RECEPTOR UNITS RESPONDING TO MOVEMENT IN THE OCTOPUS MANTLE

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SUMMARY

1. A preparation of the mantle of Octopus which is inverted over a solid support and which exposes the stellate ganglion and associated nerves is described.

2. Afferent activity can be recorded from stellar nerves following electrical stimulation of the pallial nerve. The latency and frequency of the phasic sensory response is correlated with the contraction of the mantle musculature.

3. It is proposed that receptor cells located in the muscle, and their activity following mantle contraction, form part of a sensory feedback system in the mantle. Large, multipolar nerve cells that were found between the two main layers of circular muscle in the mantle could be such receptors.

INTRODUCTION

Mechanoreceptors associated with locomotory systems are well known from many groups of animals, particularly the vertebrates and Crustacea. These receptors monitor movement, position or stress in skeletal elements and muscles. Their role is apparently to provide the feedback of information to the nervous system that is necessary for motor control.

In the Mollusca, there are several reports of mechanoreceptors which are sensitive to movements of the animal. These mechanoreceptors are associated with the buccal musculature (Laverack, 1970; Kater & Rowell, 1973), and diverse locomotory areas such as the columnellar muscle of Helisoma trivolvis (Heyer, Kater & Karlsson, 1973), parapodia of Aplysia fasciata (Hughes, 1971) and body wall of Buccinum (Laverack & Bailey, 1963). In such soft-bodied animals it may be difficult to distinguish mechanoreceptor activity having a proprioceptive function (Laverack, 1968) from superficial tactile receptors associated with the body wall (Rowell, 1966). Although some molluscan central nervous systems are capable of rhythmic motor outputs appropriate to a behaviour pattern in the absence of sensory feedback (Dorsett, Willows & Hoyle, 1969), modification of motor output by afferent input has been demonstrated for Helisoma (Kater & Rowell, 1973) and the bivalve Aequipecten (Mellon, 1969).

The cephalopod mantle is responsible for regular respiratory movements and also for fast locomotion by means of jetting. To achieve these movements in the absence of a hard skeleton, the musculature and connective tissue layers are necessarily
complex (Ward & Wainwright, 1972; Ward, 1972; Packard & Trueman, 1974). In the octopus the mantle consists of an inner and outer layer of longitudinal muscle, two layers of circular muscle separated by connective tissue, and bundles of transverse or radial muscle at regular intervals. The control of this musculature by the brain is mediated by the paired stellate ganglia where the final motoneurones are located. Although anatomically there appears to be a division of function within each stellate ganglion (Young, 1972), it is not possible to distinguish nervous elements associated with a particular muscle layer. The connexion between the stellate ganglion and the brain is necessary for co-ordinated movements of the mantle, but the stellate ganglion itself may act as a reflex centre (Fröhlich, 1910; ten Cate, 1929; Wilson, 1960).

Mechanically excitable sensory units in the mantle musculature and skin have been demonstrated physiologically (Gray, 1960). Phasic and tonic units responded to light touch or pinch of the mantle, but activity could not be recorded during normal respiratory movements. The latter finding has led to the association of the ventral and dorsal divisions of the ganglion with the functions of respiration and locomotion respectively, because only the dorsal division receives afferent fibres from the periphery (Young, 1972). The only sensory cells in the mantle that have been described anatomically are multipolar cells sited on a special area of muscle close to the stellate ganglion (Alexandrowicz, 1960). They are presumed to form a muscle receptor organ distinct from the units recorded by Gray (1960).

The present study describes sensory activity in a new preparation of the mantle musculature of Octopus vulgaris and Eledone cirrosa in response to movements of the mantle.

**PREPARATION AND METHODS**

The work on Octopus vulgaris was carried out at Laboratoire Arago, Banyuls-sur-Mer, France, and preliminary experiments on Eledone cirrosa at Aberdeen. The animals were killed by a midline incision into the brain and the entire mantle with its contained organs rapidly severed from the head and arms. The midventral septum was cut, and the adductor pallii muscle severed close to its anterior insertion, leaving a considerable length of the pallial nerve (or connective) with the muscle and attached to the stellate ganglion.

The anterior rim of the mantle was reflected, exposing the viscera. Each branchial membrane was carefully cut, freeing the gills. The funnel retractor muscle of each side was then cut close to the mantle allowing the entire mantle to be detached from the visceral mass. Finally, unless otherwise stated, the skin was removed from the external surface, cutting the connective tissue close to the mantle muscle surface.

The preparation was mounted by inverting the mantle musculature over an egg-shaped cork support, 10 cm in length and 8 cm in diameter (Fig. 1). In this way the stellate ganglion and associated pallial and stellar nerves of each side were exposed on the surface and one of them could be prepared for recording. Usually stellar nerves from the groups numbered 17–22 by Bühler et al. (1975) were used. The muscle was pinned to the cork around the mantle opening, and also in some places around the ganglion itself, to reduce movement artifacts.

Extracellular recordings were made with etched silver hook electrodes from dissected stellar nerves. Signals were amplified and displayed by conventional a.c.-
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Fig. 1. Diagram of the mantle preparation inverted over a cork support, showing hook electrodes placed under the pallial nerve (right) and under a centrally severed stellar nerve (left).

coupled methods, and recorded on magnetic tape or Polaroid film. Electrical stimuli were applied to the pallial or stellar nerves through a pair of silver electrodes. Single or multiple squarewave pulses were supplied from a Devices isolated stimulator driven by a gated waveform generator (Devices) which could also be used to trigger the oscilloscope. Mechanical movements of the muscle were registered with a wire strain gauge (Electrophysiological Instruments) attached to the muscle by a small hook. The amplified signal was displayed and recorded as above.

The inverted mantle was immersed in a bath of flowing sea water at 14.5–16.5 °C (Banyuls) or static sea water maintained at 10–12 °C by a constant temperature jacket (Aberdeen). The stellate ganglion and the nerves required were allowed above the water surface. A silver earth electrode was often used between the stimulating and recording pairs in addition to the usual earth to the bath.

Methylene blue staining was employed intra-vitally for the visualization of sensory cells. Pieces of mantle muscle were cut in segments radially from the ganglion. The distal end of the segment was split horizontally, with a razor blade, between the two layers of circular muscle, and the two were pulled slowly apart. The pieces of muscle tissue thus obtained were pinned on to wax plates with the newly exposed muscle surface uppermost. They were then placed muscle downwards into bowls of filtered sea water at room temperature (about 19 °C), to which a 1% solution of methylene blue in distilled water was added to give a final concentration of 1:30000 (Alexandro-wicz, 1960). The muscle surface was examined periodically under a microscope with incident light and some dissection of the superficial connective tissue undertaken,
particularly along the line of visibly staining nerves. When staining of nerve cells was suspected the muscle was examined at higher power with light transmitted through the wax and muscle. Under these conditions staining was optimal after 3–5 h. Photographs of stained cells were taken from the fresh preparation, using a Leitz Orthomat photomicroscope with normal or ultraviolet transmitted illumination and a range of yellow and orange filters.

RESULTS

The presence of mechanically excitable receptor units in the octopus mantle could easily be demonstrated in a simple nerve-muscle preparation made from a piece of mantle tissue and its associated stellar nerves pinned out, in air, on a cork board. A rounded metal probe driven from a function generator was used to apply mechanical stimulation to the mantle musculature. Mounted vertically the probe applied a fluctuating load to the mantle thickness, pinching it against the board; mounted horizontally it applied a shear force. Examples of records made from the centrally severed stellar nerves of *Eledone cirrosa* in response to such stimulation are shown in Fig. 2. Typically, short phasic bursts of impulses were elicited which adapted rapidly to repeated stimulation. Where identification of unit responses was possible, no clear correlation of response frequency with stimulus intensity was found. Bursts of impulses from tonic units were seen in preparations with or without the skin present, but these were more common in ageing preparations and may indicate damaged tissue. Length changes imposed on the mantle musculature by stretching or pulling it also stimulated activity in the stellar nerves, but such activity could not be elicited with any consistency.

In the fresh inverted mantle preparation of *Octopus vulgaris*, electrical stimulation of the pallial nerve of one side elicited a powerful contraction of the mantle musculature of that side. From the distal end of a cut stellar nerve a phasic burst of impulses followed this stimulation with a latency of between about 100 and 240 ms. For any one preparation the latency was relatively constant. An increase in the amplitude of the stimulus to the pallial nerve resulted in the recruitment of additional units into the burst in the stellar nerve (Fig. 3). With a flat nerve-muscle preparation of the mantle, afferent activity did not usually follow stimulation of the pallial nerve even though muscle movement was elicited.

Separation of the mantle from the skin and most of the intervening connective tissue made no consistent difference to the presence of the sensory activity recorded following stimulation of the pallial nerve. Again, increase in the intensity of stimulation resulted in further units becoming active in the stellar nerve (Fig. 4), which naturally increased the phasic burst frequency up to a point. However, where identification of an individual unit in the burst was possible, by the identification of a characteristic spike amplitude, its frequency was rather variable and not clearly related to the degree of stimulus intensity applied to the pallial nerve. In some preparations the increase in stimulus voltage resulted in an initial progressive decrease in the latency of the phasic burst, after which it became relatively constant.

Records of mantle movement showed that movement always preceded the burst of activity in the cut stellar nerve. Increase in the intensity of stimulation to the pallial nerve did not significantly alter the latency of the onset of contraction, but had a
Fig. 2. Sensory activity recorded from severed stellar nerves in a flat mantle preparation of *Eledone*, in response to mechanical stimulation of the mantle musculature with a round-ended probe driven from a waveform generator (lower traces). In all preparations, except (a) and (b), the skin and outer connective tissue was removed. In (a)–(d) and (f) the probe applied load vertically downwards (pinch) to the mantle, downwards deflection of the mechanical record indicates increasing pinch. In (e) the probe applied a horizontal movement.

Fig. 3. Inverted mantle of *Octopus*; sensory activity (upper traces) recorded in the distal stump of a severed stellar nerve in response to a pair of stimulus pulses delivered to the pallial nerve at increasing voltage (a–c, 0.5 V steps). Stimulus pulses are shown on lower trace and as artifacts on upper trace. The shift of baseline is due to movement. The skin and connective tissue of this preparation was left intact.
Fig. 4. Inverted mantle of Octopus; sensory activity (upper traces) recorded in the distal stump of a severed stellate nerve in response to stimulation of the pallial nerve (a–c, 0.5 V increments in the stimulus). The skin and majority of connective tissue was removed from the outer surface of the mantle.
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Fig. 5. Inverted mantle of Octopus; mechanical record of mantle movement (upper traces) and correlated sensory activity recorded in the distal stump of a severed stellar nerve (lower traces); (a) and (b) are separate experiments on the same preparation. The stimulus pulses are shown as artifacts on the lower traces.

marked effect in increasing the rate at which movement proceeded, as arbitrarily indicated by the slope of the record. The latency of the onset of contraction varied slightly with recording position.

These experiments suggested that the sensory responses recorded in the cut stellar nerve were the result of muscle contraction elicited by stimulation of the pallial nerve. In a few preparations records of mechanical movement were made simultaneously with recordings of sensory response from a cut stellar nerve (Fig. 5). The sensory response occurred while the mantle was contracting and usually ceased when the maximum movement for that stimulus had been obtained. The exact form and latency of the mechanical response depended on where the strain gauge was attached to the mantle. The muscle contraction, however, always preceded the sensory response.

Sensory units in the cut stellar nerve were also active during the relaxation of the mantle (Fig. 6). Activity occurred more often after a powerful contraction had been elicited by the summation of stimuli to the pallial nerve (Fig. 6a). There was no way of knowing whether this activity during relaxation resulted from the same units as those active during contraction. Strong or prolonged stimulation of the mantle sometimes elicited activity of tonically firing units in the stellar nerve which continued for some seconds.

There are two thick layers of circular muscle running round the circumference of the mantle, which are separated by a sheet of connective tissue. Methylene blue staining of the living tissue revealed the presence of multipolar nerve cells on the surface of the circular muscle at the interface between the two layers (Fig. 7). Often densely stained when first seen, the cells lost their blue colouration rapidly in the strong light of the photomicroscope, leaving only a short period when the nucleus was clearly visible. The photographs reproduced in Fig. 7 were selected to show either the nucleus or branching processes. Usually one of the processes was longer and thicker than the others and was identified as an axon. Sometimes the axon could be seen joining small bundles of fibres running across the muscle surface, believed to be fine branches of the stellar nerves. Although preparations were made of both sides of the split muscle interface, as well as interior and exterior faces of the whole mantle, these cells were only observed on the outer face of the inner layer of circular muscle (Fig. 8).
Sometimes two or three such cells were seen grouped together, but no estimate can be given yet of their average density or orientation.

**DISCUSSION**

Afferent activity in the stellar nerves of octopuses can be easily elicited by touching, pinching or loading the mantle musculature (Gray, 1960). Preliminary experiments for this study, carried out on a flat mantle preparation, confirmed that while many units having a phasic or tonic discharge responded to a light touch with a probe, others required considerable mechanical force. With such local stimulation, pinching of the mantle was very effective, and so was lateral movement (producing shear forces within the mantle). The question arose as to whether these mechanically responsive units were tactile receptors responding to simple deformation of the body wall, or components of a proprioceptive feedback system. The mantle is a complex mass of tissue with at least three sets of antagonistic musculature. Imposed mechanical stimuli necessarily affect all three layers as well as relative movements between them, and perhaps it is not surprising that length changes applied to the musculature were stimuli with not very consistent responses. Electrical stimulation of the pallial nerve in the flat nerve-muscle preparation, although causing muscle movements, did not usually elicit sensory activity in the stellar nerves.

The experiments with the inverted mantle preparation, where mantle contraction resulting from stimulation of the pallial nerve occurs against the resistance of the cork ball, demonstrate the presence of sensory units responding to muscle contraction alone. In these experiments there are several forms of stimulation occurring within the mantle. There is change in the length and tension of the muscles themselves as well as relative movement between various components, but it is in the development of tension within the mantle that this preparation differs most significantly. The results obtained from the stellar nerve correlate the latency and frequency of the phasic response burst with the rate and extent of muscle contraction. The ease with which these responses are elicited indicates that these conditions of stimulation are more appropriate than a loading of the flat mantle, and resemble to some extent the natural condition of mantle contraction around an enclosed volume of water.

There are other possible ways in which these units could have been stimulated. The mantle musculature is inverted, so that the exterior surface is applied to the ball during muscle contraction. Most of the tactile units seem to be located in the skin (Gray, 1960), but it is possible that some are functional in the connective tissue remaining after removal of the skin. However, it does not seem likely that the responses obtained are due to chance stimulation of such units in the connective tissue by pressure against the cork or movement over irregularities in the surface. Intra-vital methylene blue staining failed to show nerve cells convincingly on this damaged surface. The ball itself is not perfectly smooth, so that the contraction of the mantle over small irregularities could also have the effect of a pinch, so the possibility remains that the activity recorded could have resulted from tactile receptors.

The widely spaced dendrites of the multipolar nerve cells at the interface of the two layers of circular muscle would be easily deformed by length changes in the muscle, or relative movement between the fibres and connective tissue elements. The morpho-
Fig. 6. Inverted mantle of *Octopus*; mechanical record of mantle movement (upper traces) and correlated sensory activity recorded in the distal stump of a severed stellar nerve (lower traces) showing responses both to contraction and relaxation of the muscle. The pair of stimulus pulses is shown as artifact on the lower traces. The stimulus was repeated in (a); (b) results from a separate preparation.
Fig. 7. Examples of multipolar nerve cells located between the circular muscle layers of the Octopus mantle. The fresh cells are stained with methylene blue. The two top photographs show the same cell at different magnifications and stage of staining. Scale mark represents 10 μm.

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Fig. 8. Schematic representation of the Octopus mantle showing the location of multipolar nerve cells between the circular muscle layers.

logy of the nerve cells is very similar to that of muscle receptor cells identified in cephalopods by Graziadei (1964) who has compared them to crustacean stretch receptors. There seems to be no positive way of identifying their function, short of recording directly from one of them, but in view of their position in the mantle and the absence of other sense organs it is proposed that these cells could respond to length or tension changes in the muscle. Of these two possibilities, length change alone is unlikely because of the negative results obtained with the flat preparation. The only sensory cells previously described from the mantle which could be acting as a muscle receptor organ are those of Alexandrowicz (1960). These cells are located on a specialized area of muscle close to the ganglion and beneath the stellar nerves, but their connections with the nerves should have been severed during the preparation for recording and they are unlikely to have contributed to the activity recorded here.

Although Gray (1960) could not record afferent activity resulting from normal breathing movements in the octopus, he showed that clamping of the funnel caused a prolonged expiratory effort and an increased discharge of expiratory motor units from the brain. Thus, if the normal expiratory movement is blocked, the centrally generated breathing rhythm is interrupted and the presence of sensory feedback is implicated. Mellon (1969) showed that the sequence of swimming adductions by the scallop Aequipecten relied on adequate stretch of the adductor muscle between adductions. This led him to describe the control of swimming in the scallop as basically a myotactic stretch reflex and to postulate the presence of stretch receptors in the adductor muscle.

In the present study, the responses to stimulation of the pallial nerve seem likely
to be related to the more powerful contractions of the whole mantle required for jetting rather than respiratory movements. Large numbers of afferent nerve fibres are known to enter the stellate ganglion and terminate there or pass through to the pallial nerve and into the brain (Young, 1965, 1972). Co-ordinated motor commands to the mantle arise from the palliovisceral lobe of the brain and are relayed to the musculature via the motor neurons of the stellate ganglion. There are two sites at which sensory feedback from the mantle could reasonably modify the motor control. Alterations to the frequency and rate of the contraction/relaxation cycle would have to take place within the brain, but more detailed control of muscle tension could well be effected at the level of the stellate ganglion, ensuring, for example, even and symmetrical contractions. Unlike the brain, the cells of the stellate ganglion are relatively accessible to microelectrodes. The activity of these cells is currently under study (Fig. 9) but as yet there is no proof of its modification by afferent input. Although several categories of cells are present in the ganglion, individual somata cannot be routinely identified and characterized in the way possible for many gastropods (Kater & Rowell, 1973; Dorsett 1975).

Many questions arise as to the precise function of any sensory feedback in the co-ordination of antagonistic muscle groups. A study of the responses of the mantle receptors to controlled conditions of mantle stimulation is really essential, but difficult in the absence of a means of reliably stimulating one particular muscle type by imposed length changes. All of the stellar nerves appear to carry a mixed population of motor fibres to the several muscle layers, as well as sensory fibres. Both the pattern of muscular innervation and the functional organization of the stellate ganglion are largely unknown. Better pictures of the branching of the sensory cells might illustrate their relationship to particular muscle blocks or supportive elements.

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REFERENCES


Fig. 9. Examples of continuously firing units recorded within the stellate ganglion of *Eledone*.
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