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SYNAPTIC ACTIONS OF OESOPHAGEAL PROPRIOCEPTORS IN THE MOLLUSC, *PHILINE*

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SUMMARY

The buccal ganglia of *Philine* each contain a group of mechanoreceptors, consisting of 1 large and 3 small cells, with receptive fields in the oesophagus.

Synaptic contacts occur between the receptors; the large cell providing an EIPSP input to its contralateral partner and to the two groups of smaller receptors. The small receptors make weak excitatory contacts with both the large receptors.

The sensory cells synapse with other buccal motoneurones and interneurones, some of which show periodic activity associated with the feeding movements.

Protraction phase neurones are divisible into two groups, one of which receives EPSPs from the receptors, while the other group receives IPSPs. Retraction phase neurones receive a biphasic EIPSP.

The receptors provide excitatory synaptic input to a pair of interneurones which 'gate' the feeding cycle. A third class of neurones which are not rhythmically active during feeding receive a predominantly inhibitory EIPSP.

INTRODUCTION

Primary sensory neurones have been identified in the ganglia of several molluscs (Kater & Rowell, 1973; Siegler, 1977; Audesirk, 1979; Spray, Spira & Bennett, 1980*a*), but little is known concerning the connexions made by these cells. A large population of mechanoreceptors has been identified in *Navanax* (Spray, Spira & Bennett, 1980*b*) although few synaptic interactions were found within the group. Audesirk (1979) found no evidence of synaptic contacts between oral mechanoreceptors of *Tritonia diomedea*.

Data on the synaptic action of buccal sensory cells with other neurones is also limited. Kater & Rowell (1973) found sensory feedback from buccal receptors of *Helisoma* alters the duration and intensity of motoneurone bursts during feeding, but does not affect the period of the rhythm. In contrast, the abolition of sensory feedback in *Pleurobranchaea* resulted in a reduction in the frequency of feeding cycles and in the maximum spike rate of individual motoneurones (Siegler, 1977).

In this paper we describe the interactions between a group of proprioceptors serving the oesophagus in the mollusc *Philine aperta*, and their synaptic action on

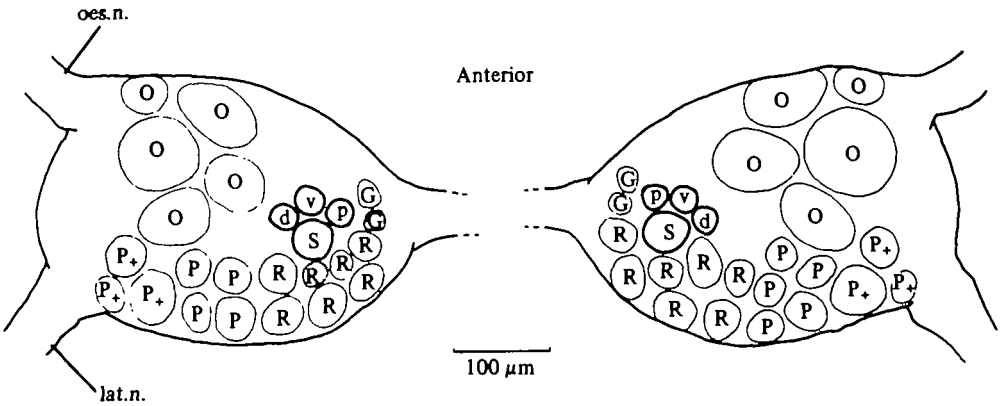

Fig. 1. Diagram of the dorsal surface of the buccal ganglia. The buccal commissure and median nerve have been omitted. *S*, *d*, *v*, *p*, Oesophageal proprioceptors; *G*, gating cells; *P*, *R*, protraction and retraction phase neurones; *O*, cells with axons in the oesophageal nerve.

motor and interneurones involved in the feeding cycle. A previous paper (Dorsett & Sigger, 1981) contains details of the electrophysiological properties and receptive fields of the receptors.

MATERIALS AND METHODS

Details of the preparation used in these experiments are as given in the previous paper. Briefly, it consists of the oesophagus, buccal mass and the complete oral region which were separated from the rest of the body by a cut around the mouth. The preparation is mounted on a wax coated platform inserted down the oesophagus until its tip lay beneath the buccal ganglia. Pins were inserted to immobilize the ganglia while leaving the buccal mass free to make limited movements. All the buccal nerves and the connexions with the cerebral complex were left intact. Microelectrodes were filled with 2 M-KAc and normally had resistances in the range of 30–60 MΩ. Conventional recording techniques were employed.

Synaptic input to the oesophageal proprioceptors

In the buccal ganglia of *Philine* two symmetrically located groups of mechanoreceptor neurones innervate the wall of the oesophagus (Fig. 1). The axons project bilaterally and the receptive fields of individual cells overlap with those of their contralateral homologues. A feature of centrally located molluscan sensory neurones is the relative absence of synaptic input, although a number of exceptions to this rule are known from other invertebrate groups. In the large population of mechanoreceptors found in the buccal ganglia of *Navanax* (Spray *et al.* 1980*b*) only 4% show excitatory and 16% inhibitory interactions within the group.

Recordings from the proprioceptors of *Philine* have shown the receptors receive both simple and compound synaptic inputs which, with one exception, originate from other members of the group.

Impulse activity in either of the two S cells produced by intracellular depolarization generates a postsynaptic response in the opposite partner. Where the resting potential of the postsynaptic neurone is high (70 mV) the psp appears as a simple excitator

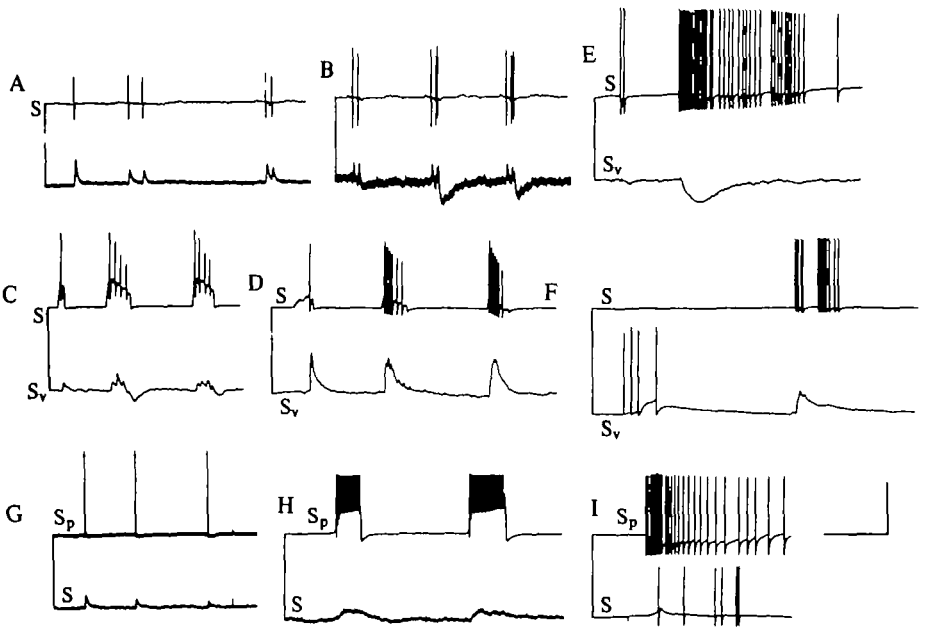

Fig. 2. Interactions between the proprioceptors. (A) The two large S cells; membrane potential of the postsynaptic cell is −72 mV. (B) Similar to (A) but with postsynaptic cell slightly depolarized showing excitatory and inhibitory components of the EIPSP. (C) Action of S cell on a small receptor; postsynaptic cell slightly depolarized to show late inhibitory component of the EIPSP. (D) As (C), but with postsynaptic cell at resting potential of −72 mV. (E) S cell (upper trace) fired by touch to oesophagus; note hyperpolarization of S_p cell (lower trace). (F) As (E); the input to the postsynaptic cell (S_v) is seen as a depolarization due to the high resting potential. (G, H) Excitatory input to the large S cell from a small ipsilateral receptor (S_p). (I) A burst in the left S_p cell, generated by mechanical stimulation, causes a depolarization in the large ipsilateral S cell. Upper traces (except B), 50 mV; B, 25 mV. Lower traces: (A, B, G, H), 10 mV; (C, D) 25 mV; (E F) 50 mV. Time scale, 5 s.

response with an amplitude of around 9 mV, but where it is naturally or artificially lowered to more depolarized levels (−55 mV) the excitatory component is reduced to around 5 mV and a slower inhibitory component is revealed (Fig. 2A, B). The postsynaptic response follows the impulse 1:1 with a constant latency of 6 ms and is reversibly abolished by raising the magnesium concentration in the bathing fluid. On these criteria the pathway is judged to be monosynaptic and the excitatory-inhibitory potential (EIPSP) a chemically transmitted compound event.

During a burst of impulses in the presynaptic cell the excitatory component antifacilitates or desensitizes so that the psp becomes predominantly inhibitory. Repeated activation of the pathway fatigues the response, but does not affect transmission in the opposite direction.

In the unfatigued preparation a single impulse does not normally generate a spike in the postsynaptic S cell. The large S cells also synapse with the smaller S_v, S_p and S_d receptors, generating a compound postsynaptic potential whose configuration is determined by the resting potential of the postsynaptic cell (Fig. 2C, D). The characteristics of the EIPSP are similar to those of the synapse between the two S cells, with rapid decline of the excitatory component during a burst of spikes,

producing a net inhibitory response (Fig. 2E). The amplitude of the EIPSP in the smaller receptors may attain values of 20 mV, depending on the membrane potential, and appears to be larger than that resulting from the S–S cell interaction. The S cells interact identically with the small receptors in their own and in the opposite ganglion.

Tactile stimulation of the oesophageal wall outside the receptive field of any one of the small receptors produces a burst of spikes in the S cells and a pronounced inhibitory wave in the S_v, S_d and S_p cells (Fig. 2E). If the stimulus is applied within the receptive field of the smaller cell there is an initial burst of 2–3 impulses before the cell is inhibited by the S cell input. Hyperpolarizing the S cell so as to reduce or prevent impulse activity prolongs the response in the smaller cell.

In the reverse direction, each of the small receptors generates a weak psp in the S cells (Fig. 2G, H). The EPSPs follow the presynaptic impulses 1:1 and with a constant latency, and are abolished by raised magnesium concentrations in the bath. The pathway appears to be both chemically mediated and monosynaptic. The maximum amplitude of an EPSP resulting from a single presynaptic spike is around 3 mV and insufficient to generate an impulse in the S cell. The S_v, S_d and S_p cells do not synapse with each other or with their partners in the opposite ganglion. The only other synaptic input observed in the S cells are occasional inhibitory potentials, the source of which has not been identified.

Synaptic contacts with the motor network

Before describing the contacts of the proprioceptors with buccal neurones outside their group it will be useful to provide a short summary of data, as yet unpublished, relating to the interneurone and motor network within the ganglia, details of which are outside the scope of the present paper.

Along the posterior border of the buccal ganglia there lie groups of neurones which undergo periodic sequences of patterned bursting. Individually recognizable neurones within these groups have been tentatively classified as motoneurones on the basis that when driven, they cause consistent and reproducible movements of muscles within the wall of the buccal mass, similar to those observed during spontaneous activity cycles. Four of these cells have been positively identified as innervating the muscle fibres of the extrinsic retractor muscles 4 and 5 (Dorsett & Sigger, 1981; Dorsett, unpublished observations), and their activity can be used to define the retraction phase of the buccal cycle. The motoneurones can be divided into two principal groups on the basis of their phase relationships within the cycles of patterned bursting (Fig. 3A), and the latter can be visually correlated with the feeding sequence described by Hurst (1965). The two activity periods of the motoneurones have been provisionally identified with the protraction (P) and retraction (R) phases of the buccal cycle.

Neurones exhibiting P-phase activity are located in a group close to the entry of the lateral nerve trunk, while R-phase neurons lie close to the buccal commissure (Fig. 1). A further group of cells with small transparent somata, which also synapse with the buccal motoneurones, appear to be interneurones capable of 'gating' the feeding cycle. Prolonged activity of the gating cells caused by an applied depolarizing current to the soma drives the motoneurones in a rhythmic sequence which stops on cessation of the current (Fig. 3C) and gating cell activity.

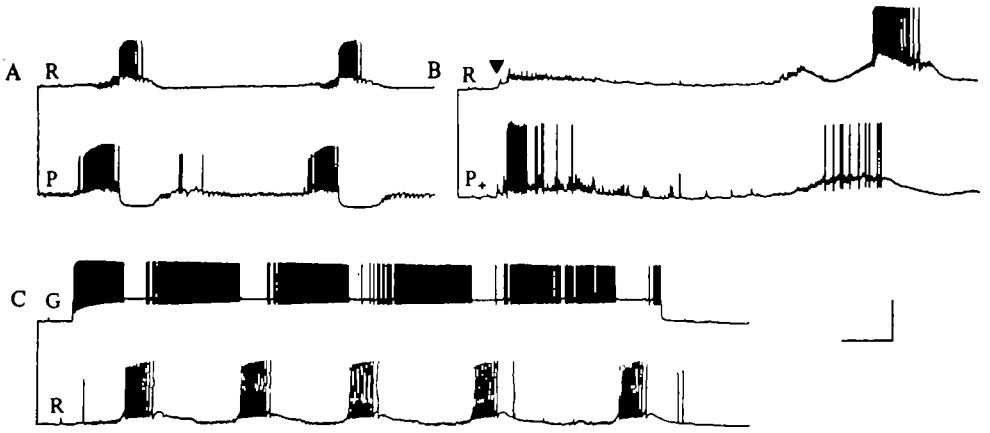

Fig. 3. Cyclic activity in motoneurones and an interneurone. (A) Two cycles of spontaneous activity recorded in an R cell (upper trace) and a P cell (lower trace). (B) Synaptic inputs to an R cell (upper trace) and a P_+ cell (lower trace) following mechanical stimulation of the oesophagus (arrow). Compare the input to this R cell, which has a high resting potential to that of Fig. 5A. Note that the stimulus triggered a complete cycle of activity in the two neurones. (C) Prolonged activity in a gating cell (upper trace) caused by a steady depolarization drives the feeding cycle which is recorded in an R phase motoneurone on the lower trace. Note that the gating cell is inhibited during the R phase burst, and that rhythmic activity stops when the depolarizing current ceases. Upper traces (A, C) 50 mV, (B) 25 mV. Lower traces (A, C) 50 mV, (B) 25 mV. Time scale, 5 s.

Cyclic activity in the buccal motoneurones can be elicited from a quiescent preparation in two other ways. The first is by tactile stimulation of the oesophageal wall, which initiates a period of intense synaptic input in both P and R cells and may be followed by one or more cycles of impulse activity after a latency of about 25 s (Fig. 3B). The second method involves a short electrical stimulus applied to an oesophageal nerve, which can cause a burst cycle in neurones of an otherwise isolated pair of ganglia. Both types of stimulus presumably activate the axons of the proprioceptors, and it was therefore anticipated that part of the synaptic activity observed in the P and R cells prior to the patterned sequence was derived from sensory input.

Synapses with gating cells

The two identified gating cells (G) in the buccal ganglia each receive excitatory inputs from both the large S cells (Fig. 4A). The EPSPs follow the impulse in the presynaptic cell with constant latency and have an amplitude of 7 mV. The synapse fatigues rapidly with repeated activation, and does not appear to be capable of generating an impulse in the G cells. The proximity of the small proprioceptors to the G cells has prevented concurrent recording from these groups and their synaptic action is unknown. Tactile stimulation of the oesophagus results in a barrage of small EPSPs which depolarizes the gating cells by approximately 5 mV, but does not in itself regularly generate prolonged impulse activity.

Synapses on P-phase neurones

The neurones showing P-phase activity are divisible into two groups according to whether they receive excitatory or inhibitory input from the oesophageal proprioceptors. The three neurones which receive excitatory input are grouped at the entry

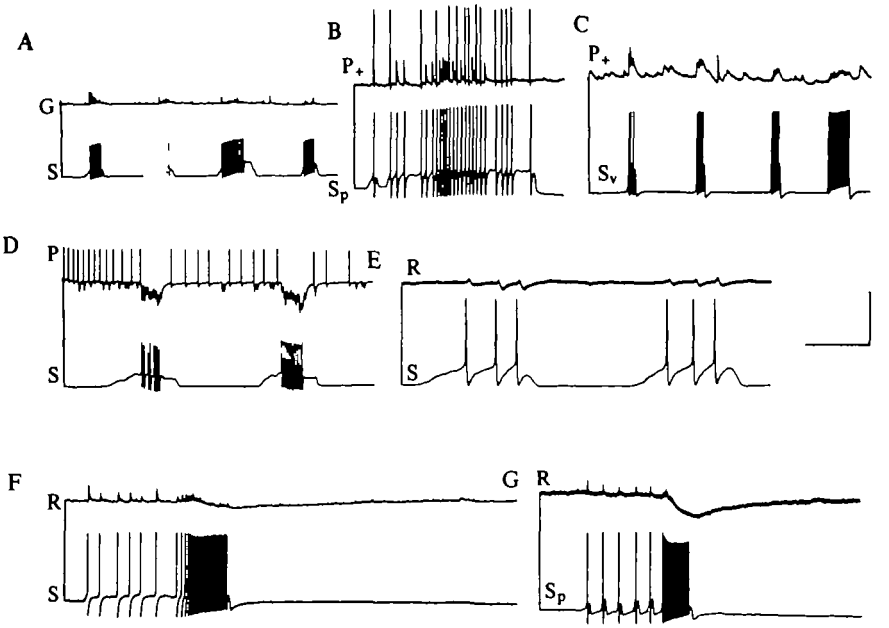

Fig. 4. Synaptic actions on buccal neurones. The upper trace indicates the postsynaptic response. (A) Excitatory input to a gating cell from the ipsilateral S cell. (B, C) EPSPs in a P_+ neurone generated by the ipsilateral S_p and S_v receptors. (D) IPSPs in a P motoneurone from the ipsilateral S cell. (E, F) EIPSPs in a retractor phase motoneurone (upper trace) generated by impulses in the contralateral S cell. Note that the predominant effect of the EIPSP changes from excitation to inhibition with increasing frequency. (G) Similar to (F), but in this case the EIPSPs are generated in the R neurone by the ipsilateral S_p cell. Upper traces: (B, D) 50 mV; (A, F) 25 mV; (C, E, G) 10 mV. Lower traces: (A, D) 125 mV, (B, C, E, F, G) 50 mV. Time scale, 5 s; except E, 1 s.

of the lateral nerve, and their function is unknown (Fig. 1, P_+). The EPSPs generated in these P_+ cells by the S neurones attain amplitudes of 25 mV, which is sufficient to trigger impulses (Fig. 4B), and the amplitude of the EPSP only diminishes slightly with repeated activation of the S cell. The S_v, S_d and S_p receptors generate smaller EPSPs with a maximum amplitude of 5 mV in this group of P cells. The synaptic potentials decline rapidly with repetition and are insufficient to reach spike threshold (Fig. 4C).

The second class of P-phase neurones are located mid-way along the posterior border and overlap the dorsal and ventral surfaces of the ganglion. When stimulated these cells cause movements of the buccal mass and oral tube which cannot be clearly resolved in this type of preparation. Impulses in the S cells are followed 1:1 by inhibitory postsynaptic potentials with an initial amplitude of 15 mV, which declines to 3–4 mV at the end of a 10 s burst (Fig. 4D). A brief tactile stimulus to the oesophageal wall inhibits spike activity in these P neurones for some 6–7 s. The small mechanoreceptors have not been found to synapse with this group, although this detail has not been checked with the P neurones on the more ventral aspect of the ganglion.

Synapses on R-phase neurones

The R-phase neurones are found on both the dorsal and ventral surfaces of the ganglia adjacent to the buccal commissure. All identified R-phase neurones recei

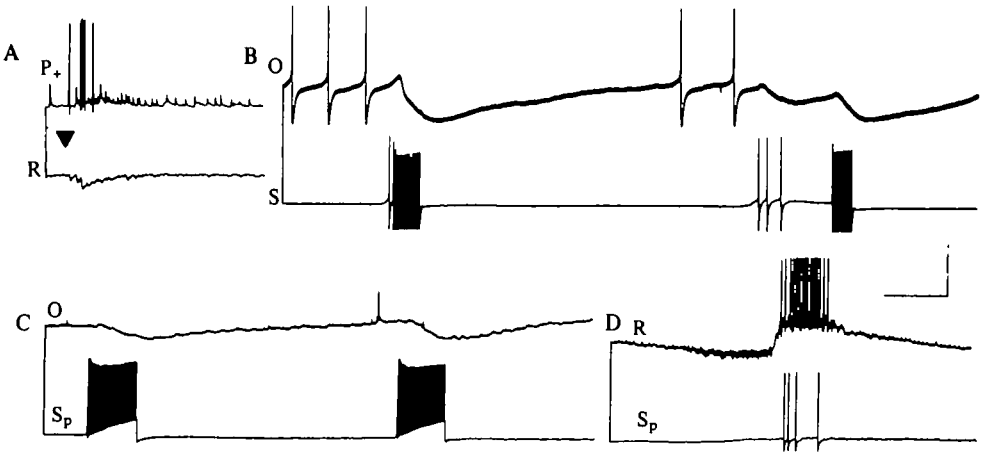

Fig. 5. Actions on buccal motoneurones continued. (A) Synaptic activity in a P_+ cell (upper trace) and an R cell (lower trace) following mechanical stimulation of the oesophagus (arrow). (B) EIPSPs generated in an O cell (upper trace) by the ipsilateral S cell. The excitatory component is weak, and the inhibitory component large and long-lasting in comparison to the EIPSP to the R cells. (C) EIPSPs in an O cell (upper trace) generated by a burst in the ipsilateral S_p cell. The action of the small receptors on postsynaptic neurones is weaker than that of the S cells. (D) Spontaneous activity in an S_p cell (lower trace) during the retraction phase of the feeding cycle, seen in an R phase motoneurone on the upper trace. Upper traces: (A, B, D) 25 mV, (C) 10 mV. Lower traces: (A) 25 mV, (B–D) 50 mV. Time scale, 5 s.

a biphasic EIPSP from both the large S cells, and the ipsilateral group of small oesophageal receptors (Fig. 4E), the psp's from the small cells having a smaller amplitude than those of the large S cells.

The effect of the EIPSP on the R cells is determined by two factors: the firing rate of the receptor, and the membrane potential of the postsynaptic cell. At low firing frequencies the excitatory component of the psp predominates and activity in the S cells frequently elicits action potentials in the postsynaptic R neurone. With an increase in the firing rate of the receptor the excitatory component anti-facilitates, so that at frequencies above 6 s^{-1} the inhibitory component summates as a smooth hyperpolarizing wave which can inhibit ongoing activity in the R cell (Fig. 4F, G). A high resting potential in the postsynaptic cell may cause reversal of the inhibitory component which is then seen as a depolarizing wave (Fig. 3B). Such high values of the resting potential are rarely encountered in the R group, the normal response to a touch on the oesophagus being a brief depolarization followed by a more prolonged hyperpolarization, presumably resulting from direct synaptic input from the proprioceptive group (Fig. 5A).

Synapses on the O-cells

The mid-dorsal region of the ganglion is occupied by a group of five large cells whose activity does not result in any movement of the buccal mass. Cobalt backfills and external recordings made from the oesophageal nerves have shown that each of these cells sends an axon into the ipsilateral oesophageal trunk, hence the designation O-group. When recorded from unstimulated preparations these cells are normally silent or fire irregularly at low frequencies. They do not show patterned

bursting in phase with the motoneurones. Each O-neurone receives a synaptic input from the two S cells and the ipsilateral groups of S_v, S_d and S_p receptors. The action of the mechanoreceptors on the O-cells is predominantly inhibitory, but a small excitatory component can often be detected preceding the inhibitory wave (Fig. 5B). Individual EIPSP's have little effect on the membrane potential and a rapid burst is necessary to generate significant hyperpolarization in the postsynaptic cell. The synaptic potentials correspond 1:1 with impulses in the presynaptic receptor and the connexion is considered to be monosynaptic. A 2 s burst of impulses in the S cell, firing at a rate of 6 s^{-1} generates a 15 mV hyperpolarization in the O-cell which may cause spontaneous firing to cease for up to 20 s (Fig. 5B). Comparable but smaller amplitude responses are obtained following activity in the ipsilateral S_p cell (Fig. 5C).

Activity of the proprioceptors during buccal cycles

The method of stabilizing the buccal ganglia to permit intracellular recording prevents the buccal mass and oesophagus from making unrestricted movements during spontaneous and stimulus evoked buccal cycles. Thus in many preparations the oesophageal proprioceptors do not show activity which is phase-linked to any particular part of the cycle. On several occasions preparations have been obtained where the S cells respond with a burst of impulses during the R-phase activity, coinciding with visible contractions of muscles in the proximal region of the oesophagus and the radula sac (Fig. 5D). Spontaneous bursts of this type have not been noted in conjunction with any other period during the cycle.

DISCUSSION

Mechanoreceptors have now been identified in the buccal mass of several molluscs, their somata typically forming aggregates of 40–75 small, individually unidentifiable cells, each with a restricted sensory field (Audesirk, 1979; Spray *et al.* 1980*a*). By way of contrast, the oesophageal proprioceptors of *Philine* are arranged in a hierarchy of two large and six small neurones, the receptive fields of the larger cells encompassing the smaller ones with an apparent high degree of redundancy in the network. The subordinate role of the smaller receptors may also be reflected in the pattern of synaptic connexions established between the cells, the small ones exciting the larger, while the reciprocal synapses are biphasic but effectively inhibitory. The synaptic influence of one proprioceptor on another is weak, acting on the overall level of excitability of the postsynaptic cell rather than by generating impulse activity.

In the previous paper (Dorsett & Sigger, 1981) we described the tendency of the centripetal impulse to block at major branch points of the axon as it enters the ganglion. If sites of synaptic interaction between receptors occur close to these critical points on the axon, they may exert a powerful influence on the passage of the centrally propagating impulse, determining whether it reaches the efferent synapses on the interneurones and motoneurones of the feeding network.

The monosynaptic connexions between the oesophageal mechanoreceptors is not a common feature of sensory neurones described in other molluscs, although comparable interactions are seen in the T, P and N cells of the leech (Baylor & Nicholls

1968). Among the mechanoreceptors of *Navanax*, a small percentage show excitatory or inhibitory connexions (Spray *et al.* 1980*b*), which in conjunction with other polysynaptic pathways combine to induce prolonged synaptic activity and spiking among members of the group following stimulation of a single cell.

The mechanoreceptors of *Philine*, and the two S cells in particular, make widespread synaptic contacts among the buccal neurones, including the interneurones and motoneurones active during the feeding sequences. As in other molluscs the patterned bursting in these groups is generated by intraganglionic networks which can be activated by tactile stimulation of the oesophageal wall (Siegler, 1977; Bulloch & Dorsett, 1979*a*, *b*). Prolonged stimulation of individual oesophageal proprioceptors has so far failed to duplicate this effect, which may require the convergent input from several sources to trigger successfully the interneurones which initiate the activity. The gating neurones, which receive excitatory input from the oesophageal proprioceptors, do not represent the first stage in the initiation process as they themselves receive an accelerating barrage of postsynaptic potentials following tactile stimulation of the oesophagus (Sigger, unpublished observation).

What is the role of the oesophageal proprioceptors? As this particular preparation is unable to perform the full range of movements which comprise the feeding cycle (Hurst, 1965), it is unlikely that the activity recorded during spontaneous sequences reflects the natural activity of the receptors. Where such activity has been recorded, it occurs during the retraction phase of the cycle. The distribution of synaptic input to the P- and R-phase neurones is complex, due partly to the biphasic nature of some potentials and because the precise role of several motoneurones has yet to be resolved. Other sources of sensory input, such as the mechanoreceptors in the wall of the buccal mass, which are currently under investigation, also provide feedback to the buccal motoneurones.

Sensory input has been shown to have a purpose in modulating the feeding pattern in *Helisoma* (Kater & Rowell, 1973) and Siegler (1977) found a significant reduction in the frequency of the cyclic activity of buccal motoneurones of *Pleurobranchaea* following de-afferentation. The activity resulting from stimulation of the oesophageal wall of *Philine* indicates that the receptors contribute some form of positive feedback to the pattern generating network, both at interneurone and motoneurone levels. It is clear that they can provide little by way of spatial information, which suggests that their function is more concerned with timing.

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REFERENCES

AUDESIRK, T. E. (1979). Oral mechanoreceptors in *Tritonia diomedea*. Electrophysiological properties and location of receptive fields. *J. comp. Physiol.* **130**, 71–78.

BAYLOR, D. A. & NICHOLLS, J. G. (1968). Receptive fields, synaptic connections and regeneration patterns of sensory neurones in the CNS of the leech. In *Physiological and Biochemical Aspects of Nervous Integration* (ed. F. D. Carlson). London, New Jersey: Prentice-Hall.

BULLOCH, A. G. M. & DORSETT, D. A. (1979*a*). The functional morphology and motor innervation of the buccal mass of *Tritonia hombergi*. *J. exp. Biol.* **79**, 7–22.

BULLOCH, A. G. M. & DORSETT, D. A. (1979*b*). The integration of the patterned output of buccal motoneurones during feeding in *Tritonia hombergi*. *J. exp. Biol.* **79**, 23–40.

DORSETT, D. A. & SIGGER, J. N. (1981). Sensory fields and properties of the oesophageal proprioceptors of *Philine*. *J. exp. Biol.* **94**, 77–93.

HURST, A. (1965). Studies on the structure and function of the feeding apparatus of *Philine aperta* with a comparative consideration of some other opisthobranchs. *Malacologia* **2** (3), 281–347.

KATER, S. B. & ROWELL, C. H. F. (1973). Integration of sensory and centrally programmed components in generation of cyclical feeding activity of *Helisoma trivolvis*. *J. Neurophysiol.* **36**, 142–155.

SIEGLER, M. V. S. (1977). Motor neuron coordination and sensory modulation in the feeding system of the mollusc, *Pleurobranchaea californica*. *J. exp. Biol.* **71**, 27–48.

SPRAY, D. C., SPIRA, M. E. & BENNETT, M. V. L. (1980*a*). Peripheral fields and branching patterns of buccal mechanosensory neurones in the opisthobranch mollusc *Navanax inermis*. *Brain Res.* **182**, 253–270.

SPRAY, D. C., SPIRA, M. E. & BENNETT, M. V. L. (1980*b*). Synaptic connections of buccal mechanosensory neurones in the opisthobranch mollusc *Navanax inermis*. *Brain Res.* **182**, 271–286.