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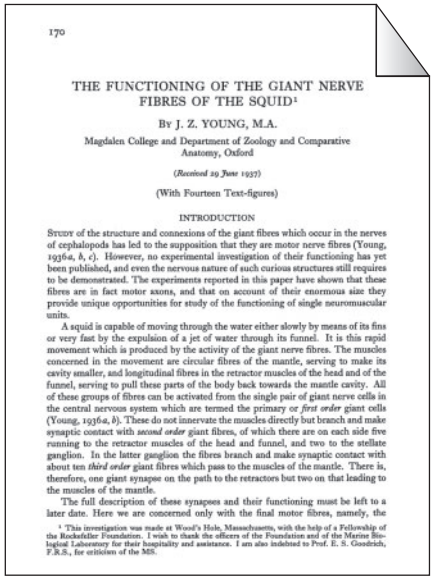
JEB Classics

J.Z. AND THE DISCOVERY OF SQUID GIANT NERVE FIBRES



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THE FUNCTIONING OF THE GIANT NERVE FIBRES OF THE SQUID[1]

By J. Z. YOUNG, M.A.

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(*Received 29 June* 1937)

(With Fourteen Text-figures)

INTRODUCTION

Study of the structure and connexions of the giant fibres which occur in the nerves of cephalopods has led to the supposition that they are motor nerve fibres (Young, 1936*a*, *b*, *c*). However, no experimental investigation of their functioning has yet been published, and even the nervous nature of such curious structures still requires to be demonstrated. The experiments reported in this paper have shown that these fibres are in fact motor axons, and that on account of their enormous size they provide unique opportunities for study of the functioning of single neuromuscular units.

A squid is capable of moving through the water either slowly by means of its fins or very fast by the expulsion of a jet of water through its funnel. It is this rapid movement which is produced by the activity of the giant nerve fibres. The muscles concerned in the movement are circular fibres of the mantle, serving to make its cavity smaller, and longitudinal fibres in the retractor muscles of the head and of the funnel, serving to pull these parts of the body back towards the mantle cavity. All of these groups of fibres can be activated from the single pair of giant nerve cells in the central nervous system which are termed the primary or *first order* giant cells (Young, 1936*a*, *b*). These do not innervate the muscles directly but branch and make synaptic contact with *second order* giant fibres, of which there are on each side five running to the retractor muscles of the head and funnel, and two to the stellate ganglion. In the latter ganglion the fibres branch and make synaptic contact with about ten *third order* giant fibres which pass to the muscles of the mantle. There is, therefore, one giant synapse on the path to the retractors but two on that leading to the muscles of the mantle.

The full description of these synapses and their functioning must be left to a later date. Here we are concerned only with the final motor fibres, namely, the

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Richard Keynes writes about J. Z. Young's 1938 ground-breaking publication on the function of squid giant nerve fibres. A PDF file of Young's paper can be accessed as supplementary data at jeb.biologists.org

J.Z., to give him the title by which he was universally known, initially acquired an interest in cephalopods when working in Naples with Enrico Sereni in 1932 on the axons in the mantle connectives and stellar nerves of octopus. This led him to further studies at the Plymouth Marine Laboratory of some structures in the mantles of squid that he tentatively identified as giant nerve fibres (Young, 1936). In the summer of 1936 he visited Woods Hole in Massachusetts, determined to prove that these 'curious structures' were in fact motor axons. With F. O. Schmitt and R. Bear, he successfully examined the axoplasm of axons from the mantle of the squid Loligo pealii with polarized light, but failed in attempts with Ralph Gerard, Detlev Bronk and Keffer Hartline to make any oscilloscope recordings of action potentials from single fibres. However, he and Hartline did better one day when they found that application of a solution of sodium citrate to one end of the supposed axons generated a rhythmic discharge at the other, showing that they were indeed nerve fibres. He then made a careful study of the anatomy of the mantles, and in his classical paper on '*The functioning of the giant nerve fibres of the squid*' (Young, 1938), he showed that the third order giant axons served to bring about the precisely coordinated contraction of the mantle causing expulsion of a powerful jet of water propelling the animals rapidly backwards or forwards according to the position of the funnel, sometimes accompanied by a slug of 'ink' to assist the animal's escape.

Having confirmed that the squid giant axons did conduct action potentials, and having with R. J. Pumphrey in 1938 (Young and Pumphrey, 1938) looked at the effect of their diameter on the rate of conduction, the only respect in which J.Z. subsequently involved himself in research on the ionic basis of conduction was to measure their electrolyte content (Young and Webb, 1945). He did, nevertheless, devote many years to an important series of observations at the Zoological Station in Naples on the mechanism of memory in octopus. And always interested in the animal as a whole he was working vigorously in the laboratory till the very end of his life on a wide range of problems. He will also be remembered as a teacher of great distinction, and as the author of two outstandingly wise and well-written textbooks on vertebrates and invertebrates.

It was, however, the introduction of giant nerve fibres by J.Z. that enabled the biophysics and biochemistry of excitable membranes to be properly studied in depth, which was said by Alan Hodgkin in 1973 to have done more for axonology than any other single advance in technique during the previous 40 years. J.Z. neatly summed up the impact that the discovery of giant motor axons would have on the field when he wrote '*on account of their enormous size [the squid's giant nerve fibres] provide unique opportunities for study of the functioning of single neuromuscular units*' (Young, 1938).

The first step in the exploitation of squid axons was taken in 1938 at Woods Hole by Kacy Cole and H. J. Curtis (Cole and Curtis, 1939) when they showed using external electrodes that during the passage of an impulse there was a rise and fall of the membrane conductance whose time course was very similar to that of the action potential. Then in the summer of 1939, both Curtis and Cole (1940, 1942) at Woods Hole, and Alan Hodgkin and Andrew Huxley (Hodgkin and Huxley, 1939) at the Laboratory of the Marine Biological Association in Plymouth, succeeded in slightly different ways in pushing long glass tubes, 0.1 mm in diameter and filled with K^+ solutions, for some distance into the axons and thus recording the potential internally from an undamaged part of the membrane. To their great surprise they found that at the peak of

the conducted impulse the membrane potential did not, as was expected, fall close to zero, but was in fact substantially reversed.

After the end of six years of war that had interrupted biological research, the problem of accounting for the reversal of potential at the peak of the spike still remained unsolved. In writing up their 1939 experiments at greater length, Hodgkin and Huxley (1945) presented four elegantly argued alternative explanations, in none of which it was obvious that they had any faith. But then Alan Hodgkin dared to suggest that the permeability of the membrane to Na^+ ions might undergo a transient increase. Working with squid giant axons at Plymouth in 1947, he and Bernard Katz were able to establish that the sodium theory was sound (Hodgkin and Katz, 1949). As has been described vividly by Hodgkin in his autobiography *Chance & Design* (Hodgkin, 1992), the great experimental triumph that came next was his and Huxley's development at Plymouth of the voltage-clamp technique for the quantitative analysis of the relationship between current and voltage in an excitable membrane (Hodgkin and Huxley, 1952).

There followed a series of research projects on related questions, for example the measurement of the net movements during the nerve impulse of sodium and potassium by Keynes and Lewis (1951); the establishment by Hodgkin and Keynes (1955a) of the existence of the sodium pump; studies by Caldwell, Hodgkin, Keynes and Shaw (1960) on the dependence of the sodium pump on a supply of phosphate-bond energy from ATP and arginine phosphate; the discovery of Hodgkin and Keynes (1955b) in Cambridge, using cuttlefish giant axons, of the manner in which K^+ ions diffused in single file through the voltage-gated potassium channels in nerve membranes; and to crown Hodgkin's direct participation in experiments on squid axons, the development of a method for perfusing them with a variety of solutions after squeezing out the axoplasm as described by Baker, Hodgkin and Shaw (1962), in order to carry out further rigorous tests of the ionic theory.

During the 1960s and 1970s, experiments on squid giant fibres continued to occupy many axonologists, an advance of particular interest being the records made for the first time independently at Woods Hole by Armstrong and Bezanilla (1974) and at Plymouth by Keynes and Rojas (1974), of the sodium gating current. The existence of such currents generated by the transmembrane movements of the charged gating particles had been predicted by Hodgkin, but they had not previously been recorded because of their small size relative to the ion currents. Then in 1984 Numa and his colleagues in Kyoto had succeeded, as described by Noda et al. (1984), in cloning the sodium channel gene of the electric eel, and soon the primary amino acid sequences of the voltage-gated sodium, potassium and calcium channels in a great many animals were known. What is more, it turned out that the channel proteins could readily be expressed in *Xenopus* oocytes, where their properties could conveniently be examined by the patch-clamping techniques first developed by Neher and Sakmann (1976). Research on these lines is now being vigorously pursued in many laboratories all over the world on the properties of ion channels gated not only by membrane potential, but also by other agents.

Such work could be regarded as the ultimate offspring of J.Z.'s introduction of giant axons to biologists, though few of its practitioners have ever seen a squid. But as a postscript it may be added that for technical reasons the time resolution obtained when voltage-clamping a squid giant axon is appreciably better than when voltage-clamping a patch of oocyte membrane, and for the best records yet made of the time course of the rise and fall of the sodium gating current in a squid axon the reader should refer to those obtained in the old-fashioned way by Keynes and Elinder (1998) and their colleagues.

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A squid is capable of moving through the water either slowly by means of its fins or very fast by the expulsion of a jet of water through its funnel. It is this rapid movement which is produced by the activity of the giant nerve fibres. The muscles concerned in the movement are circular fibres of the mantle, serving to make its cavity smaller, and longitudinal fibres in the retractor muscles of the head and of the funnel, serving to pull these parts of the body back towards the mantle cavity. All of these groups of fibres can be activated from the single pair of giant nerve cells in the central nervous system which are termed the primary or *first order* giant cells (Young, 1936*a*, *b*). These do not innervate the muscles directly but branch and make synaptic contact with *second order* giant fibres, of which there are on each side five running to the retractor muscles of the head and funnel, and two to the stellate ganglion. In the latter ganglion the fibres branch and make synaptic contact with about ten *third order* giant fibres which pass to the muscles of the mantle. There is, therefore, one giant synapse on the path to the retractors but two on that leading to the muscles of the mantle.

The full description of these synapses and their functioning must be left to a later date. Here we are concerned only with the final motor fibres, namely, the

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second order axons running to the retractors and the third order axons running to the mantle muscles.

The first aim of the investigation was to discover whether these large structures are in fact motor nerve fibres. This having been shown the second aim became to discover whether each fibre with its muscles behaves as a single neuromotor unit in the manner of vertebrate motor neurons (see Sherrington, 1929; Kato, 1936). This latter problem is of particular interest since the third order giant axons have an unusual syncytial structure, being formed by fusion of the processes of numerous nerve cells (Young, 1936*a*).

STRUCTURE AND INNERVATION OF THE MANTLE

The mantle of *Loligo* is used for respiration as well as for locomotion. It is a deep sack, closed at the hind end, water being taken in anteriorly at the sides and expelled through a narrow tube, the funnel, also at the front end, suitable valves operating to close the lateral inlets during expulsion. During life a continual slow rhythmical expansion and contraction of the cavity of the mantle is kept up, providing a flow of water over the gills. In addition to this rhythmical movement the sack can also be suddenly contracted, causing expulsion of the powerful jet which propels the animal rapidly along, either forwards or backwards according to the position of the funnel, a stream of "ink" being sometimes ejected at the same time to provide cover for the animal's escape.

The muscles which produce the movements of the mantle include circular fibres running around the sack and radial fibres running through its thickness from the inside to the outside (Fig. 1). There is also a thin layer of longitudinal muscles on the outer surface of the very front end of the mantle, but this layer extends only over a very small part of the surface and has been neglected in the present account. The circular muscles have been described by Marceau (1906) and Burian (1908), but the radial fibres appear to have been overlooked by previous workers.

The circular fibres are uninucleate and very long. They are joined to each other end to end, the whole mass being attached dorsally to the pen. The radial fibres form sheets which divide the circular fibres into blocks. These sheets of radial fibres, however, are not continuous, but broken up, especially laterally, as shown in Fig. 1, in which the section is thick enough to include both circular and radial fibres.

The presence of two sets of antagonistic fibres mixed in the same mass of muscles appears to be characteristic of the cephalopods, since it is found also in the retractor muscles of the head and funnel (see p. 181). For proper working of such a system particular conditions of rigidity and elasticity are necessary and these are apparently supplied by a very complex system of connective tissue fibres found in these muscles. There is one set of such fibres lying in the plane of the circular muscles, but running obliquely in all directions across the latter. A second set of connective tissue fibres lies in the plane of the radial muscles.

According to Plenk (1933), who investigated the fine structure of the muscle fibres of various organs in cephalopods, a true cross striation is present in all of them.

It appears from the structure of the mantle, therefore, that water is taken into the sack by contraction of the radial muscles, serving to make the wall thinner and the cavity greater. The mantle is like a balloon which has the power of blowing itself up by stretching the wall. Such a mechanism of intake is highly peculiar, and does not seem to have been reported from any other group of animals. There is in this paper very little evidence as to the functions of the radial fibres (see p. 178), but

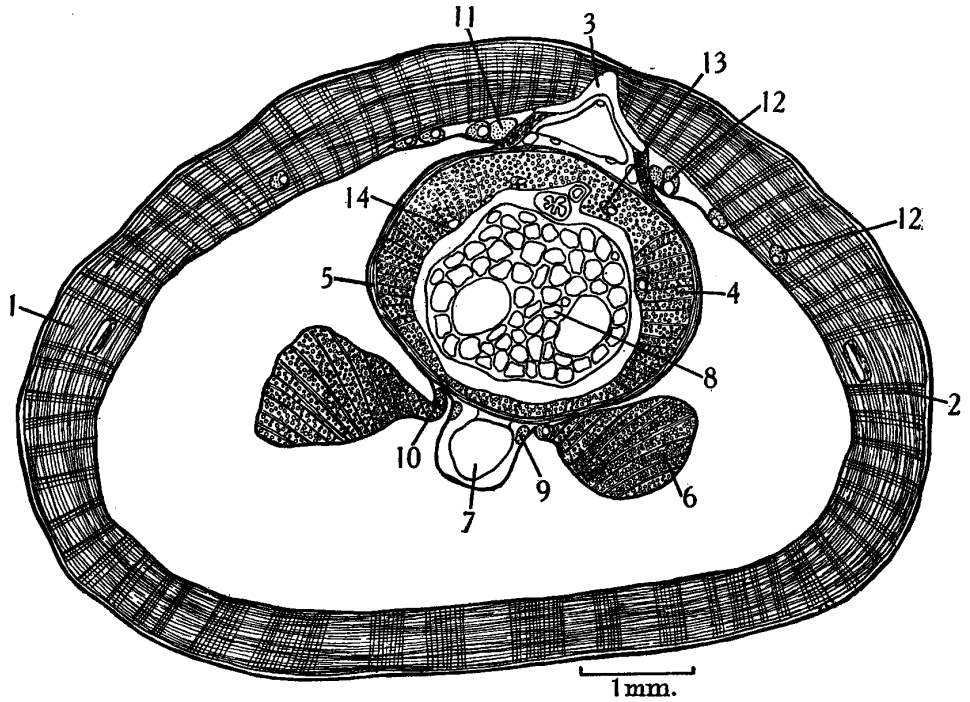

Fig. 1. Diagram of a transverse section through the mantle of a young *Loligo pealii*. The irregular shape is due to distortion during fixation. 1, circular muscle fibres of mantle; 2, radial muscle fibres of mantle; 3, pen; 4, longitudinal muscle fibres of m. retractor capitis; 5, circular muscle fibres of the same; 6, longitudinal muscle fibres of m. retractor (depressor) infundibuli; 7, vena cava anterior; 8, digestive gland; 9, n. visceralis; 10, branch of same to m. retractor infundibuli; 11, fin nerve; 12, stellar nerves; 13, 14, branches of n. retractor capitis posterior; 10, 12, 13 and 14 contain one giant fibre each. The large fibre in 14 is the one which supplies the hind part of m. retractor capitis and of m. retractor infundibuli.

their direction of action, and the absence of any other antagonists for the circular muscles, leaves little doubt as to their mode of action.

Whatever may be the mechanism of expansion of the mantle cavity it is certain that contraction of the circular muscles expels the water, either gently, as in respiration, or violently in sudden movements. It is the nerves producing this contraction of the circular muscles which have been principally studied in the present work.

In *Loligo pealii* (Leseuer), the species of squid most commonly found at Wood's Hole, the mantle is supplied by between nine and eleven stellar nerves, which radiate

from the stellate ganglion (Fig. 2). Each of these nerves contains one giant fibre and numerous smaller ones, the diameters of the giant fibres increasing progressively

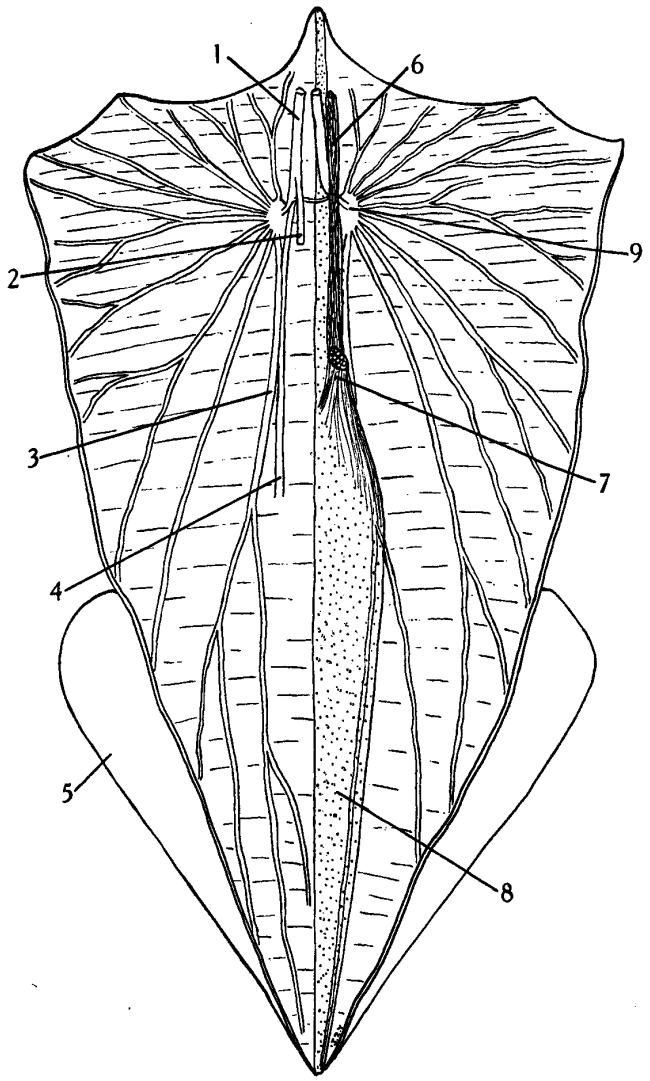

Fig. 2. Drawing of mantle of *Loligo*, cut open ventrally and seen from the ventral side. About one-third natural size. The pen and retractor muscles have been removed on the animal's left side. 1 pallial nerve; 2, n. retractor capitis posterior; 3, hindmost stellar nerve; 4, fin nerve; 5, fin; 6, m. retractor capitis posterior; 7, m. retractor (depressor) infundibuli (cut across); 8, pen; 9, stellate ganglion.

from 100μ or less in the anterior and shorter stellar nerves to 800μ or more in the hindmost and longest nerve. The giant fibres branch repeatedly among the muscle fibres but the nature of their final terminations has not yet been made out.

METHODS

For investigation of the contractions of the circular muscles of the mantle the hindmost stellar nerve and the territory which it innervates proved to be convenient (Fig. 3). The animal is opened by cutting with scissors along the mid-ventral line and the pallial nerve, which joins the stellate ganglion to the central nervous system, severed on both sides. With fine scissors or scalpel the hindmost stellar nerve is then freed from the visceral mass on each side, after which the whole of the viscera can readily be removed, starting from the anterior end. This leaves only the stellate ganglia, mantle and pen, which latter can then also be carefully lifted away, starting from the hind end.

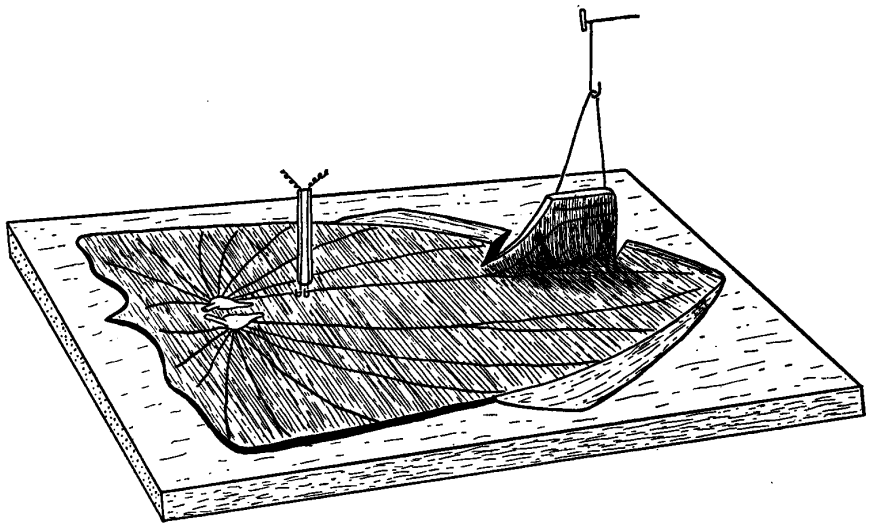

Fig. 3. Diagram of arrangement of mantle as prepared for recording contractions of the circular muscle fibres after stimulation of the hindmost stellar nerve.

The portion of the hindmost stellar nerve which is to be stimulated can next be isolated by dissecting the fin nerve away from it, tying it with cotton close to the stellate ganglion and cutting it away from the latter. About 3 cm. of nerve can very easily be prepared in this manner, and if required a further 5 cm. or more can be added without great difficulty by further dissection of the nerve as it runs through the muscles.

The piece of muscle chosen for recording is finally cut free with a scalpel as shown in Fig. 3 and attached to a lever with glass or silver hooks, or, quite satisfactorily, by threading a piece of cotton through it with a needle. The preparation is pinned down to a piece of cork, and the thread attached so that the circular fibres of the mantle pull vertically. Both isotonic and semi-isometric levers were used, recording on a smoked drum.

Stimulation of the nerve was usually by means of single condenser discharges through chlorided silver electrodes placed under the nerve. Make and break induction shocks were also tested. Sea water was used throughout as a medium.

The whole preparation can be completed in about 10 min. Unfortunately the muscles do not last well after isolation and the responses obtained gradually decline. However, with animals in good condition preparations which last for half an hour or more can be obtained.

STIMULATION OF INTACT STELLAR NERVES

If an entire stellar nerve be stimulated at 15 sec. intervals with discharges of a 0·04μF. condenser and progressively increasing voltage, then it is found

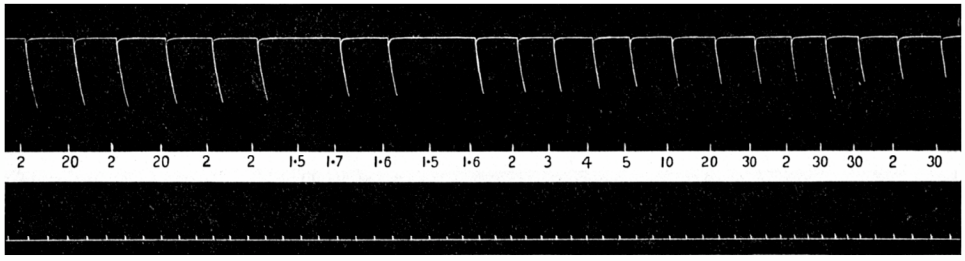

Fig. 4. Stimulation of intact stellar nerve. Figs. 4, 6, 7 and 8 all show semi-isometric contractions of the circular fibres of the mantle when arranged as in Fig. 3, the nerve being stimulated with discharges of a 0·04μF. condenser, previously charged to the voltages shown on the figures. Time intervals 6 sec.

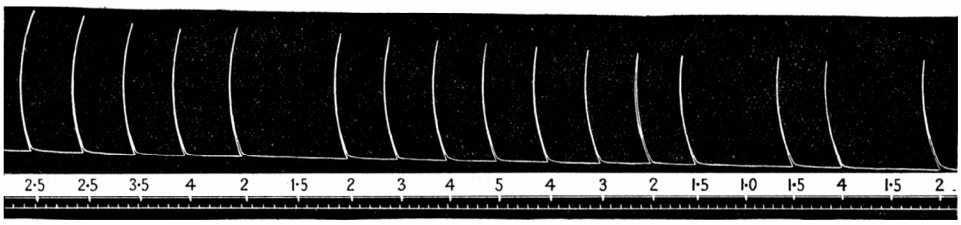

Fig. 5. Isotonic contractions of circular fibres of mantle, stimulation of intact stellar nerve. Time intervals 3 sec.

that at about 1 V., after a small increase of voltage, a very large twitch of the circular fibres of the muscle appears (Figs. 4, 5 and 6). Lesser voltages produce no contraction at all, and further increase of the voltage applied does not lead to any increase of the tension developed isometrically or the amplitude of contraction isotonically. The only exception to this rule is that at about ten times threshold there is often a slight increase in the tension above that produced at lower voltages (Figs. 4 and 6).

The experiments described in the next section will show that this contraction of the circular muscles is due to the activity of the giant fibre in the nerve. Since at all voltages above threshold a constant response is produced, it seems probable that the effect of stimuli such as those used is to set up a single impulse in the giant fibre which in turn excites all the muscle fibres which it reaches. Presumably the increased response obtained at about ten times threshold voltage is due to the setting up of two or more impulses in the nerve, but the proof of this requires investigation of the action potentials in the nerve. Preliminary studies (Bronk, Gerard, Hartline and Young unpublished) have shown that condenser discharges usually set up single impulses in the nerve, but the conditions for repetitive firing have not yet been investigated. Kato (1936) found closely similar increases in response with strong stimuli during his investigation of single neuro-motor units in the frog.

Using break induction shocks exactly similar results are obtained. At the threshold, which is very low, the stimulus produces a large response, which is not further increased even by great increase in the intensity of stimulation.

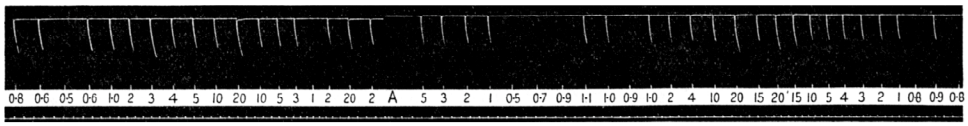

Fig. 6. During the first part of the record the whole of the stellar nerve was stimulated, then at *A* all of the fibres in the nerve except the giant fibre were cut at a point 2 cm. from the electrodes. The responses to the right of *A* are therefore due to the activities of the giant fibre alone.

STIMULATION OF GIANT FIBRE ALONE

By careful preparation under a low-power binocular microscope all the smaller nerve fibres can be removed from a stretch of stellar nerve, leaving only the giant fibre. Stimulation of this with condenser discharges gives results similar to those from stimulation of the intact stellar nerve (Fig. 6), there being a threshold at which nearly maximal contractions appear. This threshold can be determined very accurately so that an increase of 0·01 V. applied to the condenser will produce the response. With further increase in applied voltage the tension developed remains constant except for a slight increase at voltages greatly above threshold, presumably due to repetitive firing.

To avoid the rather difficult task of cleaning a long section of the giant fibre without damaging it, all the smaller fibres can be cut at some point between the stimulating electrodes and the muscle. In this way single fibre preparations of the giant fibre can very easily be obtained.

STIMULATION OF SMALLER FIBRES IN THE STELLAR NERVES

If the giant fibre be destroyed, by pricking with a needle, at some point between the stimulating electrodes and the muscle, leaving the other fibres in the nerve intact, then stimulation will still produce contraction of some of the circular muscles of the mantle. The tension which can be developed in this way is always very much

less than that produced by the activity of the giant fibre, and unlike the latter it *can be produced in graded steps*, increasing voltages producing increased tension up to a maximum (Figs. 7 and 8). Presumably increasing numbers of nerve fibres are being brought into action. The threshold for stimulation of the smaller fibres in the nerve is usually higher than that for stimulation of the giant fibre, but in a few cases a small graded contraction appears on stimulation of the whole stellar nerve, at voltages lower than that at which the large response due to the giant fibre appears (Fig. 8). Presumably in these cases the large fibre is unfavourably placed for receiving the current, so that enough of the small fibres are stimulated to produce some contraction at voltages still too low to stimulate the giant fibre.

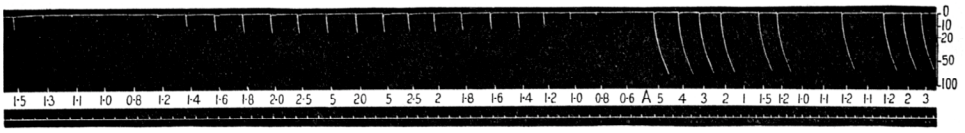

Fig. 7. Stimulation of small fibres in a stellar nerve. During the first part of the record stimulation was through electrodes placed central to a point at which the giant fibre had been pricked, the responses are graded. Then at *A* the electrodes were moved to a point peripheral to the prick, and subsequent responses therefore involve the activity of the giant fibre. The calibration of the lever, in grams, is shown at the right. Figs. 4, 6, 8, 9 and 14, are from the same lever but have been somewhat less reduced in reproduction.

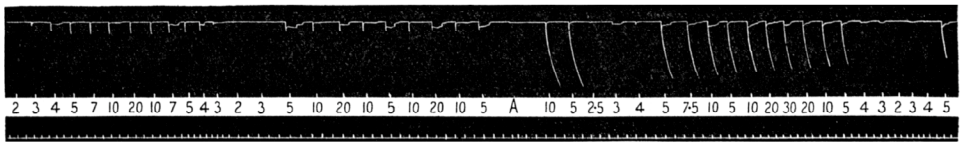

Fig. 8. Experiment exactly as in Fig. 7. During the part of the record lying to the left of *A* responses are to stimulation of the smaller fibres only, to the right of *A* the giant fibre was being stimulated. Note the persistence of tension after some of the twitches and also that the threshold for the giant fibre was, in this experiment, higher than that of some of the smaller fibres in the nerve.

It cannot at present be decided whether these graded contractions produced by stimulation of the smaller fibres in the nerve are due to the shortening of muscle fibres which are also innervated by the giant fibre, or to a different set of muscles. It has not yet been possible by histological studies to differentiate two types of circular muscle fibre or to decide whether any one fibre receives nerve endings both from the giant and from smaller nerve fibres. However, the physiological analysis has already been sufficient to show that the neuromuscular mechanism allows of the production of two types of response on the part of circular fibres in the mantle, one graded and smaller and the other much larger, and total, involving the simultaneous contraction of all the fibres. The presumption is that the lesser contraction, operated by the smaller fibres in the nerve, is that used in the gentle respiratory movements, while the maximum contraction produced by a single impulse in the giant fibre ejects a propulsive stream of water.

TIME COURSE OF THE CONTRACTIONS

No thorough investigation has yet been made of the time course of the contractions produced by either set of fibres. The twitches set up through the giant fibre are quick (Fig. 9), the maximum tension being reached about 60 ms. after the beginning of the contraction and relaxation being over in about 200 ms. under the conditions of the experiment.

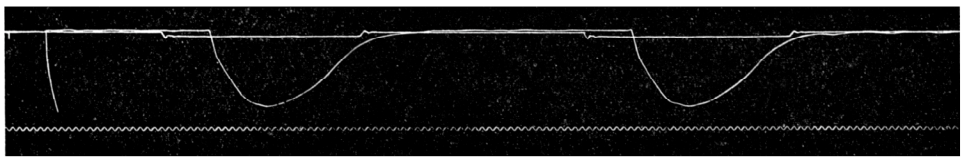

Fig. 9. Semi-isometric twitches of circular fibres of mantle muscles prepared as in Fig. 3. Response to discharge of 0·04 μF. condenser, charged to 5 V., applied to whole stellar nerve. The signal marks the throw of the discharging switch. To the left of the record the distance of the two levers at rest is shown by means of a stimulus given with the drum stationary. Tuning fork 100 d.v. per sec.

After stimulation either of the whole stellar nerve or of the smaller fibres only, the quick twitch is sometimes followed by a small further contraction which lasts for some seconds (Fig. 8). It remains uncertain whether this persisting response is due to sustained activity on the part of the nerve or muscle fibres. Probably it is of no significance in the normal life of the animal.

CONTRACTION OF THE RADIAL MUSCLES OF THE MANTLE

It has not yet been found possible to obtain a preparation in which the activity of the muscle fibres which run through the thickness of the mantle is recorded

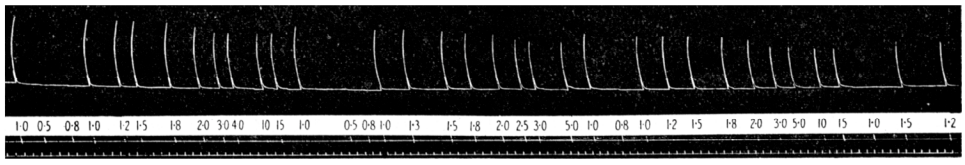

Fig. 10. Isotonic responses of circular fibres of the mantle to stimulation of the intact stellar nerve. Note the *reduction* of the response with increase of the stimulus intensity above threshold, presumably on account of the contractions of the radial fibres in the mantle. Time intervals 6 sec.

directly. However, in a few cases after stimulation of the whole stellar nerve it was found that with increase of the applied voltage above threshold the tension or amplitude of contraction of the circular fibres *decreased* progressively until it was reduced by as much as one-fifth in extreme cases. Lowering of the strength of stimulus then caused an *increase* in the response to its original threshold value (Fig. 10). This progressive reduction at higher voltages can be interpreted as the

result of the stimulation of more and more of the nerve fibres which activate the radial muscles and thus decrease the effect of the circular muscles set in action through the giant fibre.

NEUROMUSCULAR UNITS IN THE MANTLE

For purposes of maximal contraction, therefore, each side of the mantle is divided into about 10 motor units each controlled by a single nerve fibre. It must be remembered, however, that each of these giant fibres in the stellar nerves is a syncytium, produced by the fusion of the axons of a great number of cells in the stellate ganglion (Young, 1936*a*, *b*). Histological investigation has led me to suppose that this fusion is complete, producing a true single neural unit, provided with a single continuous surface membrane, and a single myelin-like sheath (Bear *et al.* 1937). This conclusion is now amply confirmed by the demonstration that each giant fibre is a functional unit which can only be set into action maximally.

The fact that the territory innervated by each stellar nerve constitutes functionally a single neuromotor unit can also readily be demonstrated in various other ways without recording its contraction with a lever. Thus if condenser discharges are applied with an electrode placed in various parts of the territory a stimulus which is strong enough to cause any contraction will always be found to cause contraction of the whole unit, irrespective of the position of the electrode, whether near to the ganglion or to the edge of the piece. In the latter case the impulse is presumably set up in the finer branches of the giant fibre and conducted antidromically towards the ganglion. By cutting up the muscle in various ways it can be shown that if one part of a unit is stimulated all other parts will always contract provided suitable connexions are left to allow the impulse to run back up the nerve and down again (Fig. 11). In this way an exact counterpart of the *gracilis* experiment can be performed with the branches of a single nerve fibre. When an electrode is placed in the muscle itself it is occasionally possible to produce local contractions by direct stimulation of the muscle fibres themselves, but usually stimulation of the finer branches of the giant fibre occurs before that of the muscle fibres, the whole unit being thus set into action.

This experiment also shows that there is no functional connexion in the ganglion between the individual giant nerve fibres, since stimulation in one unit never produces contractions in its neighbours. There has been much debate about the possibility of obtaining reflex responses or axon reflexes through the isolated stellate ganglion (see Sereni & Young, 1932). No evidence of them has been seen during the present work, but since the ganglia of the squid are even more susceptible than those of octopods to oxygen lack, the caution of Fröhlich (1910) that such reflexes must only be expected in very fresh preparations applies with especial force.

A further demonstration of the action of whole masses of muscle as single units can be given by making use of the fact that the muscles rapidly become opaque when stimulated (Sereni & Young, 1932). Under favourable conditions this opacity can be seen to develop after the application of only a few stimuli to the nerve,

and if the stimulation be continued for a short while the whole unit which is contracting will become quite opaque and sharply demarcated from the still transparent muscles on either side of it (Fig. 12). The converse of this experiment can be performed by cutting one of the stellar nerves of the animal shortly before it is

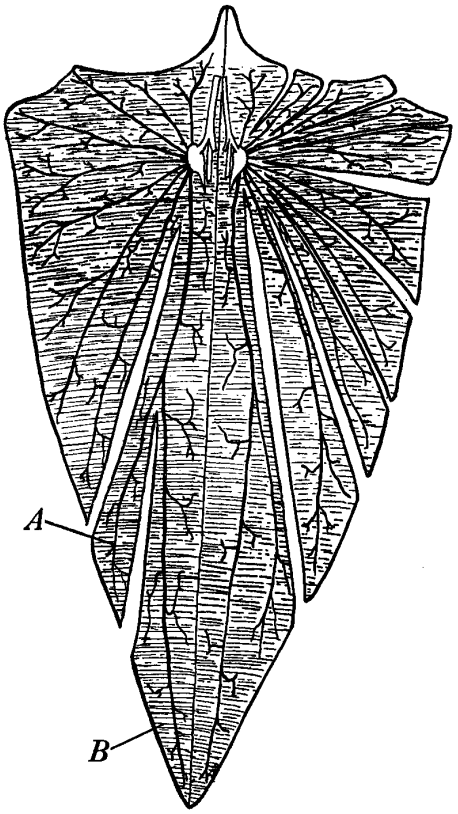

Fig. 11.

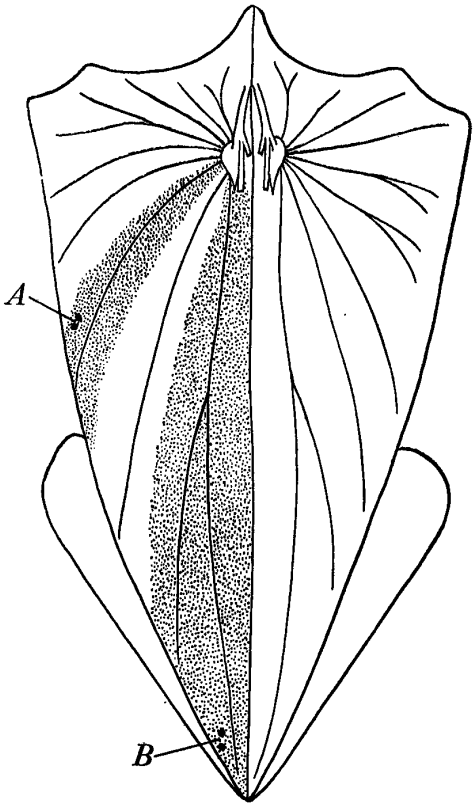

Fig. 12.

Fig. 11. Diagram of the mantle of *Loligo* cut up, on the animal's left side, into its separate motor units. Stimulation in any part of one of these causes contraction of the whole unit, but not of any part of any other unit. On the opposite side the muscles innervated by the two main branches of the hindmost stellar nerve have been separated by a cut. Stimulation in one part, say at *A*, causes contraction in the other part *B*.

Fig. 12. Diagram of the mantle of *Loligo* to show the areas which have become opaque after local stimulation. Electrodes were placed at *A* and *B*, and after the application of a few stimuli the whole of the units in which the electrodes lie become opaque.

killed. During the process of death of the animal the muscles become opaque, presumably because of the imperfect removal of products liberated during contraction. But when one of the stellar nerves has been cut the unit which it innervates does not thereafter contract at all, and hence remains as a strikingly transparent window in the otherwise opaque mantle.

INNERVATION AND RESPONSES OF THE RETRACTOR MUSCLE OF THE FUNNEL

The retractor muscles of the head and funnel are very suitable for myographic purposes. The apparatus by means of which the head is drawn backwards includes two sets of muscles, first, the short m. retractor capitis anterior, running from the cephalic to the nuchal cartilage and innervated by the n. retractor capitis anterior

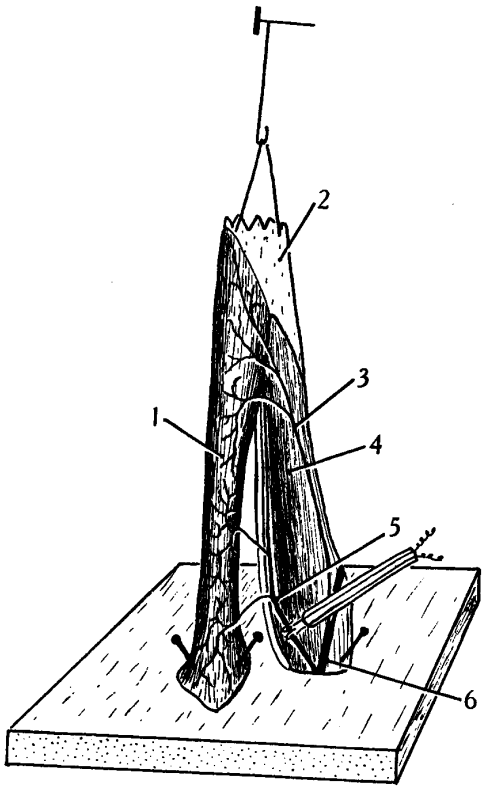

Fig. 13. Diagram of arrangement for studying the contraction of the retractor muscles of the head and funnel. The electrodes are placed for stimulation of the visceral nerve, giving the responses shown in Fig. 14. 1, m. retractor (depressor) infundibuli; 2, pen; 3, n. retractor capitis posterior, showing its branches to the retractor muscle of the funnel. The branches of this nerve to the head retractor are not shown; 4, m. retractor capitis posterior; 5, visceral nerve; 6, pallial nerve.

which does not contain any very large fibres, and secondly, the much larger m. retractor capitis posterior. This latter muscle runs from the nuchal cartilage to the pen and consists predominantly of longitudinal fibres, though in its lateral part there are also transverse fibres running through the thickness of the muscle, the contraction of these presumably causing protrusion of the head (Fig. 1).

Particularly suitable for experimental purposes is the muscle which draws in the funnel and is usually known as the m. depressor infundibuli, though m. retractor

infundibuli is a more suitable name in view of the function of the muscle and of its affinity with the retractors of the head (see below). The muscle consists mainly of parallel longitudinal fibres, but, as in the case of the mantle muscles and head retractors, antagonistic fibres are combined in the same muscle mass, being in this case transverse fibres running across the muscle (Fig. 1).

The innervation of the retractors of the head and funnel is somewhat complex since m. retractor (depressor) infundibuli, in addition to two giant fibres which it receives through branches of the visceral nerve, receives one also through n. retractor capitis posterior (Fig. 13). This latter nerve contains altogether three giant fibres, two smaller ones to the anterior parts of m. retractor capitis posterior and a large one which sends branches to the hind parts of these muscles, and also, as mentioned above, to m. retractor infundibuli. The fact that portions of the head and funnel retractors are innervated by the same axon emphasizes their close functional relationship. Both of the muscles also receive numerous smaller nerve fibres.

To prepare these muscles for myographic purposes the animal is opened ventrally and the whole of the posterior visceral mass removed. A transverse cut is made across the mantle and pen behind the attachment of m. retractor infundibuli, and a

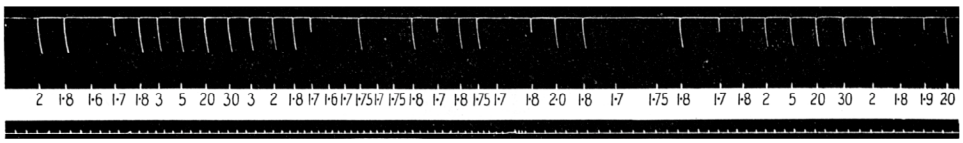

Fig. 14. Semi-isometric contractions of retractor muscle of funnel, in response to stimulation of the visceral nerve with discharges of a 0·04 μF. condenser charged to the voltages shown. There are two giant fibres in the nerve and with increasing voltage the tension develops in two steps.

portion of the pen, with the required muscles attached, can then be separated completely from the mantle. The digestive gland must be carefully removed, exposing the visceral nerve and n. retractor capitis posterior (a branch of the large pallial nerve). Finally the whole of the head region is cut away to the level of the hind end of the cephalic cartilage, the retractors of one side are completely removed and those of the other pinned down at the anterior end, and hooks attached either to the remainder of the pen or to the muscle itself. This whole preparation can be done in a few minutes, and the muscle will continue to show good responses for a further half-hour or longer.

The preliminary investigation which has been made with this preparation, and especially of m. retractor infundibuli, has shown responses to stimulation of the visceral nerves closely similar in a general way to those obtainable from the circular muscles of the mantle. There is, however, the important and interesting difference that, when stimulating the visceral nerve with condenser discharges of increasing voltage, the tension developed by the muscle increases in *two well-marked steps* (Fig. 14). This corresponds with the fact that there are two giant fibres of slightly differing diameters in this nerve. The thresholds for the two lie very close together, and in some cases it proved impossible to separate them, even by increasing the

applied voltage by very small amounts, though in many cases the two steps could be clearly distinguished.

In some preparations evidence of small graded contractions similar to those produced by stimulation of the small fibres in the stellar nerves was seen. Presumably these small fibres are responsible for the movements of the muscles during respiration.

DISCUSSION

The experiments in which single isolated giant fibres were stimulated supply proof that these structures are motor axons. It has also been shown that in spite of their syncytial nature they conduct in an all-or-nothing manner, constituting, with the muscle fibres which they innervate, single motor units, fully comparable with those into which the vertebrate muscular system is divided (Lucas, 1909; Pratt, 1917; Adrian, 1933). Each unit, however, is capable of developing an enormous tension. Even the small strips used in the above experiments develop over 100 g. and the whole of the unit of which they form a part must develop nearly a kilogram. This may be compared with the largest tension per unit found by Eccles & Sherrington (1930) in the cat, namely 30·1 g. in the median head of gastrocnemius, which is composed of 430 units. The ease with which the single nerve fibres can be isolated in the squid, and the large number of muscle fibres which each innervates, make this system ideal for showing the working of such single neuromotor units. The demonstration is particularly clear since preparations with one, two and many units can be obtained, and the all-or-nothing, two-step and graded twitches which they produce compared.

No evidence has been seen in the squid for variations in the response of the single nerve fibres such as those which are supposed by Jordan & Lullies (1933) to occur in the nerves of another mollusc, *Aplysia*, contrary to the all-or-nothing "law". There are, however, undoubtedly great differences between animals in the number of nerve impulses which are needed to set the effector cells into action. In the frog and in mammals a single impulse is normally adequate to excite all of the striped muscle fibres which it reaches. In the muscles of Crustacea and coelenterates, however, repetitive impulses produce contraction in muscle fibres which fail to respond to single impulses, the tension developed therefore varying with the frequency of stimulation (Pantin, 1934–6; Katz, 1936).

The circular mantle muscles of the squid resemble vertebrate striped muscle in that a single impulse normally produces a twitch in all of the muscle fibres which it reaches, and it has been shown (Prosser & Young, 1937) that repetitive stimulation of a giant fibre, even at high frequencies, does not increase the tension developed by the muscle.

The value of systems of giant nerve fibres to the animals possessing them lies presumably either in the greater speed of conduction in larger axons, or in that such large fibres make it possible to call into action a large number of effector agents nearly simultaneously. Studies of the conduction rates in the giant fibres of the

squid are being undertaken in order to discover whether these axons make possible a significant saving of time for the animal. The investigations reported in the present paper show that the second of the above factors is certainly significant since each of the large nerve fibres connects with great numbers of muscle fibres, all of which are caused to contract by a single nerve impulse. Such a system is very well suited to the performance of the sudden movements by means of a jet of water which play such a large part in the life of the squid, and are no doubt partly responsible for the evolutionary success of this type of animal.

SUMMARY

1. Stimulation of single giant nerve fibres in the stellar nerves of the squid (*Loligo pealii*) shows them to be motor axons which produce contraction of the circular fibres of the mantle muscles.

2. When a stellar nerve is stimulated with condenser discharges a maximal response is obtained at threshold voltage. No increase of response is obtained by further increase in the strength of stimulation except for an occasional slight increase at about ten times threshold voltage probably due to repetitive firing. It therefore appears that the stimulus produces a single impulse in the giant fibre, and that this is capable of exciting contraction in all the muscle fibres which it reaches. This confirms the conclusion reached on histological grounds that in spite of their syncytial nature each of the giant nerve fibres is a single functional unit.

3. Since there are about ten giant fibres on each side the mantle is divided into 20 neuromotor units, each nerve fibre innervating an enormous number of muscle fibres. The existence of these units can also very readily be demonstrated by the fact that threshold electrical stimulation at any point within the territory innervated by each single giant fibre sets up a contraction of the muscle fibres of all parts of the territory with which the stimulated area is in connexion through the nerve.

4. Stimulation of the smaller fibres in a stellar nerve after destruction of the giant fibre also causes contraction of the circular muscles of the mantle. The amount of this contraction increases progressively with increased voltage, presumably on account of the stimulation of more and more nerve fibres. The maximum tension developed in this way is always very much less than that produced by stimulation of the giant fibres.

5. The mantle is therefore provided with a double mechanism of expiratory contraction, maximal contractions being produced by single impulses in the giant fibres and graded contractions by those in the smaller fibres of the nerve. Presumably the former contractions are those involved in rapid movement, the latter in respiration.

6. There are also radial muscles, running through the thickness of the mantle, whose contractions effect the inspiration by making the cavity larger.

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