV) and did not decline with time. Also, there was little or no decline in amplitude with increase in duration. (5) Similar increases were never found in equally small cells that did not belong to the system. (6) During low-frequency firing the spikes had a normal time course (*c.* 10 msec). Although these criteria do not rule out damage they are strong evidence against it.

The increase in spike duration coincides with and presumably causes increase in the size and duration of the electrotonic EPSP produced in the post-synaptic (coupled) cells. This occurs at a time when the threshold of the cells is high as a result of the repetitive firing. The increase therefore tends to overcome the rise in threshold, or fatigue, and enables activity to continue to spread through the system. Whether or not this occurs to any degree under natural circumstances is not known because the normal firing pattern is unknown; activity usually had to be elicited by stimulation. Nevertheless, the size of the effect under conditions of repetitive stimulation suggest that it is important.

Besides the greater possibilities for summation and facilitation that are offered by the increase in spike duration there are other integrative actions which could result; for example, transmission of spikes only at particular frequencies. Consider two coupled cells, one of which is firing at constant low frequency and driving the other. The frequency of firing is too low for fatigue to occur, and the post-synaptic cell follows each spike. As the frequency increases, the threshold of the follower rises and it does not follow every pre-synaptic spike. Eventually it ceases to follow at all. As the frequency rises still further the pre-synaptic spike becomes longer and the resulting EPSP becomes larger. It can then reach threshold and the post-synaptic cell begins to

follow once again. If the frequency is sufficiently high summation of EPSP's also occurs. Effects such as these have occasionally been observed in the trigger system during antidromic stimulation of the driver cell.

Another possibly significant effect resulting from increase in spike duration could occur during spontaneous bursting. Suppose, for example, that the burst starts at high frequency and then declines, with the duration of the spikes gradually increasing (this occurs in a burst described by Strumwasser, 1967). At the start of the burst summation of EPSP's to threshold might occur in the follower cell, but as the frequency falls this can no longer happen. If, however, the EPSP's become larger they may still reach threshold without summating or with less summation. The configuration of activity in the follower cell will then be different from that which would occur if the EPSP's remained the same size. It could enable the follower to fire for the duration of the driver cell burst even though the driver cell spikes decline in frequency.

Is the system a trigger for feeding?

At this stage there is little to be gained by discussing in any detail possible functions for the system. Experiments using intact preparations should provide an answer.

It has been mentioned earlier that the rapidity of fatigue in the system would seem to preclude a function in continuous feeding. A possible answer is suggested by a comparison with the pleural ganglion network in *Tritonia*, namely that in the experiments performed here an insufficient number of trigger cells were being stimulated. Willows & Hoyle stress the necessity for simultaneous activation of a number of cells in the group. In the intact animal perhaps the required excitation is present.

However, the few preparations showing spontaneous activity in the trigger system suggest that it does not necessarily function like the network in *Tritonia* or in the manner suggested for the coupled cells in *Navanax*, i.e. by the receipt of synaptic input. Rather, it appears that there may be an intrinsic pacemaker. This would accord with observations that feeding movements occur spontaneously even when the buccal mass and ganglia have been removed from the snail. Snails may continue to rasp even when picked out of the water; no stimulation, or at least no specific stimulation, is required. This contrasts with the specific stimuli required to elicit escape in *Tritonia* and feeding in *Navanax*.

Whatever the role of the system it is evidently an interesting subject for study and shows that attention should not be focused exclusively on large identifiable cells. The recent findings of similar groups of cells in *Aplysia* and *Tritonia* (and the large cells in *Navanax*) suggest that such groups may be widespread in molluscan ganglia.

SUMMARY

1. The buccal ganglia of *Planorbis* contain a population of electrically coupled small cells. This has been studied, in preparations of isolated ganglia, by recording intracellularly from the cells two at a time.

2. The population is usually silent but activity initiated in any one of its members tends to spread to the rest of the population in both ganglia. Failure of spread, or fatigue, gradually occurs on repetition.

3. The group has the properties of a trigger system, initiating prolonged patterned

activity in large numbers of neurones in the buccal ganglia. This may normally initiate feeding.

4. In addition to central processes, both in the buccal ganglia and to the rest of the CNS, the system has peripheral axons in most of the buccal nerves. No synaptic input could be demonstrated.

5. Action potentials in some of the cells increase greatly in duration with repetition. The resulting electrotonic EPSP's, recorded in closely coupled trigger cells, correspondingly increase in size. The possible integrative significance of this is discussed, especially its effect in offsetting fatigue.

6. In some preparations spontaneous bursting occurred in trigger cells and this elicited burst activity in large neurones, including motoneurones. The system may have an intrinsic pacemaker.

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