

SENSORY FIELDS AND PROPERTIES OF THE OESOPHAGEAL PROPRIOCEPTORS IN THE MOLLUSC, *PHILINE*

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(Received 19 January 1981)

SUMMARY

1. The buccal ganglia of *Philine* each contain four mechanosensory neurones that are proprioceptors in the oesophageal wall. The sensory fields of the three small receptors are bilateral and separate from each other, but two are overlapped by the more extensive field of the largest cell.

2. The sensory field of each receptor overlaps that of its contralateral homologue.

3. The receptors respond to touch or stretch of the oesophageal wall or certain nerve trunks with a rapid burst of impulses, which adapt quickly to repetitive stimulation.

4. The soma spike is characterized by a large undershoot that summates at high-impulse frequencies to hyperpolarize the cell.

5. Repetitive stimulation leads to the axon spike failing to propagate to the soma. Blocking most probably occurs at a branch point on the main axon within the ganglion.

6. Impulses generated in one part of the axon normally propagate to all parts of the cell. Conduction velocity increases in the axon as the soma is approached. Conduction velocity may vary in different branches of the axon.

INTRODUCTION

In recent years several reports have appeared which describe the occurrence of primary sensory neurones in the central ganglia of annelids and molluscs (Nicholls & Baylor, 1968; Byrne, Castellucci & Kandel, 1974; Audesirk, 1979; Byrne, 1980; Spray, Spira & Bennett, 1980). In molluscs these neurones are generally small and clustered into groups within the ganglia, and may consist of 25-75 cells few, if any, of which interact with each other. The mechanoreceptors innervating the buccal mass of *Tritonia diomedea* and *Navanax* (Audesirk, 1979; Spray, Spira & Bennett, 1980) have small single or multiple receptive fields which may partly overlap with those of other receptors. They respond to pressure or stretch of muscle or epithelial tissue with a rapidly adapting burst of impulses. Although we now have good evidence that the fundamental feeding cycles in molluscs with both grazing and omnivorous habits are determined by central pattern-generating networks in which

the timing of the motor output is dependent upon interneurons (Kaneko, Merikel & Kater, 1978; Siegler, 1977; Bulloch & Dorsett, 1979*b*; Benjamin & Rose, 1979), in several instances sensory input has been shown to have an important role in modulating the output from these systems. In *Tritonia*, stimulation of mechanoreceptors in the buccal mass or oesophagus can initiate several cycles of buccal activity, whereas in other types excitatory and inhibitory interactions have been established between the proprioceptors and motoneurons serving the buccal musculature.

Philine aperta is a tectibranch mollusc living in sublittoral sands. A detailed study of the functional morphology of its feeding apparatus has been made by Hurst (1965), who describes how it seizes its prey by protruding the specialized radula through the mouth to grasp it between two rows of teeth. The prey is then withdrawn into the lumen of the buccal mass during the retraction cycle, released and transferred to the oesophagus and hence to the gizzard. The feeding action differs from grazing gastropods and opisthobranchs such as *Tritonia* (Bulloch & Dorsett, 1979*a*) in that it normally involves only one or two sequences of buccal activity. In this and the accompanying paper we describe the receptive fields and electrophysiological properties of a group of mechanoreceptors innervating the oesophageal wall of the mollusc *Philine aperta*. They differ from the receptors associated with the buccal mass of other molluscs in a number of ways, but most importantly in the small number of neurones, the size and overlap in their receptive fields, and in the connexions they make with each other and the interneurons and motoneurons involved in generating the patterned output to the buccal musculature.

MATERIALS AND METHODS

Philine aperta were maintained in tanks with circulating sea water for periods of about 1 month. They were not fed and appeared to survive without harm. For this preparation the oesophagus and gizzard were exposed by a median longitudinal incision through the cephalic shield, and the oesophagus was cut through close to the gizzard. The paired retractor muscles 4 and 5, the columella muscle and the visceral nerve loop were sectioned (Hurst, 1965), and a circular cut was made through the body wall surrounding the mouth so that the buccal mass, oesophagus and entire circumoesophageal nerve ring could be removed with minimal disturbance. A tapered wax-covered platform was then inserted down the oesophagus until its end lay under the buccal ganglia. Pins placed at strategic points around the ganglia effectively immobilized the oesophagus and buccal wall around the ganglia, but allowed limited movements of the remainder of the buccal mass.

The oesophageal wall was stimulated mechanically by means of hand-held probes or by a tapered tungsten-wire probe mounted on the end of a loudspeaker coil. The coil was driven by pulses from a pulse generator (Farnell). The movement of the tip was directly proportional to the pulse amplitude over the range employed in these experiments. Cell morphology and axon distribution were studied by intracellular injection of CoCl_2 (Pitman, Tweedle & Cohen, 1972), which was precipitated and subsequently intensified with silver (Bacon & Altmann, 1977). Alternatively cells were injected iontophoretically with horseradish peroxidase using electrodes filled with a 2% solution of HRP in 0.1 M-KCl, and 5 nA positive current. The tissue

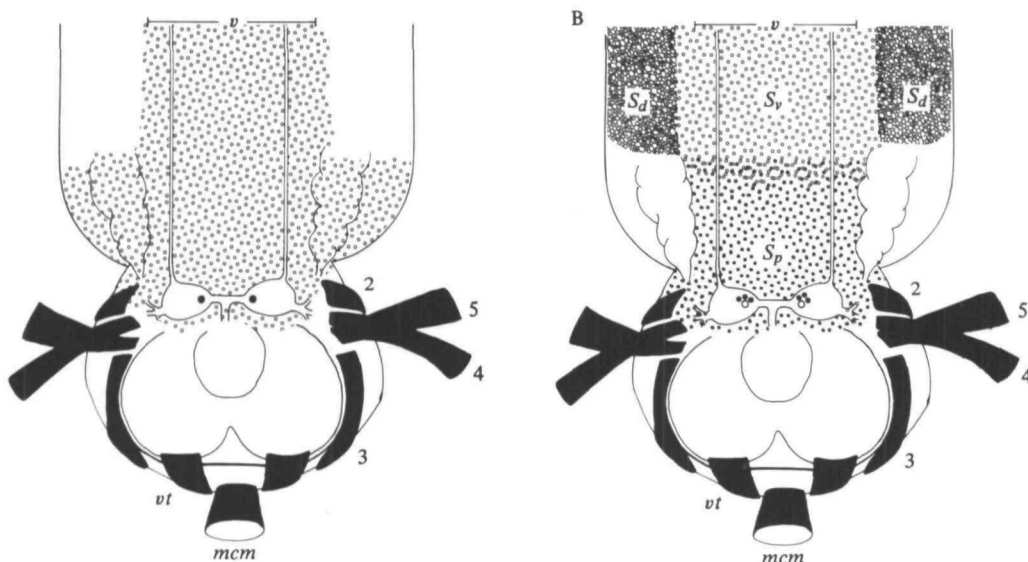


Fig. 1. Receptive fields of the S cells. The oesophagus is represented as having been cut along the dorsal mid-line and spread laterally. The ventral boundary is indicated by *v*. (A) Sensory fields of the S receptors. The fields are generally symmetrical and overlap. (B) The fields of the small mechanoreceptors. That of the *S_d* cell has been divided by the cut. *S_v*, Ventral receptor; *S_p*, proximal receptor; 2-5, extrinsic muscles; *vt*, ventral tensors; *mcm*, median columella muscle.

was fixed in Tris-buffered glutaraldehyde at pH 7.6. Subsequent processing followed the technique of Muller & McMahan (1976).

The experiments reported here were made on approximately 150 animals. Measurements of conduction velocity, latency and adaptation characteristics were accumulated over many experiments, but in presenting the results we have selected single experiments which seemed typical of the range of data recorded.

Location of the receptors

The oesophageal mechanoreceptors form two symmetrical clusters of four neurones on the dorsal surface of each buccal ganglion. The largest cell in each group (called the S cell) has a diameter of $60\ \mu\text{m}$ and can normally be identified on the mid-line of the ganglion close to the junction with the buccal commissure. It also has a bright yellow fluorescent appearance. The remaining three receptors in each group are small, not exceeding $40\ \mu\text{m}$, and are found around the anterior border of the S cells (Sigger & Dorsett, 1981). The smaller receptors cannot confidently be identified individually on visual or topographical criteria, but each has its own receptive field on the oesophageal wall.

Receptive fields

The sensory fields of all the receptors extend symmetrically to the left and right sides of the oesophageal wall, and so far as we can determine, each receptor presents a mirror image of its contralateral partner. Additionally, the sensory field of the S cells is larger and overlaps those of two of the smaller receptors, so that some areas of the wall are innervated by at least four cells.

The large S cells can be excited by stimulation of the oesophageal wall over an area extending ventrally from the buccal ganglia and bases of the salivary ducts, to where the oesophagus joins the gizzard. In our preparations the gizzard is cut away and it is not known if the sensory field includes this organ. The S cells also respond to tactile stimulation of the ipsilateral salivary glands and to stimulation of the dorsal surface of the oesophagus from the buccal mass to about half-way to the gizzard (Fig. 1*a*). The receptive fields of the two S cells are symmetrical and overlapping.

Two of the smaller mechanoreceptors innervate the ventral and lateral walls of the oesophagus, the S_p cell serving the proximal region close to the ganglia, and the S_v cell the distal region as far as the gizzard (Fig. 1*b*). The boundary between these sensory fields lies midway between the buccal mass and the gizzard, and there is a small overlap between them.

The third pair of receptors, the S_d cells, innervate the distal oesophageal wall on the dorsal side – an area that has so far not been shown to be covered by the S cells.

The receptors respond to tactile stimulation of the oesophageal wall on both sides of the body, which suggests that their axons may be present in nerves arising from both left and right buccal ganglia. To test this possibility, experiments were made to determine the effects of cutting various buccal nerves on the sensory responses of the S cells. These experiments showed that the sensory field can be divided into two main regions: that served by the gastro-oesophageal nerves, and the second served by the first major branch of the combined lateral nerve trunk. This branch (BN 3) was shown by Hurst (1965, fig. 17*A*) to divide into buccal nerves 3, 4 and 5, and be distributed to the upper parts of the buccal mass and the oral tube.

Cutting both gastro-oesophageal nerves restricts the receptive field of the S cell to the lateral and dorsal areas of the oesophageal walls close to the buccal mass. Further sectioning BN 3 on the left and right sides will then eliminate all remaining responses of the receptor to stimulation of the oesophageal wall. Experiments in which one of these 4 nerve trunks was left intact indicate that the two broad divisions of the sensory field (innervated by the gastro-oesophageal and buccal nerves 3) are further divided into left and right halves served by the nerve trunks of the respective side.

In addition to the oesophageal wall, responses are also obtained to careful tactile stimulation of the surface of the buccal commissure in the region of the radula nerve BN 1, and of the basal region of the combined lateral nerve trunk on the same side as the impaled S cell. These responses appeared to be genuine receptor discharges to stimulation of sensory endings, and not caused by electrode movement resulting from the stimulus. The same stimulus applied to adjacent areas of nerve trunk did not activate the cell. The observation is also supported by anatomical evidence of sensory endings in these regions of the nerves.

Comparable experiments were not completed with each of the smaller mechanoreceptor neurones, but it was found that in the case of the S_p and S_v cells, cutting the gastro-oesophageal nerves of one side restricts the sensitivity to the complete side. This provides the only evidence of the bilateral axon distribution of these cells, as intracellular dye injections failed to penetrate the axon in the contralateral ganglion.

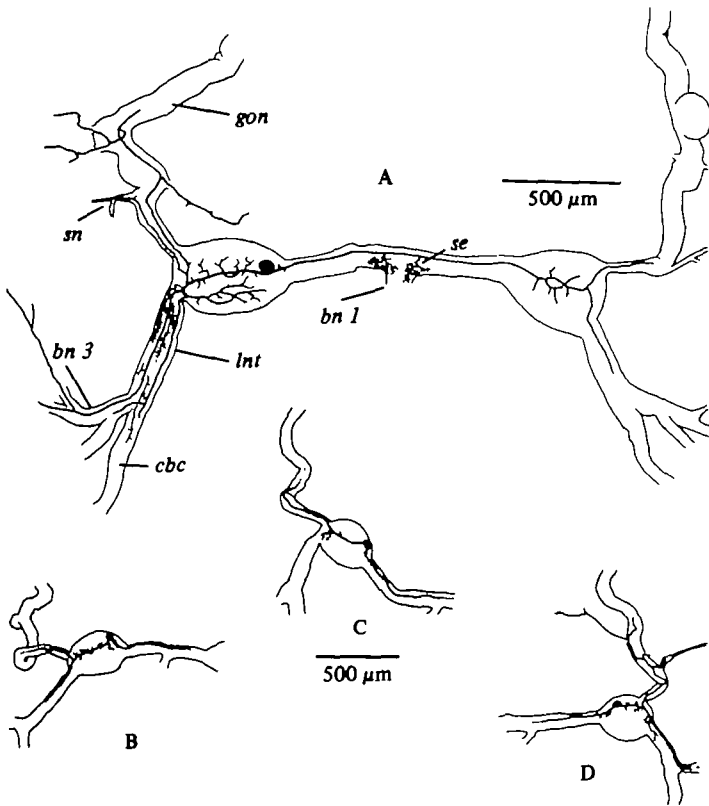


Fig. 2. Dye-injection profiles of the mechanoreceptors. The figures represent single preparations with details added from others. (A) The S cell in the left buccal ganglion. Note the sensory endings (se) in the commissure and lateral nerve trunk. (B) S_v , The ventral receptor. (C) S_d , The dorsal receptor. (D) S_p , The proximal receptor; gon , gastro-oesophageal nerve; sn , salivary nerve; lnt , lateral nerve trunk; bn 1, 3, buccal nerves; cbc , cerebro-buccal connective.

The morphology of the S cells

Dye-injection studies show two axons arising from a single point on the soma of the S cells, one entering the buccal commissure while the other crosses the neuropil of its own ganglion towards the lateral nerve trunk (Fig. 2A). Several branches occur in this region, the first entering the gastro-oesophageal nerve while the main axon continues for a short distance before dividing again. These two axons run in the combined lateral trunk, one eventually passing into BN 3 while the other seems to end around the origin of the cerebro-buccal connective. Electrical stimulation of the connective does not excite the S cell antidromically, so presumably it does not contain a branch of the axon. This axon also gives rise to a recurrent process which re-enters the buccal ganglion to ramify in the neuropil close to the motoneurons along the posterior border.

Both the axons entering BN 3 and the gastro-oesophageal nerve send processes into fine branches which enter the oesophageal wall, salivary glands and the buccal wall around the ganglia.

The second major axon, which enters the buccal commissure, crosses the neuropil

of the contralateral ganglion, giving rise to numerous fine branches which presumably establish the synaptic contacts made by the S cell with other buccal neurones (Sigger & Dorsett, 1981). On this side the main axon divides only once to send a branch to the gastro-oesophageal nerve and another into the root of BN 3.

Two observations made on dye-injected preparations are of particular interest in the light of the experiments that showed the S cell responded to tactile stimulation of the radula nerve and the lateral nerve trunk of the ipsilateral ganglion. In both these regions numerous small branches are observed arising from the axon and forming clusters of fine terminals within the nerve. It could not be clearly established whether the endings were on the surface sheath or among the axons, nor was there evidence of terminal specializations. These endings do not resemble those of neuro-secretory cells (Dorsett & Taylor, 1980).

Dye-injection studies on the smaller mechanoreceptors have provided a less complete anatomical picture than was obtained for the S cells (Figs. 2B-D). In all three small cells the axon bifurcates as it arises from the soma, but the branch entering the commissure fails to stain beyond the mid-point. On the ipsilateral side the S_p , S_v and S_d receptors all have axons in the gastro-oesophageal nerve and branches which pass out towards the oesophageal wall. The S_p cell has an axon in BN 3 but not in the cerebro-buccal connective while the S_v cell has an axon in the BN 3 fibre tract, which does not quite reach the nerve. The S_d cell axon also branches towards the lateral nerve trunk, but has failed to fill beyond this point.

Thus the anatomical picture obtained for the S cells and the smaller mechanoreceptors reveals a pattern of axon distribution that is not inconsistent with the experimental results to tactile stimulation which determined the receptive fields. They support the picture of neurones with two highly branched arrays of peripheral axons distributed to the oesophageal wall on the left and right sides.

Responses of the S cells

In the preparations used for the present series of experiments the most effective means of stimulating the S cells was found to be a light touch or stroke of the oesophageal wall with a fine probe. The neurones did not respond readily to small jets of water directed at the oesophagus.

A typical response to such a stimulus is a rapidly adapting burst of impulses lasting 1–2 s, although the cell may continue sporadic firing for up to 10 s after cessation of the stimulus (Fig. 3A). The overlap in the sensory fields means that several receptors may respond to the same stimulus, the initial high-frequency component of the burst being enhanced by the excitatory synaptic connexions between the two S cells and inputs from the smaller mechanoreceptors (Sigger & Dorsett, 1981). Sensitivity to touch is not even throughout the receptive field, some areas generating much more vigorous responses than others.

The action potentials arise abruptly from the baseline with no indication of any underlying pre-potential or synaptic activity. The spike has a pronounced undershoot which has a duration of about 150 ms, and during a rapid burst of activity the soma may experience a transient hyperpolarization of up to 10 mV (Fig. 3A) which may last up to 10 s after the last impulse. In some S cells which show high resting potentials the hyperpolarization is reversed (Fig. 3B). Such cells still respond to touches but do not show prolonged intermittent firing.

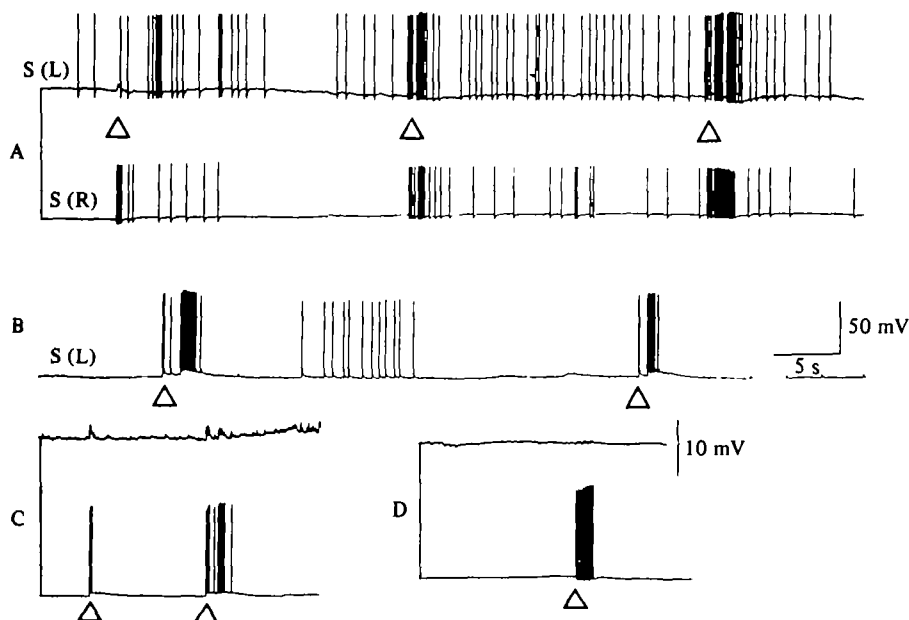


Fig. 3. Responses of the oesophageal proprioceptors to tactile stimulation of the oesophageal wall. (A) Concurrent recordings from left and right S cells. (B) Touches recorded in a cell with a high resting potential. Note the depolarizing shift during the burst. (C) Synaptic potentials in an S cell (upper trace) derived from a burst of impulses in its partner (lower trace). Stimulus was a touch to the oesophagus. (D) Response to an identical stimulus after 30 min in high Mg^{+} saline. Synaptic potentials are abolished. Δ , Tactile stimulus.

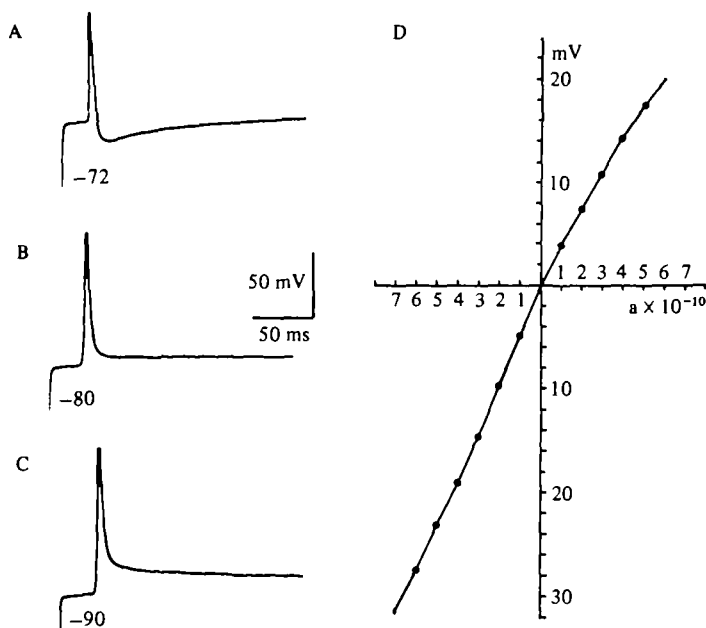


Fig. 4. Properties of the receptors. (A-C) Configuration of the action potential at three values of resting potential. Progressive hyperpolarization converts the undershoot to a depolarizing potential (C), with an equilibrium value at -80 mV. (D) Current-voltage plot of the S cell.

The absence of spontaneous synaptic activity in the S cells, taken in conjunction with their anatomical and response characteristics, are features which have been previously associated with centrally located mechanoreceptors (Nicholls & Baylor, 1968; Byrne *et al.* 1974; Spray *et al.* 1980). Driving the cells by depolarizing the soma through the microelectrode produces no consistent response in the peripheral musculature. In order to preclude the possibility of peripheral synaptic activity being responsible for the S cell response the preparation was soaked for 30 min in sea water containing 200 mM-Mg, which has been shown in this and other molluscan preparations to eliminate known synaptic actions. Following such treatment the S cell continued to respond to tactile stimulation of the oesophageal wall, although the post-synaptic response to the S cell spike in an identified neuron in the buccal ganglion was abolished (Fig. 3 C, D).

Properties of the S cells

An unusual feature of the S cells is the variable configuration of the action potential, which is associated with a wide range of values recorded for the resting potential. In different preparations the resting potential varied between -52 and -72 mV (average -57 mV, $n = 12$). At the lowest resting potentials cells often show irregular spontaneous firing, and have an undershoot with an amplitude of 20 mV. At higher values of the resting potential the neurones are normally silent and the undershoot has values of 5–6 mV or is occasionally reversed (Fig. 3 B). In the experiments represented by Fig. 4, two electrodes were inserted in the same cell, one used for recording the membrane potential and second for passing current. Progressive hyperpolarization of the cell membrane reduced the amplitude of the undershoot and eventually reversed it, the equilibrium value being -80 mV (Fig. 4 A–C). This value has been shown to represent the equilibrium potential for the K ion in several molluscan neurons (Gerschenfeld, 1973).

The current–voltage relationship plotted for a cell with a resting potential of -72 mV is essentially linear (Fig. 4 D), the slope indicating an input resistance of $40\text{ M}\Omega$ for a cell $60\text{ }\mu\text{m}$ in diameter.

Sensory adaptation

A controlled stimulus was applied to the oesophageal wall by means of a tungsten-wire probe with a cross-sectional area of $350\text{ }\mu\text{m}^2$, mounted on a mechanical prodder. The tip of the probe was manipulated close to the oesophageal wall and then advanced linearly by small increments until the receptor responded.

Adaptation to a tactile stimulus, well above threshold, delivered to a point on the oesophageal wall is rapid, but does not spread to the endings in adjacent areas of the sensory field (Fig. 5). The first tactile stimulus of a series generated a burst of 16 impulses (Fig. 5 A) that adapted to a single impulse by the 4th touch. Moving the probe tip to a site about $250\text{ }\mu\text{m}$ away from this position restored the response of the receptor to its original intensity, but it again declined to a single spike by the 4th stimulus prod (Fig. 5 B).

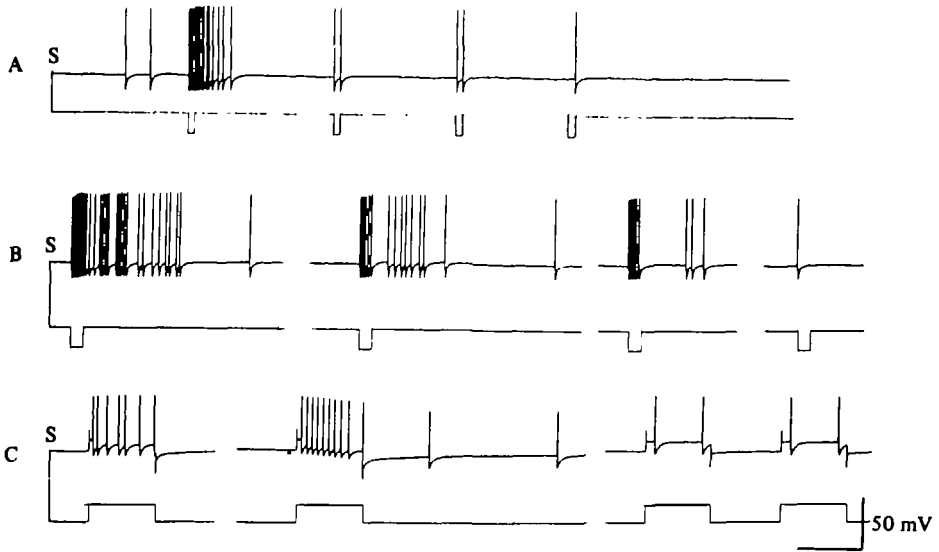


Fig. 5. Adaptation of the S cell. (A) Adaptation to four successive tactile stimuli. (B) Moving probe to an adjacent area of sensory field restores the response which then rapidly adapts. (C) Electrical stimulus of either polarity to oesophageal wall induces a train of impulses proportional to the current intensity. The response adapts over a 10 s period of stimulation. Cal. (A, B) 5 s; (C) 1 s.

Electrical stimulation of the wall

Electrical pulses of either polarity, above a threshold intensity, delivered to the oesophageal wall were capable of stimulating the S cells (Fig. 5C). A 1 s pulse elicits a burst of impulses with a duration corresponding to the length of the stimulus. The nature of the response differs from that of a tactile stimulus in that the impulses are more regularly spaced and do not show such rapid adaptation. Increasing the intensity of the stimulus or changing the polarity increases the discharge frequency. With repetition the number of spikes slowly declines so that after 10 s it is reduced to a single impulse at the onset and decay of the stimulus. Similar on-off responses are sometimes noted with a mechanical stimulus (Fig. 5A).

Blocked impulses and firing frequency

The S cells respond to the first tactile stimulus of a series, delivered at intensities just above threshold, with a short burst of impulses, but adapt rapidly to subsequent stimuli, which may only generate a single spike. As the interval between each stimulus is reduced to less than 400 ms, the response either failed completely or the axon spike was blocked at some distance from the soma.

The inability of the receptor to follow a mechanical stimulus repeated at rates above 2.5 times/s is not related to the maximal impulse frequency of which the cell is capable. Estimates of the natural maximal impulse frequencies in response to a tactile stimulus suggest that the receptor may normally fire at rates up to 25 impulses s^{-1} . It was previously shown that a distinct hyperpolarization of the S cell soma occurs at higher impulse frequencies, partly due to summation of the undershoot, and there is a possibility that the spread of this potential could play some part in blocking the axon spike at major branch points within the ganglion, or limit the firing rate of the cell in some other way.

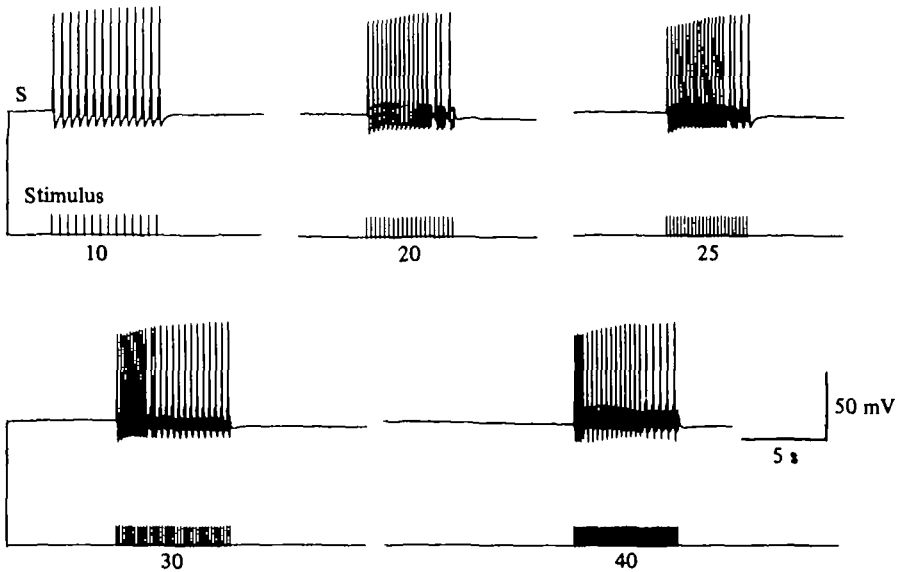


Fig. 6. Maximum firing frequency of S cell. Action potentials recorded in the soma to supra-threshold stimulation of the ipsilateral nerve trunk. Above 25 s^{-1} the cell rapidly loses the ability to follow individual stimuli.

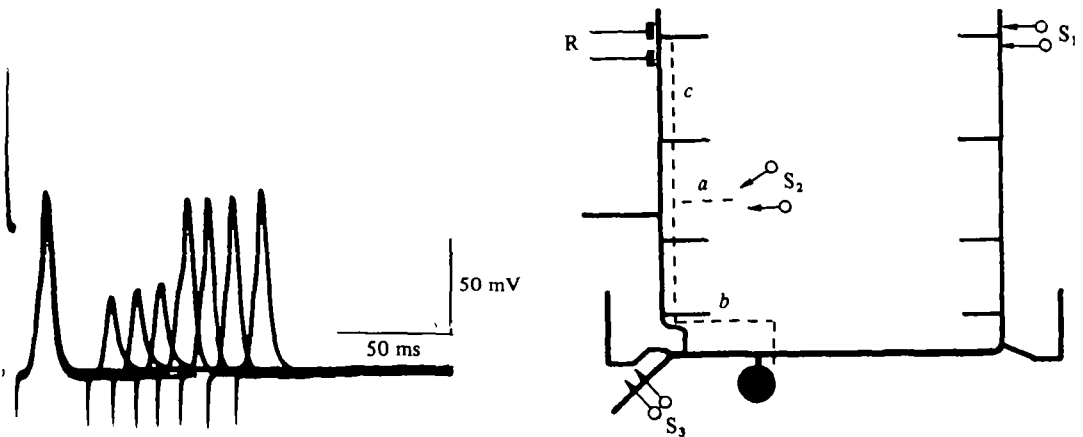


Fig. 7. Responses of the S cell to paired stimuli applied to the lateral nerve trunk. The figure consists of successive sweeps superimposed, in which the pulse interval was reduced from 90–30 ms. At intervals below 60 ms the axon spike is blocked at some distance from the soma. Below 30 msec the second pulse falls within the refractory period of the axon.

Fig. 8. The experimental arrangement to investigate the conduction properties of the S cell axon. S_1 – S_3 represent stimulation sites on the nerves and oesophageal wall. R is a recording site on the gastro-oesophageal nerve. (a–c) Sectors of the conduction pathway in the oesophageal wall (a), to the recording sites on the gastro-oesophageal nerve centripetal (b), and centrifugal (c) to the junction.

To investigate the maximum impulse frequencies which can be sustained by the receptor, a suction electrode was used to stimulate the lateral nerve trunk close to the origin of BN 3, while simultaneously recording from the ipsilateral S cell (Fig. 6). At frequencies up to 10 s^{-1} the antidromic spike in the S cell followed each stimulus pulse. At 25 s^{-1} the cell could follow the first 15 pulses without loss. The maximum frequency the receptor could follow was 40 s^{-1} , and then only the first four pulses of the series. After missing a pulse the cell followed alternate stimuli.

A second estimate of the maximum impulse rate that could be sustained by the cell was made by stimulating the lateral trunk with pairs of impulses at reducing intervals (Fig. 7). A full spike followed the second pulse down to intervals of 60 ms, after which the impulse blocked at some distance from the soma. At intervals of less than 30 ms the second stimulus fell within the refractory period of the axon. Both these experiments suggest an upper limit of about 30–40 impulses s^{-1} for both the soma and the axon.

Conduction of the axon

The morphological and physiological evidence confirms that the mechanoreceptors have a bilaterally distributed sensory field, with the axons having a high degree of terminal branching within the oesophageal wall. Precise details of the site of the impulse generating mechanisms are not known, but action potentials associated with the S cells are recorded at all points along the gastro-oesophageal nerve as far as the gizzard. With this configuration of the axon it is probable that an impulse generated in one part of the sensory field will propagate to all parts of the cell, where it may collide with impulses originating in these regions or render them refractory.

To investigate these possibilities an electrode was inserted into the S cell of the left buccal ganglion, and recording and stimulating electrodes were placed on the left and right gastro-oesophageal nerves respectively (Fig. 8, R, S 1). A stimulus to the right nerve was followed by an action potential in the soma and then at the recording site in the left gastro-oesophageal nerve (Fig. 9A).

The impulse had a mean conduction velocity of approximately 30 cm s^{-1} for this preparation. Hyperpolarizing current applied to the cell caused an inflexion to develop in the leading edge of the spike that eventually failed to invade the soma, leaving an axon spike that probably blocked at the junction of the main axon with the branch in the right gastro-oesophageal nerve (Fig. 9B, C). The axon spike is not transmitted to the left side (Fig. 9C).

If the stimulus is now delivered to the lateral nerve trunk of the left ganglion (Fig. 8, S 3), the impulse propagating towards the soma also invades the left gastro-oesophageal nerve (Fig. 9D). Hyperpolarization of the soma blocks the impulse at a point beyond the origin of the gastro-oesophageal nerve which is no longer invaded, while stronger hyperpolarization can prevent excitation of the axon by the stimulus (Fig. 9E).

Stimulation of the oesophageal wall

In the experiment represented by Fig. 10 the stimulating electrode was placed on the left side of the oesophageal wall (Fig. 8, S 2), the contralateral gastro-oesophageal nerve being cut to eliminate the alternative pathway to the receptor. The stimulus to the wall was followed by an impulse in the soma with a 15 ms latency and another in the distal region of the ipsilateral gastro-oesophageal nerve

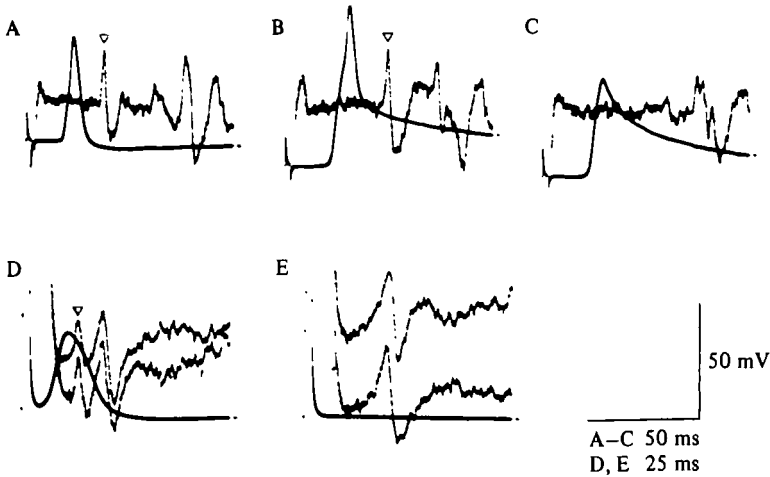


Fig. 9. Conduction in the S cell axon. (A) The stimulus to the contralateral gastro-oesophageal nerve leads to an impulse in the S cell soma (lower) and in the contralateral gastro-oesophageal nerve (upper). (B) Hyperpolarization of the soma does not affect conduction. (C) Further hyperpolarization blocks the soma spike, and the axon spike is not conducted to the opposite side. (D) A stimulus to the lateral nerve trunk activates the S cell spike (lower) and an impulse in the ipsilateral gastro-oesophageal nerve (two sweeps recorded). (E) Hyperpolarization of the soma abolished both the spike and nerve impulse. A large unit in the nerve (possibly the contralateral S cell) was not affected. The two sweeps of the external recording are registered separately.

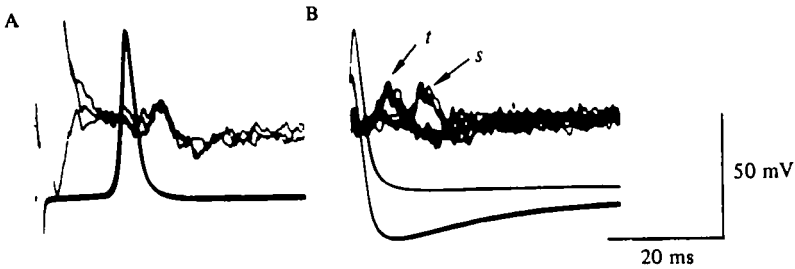


Fig. 10. (A) Responses to stimulation of the oesophageal wall recorded in the ipsilateral S cell (lower) and the gastro-oesophageal nerve (upper). (B) Superimposed sweeps triggered on the S cell spike evoked by a touch (*t*) and by depolarizing the S cell (*s*). The touch-generated impulse reaches the recording site on the gastro-oesophageal nerve with a shorter latency than that resulting from the soma spike.

after a further 6 ms (Fig. 10A). A tactile stimulus generates a similar response to the oesophageal wall close to the stimulating electrode with the same latencies (Fig. 10B). A centrifugal impulse generated in the soma by depolarization through the microelectrode reached the recording site in the gastro-oesophageal nerve after a latency of 12 ms (Fig. 10B). From these intervals it is clear that the impulse generated in the oesophageal wall reaches the oesophageal nerve, where it then propagates both towards the soma and towards more peripheral regions of the axon.

Referring to Fig. 8, pathways

$$a + b = 15 \text{ ms}, \quad a + c = 21 \text{ ms}, \quad b + c = 12 \text{ ms},$$

from which $a = 12 \text{ ms}$, $b = 3 \text{ ms}$, $c = 9 \text{ ms}$.

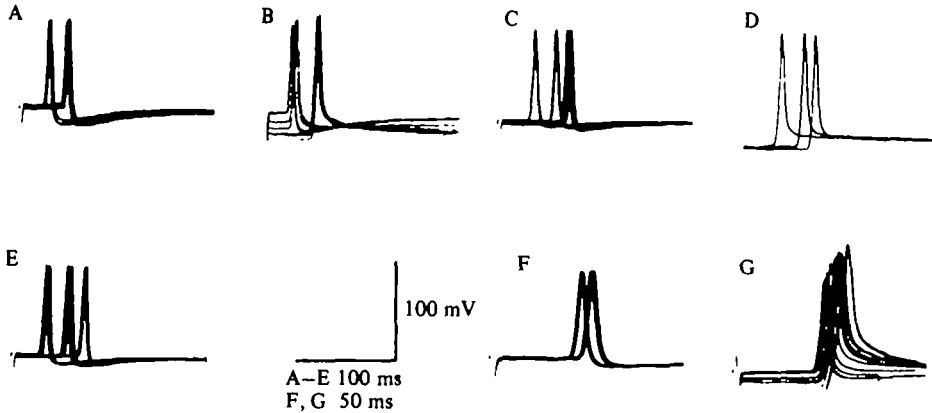


Fig. 11. Variable latency responses. (A) A stimulus to the lateral nerve trunk generates a soma spike. Increasing the stimulus intensity causes a sudden reduction in the latency. A number of sweeps superimposed. (B) Increasing hyperpolarization of the S cell causes a transition from short-latency to long-latency spike. (C) At intensities just above threshold for the short-latency component, increasing stimulus frequency reveals two longer-latency components. (D) With cell soma hyperpolarized, regulation of stimulus intensity also separates the three components. (E) At stimulus repetition frequency of 4 s^{-1} , increasing stimulus intensity causes transition to short-latency spike. (F) Electrical stimulus to oesophageal wall. Varying intensity separates long and short latency responses. (G) Hyperpolarizing the soma produces range of intermediate values between long and short latencies. Cal. (A-E) 100 ms; (F, G) 50 ms.

These results, which are comparable to those obtained in four other experiments, indicate a considerable increase in the conduction velocity of the spike as it approaches the soma. The conduction velocity of the impulse in the oesophageal wall is uncertain as the path length is unknown, as is the delay in the impulse arising from a generator potential.

Variable latency responses

A stimulus to the lateral nerve trunk a short distance from the buccal ganglion, delivered with gradually increasing intensity, eventually evoked an impulse in the S cell with a latency of 40 ms. Increasing the stimulus intensity still further caused a sudden drop in the latency to 20 ms (Fig. 11 A). Similar results have been reported by Spray *et al.* (1980), who interpret them as evidence of threshold differences between two branches of an axon with different conduction velocities. The impulse in the faster conducting axon is initiated at a higher threshold and collides with and eliminates the slower spike.

If the soma of the S cell is hyperpolarized, the short-latency spike is blocked and the slower spike reappears (Fig. 11 B), the difference in the latency remaining unchanged.

These experiments were made with individual pulses delivered at intervals greater than 1 s. The preparation was then stimulated with a series of pulses at repetition frequencies ranging from $1\text{--}5 \text{ s}^{-1}$ at an intensity which was just sufficient to elicit short-latency spike to a single stimulus. As the repetition rate of the stimulus was increased to $2\text{--}3 \text{ pulses s}^{-1}$, the short-latency spike gave way to the previous slower component. Upon further increase to 5 pulses s^{-1} the slower component was in turn replaced by a third spike whose latency was 40 ms slower than the fastest component spike (Fig. 11 C). When the cell is hyperpolarized, or when stimulated at the higher

repetition rates, the three components of the response show a consistent relationship to the intensity of the stimulus (Fig. 11 D, E). The slowest component has the lowest threshold, while the intermediate and fast components are introduced as the intensity is progressively increased.

These experiments were repeated with the stimulating electrode placed on the oesophageal wall. Following a response obtained at a lower threshold, increasing the intensity caused a sudden reduction of 5 ms in the latency of the impulse in the receptor (Fig. 11 F). In contrast to the experiments where the stimulus was applied to the nerve trunk, hyperpolarization of the soma produced a continuous increase in the latency of the fast spike, until it merged with the slower component (Fig. 11 G). Further hyperpolarization blocked all activity in the cell. In this situation the 5 ms latency difference may represent the separate excitation of the sensory terminals and the axons in the oesophageal wall by stimuli of different intensities.

DISCUSSION

Two fundamental questions are posed in relation to the role of the oesophageal mechanoreceptors; to what stimulus do they respond or how are they excited naturally within the animal, and in what phase of the feeding cycle are they active? The most natural stimulus would appear to be the passage of food along the oesophagus from the buccal mass towards the gizzard, but two observations suggest that excitation may not arise from stimulation of the mucosal surface. The receptors respond vigorously to stimulation of the outer wall of the oesophagus, which produces no visible indentation at the surface while remaining unresponsive to comparable stimulation of the mucosa at the cut end of the oesophagus. Secondly, the sensory endings in the lateral nerve trunks and the buccal commissure at the junction with BN 1 are unlikely to be stimulated by the movement of food within the oesophagus.

An alternative stimulation could be stretching or sliding actions of the oesophagus against the body wall during the vigorous protraction and retraction movements or during peristalsis. The movements of the radula sac and buccal mass during spontaneous feeding cycles are transmitted to the ganglia and nerve trunks, although these are normally restrained in making the preparation. Other mechanoreceptors serving the wall of the buccal mass are known to fire during the protraction phase of the cycle (unpublished observations).

Sensory adaptation and conduction block

The extensive and overlapping sensory fields of the receptors are poorly designed to provide precise spatial or temporal information related to events occurring along the oesophageal wall. This is compounded by the possibilities of impulse occlusion and refractoriness resulting from impulses originating in one part of the field invading all parts of the axonal system.

Activation of the receptor by touch to one small part of the field leads to rapid sensory adaptation of the underlying endings, which is limited to the area of stimulation and not transferred to immediately adjacent areas.

Although no information is available on the structural details of the sensory terminals,

the inability of the receptor to follow repetitive mechanical stimulation at rates greater than 2.5 s^{-1} suggests the coupling system incorporates a low-pass filter. In other centrally located mechanoreceptors with highly branched axon systems, repeated mechanical or electrical stimulation often results in the centripetal spike failing to reach the soma (Yau, 1976). In the leech the conduction block occurs in regions of low safety factor where peripheral branches expand into the main axon. The block may also be associated with a prolonged hyperpolarization that develops following repeated firing, and is a result of an increase in the potassium conductance of the membrane (Van Essen, 1973). The mechanoreceptors of *Philine* show a more transient hyperpolarization than those of the leech, which declines over 10–15 s following a burst of impulses. The hyperpolarization has a reversal value similar to that of the undershoot, and presumably also represents a delayed potassium conductance. Conduction block frequently follows repetitive activity and may occur concurrently in different branches of the axon, the different amplitudes of the recorded axon spikes depending on the distance of the blocked branch from the soma. It has not proved possible to relate the occurrence of blocked spikes to the degree of membrane polarization in any consistent manner, conduction failure sometimes occurring in response to single spikes in the absence of any detectable increase in cell resting potential. Shortening the interval between pairs of shocks delivered to a nerve trunk leads to blocking of the second spike, again with no appreciable hyperpolarization of the membrane.

Latency and conduction velocity

Two experiments in the current series have indicated considerable differences in the conduction velocity in different regions of the axon. A stimulus to the oesophageal wall initiates an impulse which propagates centripetally and centrifugally along the gastro-oesophageal nerve, but the velocity is greater in the direction of the soma than away from it. These findings are supported by the changes that are registered in the latency of the somatic impulse when the lateral nerve trunk is stimulated with shocks of varying intensity. Three distinct components may be separated in this way, and it has been suggested (Spray *et al.* 1980) that the different latencies may be correlated with branches of the axon having different diameters and conduction velocities.

Dye injection studies of the S cell show two principal branches of the axon enter the lateral nerve trunk, but an interesting new observation is the presence of sensory terminals within the nerve trunk itself. These may contribute the third component of the variable latency responses of this nerve. It is conventionally accepted that, in the absence of other considerations, the fibres with the largest diameter have the greatest conduction velocity and show the lowest threshold to stimulation. This premise does not apparently hold for the buccal nerves of *Philine* or *Navanax*, and one suspects its applicability to a number of other preparations. In our experiments the shortest latency spike consistently showed the highest threshold, but as the soma was hyperpolarized, the fast-conducting component was replaced by one of intermediate velocity, which in turn gave way to the slowest conducting spike. These results are consistent with the current distributing in the different branches of the axon in proportion to their diameters, so that the largest branch is the first to be

inhibited. The results are complicated by experiments in which the lateral nerve trunk was stimulated at different frequencies. Increasing the rate from 1 to 5 s⁻¹ caused a switch from the fastest to the intermediate and then the slowest-conducting component.

This firing rate is well within the capability of all parts of the axon as determined by stimulation of the sensory fields, and at the moment there is no ready explanation for the dominance of the slower components as the frequency is raised.

The same technique was used on the oesophageal wall in an attempt to resolve the delay in activating the cell by direct stimulation of the axon and through the sensory terminals. Two impulses with a latency difference of 5 ms were obtained, but in contrast to the results obtained using the lateral nerve, hyperpolarization caused a progressive shift in the latency from the fast to the slow value, the continuous change in the latency suggests that in the oesophageal wall we are dealing with a single conducting system with two components having different susceptibilities to the hyperpolarizing current. The fast component, which might be the spike initiating mechanism, can be delayed, whereas the slowest component, dependent on the generator potential once affected by the hyperpolarization will no longer excite the active membrane.

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