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COMPONENTS OF A RESPONSE PROGRAMME INVOLVING INHIBITORY AND EXCITATORY REFLEXES IN THE SURF CLAM

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INTRODUCTION

The central nervous system of the clam, *Spisula solidissima*, contains neurones which respond to volleys in some presynaptic pathways by generating compound biphasic post-synaptic potentials. These are excitatory-inhibitory sequences which, at maximum amplitude, evoke an action potential in the post-synaptic cell and then hyperpolarize its membrane for periods of up to 2 sec. (Mellon, 1967). Such responses resemble those reported to occur in various vertebrate (Phillips, 1961; Anderson & Eccles, 1962) and invertebrate (Hughes & Tauc, 1968; Tauc, 1958) preparations. Thus, an elucidation of their role in the behaviour of the clam would have some comparative interest, as well as a more parochial value in comprehending bivalve nervous mechanisms

The present study of these biphasically responding neurones includes an examination of their peripheral connexions, their responses to input over intact sensory pathways, and their control of some reflex behaviour in this animal. The results indicate that these cells are motoneurones to the fast portion of the posterior adductor. The polarity and complexity of the post-synaptic response of these motoneurones to stimulation of the peripheral sensory structures is dependent upon stimulus strength; biphasic post-synaptic potentials may occur only in response to large stimulus intensities.

The dramatic changes which occur in the response characteristics of these cells indicate the presence of an effective discriminatory mechanism operating within the nervous system of the clam.

MATERIALS AND METHODS

1. *Recording techniques*

Experiments were performed on mature individuals of the surf clam, *S. solidissima*. Animals were obtained from the Marine Biological Laboratory, Woods Hole, Massachusetts, and maintained in artificial sea water at 10 °C.

Intracellular records from nerve and muscle cells utilized glass micropipette electrodes filled with 2·5 M KCl and having resistances of 20–50 MΩ. Signals were monitored with high-impedance D.C. amplifiers and displayed on a multi-trace oscilloscope for visual inspection and photography.

Extracellular records from muscle preparations were obtained using a variety of

procedures. With intact animals, a flexible insulated wire was inserted directly into the fast portion of the posterior adductor muscle through a small hole drilled in one of the valves. Small electrical signals from dissected portions of the adductor were obtained with a tapered stainless-steel electrode insulated to within 50 μ of its tip. Electrical signals were amplified with high-gain a.c. equipment before being led to the monitoring oscilloscope.

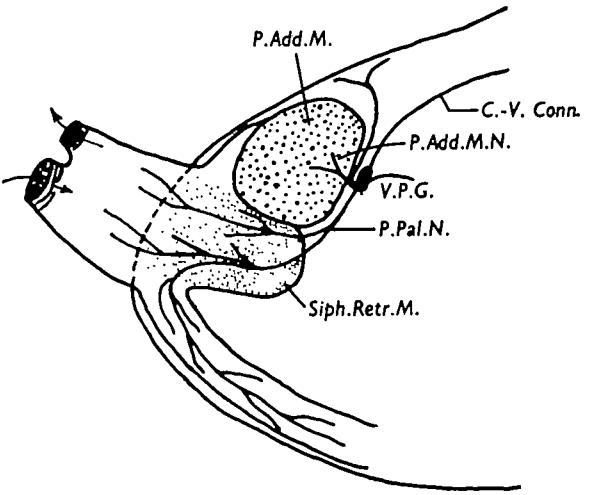


Fig. 1. A diagram of the posterior half of the surf clam to illustrate the muscles and nerve pathways involved in the behaviour examined in the present paper. The animal appears as it would after removal of the right shell valve. The exhalent and inhalent siphons are shown, along with the right posterior pallial nerve (*P.Pal.N.*) and its branches, the right posterior adductor muscle nerve (*P.Add.M.N.*), the right cerebrovisceral connective (*C.-V. Conn.*), the right siphon retractor muscle (*Siph.Retr.M.*), the posterior adductor muscle (*P.Add.M.*), and the visceroparietal ganglion (*V.P.G.*).

2. *Types of preparation utilized*

Fig. 1. is a diagram of the posterior region of the surf clam and indicates the nerve pathways and muscles examined in the present study. Both siphon apertures, as well as the flanking regions of mantle, contain large numbers of tactile afferents which run to the visceroparietal ganglion via the various branches of the posterior pallial nerves. These latter are mixed nerves and contain the motor supply to the mantle and the siphon retractor muscles. In this diagram no distinction is made between the fast and slow regions of the posterior adductor muscle. The adductor muscle nerve presumably supplies both regions, but it most certainly branches very extensively in the fast region, which comprises more than 75% of the bulk of the muscle.

It was necessary to examine several different types of preparation in order to attack the various questions related to the control of the fast portion of the adductor muscle. The different procedures utilized are catalogued below.

Intact preparations. Clams were fastened to the floor of a recording vessel by the right-hand valve. A stainless-steel wire attached to the left valve ran vertically to a Harvard Instrument Company heart-muscle transducer. This device measured excursions of the left valve following stimulation of the animal's siphons. Electrical activity in the fast portion of the posterior adductor was recorded with flexible insulated wire, as described above.

In some cases we wished to monitor, simultaneously, electrical activity both of the posterior adductor muscle and of one of the siphon retractor muscles. To accomplish this it was found easiest to remove the entire animal carefully from its shell valves and place it in a shallow dish moistened with sea water. Stainless-steel electrodes were then thrust into the fast portion of the posterior adductor and also into the siphon retractor muscle on one side. Siphon stimulation was accomplished by stroking with a camel-hair brush.

Visceroparietal ganglion—adductor muscle preparation. In order to examine the correlation of electrical activity in the viscoparietal ganglion and the fast portion of the adductor muscle it was necessary that the nervous connexions between these two tissues remained intact. A bundle of muscle fibres was cut from its insertions on the shell valves, and, with the adductor nerve branches still attached to the visceroparietal ganglion, the muscle tissue was pinned out in a shallow dish of sea water. The connective tissue surrounding the ganglion was then carefully stripped away, and the ganglion was pinned to the floor of the dish as close as possible to the muscle. Electrodes were arranged to stimulate the posterior pallial nerves and the adductor nerves. An insulated stainless-steel electrode was used to search for electrical activity in the muscle tissue, while recordings from the ganglion cells were made with glass micropipette electrodes.

Visceroparietal ganglion-siphon preparation. Threshold differences in different motoneurone populations and the waveform characteristics of motoneurone responses were monitored using pairs of intracellular recording electrodes to record activity from two individual nerve cells simultaneously. It was necessary in these studies to dissect preparations in a manner which maintained the integrity of the sensory pathways to the visceroparietal ganglion from the siphons, and the entire siphon structure, including a small region of flanking mantle margin, was cut from the clam so as not to disturb the ganglion or its pallial nerve connexions. The connective tissue surrounding the nervous structures was then dissected away. Some slack was allowed in the nerves connecting the ganglion to the siphons so that reflex activation of the retractor muscles would not disturb the recording situation. Stimulation of the siphons was accomplished by stroking them with a camel-hair brush, and electrical stimuli were delivered directly to the posterior pallial nerves by pairs of chlorided silver electrodes.

Isolated muscle preparations. Usually the entire posterior adductor muscle with its valve insertions was isolated and clamped in a sea-water bath for recording. The clamp assured that the muscle fibres remained at approximately resting length during stimulation. The two adductor nerves—one emerging from either side of the ganglion—plunge into the main body of the fast portion of the muscle between two prominent fibre bundles on the ventral surface. One or both of these nerves was picked up on paired chlorided silver electrodes for purposes of stimulation. Extracellular records from these preparations were obtained using a sea-water-filled glass capillary electrode. This served as a convenient roving recording element and was used to measure differences in signal polarity and amplitude in different regions of the muscle.

Movements of selected regions of the muscle along its length following nerve stimulation were obtained using a sensitive force-displacement transducer (Grass Instrument Co., Model FT. 03 C). A rigid steel needle was clamped to the transducer, and the latter was lowered at right angles to the muscle until the needle penetrated the

muscle surface to a depth of approximately 1 mm. Movements at this point on the muscle surface along its axis of contraction were registered as voltage changes in the carrier amplifier which supplied the activation energy for the transducer resistance bridge.

Intracellular records from individual muscle fibres in the fast portion of the adductor were, at first, also obtained using this type of preparation. However, it was later found that a larger degree of success in penetrating the small fibres was obtained if individual bundles of fibres were cut from their insertions on the valves and pinned out in a shallow dish of sea water. If care was taken to preserve the adductor nerve branches to the bundle in questions, neuromuscular transmission was maintained in most of the individual fibres penetrated by the electrode.

RESULTS

1. *Behaviour*

A primary objective of the present study was to obtain a detailed account of the neural control of the posterior adductor muscle. It was thus necessary to collect a certain amount of data from intact clams, responding to stimuli over the normal sensory and motor routes. Accordingly, electrical and mechanical records were obtained from whole animals which had been fastened to the substrate by the right-hand valve. Reflex activation of the fast portion of the posterior adductor muscle and the siphon retractor muscle was examined following mechanical and chemical stimulattion of the siphons.

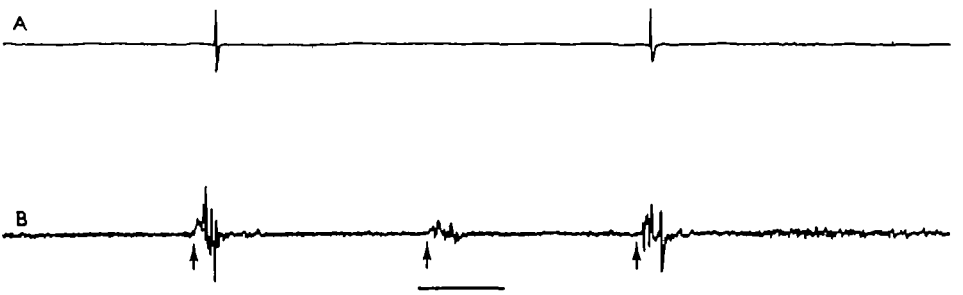


Fig. 2. Simultaneous electrical responses from adductor and siphon retractor muscles following tactile stimulation of the siphons. A, single electrical transients evoked in the fast portion of the posterior adductor of a clam in response to strong tactile stimulation of the siphonal tentacles (first and third arrows). B, Repetitive electrical activity from the right-hand siphon retractor muscle in response to the tactile stimuli. The stimulus at the second arrow was less intense than the others and evoked a smaller electrical response from the retractor muscle. Calibration line is one second. Records lightly retouched for clarity.

Gentle tactile stimulation of the tentacles on the extended siphons of a resting clam evokes behavioural responses which are confined to the siphons themselves. On occasion, only the apertures of the inhalent and exhalent siphons are involved, closing transiently following the stimulus. With slightly stronger stimulation the siphon retractor muscles respond, and both siphons are withdrawn into the mantle cavity. The degree of response appears to be graded. With stronger or protracted stimulation to the tentacles, the electrical activity of the retractor muscles is greater and the siphons are drawn increasingly further into the mantle cavity.

The characteristics of the adductor muscle response to tactile stimulation of the siphons contrasts sharply with that of the siphonal retractors. While the retractors exhibit progressive gradation of response magnitude as stimulus strength is increased, the adductor response possesses a sharply defined threshold and is strictly transient. This is clearly illustrated in the records of Fig. 2A and Fig. 3. Light stroking of the siphonal tentacles caused progressive withdrawal of the siphons. A stronger application of the camel-hair brush then evoked single transients in the fast portion of the adductor (Fig. 3A) followed by a rapid adduction of the shell valves. A similar response sequence occurred as a result of dropping crystals of sea salt directly onto the siphonal apertures (Fig. 3B). The non-repetitive nature of the response of the fast portion of the adductor to input over the pallial nerve pathways is significant, and it will be further considered when the response characteristics of Type I ganglion cells are discussed in a later section.

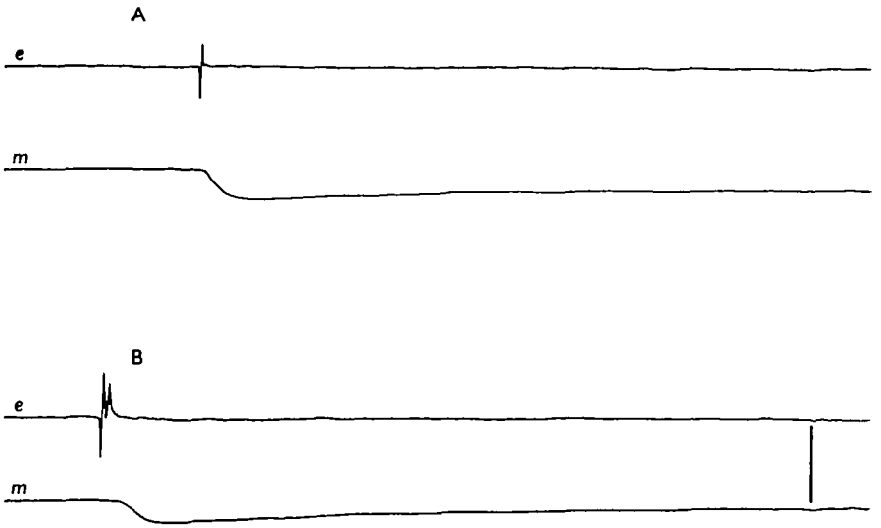


Fig. 3. Electrical (*e*) and mechanical (*m*) records from an intact clam in response to siphonal stimulation. In A the siphonal tentacles were vigorously jabbed with a camel-hair brush, evoking a single muscle action potential in the fast portion of the posterior adductor and a transient adduction of the shell valves. A double electrical response occurred in B as a result of dropping crystals of sea salt in the siphonal apertures. The vertical calibration line applies to both A and B and indicates 1 cm of movement of the ventral margin of the left valve relative to the right valve. The time calibration is 1 sec.

2. *Response correlation between adductor muscle and visceroparietal ganglion*

Three previous papers have dealt with the response characteristics of cell populations in the visceroparietal ganglion of *Spisula* (Mellon, 1965; Mellon, 1967; Mellon & Mpitsos, 1967). Cells in the anterior lobes designated as Type I respond to input volleys over the pallial nerve pathways. Typically, these responses take the form of compound biphasic post-synaptic potentials, in which the initial phase is depolarizing and may generate an action potential. The secondary phase of the response is a prolonged inhibitory potential, during which the conductance of the post-synaptic membrane increases dramatically (Mellon, 1967).

Afferent pathways in the posterior adductor nerves also drive Type I ganglion cells. However, the post-synaptic responses generated by volleys in these pathways are purely excitatory, and the response to succeeding input volleys shows facilitation and produces brief trains of impulses. Since it is known that the adductor muscles contract reflexly following passive stretch and that the axons of Type I cells are found exclusively in the two adductor nerves, it seemed possible that these ganglion cells are motoneurones to the adductor.

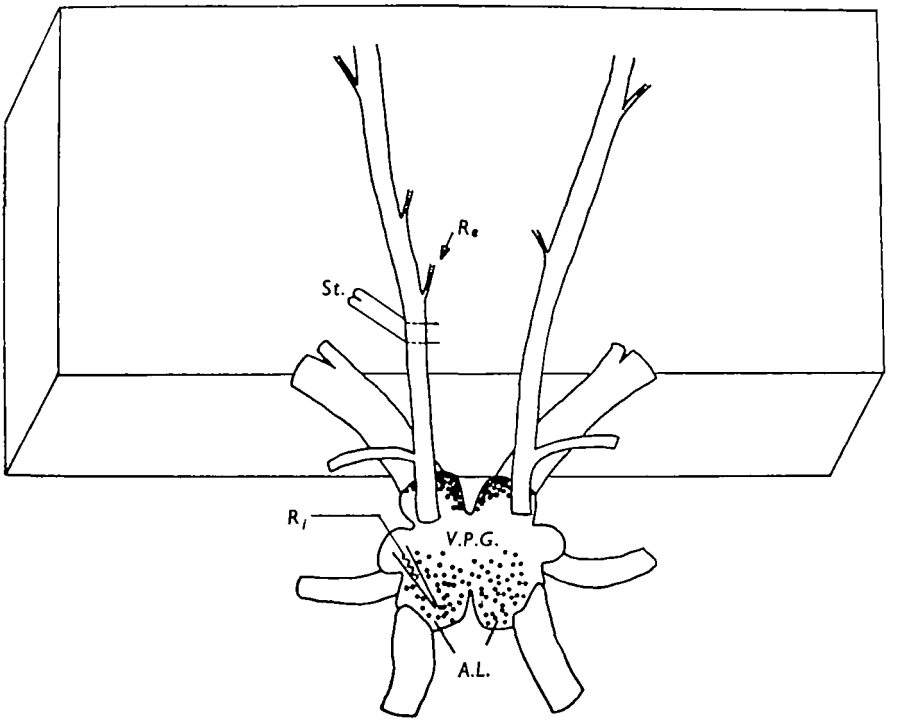


Fig. 4. A diagram of the preparation used to obtain correlations between electrical activity in single Type I neurones and in the fast portion of the posterior adductor muscle. A small region of the muscle is shown, along with its intact branches from the adductor nerves and the visceroparietal ganglion (*V.P.G.*) The extracellular electrode (R_e) was used to search for activity in the muscle in the vicinity of ipsilateral nerve branches. A stimulating electrode pair (*St.*) was placed under the adductor nerve to check antidromic activation of Type I neurones. The intracellular electrode (R_i) was used to record from Type I cells in the ipsilateral anterior lobe of the ganglion, and also to pass current as the means for exciting single Type I cells. The filled circles indicate the approximate extent and location of presumed adductor motoneurone and siphon motoneurone populations, occurring, respectively, in the anterior lobes (*A.L.*) and the pallial lobes of the ganglion.

To test this possibility we decided to look for correlations between the electrical activity of Type I ganglion cells and electrical activity on the fast portion of the posterior adductor muscle. The preparation utilized in this search is illustrated by the diagram of Fig. 4. The Type I neurones are found in the anterior lobes of the ganglion in the regions labelled '*A.L.*' in Fig. 4 (Mellon, 1967). Intracellular micropipette electrodes were used to record transmembrane potential changes, and simultaneously, to pass current across the membrane of cells at the surface of the ganglion. When it had been established that a cell responded to pallial nerve volleys in a manner typical

of Type I neurones, outward current pulses were applied to the soma membrane by means of a bridge circuit built into the pre-amplifier. Currents of sufficient strength generated action potentials in the impaled soma. During spike trains, extracellular electrical records from the region of the fast adductor near the entrance of an ipsilateral nerve branch were carefully scrutinized for any sign of activity which might be correlated with the evoked activity in the Type I cell. In most instances no activity of any kind was seen in the muscle during stimulation of a ganglion cell. Often, the extracellular recording electrode was moved from place to place on the muscle surface, and each new recording site was examined for some response to neuronal stimulation. A new ganglion cell was then penetrated, and the procedure was repeated. Seven different preparations were tested in this manner, and a positive correlation between activity occurring in a Type I neuron and the muscle was seen in one instance. The records from this case are shown in Fig. 5A–C. Each nerve action potential precedes a

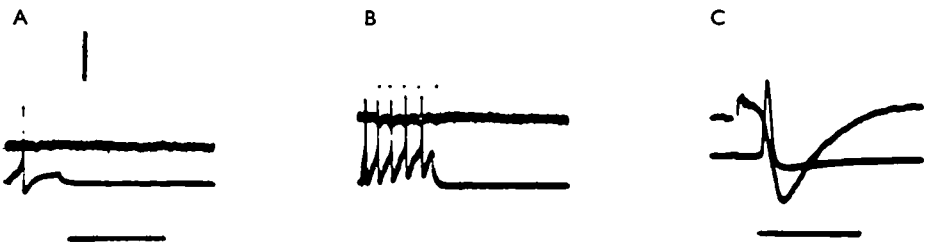


Fig. 5. Electrical records obtained simultaneously from a Type I cell in the visceroparietal ganglion (lower traces) and from a region of the fast portion of the posterior adductor (upper traces). The impulses generated in the ganglion cell in A and B were evoked by passing current through the intracellular recording electrode. Small electrical transients (indicated by the position of the dots above the upper trace) can be observed in the muscle record following each nerve impulse at a constant latency. In C a single strong shock to the whole adductor nerve produced an antidromically propagated impulse in the axon of the impaled Type I neurone and a large compound action potential in the muscle record. The gain of the extracellular recording channel in C was reduced from its level in A and B. Calibration: A–C (intracellular), 50 mV; A–B, 1 sec; C, 100 msec.

very small negative-going deflexion recorded from the muscle. The latency of the muscle response following each nerve impulse is constant, although the later responses to a brief train of neurone spikes are reduced in amplitude. By analogy with the much larger muscle potentials evoked by volleys set up in the whole adductor nerve (Fig. 5C) the small responses are probably unitary excitatory junction potentials evoked by individual action potentials arriving over one axonal pathway. These records indicate that Type I neurones do innervate muscle fibres in the fast portion of the adductor. The classification of these ganglion cells as (excitatory) motoneurones is an assumption based primarily upon the polarity of the electrical response which they evoke in the muscle. As will be elaborated in the Discussion section below, this assumption must be made with caution.

3. *Response of Type I cells to tactile input*

In the light of the non-repetitive nature of the fast adductor response following stimulation of the siphonal tentacles it was of interest to examine the electrical activity of Type I cells following similar sort of stimulation over the same sensory pathways. Both pallial motoneurones and Type I cells were penetrated with micropipettes, in a

visceroparietal ganglion which was attached to the siphonal structures by means of the pallial nerve branches. Simultaneous electrical records were thereby obtained from both kinds of neurones during tactile stimulation of the siphonal apertures and flanking regions of the mantle. Typical results are shown by the records of Fig. 6. Low-intensity stimulation of the pallial regions by means of camel-hair brush evokes large EPSPs and groups of impulses in the pallial motoneurones; however, the response of Type I cells is strictly hyperpolarizing (Fig. 6A, D). A slightly increased level of stimulus intensity (Fig. 6B) evokes a slight depolarization prior to the onset of the inhibitory response, but it is only with intense stimulation that the initial excitatory phase reaches levels which are threshold for impulse generation (Fig. 6C, F). In contrast, the levels of hyperpolarization generated by weak and strong tactile stimulation, respectively, were usually extremely close, if not identical (e.g. Fig. 6A–C). Stimulus intensities which are insufficient to generate impulses in the Type I cells may nonetheless produce maximal inhibitory responses. This point is of importance and it suggests a mechanism for the suppression of subthreshold depolarizing responses which otherwise, through temporal summation, might have generated impulses in Type I neurones.

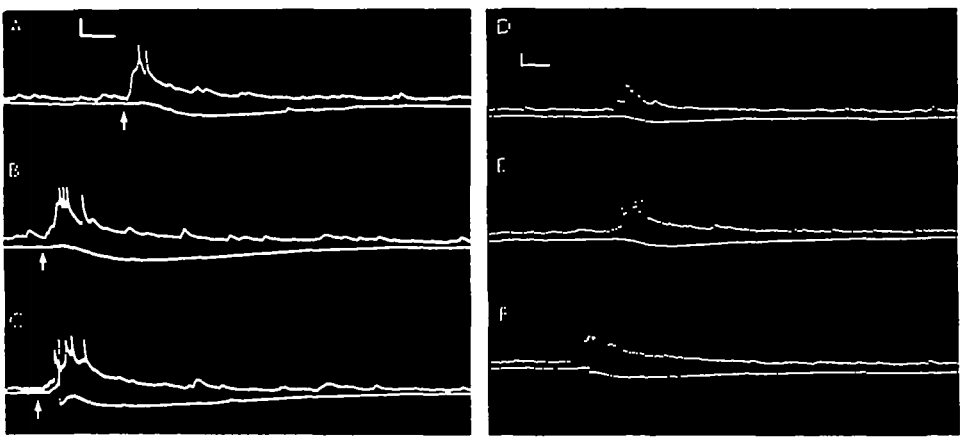


Fig. 6. Simultaneous intracellular records obtained from siphon motoneurones (upper traces) and Type I cells (lower traces) in two different preparations in response to tactile stimulation of the siphonal tentacles. The intensity was increased progressively in A–C and D–F. A rather slight stimulation, as in A and D, routinely produced excitation in the pallial motoneurones but only a hyperpolarizing response in the Type I cells. Stronger stimulation resulted in increased pallial motoneurone output and a definite depolarizing initial phase in the Type I cell record (B). In C and F, a strong stimulus to the siphons generated a large EPSP and a spike in the Type I cells. However, note that the amplitude of the inhibitory phase of the Type I cell response is nearly identical in all records from each cell. Calibration: 20 mV and 350 msec.

4. *Muscle responses*

Mechanical and extracellular electrical recordings were made from preparations consisting of an entire isolated posterior adductor muscle and its shell-valve insertions. These portions of shell were held in a clamp which kept the muscle under slight tension. Contractions evoked by adductor nerve stimulation were thus isometric. Under such conditions, while the overall length of the individual muscle fibres does not change during a contraction, some relative motion is seen to occur along the length of the

muscle when one or both adductor nerves are stimulated. The most reasonable explanation for this observation is that only selected regions of each fibre take part in the contraction process. For example, by visual inspection of the surface of the fast adductor following a low-frequency train of electrical shocks delivered to the right-hand adductor nerve, regions of the muscle on both sides of the point of entry of the right-hand adductor nerve move toward that point of entry, as indicated in Fig. 7. Conversely stimulation of the left-hand nerve evokes a general movement of the muscle surface toward the point of entry of the left-hand nerve into the muscle.

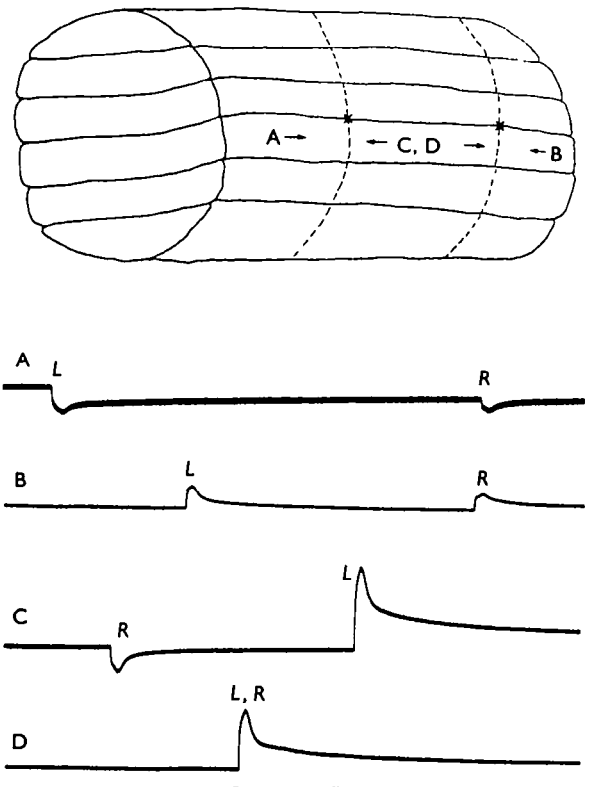
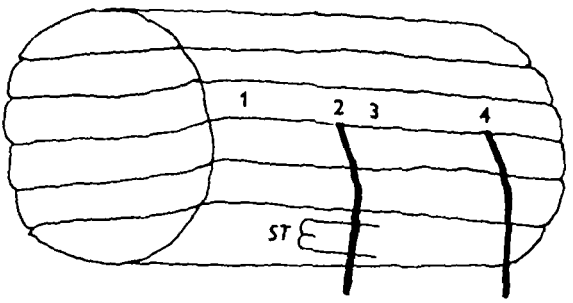


Fig. 7. A diagram of the posterior adductor muscle and (A–D) mechanical records obtained from the muscle surface in response to volleys evoked in the right (*R*) and left (*L*) adductor nerves. Lettering on the diagram indicates the positions at which the various records were obtained; the arrows show the direction of movement of the muscle surface following ipsilateral nerve stimulation. In A–D, an upward deflexion indicates surface movements to the left, and downward indicates movements to the right. Calibration: 25 sec.

The visible movements of the surface muscle fibres can be recorded by coupling one region of the intact muscle to a sensitive strain gauge. In the present study this was done by clamping a sharp needle to the transducer and inserting the needle into the muscle at right angles to its longitudinal axis. Some of the records obtained by this technique are shown in Fig. 7A–D. The index letter of the upper left-hand corner of each trace refers to the position of the needle on the muscle. It can be seen that relative motion of the needle occurs when it is placed on either side of an active region, and the direction of this motion is always toward the active nerve. When both adductor

nerves are stimulated simultaneously, the movement at the midline of the muscle is an algebraically summated resultant (Fig. 7D).

Electrical activity recorded extracellularly along the length of the adductor muscle also reflects foci at the two innervated regions. As indicated by the records in Fig. 8, the polarity of the electrical muscle transients recorded in the vicinity of one of the nerves is negative—going with respect to an indifferent electrode (Fig. 8C). As the recording



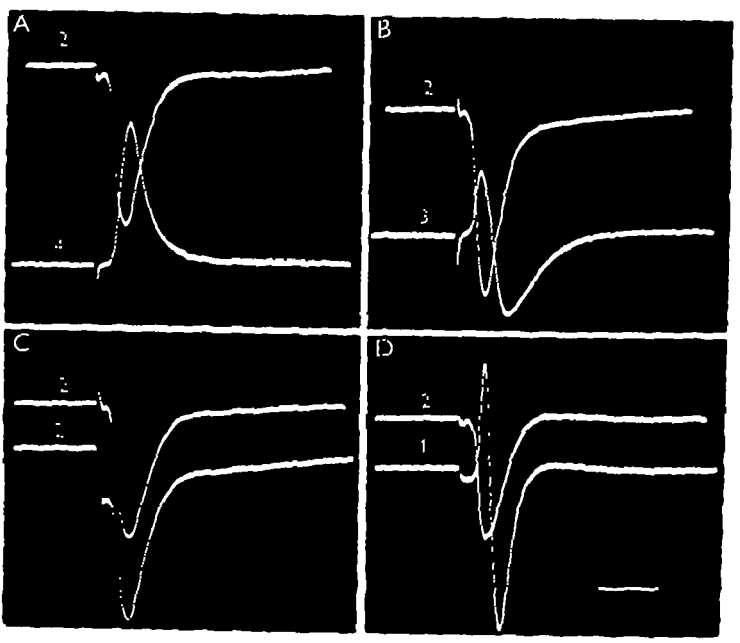


Fig. 8. Simultaneous electrical records from pairs of separate extracellular electrodes on the surface of the fast adductor in response to maximal volleys evoked in an adductor nerve (*ST*). The index number on each trace indicates the position of the electrode on the muscle surface when the record was obtained. Calibration: 100 msec.

electrode is moved to either side of the innervated region, the electrical record becomes triphasic with an initial positive-going excursion (Fig. 8B). Movement of the electrode still farther from the point of innervation (e.g. at the midline of the muscle or at the valve insertions) results in a signal which is predominantly positive-going and biphasic (Fig. 8A). It is not yet clear what pattern of current flow can account for the variations in extracellular potential along the muscle (see discussion), but it

is interesting that changes in mechanical activity of the muscle are paralleled by variations in externally recorded electrical activity. Neither set of observations is inconsistent with the hypothesis that regions of electrical and contractile activity occur only in areas adjacent to the entry of the two adductor nerves.

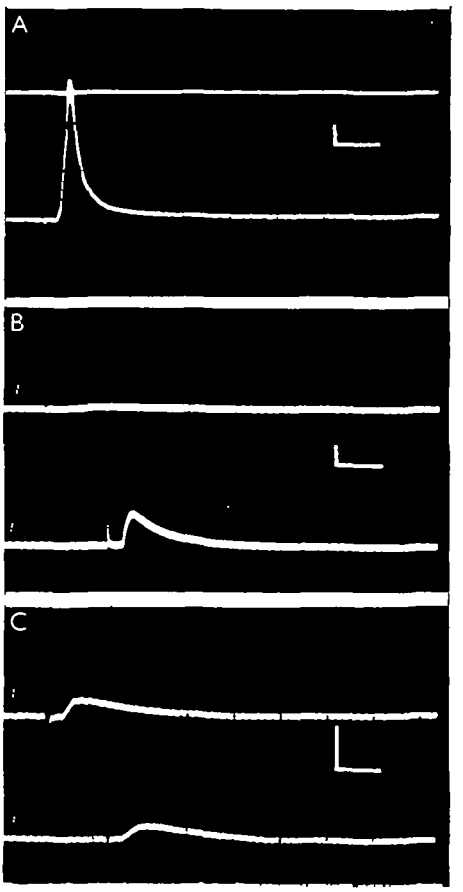


Fig. 9. Intracellular records obtained with glass capillary micro-electrodes from individual muscle fibres in the fast portion of the posterior adductor in response to single motor nerve volleys. In A the position of the recording electrode was close to the point of entrance of the left adductor nerve into the muscle, and a maximal volley set up in the left nerve evoked an overshooting muscle action potential. Zero potential level is indicated by the top horizontal trace. In B, the recording electrode was situated in another fibre, near the entrance point of the right adductor nerve. Volleys set up in this nerve (*r*) and in the left adductor nerve (*l*) produce excitatory junction potentials of disparate amplitudes, as discussed in the text. An electrode placed in a muscle fibre at a point approximately midway between the two nerves (C) records junction potentials which are of similar amplitudes in response to volleys set up in either nerve. Calibration: 10 mV and 100 msec.

The possibility that electrical and mechanical activity was confined to certain regions of each individual muscle fibre prompted an examination of these cells using intracellular recording techniques. In particular, it was important to discover whether each muscle fibre receives innervation from right- and left-hand adductor nerves, or whether two parallel populations of fibres exist, each population receiving its nerve

supply from one side alone. Portions of the muscle and its nerve supply were cut from the shell-valve insertions and placed in cold sea water. Penetrations were made with KCl-filled micropipettes in the vicinity of the entrance of the two adductor nerves and at points roughly midway between the nerves. The results of electrical stimulation of the nerves are shown in Fig. 9. Stimulation of the nerve closest to the recording site on the muscle evokes large excitatory junction potentials (EJPs), while the response to the excitation of the more distant nerve is always of much lower amplitude, undoubtedly due to electrotonic degradation (Fig. 9B). Nonetheless, the evidence is clear that both nerves make functional connections with the individual muscle fibres. Electrical records obtained from a position on the muscle midway between the entry points of the two nerves exhibit EJPs of moderate amplitude in response to electrical stimulation of either nerve (Fig. 9C), and this observation confirms the dual nature of the motor innervation of these muscle cells.

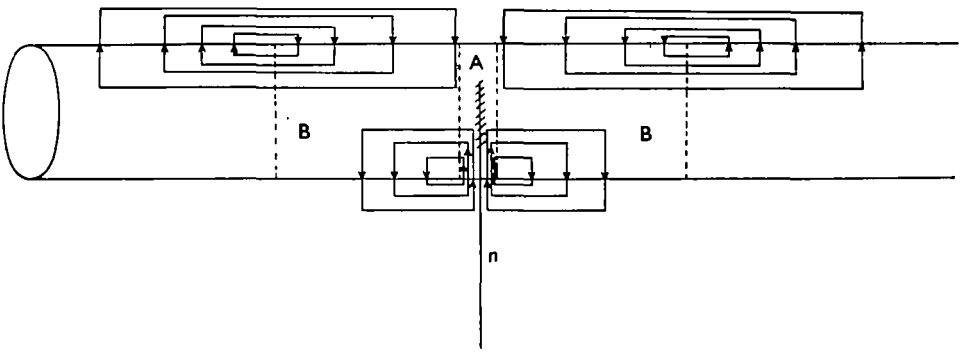


Fig. 10. A hypothetical scheme for major paths of current flow in single muscle fibres of the fast adductor following a motor nerve impulse. Adductor nerve motor axons are assumed to synapse with an individual muscle fibre at a rather limited zone, delineated in the diagram by the area labelled A inside the closest pair of dashed lines. The impulse supporting membrane is assumed to extend on either side of this region, and is delineated by the regions labelled B inside the outermost dashed lines. An extracellular recording electrode over the region marked A would be located at the centre of a current sink during both EJP and spike electrogenesis and would record only negative-going transients in response to ipsilateral nerve volleys. A recording electrode over the region marked B, however, would record a positive-going potential during the rising phase of the EJP, or until an action potential was evoked and its own current sink became the dominant electrical influence. Areas outside both regions would always act as sources of current, and an electrode would record only positive-going activity following nerve stimulation.

The membrane of fibres in the fast portion of the adductor is capable of regenerative electrical activity, and action potentials which overshoot the zero potential level may be seen in response to maximal adductor nerve volleys (Fig. 9A). Overshooting spikes were never recorded from mid-regions of the individual muscle fibres, however, nor were they ever seen in response to stimulation of the contralateral adductor nerve. This is suggestive that muscle spikes are not propagated from one end of the fibre to the other, and it is consistent with the view that contraction is confined to zones at the separate ends of a muscle fibre.

DISCUSSION

The data obtained above pertain only to the control of the fast portion of the posterior adductor muscle of *Spisula*. From observations involving the scallop adductor (e.g. Mellon, 1968) it is clear that the slow muscle utilizes separate nervous pathways and may be involved in different reflexes from those involving the fast muscle. The data and the following discussion thus refer only to the fast portion of the muscle.

We have attempted to compare records obtained in intact or nearly intact preparations with those obtained from isolated regions of the animal. In the first instance electrical and mechanical responses to tactile stimulation of the siphonal tentacles were recorded from two different muscles, the fast portion of the posterior adductor and one of the siphon retractor muscles. The second related group of observations were obtained from the motoneurones to these two muscles, in response to a similar method of tactile stimulation. A qualitative comparison of the electrical responses in the nervous and muscle tissues has shown that while the siphon retractor muscles and the efferent neurones to the siphon respond repetitively to tactile stimulation of the siphonal tentacles both the fast portion of the adductor muscle and the Type I ganglion cells have relatively high thresholds for response to tactile stimulation of the siphons, and both generate only transient excitation (usually a single action potential) following suprathreshold stimulation. These observations have provided support to the conclusion, drawn from the results of simultaneous electrical recording in nerve and muscle that Type I cells are motoneurones to the fast portion of the posterior adductor.

While we regard the positive correlation of electrical transients in the Type I cells and the muscle as the most convincing evidence that the former are motoneurones, the difficulty in obtaining these records needs some comment. From previous work on the fast portion of the scallop adductor (Mellon, 1968), it is clear that the muscle is functionally divided into large numbers of motor units, as occurs in vertebrates. If this pattern of motor innervation is applicable to the mactrid bivalves as well, it is apparent that each adductor motoneurone in *Spisula* supplies just a few individual muscle fibres out of the many thousands which are present. The electrical field set up by simultaneous junction potentials in the fibres of a motor unit must, therefore, be small relative to the potential generated following the stimulation of all available motor axons in the adductor nerve. Moreover, since in each preparation used only a small portion (less than 15%) of the fast adductor muscle was examined for correlative electrical activity, it should be expected that most of the Type I cells penetrated by the current-passing micro-electrode would have had their axons severed at the periphery during the dissection procedures.

The assumption that Type I neurones are excitatory to muscle activation is supported mainly by the electrical sign of the muscle response following impulse generation in the Type I cells. Liminal-to-maximal volleys evoked in the adductor nerve produce negative-going extracellularly recorded electrical responses in the muscle at the point of entrance for that nerve, as described above. Intracellular records from muscle fibres exhibit depolarizing responses as a result of ipsilateral nerve stimulation; it is reasonable therefore, to assume that the small negative-going transients recorded from the muscle in response to impulses evoked in single Type I cells represent localized depolarization of a group of muscle fibres. The data do not exclude the possibility

that such depolarizations in the muscle represent inhibitory postjunctional potentials, as routinely occur in certain crustacean skeletal muscle fibres (Dudel & Kuffler, 1961). However, neither the electrical nor the mechanical records from fast adductor muscle preparations have indicated the presence of a peripheral inhibitory nerve supply.

The change in the waveform of the extracellular records obtained from the fast portion of the adductor as the electrode was moved along the muscle is interesting. A hypothetical scheme such as the one shown diagrammatically in Fig. 10 could account for the potential variations along each muscle fibre during activity. It is assumed that a restricted, innervated zone of each muscle fibre acts as a current sink during excitatory junctional potentials (EJPs) initiated by motor nerve impulses. This region, and perhaps adjacent regions as well, may respond to large depolarizations by regenerative electrical activity. During an action potential, regions of membrane taking part in the spike electrogenesis would appear negative to an active electrode on the muscle surface. However, regions outside of the immediate zone of innervation would appear positive during the rising phase of the EJP, since during this period the muscle fibres act as a current source for membrane regions peripheral to the synaptic loci. Areas of the muscle fibre well removed from the site of either type of electrogenesis would, for the same reason, appear electrically positive to an active electrode.

We regard as the most important finding of the present studies the fact that the functional polarity of the post-synaptic responses in Type I neurones reverses as stimulus intensity increases. Weak tactile stimuli to the siphons generate predominantly inhibitory responses, while strong stimuli evoke sequences of excitation plus inhibition. The input organization of Type I cells thus constitutes an intensity discriminator. What are the mechanisms which are involved in this polarity reversal? A simple solution would be to postulate two intermingled groups of sensory endings, having very different thresholds for activation by tactile stimuli, and each population having opposing central actions. However, previous investigations of Type I cells (Mellon, 1967) suggest that very similar or identical peripheral pathways conduct sensory impulses to both the excitatory and inhibitory synapses. These findings may mean that a functionally homogeneous population of sensory cells exists at the periphery; and therefore, other mechanisms must be considered as possible influences on the reversal of the synaptic polarity. It seems clear that as stimulus intensity increases firing frequency in the available sensory pathways will increase and additional sensory pathways will be recruited (Mellon, 1965). The overall frequency of arriving sensory impulses could therefore be used to determine response polarity. In order to determine exactly how the input organization of Type I neurons operates differentially upon frequencies of different magnitudes, it would be necessary to know not only the precise anatomical nature of both the excitatory and inhibitory pathways, but the transfer efficiencies of the different synapses as well. As an example, some excitatory synaptic pathways may require facilitation to become most effective, while inhibitory pathways may operate with equal efficacy over a broad range of the spectrum of sensory impulse frequency.

It is clear that once the inhibitory phase of the Type I cell response has become established excitatory input to these neurones is suppressed. This must be due to the

increase in membrane conductance which occurs during the inhibitory potential change (Mellon, 1967).

The important features of the proposed sensory discriminator therefore involve the initial interaction of the two response phases which, in effect, allows high-intensity input to pass through and critically depolarize the Type I cell membrane. The changes in the two response phases as a function of time following the initial arrival of sensory impulses may be of great importance. Questions such as these must be carefully examined in an attempt to describe, on the basis of known neuronal interactions, the mechanisms underlying this interesting discriminatory phenomenon.

SUMMARY

1. Electrical records from ganglion cells in the central nervous system and from intact muscle groups controlling siphon retraction and shell-valve adduction have revealed qualitative similarities in the response characteristics of two neurone-effector systems following stimulation of tactile afferents.

2. Simultaneous electrical records from neurones and muscle indicate that Type I ganglion cells are motoneurones to the fast portion of the posterior adductor muscle.

3. The waveform and polarity of the post-synaptic responses of Type I cells depend critically upon the intensity of stimulation over *intact* sensory pathways. High-intensity input transiently excites the fast portion of the adductor; low-intensity input inhibits the adductor motoneurones. The input organization of Type I neurones therefore permits discrimination of stimulus magnitude and thus controls the characteristics of the response programme.

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