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THE SENSITIVITY AND CONTROL OF THE SCALLOP MANTLE EDGE

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SUMMARY

1. Application of mechanical stimulation or crude starfish extracts to the mantle edge of *Aequipecten irradians* elicited afferent impulse activity in the radial pallial nerves and local movements of the stimulated mantle edge. The evoked afferent spike activity was not recorded from primary receptor cells. The local mantle edge movements were controlled by peripherally located neurones and resembled jet formation on the velum of intact scallops.

2. The central efferent neurones that supply the adductor muscle and much of the mantle edge are situated in the visceroparietal ganglion. Cobaltous chloride back-filling of the radial pallial nerves of the right side revealed the routes of the nerve fibres and the locations of the cell bodies in the visceroparietal ganglion.

3. One group of motor neurones has fibres that are spatio-topically arranged across the visceroparietal ganglion and play a role in jet formation on corresponding portions of the mantle edge on both valves. It is apparent that axons from this group of mantle edge efferents traverse the ganglion without chemical synaptic connection.

4. Two groups of mantle edge efferents that control concerted movements of the mantle edge on both shells appear to have cell bodies in the lateral margins of the dorso-central lobes. One group of motor neurones controls the raising of the velum curtain to an erect position around the shell margin. The output from the second group of efferents can be synchronized with the motor output to the adductor muscle to ensure that the velum folds into the mantle cavity, and thus is protected, as the shells are closed.

5. Fibres in the radial pallial nerves have conduction velocities of up to 2·35 m/s at a temperature of 25 °C.

INTRODUCTION

Swimming in the Bivalvia is confined to the monomyarians (Younge, 1936). Movement is produced by rapid contractions of the single adductor muscle which close the valves and forcefully expel water out of the mantle cavity. In scallops the mantle edge, or velum, forms a muscular curtain around much of the margin of both shells and plays an important role in movement. Local contractions in the velum curtain produce jets through which the expelled water is directed, and, since the curtain on the upper valve overlaps that on the lower valve, water that escapes around the shell margin is directed downwards and generates lift (Buddenbrock, 1911).

Scallops may move directly away from a stimulus applied to the mantle edge by using adductions that expel water out of the mantle cavity through a jet formed on the stimulated portion of the velum (Stephens & Boyle, 1978). Alternatively, the scallop swims using a rhythmic series of shell adductions that expel water through jets formed on either side of the dorsal hinge line and propel the animal through the water with its ventral margin foremost (Buddenbrock, 1911). Both types of movements appear to be centrally controlled by units in the visceroparietal ganglion (Stephens & Boyle, 1978).

The visceroparietal ganglion is located on the ventral surface of the striated adductor muscle and is differentiated into five discrete lobes. The dorso-central lobes are separated from the ventro-central lobe by the pigmented intermediate lateral groove; lateral lobes are situated on either side of the three central lobes. Connective nerves link the visceroparietal ganglion with the other main ganglionic mass, the cerebropedal ganglion. A pair of nerves that exit the visceroparietal ganglion on the dorsal surface innervate the adductor muscle, whereas the radial pallial nerves leave the ganglion laterally and carry information to and from most of the mantle edge on both valves. Dakin (1910) mapped out the routes of the main nerve tracts through the visceroparietal ganglion of the scallop *Pecten maximus* and located the cell bodies of the adductor muscle and the mantle edge efferents in the dorso-central lobes.

Scallop movements are triggered by the touch of a predatory starfish, the application of starfish extract or mechanical stimulation of the mantle edge (Thomas & Gruffydd, 1971; Stephens & Boyle, 1978). There are numerous tentacles and eyes located around the mantle edge of both shells and investigations of the anatomy and physiology of the conspicuous eyes have been reported (Charles, 1966; Land, 1966*a*, *b*). However, little work has been done on the capability of scallops to detect stimuli presented by predatory starfish. Certain scallops discriminate between the touch of predatory and non-predatory starfish and change their behaviour accordingly (Thomas & Gruffydd, 1971). Mackie (1970) demonstrated that escape movements can be evoked from scallops by stimulation of the mantle edge with a steroid glycoside extracted from predatory starfish. Thus it is apparent that scallops detect chemical, and perhaps mechanical, stimuli presented by attacking predatory starfish. In certain molluscs areas sensitive to mechanical stimulation have been described (Laverack & Bailey, 1963; Mellon, 1972), and in gastropods chemoreceptors have been located in the foot (Mackie, Lasker & Grant, 1968), the osphradium (Bailey & Laverack, 1966) and the mantle margin (Phillips, 1976). In the present paper the sensitivity of the mantle edge of the scallop *Aequipecten irradians* to crude starfish extracts and to mechanical stimulation has been investigated. Furthermore the radial pallial nerves have been back-filled with cobaltous chloride to provide an accurate map of the routes of the nerve fibres and the sites of the cell bodies in the visceroparietal ganglion. Some properties of the efferent neurones that supply the mantle edge have been investigated.

MATERIALS AND METHODS

Scallops (*Aequipecten irradians*) were obtained from the Marine Biological Laboratory, Woods Hole, Massachusetts, and maintained in constantly circulating, aerated artificial sea-water at 19 °C.

Cobaltous chloride back-filling of the radial pallial nerves

The shell and gill of both sides were removed from a scallop. The visceroparietal ganglion and the radial pallial nerves were carefully dissected away from the underlying adductor muscle and pinned down, ventral surface uppermost, in a Sylgard-lined Petri dish containing artificial sea water. The large trunk of radial pallial nerves on the right side was dissected clear of connective tissue and a petroleum-jelly well was constructed so that the nerve trunk passed through the wall and into the well. The sea-water in the well was replaced with distilled water and the nerve trunk was cut as near as possible to the well wall. After 90 s the distilled water in the well was replaced with a 1 M solution of cobaltous chloride. The Petri dish was placed in an incubator set at 12 °C and a current of 5×10^{-7} A was passed down the nerve trunk. After 24 h incubation the current was turned off, the cobaltous chloride solution was removed from the well and the preparation was transferred to a clean Petri dish. The ganglion was treated with ammonium sulphide, fixed in alcoholic Bouins and dehydrated through an ascending series of alcohols. The tissue was cleared in methyl salicylate, embedded in paraffin wax and cut into sections 15 μm. thick. Neurone profiles were intensified using a modified silver technique (Tyrer & Bell, 1974).

Stimulation of the mantle edge

1. *Afferent activity*

One valve was removed from a scallop by cutting the adductor muscle at its point of shell insertion. The removed valve was anchored to the wax bottom of the experimental dish with pins overlapping the shell margin. The branching radial pallial nerves, which run in the pallium between and adductor muscle scar and the mantle edge, were usually clearly visible against the dark background of the shell. A fine branch of a selected radial pallial nerve was dissected clear of connective tissue, cut, and its distal end was drawn into a glass suction electrode. An indifferent silver electrode was placed in the artificial sea-water bathing the preparation and afferent signals were a.c.-amplified, displayed on an oscilloscope and photographed in a conventional manner.

Mechanical stimulation was applied to the mantle edge of *A. irradians* with a fine steel pin. Crude starfish extracts of *Asterias forbesi* or *Henricia sanguinolenta* (from M.B.L., Woods Hole, Mass.) were prepared after Mackie, Lasker & Grant (1968). A sample of starfish extract was adjusted to room temperature (25 °C) and one drop of extract solution was applied to the mantle edge through a Pasteur pipette.

2. *Efferent activity*

Fig. 1 is a diagram of the experimental preparation used in this part of the investigation. A scalpel blade was passed between the pallium and the inner surface of the shell of one side, and the mantle edge and the pallium were detached from the valve. The adductor muscle was cut at its point of shell insertion and the valve was removed. The preparation, with the pallium and mantle edge of both sides intact, was anchored to the experimental dish with pins overlapping the margin of the remaining shell. Individual fine branches of selected radial pallial nerves from both sides were dissected clear of connective tissue, cut, and the proximal ends were drawn into glass suction electrodes. Mechanograms were recorded from a strain-gauge attached to the adductor

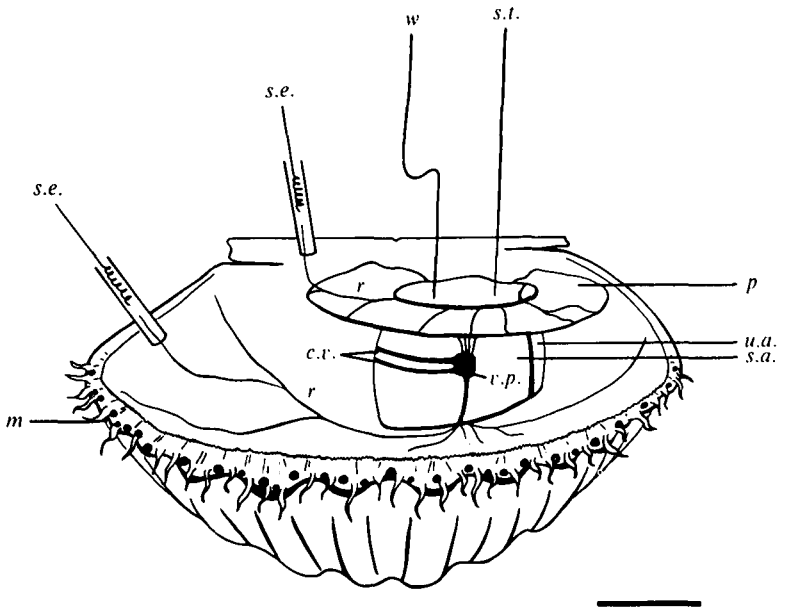


Fig. 1. A schematic diagram of the preparation used to record efferent activity, viewed from the left-ventral aspect. The shell of the left side has been removed (see text); the radial pallial nerves (*r*) on both sides and the cerebro-visceral connectives (*c.v.*) are shown entering the visceroparietal ganglion (*v.p.*). *m*, Mantle edge; *p*, pallium; *s.a.*, striated portion of the adductor muscle; *s.e.*, suction electrode; *s.t.*, suture thread attached to strain-gauge; *u.a.*, unstriated or smooth portion of the adductor muscle; *w*, wire electrode for recording electromyograms. Calibration: 1 cm.

muscle by a length of suture thread and a small metal hook. Electromyograms were recorded from a fine (about 100 μm diameter) copper wire electrode which was insulated to the tip and embedded in the striated portion of the adductor muscle. The gills and the unattached mantle edge were surgically removed and indifferent silver electrodes were placed in the artificial sea-water bathing the preparation.

Starfish extract or mechanical stimulation was applied to the mantle edge on the anchoring shell. Efferent signals were a.c.-amplified and displayed, together with the mechanograms, on the screen of a multi-trace oscilloscope for observation and photography. In such preparations activity was recorded from the adductor muscle and from the radial pallial nerves of both sides, while movements of regions of the velum with an intact nervous supply were observed.

Electrical stimulation of the radial pallial nerves

1. *Efferent activity*

The preparation was as described above (Fig. 1). Non-polarizing stimulus pulses (1 ms duration) were applied to the proximal end of a nerve and efferent impulse activity was recorded from the cut ends of other radial pallial nerves. Signals were a.c.-amplified and photographed in a conventional manner from the screen of an oscilloscope, which was triggered from the stimulus pulse.

2. *Conduction velocities*

A shell was removed from a large scallop (maximum diameter ranging from 8–10 cm) and anchored to the wax bottom of an experimental dish with pins overlapping the margin. A distal branch of a selected radial pallial nerve was dissected clear of connective tissue, cut, and the proximal end was drawn into a glass suction electrode. Non-polarizing stimulus pulses (0·1 ms duration) were applied through a second suction electrode attached *en passant* to the proximal region of the nerve, near the adductor muscle scar. An indifferent silver electrode was placed in the artificial sea water bathing the preparation and the evoked signals were a.c.-amplified, displayed on an oscilloscope screen and photographed.

The length of nerve between the two suction electrodes was measured *in vivo* from the cut distal end of the nerve attached to the mantle edge to the tip of the stimulating electrode (0·3 mm in diameter). Conduction velocities of groups of fibres were calculated from 22 radial pallial nerves of average length 15·5 mm (range 11–20 mm).

3. *Salines*

Unless otherwise specified all preparations were bathed in Instant Ocean (Aquarium Systems Inc., Ohio) at room temperature (25 °C). A calcium-free M.B.L. sea-water saline was prepared by replacing all calcium ions (9 mM) with magnesium, thereby producing a saline with the same osmolarity as standard M.B.L. artificial sea-water (after Prior, 1972*a*).

RESULTS

Cobaltous chloride back-filling of the radial pallial nerves

Examination of cleared, whole-mount preparations of ganglia with the right radial pallial nerves back-filled with cobaltous chloride offered no fine details of the geometry of single neurones. In addition, the fine processes of individual neurones could not be traced through serial sections of the ganglion. However, the routes of the perfused axons through the neuropile and the sites of the cell bodies in the different lobes of the visceroparietal ganglion were readily identifiable (Fig. 2A).

The radial pallial nerves of the right side enter the right lateral lobe and run through the neuropile as discrete tracts. Many radial pallial nerve fibres have somata in the cell rind of the lateral lobe. The nerve tracts converge as they travel through the neuropile of the lateral lobe and, upon entering the ventro-central lobe, branch to form four tracts (Fig. 2A). One bundle of fibres exits the ganglion in the right cerebro-visceral connective and a second branch of nerve fibres has cell bodies in the lateral region of the right dorso-central lobe (Fig. 2B). The remaining axons travel into the ventro-central lobe in one of the two transverse nerve tracts (Fig. 2C). The medial transverse nerve tract is a discrete bundle of nerve fibres and is readily identifiable in longitudinal sections of the ganglion (Figs. 2B, 3A, B, D). The medial transverse nerve tract in the visceroparietal ganglion of *A. irradians* may be homologous to the commissural fibre described in *Pecten maximus* (Dakin, 1910). The anterior transverse nerve tract is a collective name given to a number of nerve fibre bundles that run along the anterior margin of the ventro-central lobe (Figs. 2B, 3A, B). Fibres in both transverse nerve tracts have cell bodies situated in the mid-line region of the ganglion. Fibres in the

medial transverse nerve tract have somata in the ventro-central lobe (Fig. 3A) whereas axons in the anterior transverse nerve tract have cell bodies in the dorso-central lobes (Fig. 3A, B).

In the left lateral margin of the ventro-central lobe the transverse nerve tracts branch to form three nerve tracts (Fig. 2A). Some axons run anteriorly and leave the ganglion in the left cerebro-visceral connective (Fig. 3C) and other fibres can be traced through serial sections to the radial pallial nerves of the left side. Thus it is apparent that some axons from the radial pallial nerves of the right side travel across the visceroparietal ganglion and exit in nerves of the left (contralateral) side. Some axons that run across the ganglion in the transverse nerve tracts have somata in the left lateral lobe (Fig. 3C) and in the lateral portion of the left dorso-central lobe. From Fig. 2A it is evident that many axons that travel in the radial pallial nerves have cell bodies in discrete areas of the dorso-central lobes. These somata are confined to the lateral regions of each dorso-central lobe and are not located in the central portions, which are associated with the nerves that supply the adductor muscle (Fig. 3D and unpublished observations).

Stimulation of the mantle edge

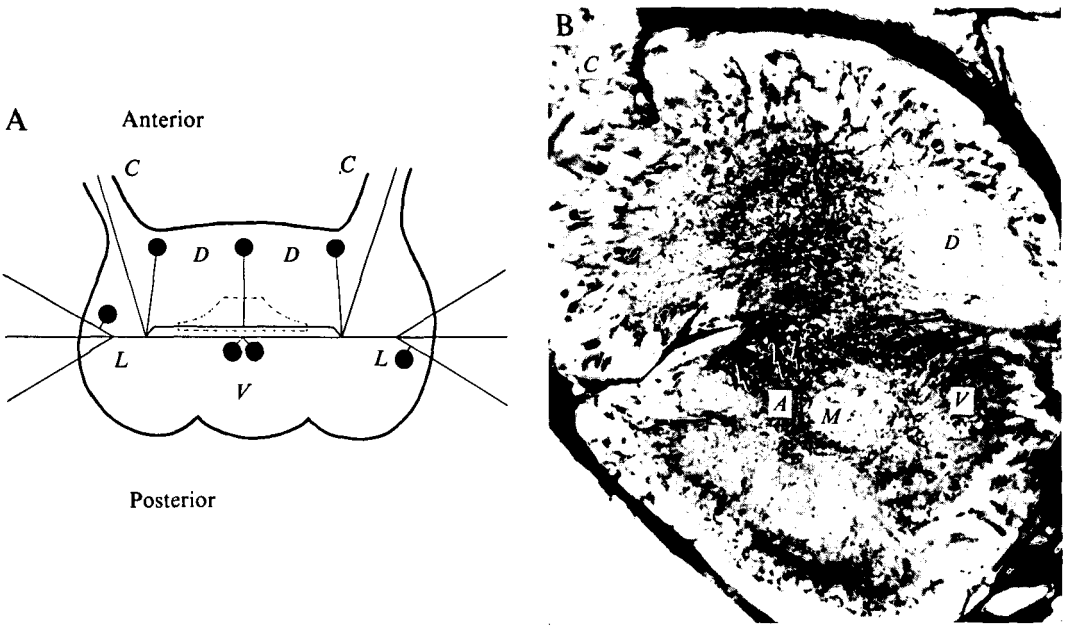
1. *Afferent activity*

Following isolation of the mantle edge from the central nervous system (CNS) the mantle tentacles became elongate and immobile, and the velum curtain stood at the shell margin at about 45° to the vertical. In the absence of specific mantle edge stimulation no afferent impulse activity was recorded from cut radial pallial nerves.

(i) *Mechanoreception.* The distal tentacles are located along the margin of the velum curtain and it proved difficult to mechanically displace single distal tentacles without also moving the curtain. However, a Perspex rod pushed against the inner surface of the velum provided sufficient tension to permit mechanical stimulation of the distal tentacles without any apparent movements of other regions of the mantle edge. Mechanical distortion of single distal tentacles produced immediate tentacle retraction and a burst of afferent spike activity in the radial pallial nerve supplying the stimulated region of the mantle edge (Fig. 4A). Mechanically displacing distal tentacles in different directions revealed no apparent response polarity.

Mechanical distortion of the tentacles in the eye region or the velum curtain evoked a multi-unit afferent response from the radial pallial nerve supplying the stimulated portion of the mantle edge (Fig. 4B, C). The initial burst of spikes was followed by further impulse activity which coincided with tentacle retraction and local movements of the velum. The stimulated portion of the mantle edge retracted away from the stimulus, the velum curtain stood erect and everted along the distal margin. These local movements of the mantle edge resembled those observed during jet formation on the velum of the clam *Lima scabra* (Stephens, 1977) and the scallop *Chlamys opercularis* (Stephens & Boyle, 1978).

(ii) *Chemoreception.* One drop of an extract solution of *H. sanguinolenta* or *A. forbesi* applied to any portion of the mantle edge on either valve elicited local movements of the mantle edge and afferent spike activity in the radial pallial nerves (Fig. 5). The recorded afferent response was characterized by an initial high discharge of impuls



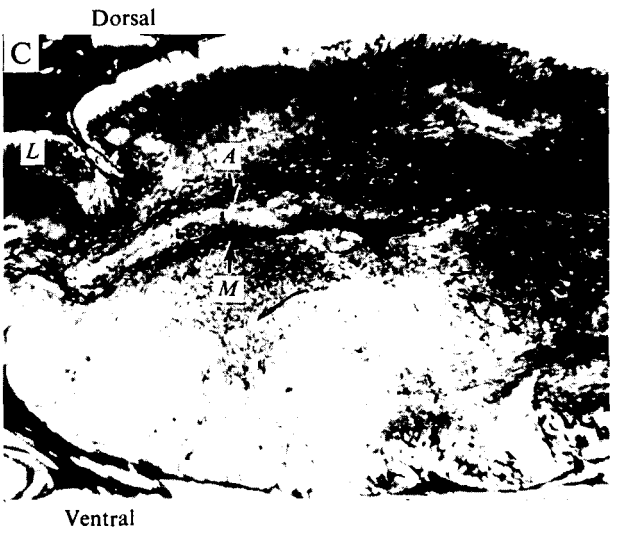
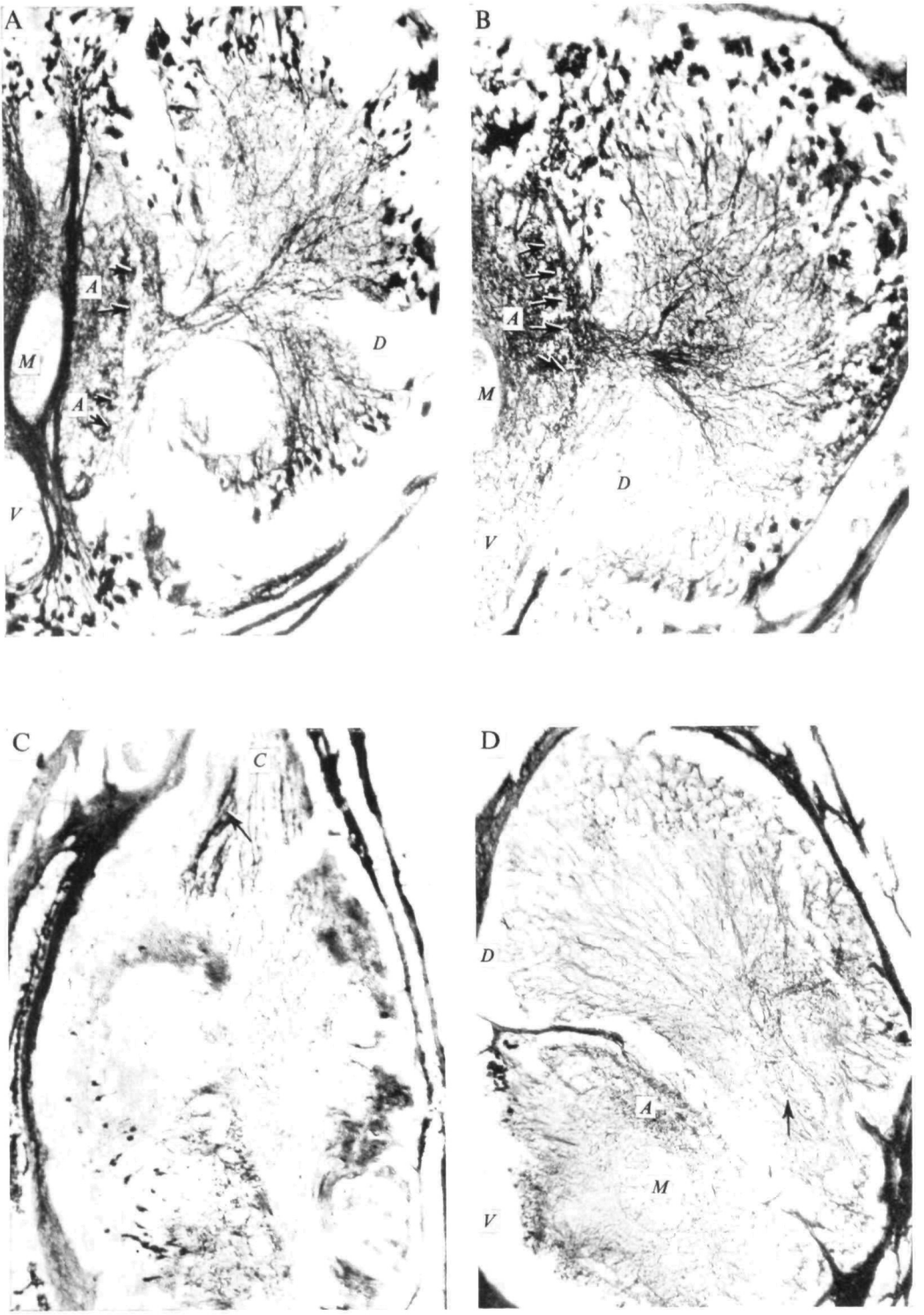


Fig. 2. The visceroparietal ganglion of *A. irradians* with the radial pallial nerves of the right side back-filled with cobaltous chloride. (A) A diagram of the ganglion viewed from the ventral aspect illustrating the routes of the fibres and the locations of the cell bodies. (B) A longitudinal section of the ganglion showing stained nerve cells in the lateral portion of the right dorso-central lobe (*D*). (C) A transverse section of the ganglion showing the medial (*M*) and anterior (A) transverse nerve tracts running across the ventro-central lobe to the left lateral lobe (*L*). *A*, Anterior transverse nerve tract; *C*, cerebro-visceral connective; *D*, dorso-central lobe; *L*, lateral lobe; *M*, medial transverse nerve tract; *V*, ventro-central lobe. Calibration: 250 μm (A), 100 μm (B, C).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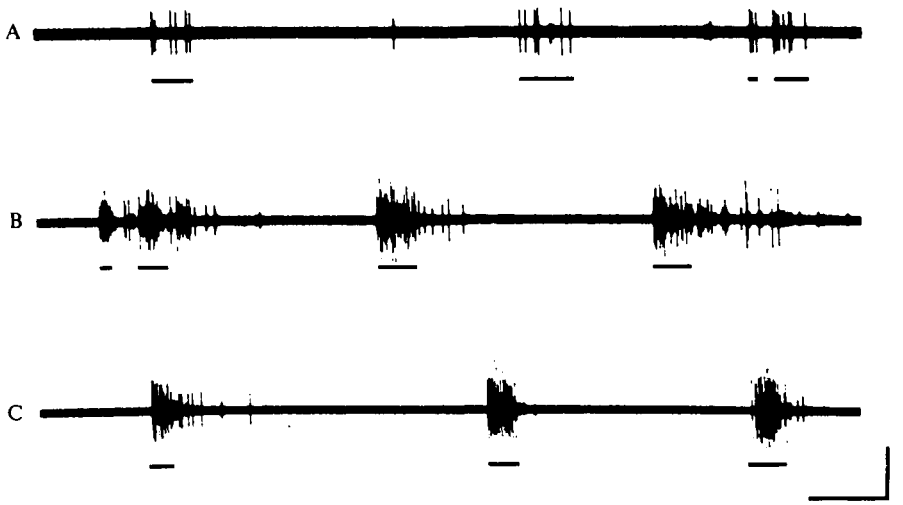

Fig. 4. Afferent impulse activity recorded from a cut radial pallial nerve following mechanical stimulation (bar) of the distal tentacles (A), the velum curtain (B) and the eye tentacles (C). Calibration: 100 μV, 1 s.

followed by a low level of spike activity. The evoked mantle edge movements were similar to those elicited by mechanical stimulation and involved a large portion of the velum – frequently more than 50% of the mantle edge on one shell formed a jet. It seems possible that the evoked movements of the velum stimulated the mechanoreceptors at the mantle edge, and that the recorded afferent response represents a combination of chemoreceptive and mechanoreceptive activity. Following starfish extract application, the stimulated mantle edge, isolated from the CNS, remained retracted and never returned to its original position at the shell margin. A second application of starfish extract produced afferent impulse activity in the radial pallial nerves without any observable movements of the velum. The rate of impulse discharge following the second stimulus was less than that recorded after the initial extract application (Fig. 5). This may indicate adaptation of the sensory receptors at the mantle edge, but it also seems likely that it reflects chemoreceptive activity in the absence of mechanoreception. Subsequent additions of extract solutions rarely

Fig. 3. Longitudinal sections of the visceroparietal ganglion with the radial pallial nerves of the right side back-filled with cobaltous chloride. (A) A section through the mid-line of the ganglion showing that axons with somata in the cell rind of the ventro-central lobe (*V*) are associated with the medial transverse nerve tract (*M*). Fibres from the anterior transverse nerve tract (*A*) have cell bodies in the dorso-central lobes (*D*). (B) A section through the mid-line of the ganglion showing that fibres from the anterior transverse nerve tract (*A*) have cell bodies in the dorso-central lobes (*D*). (C) A section through the left lateral lobe showing stained somata in the cell rind and fibres (arrow) running to the left cerebro-visceral connective (*C*). (D) A section through the central portion of the right dorso-central lobe showing only two stained nerve cells in the dorso-central lobe (*D*). The nerve tract (arrow) that is associated with the cell bodies in the central portion of the right dorso-central lobe exits the ganglion on the dorsal surface and innervates the adductor muscle (unpublished observations). *A*, Anterior transverse nerve tract; *C*, cerebro-visceral connective; *D*, dorso-central lobe; *L*, lateral lobe; *M*, medial transverse nerve tract; *V*, ventro-central lobe. Calibration: 100 μm.

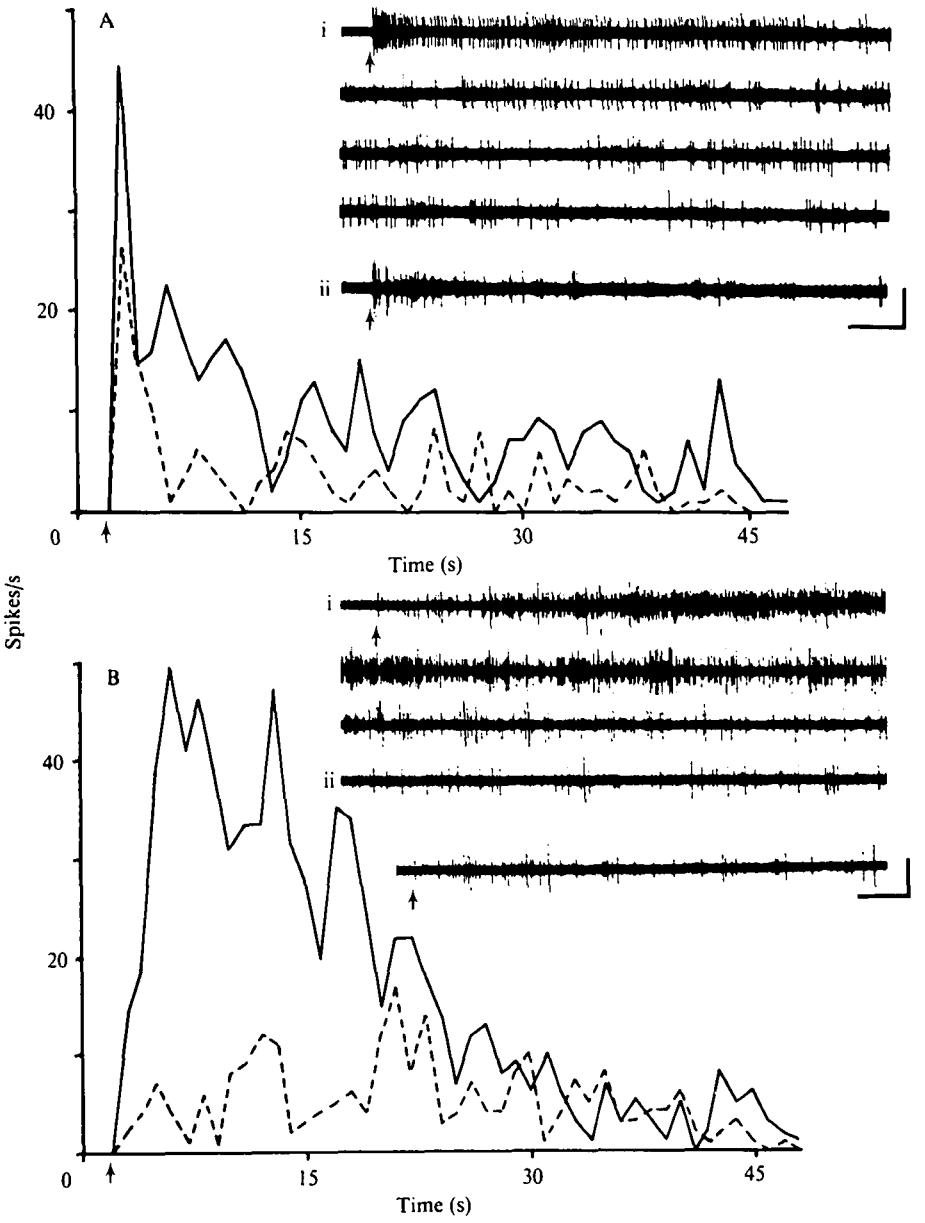

Fig. 5. Afferent nervous responses produced by application (↑) of one drop of extract solution of *A. forbesi* (A) and of *H. sanguinolenta* (B) to the mantle edge of different preparations. The graphs represent the impulse discharge frequency of a number of recognizable units and the inserts show the actual recordings taken from the radial pallial nerves. The solid graph line and (i) are the responses to the initial stimulus and the broken line and (ii) are the responses evoked by a second application of starfish extract 20 min after the removal of the first stimulus solution. Inset calibration: 100 μV, 1 s.

elicited afferent spike activity, which suggests that the chemoreceptors at the mantle edge either had become completely adapted to the stimulus or were in some way damaged by the starfish extract – as described in the foot of the whelk *Buccinum undatum* (Mackie, 1970).

(iii) *Calcium-free saline.* Preparations were bathed in an M.B.L. sea-water saline containing no calcium and a high level of magnesium. After 60 min in the calcium-free sea-water saline, application of starfish extract to the mantle edge or mechanical stimulation of the velum elicited no local movements of the mantle edge and no afferent spike activity in the radial pallial nerves. Pulse stimulation applied through a second suction electrode attached *en passant* to the distal region of the radial pallial nerve produced compound action potentials at the recording electrode. This indicates that the nerve fibres were capable of propagating spikes in calcium-free conditions. Replacing the calcium-free saline with M.B.L. sea-water containing standard levels of calcium and magnesium ions resulted in the return of afferent impulse activity and local movements in response to velum stimulation. These data suggest that the recorded afferent spike activity was mediated through at least one chemical synapse.

2. *Efferent activity*

Although the branching pattern of the radial pallial nerves on the inner shell surface varies considerably between scallops, for any one animal the layout is often similar for both valves. Therefore in some cases it is possible to identify a number of corresponding nerves on both sides. In the following section ipsilateral nerves will be taken as those that supply the mantle edge on the anchoring shell.

(i) *Photoreception.* In many scallops an increase in ambient illumination produces no behavioural response whereas a shadow passed over the animal evokes an immediate rapid adduction of the valves followed by a period of sustained shell closure (Hartline & Graham, 1938; Mellon, 1969). In the present study of *A. irradians* a decrease in ambient illumination elicited a contraction of the adductor muscle and a short burst of efferent impulses in the radial pallial nerves of both sides (Fig. 6). The recorded efferent nervous response coincided with a rapid folding of the velum curtain into the mantle cavity. Increases in ambient illumination induced no adductor muscle activity or efferent radial pallial nerve spikes (Fig. 6) irrespective of the period of dark adaptation.

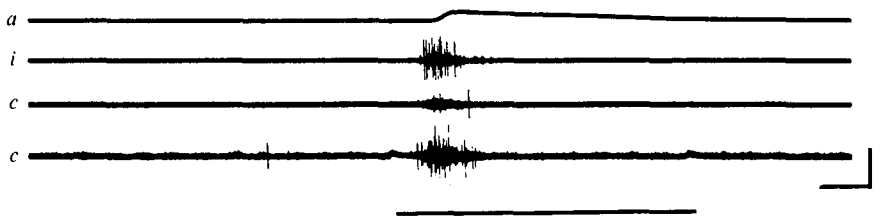

Fig. 6. Adductor muscle mechanograms (*a*) and efferent spike activity recorded from ipsilateral (*i*) and contralateral (*c*) radial pallial nerves in response to a change in ambient illumination. An illumination decrease (bar) evoked an adduction (an upward deflexion) and a short burst of efferent impulses in the nerves of both sides. Calibration: 100 μV, 500 ms.

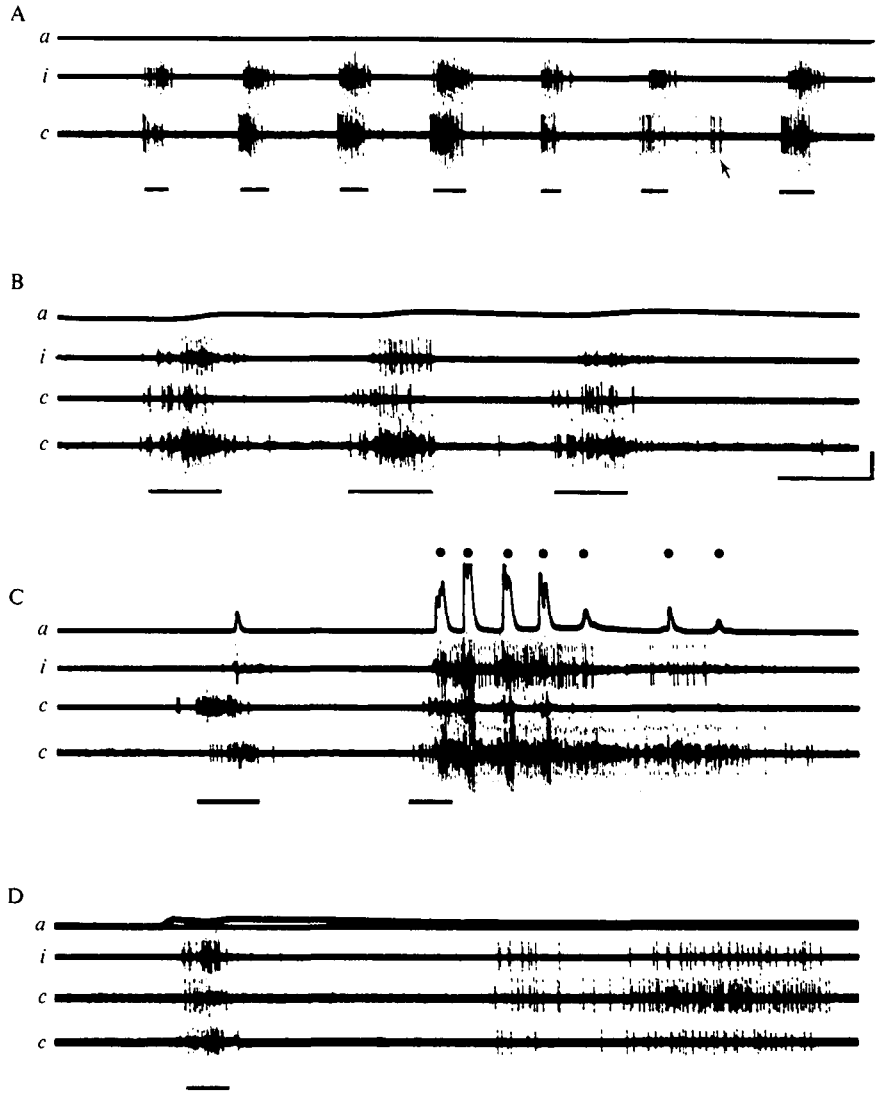

Fig. 7. Adductor muscle mechanograms (*a*) and efferent spike activity recorded from ipsilateral (*i*) and contralateral (*c*) radial pallial nerves in response to mechanical stimulation of the mantle edge (bar). (A) Stimulation usually elicited synchronous efferent bursting in the nerves of both sides (arrow denotes asynchrony – see later text). (B, C, D) Vigorous mechanical stimulation evoked efferent spike activity in the nerves of both sides and contractions of the adductor muscle (upward deflexions). The evoked adductions were slow and sustained (B), rapid twitches (C) or a fast adduction followed by a sustained contraction of the adductor muscle (D). Calibration: 100 μV, 500 ms.

(ii) *Mechanoreception.* Weak mechanical stimulation applied to the eye tentacles or to the velum curtain produced local movements of the stimulated portion of the mantle edge, but no efferent impulse activity was recorded from the radial pallial nerves. More vigorous mechanical stimulation elicited a short burst of efferent spikes in the nerves on both sides and movements of all portions of the mantle edge with an intact nervous supply (Fig. 7A). The evoked movement involved retraction of the

mantle tentacles and a rapid folding of the velum curtain into the mantle cavity. Following an efferent spike burst the velum slowly returned to its resting position after a delay of about 10 s.

Vigorous mechanical stimulation of the velum curtain frequently produced a rapid folding of the velum into the mantle cavity, synchronous efferent bursting in the radial pallial nerves and a contraction of the adductor muscle (Fig. 7B–D). The evoked adductions were either fast or slow and it seems likely that each response reflects activity in one of the two anatomically discrete portions of the adductor muscle. It is generally agreed that the striated muscle block is responsible for rapid shell adductions whereas the smooth adductor controls slow valve movements and shell posture (Mellon, 1969; Stephens & Boyle, 1978). In the present study the evoked adductions may be interpreted as slow sustained contractions of the smooth adductor (Fig. 7B) and rapid twitch responses of the striated adductor (Fig. 7C). On occasions vigorous mechanical stimulation of the velum curtain elicited a rhythmic series of fast striated muscle contractions. This is illustrated in Fig. 7C where two categories of rhythmic motor output to the striated adductor are apparent. The adductions of the slow rhythm (dots) occur at a frequency of 2·5–4 Hz and in some cases are comprised of a number of fast rhythmic muscle contractions at a high frequency (10–13 Hertz). It is of interest that the motor output to the striated adductor during the slow rhythm is similar in frequency to that recorded from intact swimming scallops, 3–5 Hz (Mellon, 1969). Mechanical stimulation of the mantle edge frequently elicited a rapid adduction followed by a prolonged contraction of the adductor muscle (Fig. 7D). This apparently represents a contraction of first the striated muscle and then the smooth. During the period of sustained smooth-muscle contraction the velum curtain slowly returned to its resting position at the shell margin. However, bursts of efferent impulses were recorded from the radial pallial nerves of both sides as the velum folded back into the mantle cavity (Fig. 7D).

On occasions when mechanical stimulation of the mantle edge produced a contraction of both portions of the adductor muscle, further velum stimulation evoked synchronous efferent bursting in the nerves of both sides (Fig. 8A). However, during a prolonged series of successive stimuli synchronous bursting in the radial pallial nerves was curtailed and impulse activity was recorded from only one nerve. When this happened efferent spike activity was always confined to a single contralateral nerve located in a corresponding position to the nerve supplying the stimulated portion of the mantle edge (Fig. 8B, C). It was possible to selectively produce impulse activity in any contralateral nerve simply by stimulating that region of the mantle edge supplied by the corresponding ipsilateral nerve. Furthermore, in certain preparations, mechanical stimulation of the velum at an intensity just below threshold for synchronous bursting elicited spikes in only the corresponding contralateral nerve (Figs. 8D and 7A – arrow).

(iii) *Chemoreception.* One drop of extract solution of either *A. forbesi* or *H. sanguinolenta* applied to the mantle edge produced a jet on the stimulated portion of the velum and efferent impulse activity in the radial pallial nerves of both sides (Fig. 9). The efferent discharge was recorded as the mantle edge around the whole shell margin retracted into the mantle cavity and the velum curtain stood erect. The rate of efferent firing increased and, immediately prior to an adduction, a short burst of large amplitude spikes was recorded as the velum rapidly folded into the mantle cavity

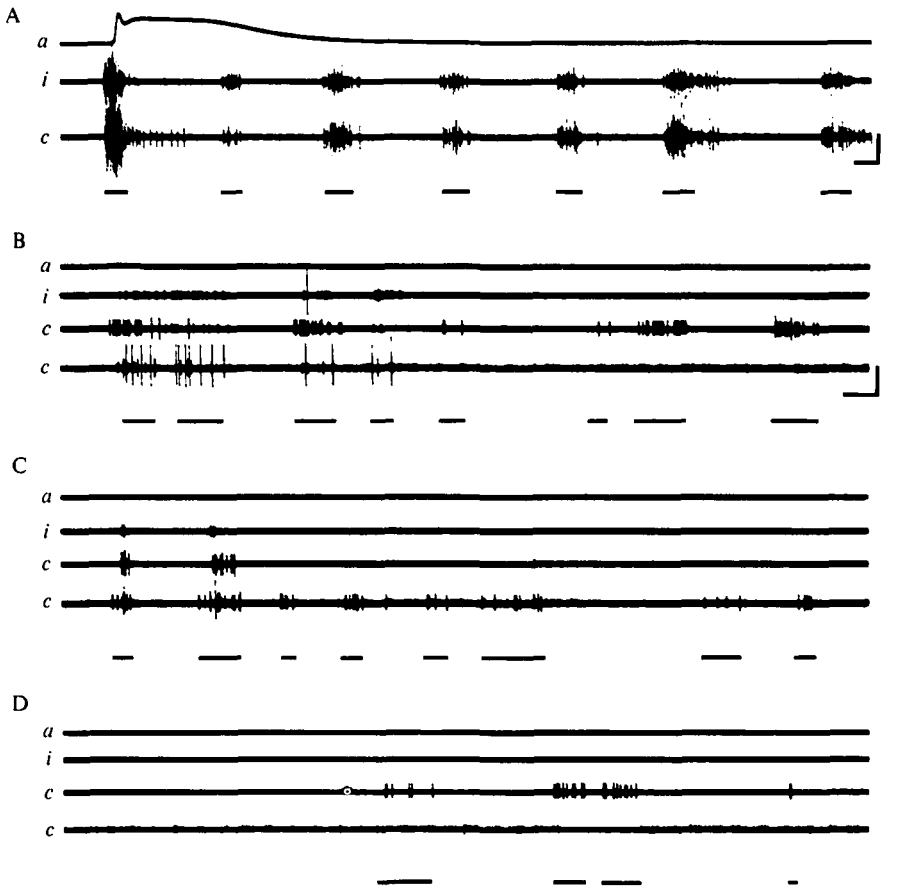

Fig. 8. Adductor muscle mechanograms (*a*) and efferent spike activity recorded from ipsilateral (*i*) and contralateral (*c*) radial pallial nerves in response to repeated mechanical stimulation of the mantle edge (bar). (A) Following the initially evoked adduction further mechanical stimulation of the velum elicited only synchronous efferent bursting in the nerves of both sides. (B, C) Synchronous efferent bursting was curtailed upon repeated stimulation and impulse activity was recorded from only one contralateral nerve located in a corresponding position to that innervating the stimulated portion of the velum. (D) Mechanical stimulation of the velum below threshold for the synchronous bursting response evoked efferent activity in only the contralateral nerve corresponding to that innervating the stimulated portion of the mantle edge. Calibration: 100 μV, 500 ms.

(Fig. 9C). Following the adduction, which apparently was comprised of a contraction of both adductor muscle blocks, the efferent spike activity in the radial pallial nerves was curtailed. Application of starfish extract to the mantle edge evoked a rhythmic series of fast adductions only when the velum was also mechanically stimulated.

(iv) *Lesion experiments*. The cerebro-visceral connective nerves were transected to isolate the two main ganglionic masses (Fig. 1). In such preparations the adductor muscle and efferent nerve activity evoked by illumination changes and by application of mechanical stimulation or starfish extract to the mantle edge were similar to the responses recorded from scallops with both connectives intact. This indicates that the motor output to the adductor muscle and much of the mantle edge must originate from connexions within the visceroparietal ganglion.

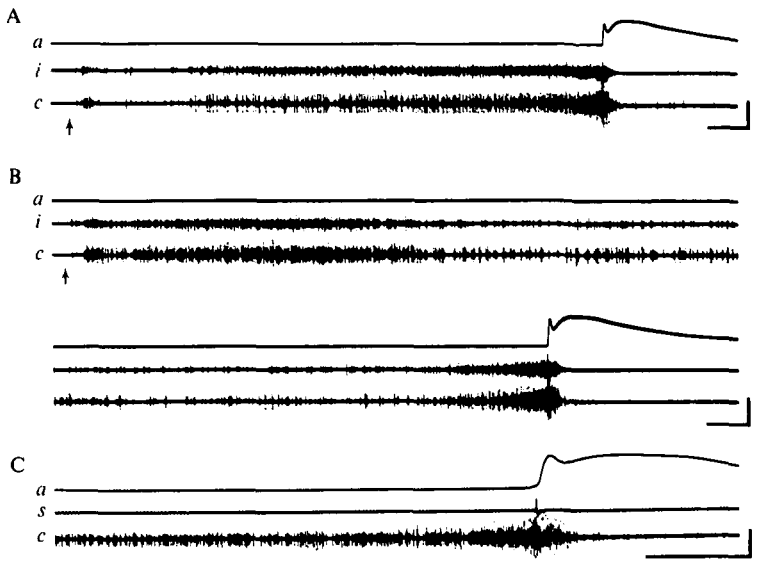

Fig. 9. Adductor muscle mechanograms (*a*), striated adductor myograms (*s*) and efferent spike activity recorded from ipsilateral (*i*) and contralateral (*c*) radial pallial nerves in response to the application (↑) of one drop of starfish extract solution to the mantle edge. (A) *A. forbesi*. (B) *H. sanguinolenta* – two traces are consecutive. (C) A high-speed trace shows large-amplitude impulses recorded from a radial pallial nerve immediately prior to and during the evoked adduction (an upward deflexion). Calibration: 100 μV, 1 s.

Electrical stimulation of the radial pallial nerves

1. *Efferent activity*

Low-intensity pulse stimulation of a radial pallial nerve did not evoke efferent impulses in the nerves of both sides, but produced activity in only one nerve. This low threshold response (LTR) was always recorded from the contralateral nerve located in a corresponding position to the stimulated nerve (Fig. 10A). In preparations where no corresponding nerve was identified, the LTR was recorded from the contralateral nerve supplying the region of the mantle edge corresponding to that innervated by the stimulated nerve. It was possible to record the LTR from a branch of the contralateral nerve and simultaneously observe any evoked movements of the mantle edge supplied by the intact branches. When repetitive, low-intensity stimulation produced an LTR following each pulse, the corresponding portion of the contralateral mantle edge retracted from the shell margin and the distal margin everted to form a jet. Thus it seems that the LTR fibres in the radial pallial nerves play a role in the formation of a jet on corresponding regions of the mantle edge on both valves.

Above an apparent threshold stimulus intensity, pulse stimulation applied to any radial pallial nerve elicited an LTR in the corresponding contralateral nerve and a synchronous efferent response (SER) in the nerves of both sides (Fig. 10B). Observations of regions of the mantle edge with an intact nervous supply revealed that the SER coincided with a rapid folding of the velum curtain into the mantle cavity. If the preparation was bathed in a calcium-free high-magnesium M.B.L. sea-water saline for about 60 min, pulse stimulation of any radial pallial nerve elicited only the LTR.

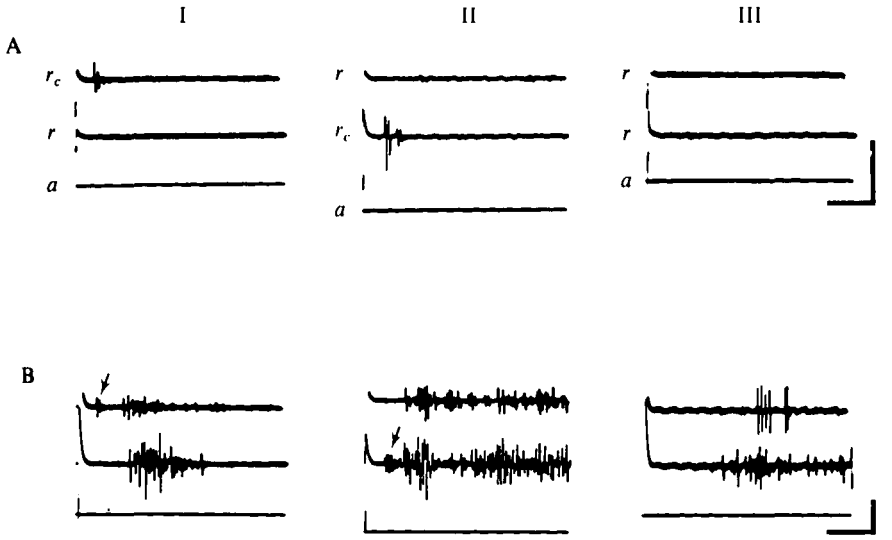

Fig. 10. Adductor muscle mechanograms (*a*) and efferent activity recorded from two contralateral radial pallial nerves (*r*) in response to pulse stimulation of ipsilateral nerves (I, II, III). Nerves I and II were located in a corresponding position to the nerves of the upper and lower traces (r_c) respectively. Nerve III was situated in a lateral position to the two contralateral nerves. (A) A low-intensity stimulus elicited a response (LTR) only in the corresponding contralateral nerve (r_c). (B) Single pulses applied at a high stimulus intensity elicited an LTR in the corresponding contralateral nerve (arrow) and an SER in both nerves. Calibration: 100 μV, 50 ms.

Moreover, the LTR was not abolished even after 200 min in calcium-free saline. When the saline was replaced with artificial M.B.L. sea-water containing standard levels of calcium and magnesium ions, after a short time period, pulse stimulation evoked an LTR, and an SER in the nerves of both sides. These results suggest that the SER is mediated through at least one chemical synaptic connexion.

A train of low-frequency stimulus pulses applied to a radial pallial nerve resulted in a progressive curtailment and, finally, the abolition of the SER. The SER was temporarily restored following a period of no stimulation or by an increase in stimulus intensity or frequency. These data seem to be indicative of fatigue at synapses in the SER pathway. The LTR was recorded even after a train of pulses ten times longer than that required to abolish the SER. Thus it is apparent that the rate of fatigue at any synapses in the LTR pathway is significantly slower than at synapses in the SER pathway.

Single pulse stimulation applied to any radial pallial nerve produced an SER in the nerves of both sides and a rapid folding of the velum curtain into the mantle cavity (Figs. 10B, 11A). Pulses of an increased stimulus intensity elicited an SER plus a contraction of the adductor muscle. This can be seen in Fig. 11B, where the slow adduction apparently represents a contraction of the smooth muscle block alone, since no electrical activity was recorded from the striated adductor. High-intensity pulse stimulation of any nerve evoked an SER, a fast contraction of the striated adductor and a sustained contraction of the smooth adductor muscle (Fig. 11C). A brief train of

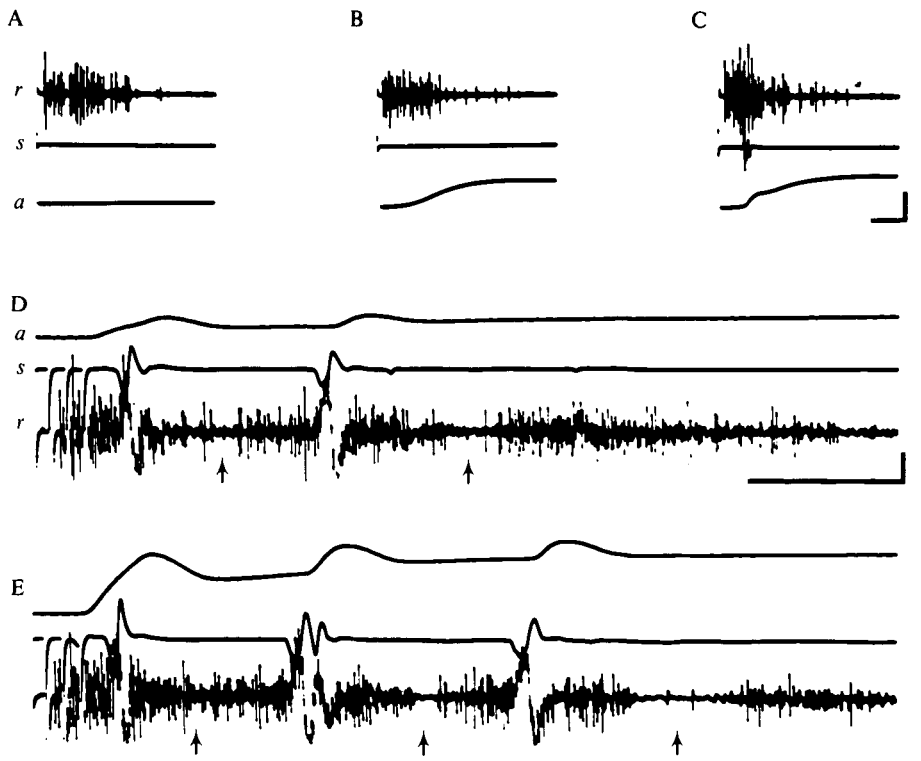

Fig. 11. Activity recorded from a radial pallial nerve (*r*) and the adductor muscle in response to pulse stimulation of a second nerve. *s*, Striated myograms; *a*, adductor muscle mechanograms. (A) Single pulse stimulation evoked an SER in the nerve but no adductions. (B) An increase in stimulus intensity resulted in an SER and a contraction of the smooth block of the adductor muscle. (C) High-intensity stimulus pulses elicited an SER and a contraction of both adductor muscle blocks. (D, E) Brief trains of stimulus pulses (at 40 Hz) elicited a contraction of the smooth adductor, rhythmic striated muscle adductions and bursts of efferent impulses in the radial pallial nerve. Arrow show periods of nerve silence between adductions. Calibration: 100 μV, 200 ms.

high-intensity pulses applied to a nerve at a high frequency (40 Hz) often elicited a contraction of the smooth muscle, rhythmic striated adductions and synchronous bursts of efferent spikes in the radial pallial nerves (Fig. 11 D, E). The frequency of the evoked adductions varied between 3 and 4 Hz and was similar to that recorded previously (Fig. 7 C). Transection of the cerebro-visceral connectives had no affect on the activity recorded from the radial pallial nerves and the adductor muscle in response to nerve stimulation.

2. *Conduction velocities*

The conduction velocities of fibres in the radial pallial nerves were calculated from the distance between the two electrodes and the time delay between the stimulus pulse and the peaks of the recorded compound action potentials. At a temperature of 25 °C one group of fibres with a conduction velocity of about 2·35 m s^{-1} was identified in all radial pallial nerves (S.D. $\pm$ 0·04). Many other nerve fibres were found to have a conduction velocity of between 2·2 and 1 m s^{-1} (Fig. 12).

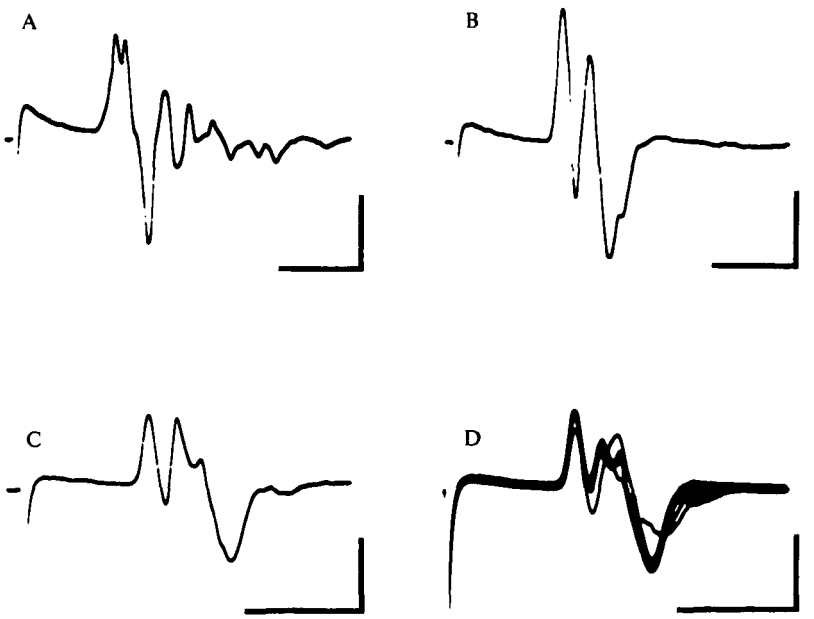

Fig. 12. Conduction velocities of fibres in the radial pallial nerves. Oscilloscope traces were triggered on the stimulus pulse. (A–C) Compound action potentials recorded from one end of a nerve in response to pulse stimulation of the other end of the nerve. Nerve lengths were 14 mm (A), 15 mm (B) and 12 mm (C). (D) Superimposed responses evoked by a short train of pulses (20 at 40 Hz) applied to a nerve 12 mm in length. Calibration: 100 μV, 5 ms.

DISCUSSION

In the present investigation it has been demonstrated that the mantle edge of the scallop *Aequipecten irradians* is sensitive to mechanical stimulation and to crude starfish extract solutions (Figs. 4, 5). Certain scallops, like *Pecten maximus*, discriminate between the touch of predatory and non-predatory starfish (Thomas & Gruffydd, 1971). However, it appears that *A. irradians* makes no distinction between extracts of predatory (*A. forbesi*) and non-predatory (*H. sanguinolenta*) starfish (Fig. 5), although no attempt was made to compare the level of chemical stimulant in the extract solutions with that presented to the scallop by the approaching intact starfish. The result of bathing preparations in a calcium-free sea-water saline indicates that the nervous activity recorded in response to starfish extract or mechanical stimulation of the mantle edge was not primary afferent but was mediated through at least one chemical synaptic connexion. A similar observation has been made for the clam *Lima scabra* (Stephens, 1977). The evoked movements of the stimulated mantle edge involved retraction from the shell margin and the formation of a jet on the velum curtain. These local movements were performed in the absence of the CNS and are evidently controlled by peripherally located neurones. Similar observations have been made for the surf clam *Spisula*, where local movements of the siphon are controlled by neurones with cell bodies located in the peripheral branches of the siphonal nerves (Prior, 1972*a*, *b*). Therefore it is apparent that in the intact scallop the formation of a jet on the stimulated velum is controlled, at least to some extent, by neurones situated at the mantle edge.

A recent study of scallop behaviour has shown that a jet is formed on the velum of both sides even when the mantle edge of only one side is stimulated (Stephens & Boyle, 1978). It has been suggested that jet formation on the unstimulated mantle edge is controlled by fibres in the radial pallial nerves that link corresponding regions of the mantle edge on both valves. In the present paper the existence of these (LTR) fibres has been established (Figs. 8B–D, 10A) and their role in jet formation has been demonstrated. Since transection of the cerebro-visceral connectives had no affect on the LTR recorded from the corresponding contralateral nerve, it is evident that the LTR fibres must be spatio-topically arranged across the visceroparietal ganglion. Cobaltous chloride back-filling of the right radial pallial nerves revealed fibres that travel in the transverse nerve tracts and exit the ganglion in the radial pallial nerves of the left side (Fig. 2A, C). Moreover, results from bathing preparations in calcium-free sea-water and from repetitive pulse stimulation of single nerves demonstrate that if there are any synapses in the LTR pathway, they have very different properties from other (SER) synapses in the visceroparietal ganglion. Therefore it seems possible that the spatio-topically arranged fibres responsible for jet formation on corresponding portions of the mantle edge on both valves may travel across the visceroparietal ganglion without chemical synaptic connexion.

Mechanical stimulation of the mantle edge or pulse stimulation of single nerves produced a short burst of efferent impulses in the nerves of both sides and a rapid folding of the velum curtain into the mantle cavity (Figs. 7A, 10B). Since activity was rarely recorded from a single unit it is evident that the response represents firing in a group of efferent neurones. Synchronous activity in functionally similar groups of motor neurones has been described previously, notably in the efferents that supply the adductor muscles of *Spisula* (Mellon & Mpitsos, 1967) and *Aequipecten* (Mellon, 1969). Activity in a second group of mantle edge efferents was evoked by application of starfish extract to the mantle edge (Fig. 9) and caused the velum to stand erect and retract from the shell margin. The efferent responses elicited by stimulation of the mantle edge or pulse stimulation of single nerves were not abolished by transection of the cerebro-visceral connectives. Therefore it is evident that the two groups of mantle-edge motor neurones that control movements of the mantle edge on both valves, must be located in the visceroparietal ganglion. Back-filling of the radial pallial nerves with cobaltous chloride stained nerve cell bodies in discrete areas of the visceroparietal ganglion (Figs. 2, 3). Although it was not possible to determine whether the perfused somata were cell bodies of afferent or efferent neurones, according to Dakin (1910) the cell bodies of the mantle edge efferents are located in the dorso-central lobes. If this is the case it is evident that the somata of the mantle-edge motor neurones are situated in the lateral margins of the dorso-central lobes (Fig. 2A) and not in the central portions, which are associated with the nerves that supply the adductor muscle (Fig. 3D, unpublished observations).

Mechanical stimulation of the mantle edge or pulse stimulation of single nerves elicited synchronous bursting in the radial pallial nerves and a rapid folding of the velum into the mantle cavity (Figs. 7A, 10B). Since contractions of the adductor muscle occurred only when very high stimulus intensities were used (Figs. 7, 11A–C), it appears that the efferents that control the folding of the velum into the mantle cavity have a lower response threshold than the adductor muscle efferents. It seems likely that these differences in response threshold ensure that the adductor muscle does not

contract without the velum curtain on both valves folding into the mantle cavity, thus protecting the mantle edge as the shells are closed.

The swimming sequence of scallops is made up of a series of fast rhythmic shell adductions that propels the animal through the water. The rhythmic motor output to the striated adductor muscle is thought to be maintained by feedback from stretch receptors that are located in the adductor muscle and are stimulated as the valves are abducted by the elastic hinge ligament (Mellon, 1969). In the present investigation the complete removal of one shell abolished the stretching influence of the hinge ligament on the adductor muscle. However, short series of rhythmic striated adductions were evoked at a frequency similar to that recorded from intact swimming scallops (Figs. 7C, 11D, E). The apparent elastic properties of the relaxing striated adductor coupled with the weak return spring in the strain-gauge resulted in the striated muscle increasing in length between adductions. Therefore it seems possible that the rhythmic motor output to the striated adductor was maintained by stretch receptor feedback. During the short series of rhythmic adductions each contraction of the striated muscle was accompanied by a burst of efferent impulses in the radial pallial nerves and a folding of the velum into the mantle cavity (Fig. 11D, E). Synchronization between the motor output to the striated adductor muscle and to the mantle edge may have been achieved simply by a common input, perhaps feedback from stretch receptors located in the adductor muscle. Moreover synchronization of the motor outputs to the striated adductor and to the mantle edge was not abolished by transection of the cerebro-visceral connectives, which suggests that coordination between the two groups of motor neurones is derived from connexions within the visceroparietal ganglion. The visceroparietal ganglion is situated on the ventral surface of the striated adductor muscle and there are great differences in length between the axons that supply the striated adductor and those that innervate the mantle edge. For example, in a scallop with a shell diameter of 9 cm the radial pallial nerves that link the visceroparietal ganglion with the mantle edge are about 3·5 cm in length. Pulse stimulation applied to certain areas of the ganglion evokes muscle potentials in the striated adductor with a latency of 18–24 ms (unpublished observations). If activity in the two groups of motor neurones is synchronous, impulses must travel down the radial pallial nerves at speeds of 1·5–2 m s^{-1} to produce simultaneous muscle potentials in the striated adductor and the velum musculature. In the present investigation it has been demonstrated that radial pallial nerve fibres of *A. irradians* have a conduction velocity of up 2·35 m s^{-1} at a temperature of 25 °C (Fig. 12). If, as in the siphon withdrawal reflex of *Spisula* (Mellon, 1965), the fast fibres are involved in the protective reflex, it seems possible that the rapid conduction velocity will offset the large distance between the ganglion and the mantle edge and ensure that the velum curtain folds into the mantle cavity before shell closure.

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