

NERVOUS CONTROL OF MOVEMENT IN CEPHALOPODS

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INTRODUCTION

The innervation of cephalopod muscle is poorly understood, although it has been the subject of many studies. From the point of view of the physiologist who needs a workable nerve-muscle preparation it is especially disappointing that most of our knowledge concerns the tentacles and suckers. The nerves and muscles of the tentacles were described early in this century by Guerin (1908), and very extensively by Rossi & Graziadei more recently (1954, 1956, 1958). The latter authors describe peripheral cell bodies, anastomosing nerve nets, two size classes of fibre with questionable diplotomic branching, enlarged neural 'tubes' with fine branches, and endings which spiral around muscle cells. Sprenkel (1929) has described loop-like endings penetrating muscle cells. Martoja & May (1956) found both spiral and bouton endings in the same animals, *Octopus* and *Sepiola*, but in different muscles. They believe that the bouton terminals penetrate the muscle cell membranes and lie near the nucleus. Cells which are regarded as both cutaneous sensory and motor to muscle but independent of the rest of the nervous system were also found by these authors. Mikhailoff (1921) described peripheral nerve cells in cephalopod muscle which he thought were involved in both peripheral and central reflexes.

From methylene-blue studies Hofmann (1907*a*) made a reserved interpretation in favour of double innervation of the muscle fibres of the chromatophores. He found (1910) no diffuse peripheral nerve net associated with the chromatophores. The question of a peripheral nerve net in the mantle and fin muscle was examined by Hofmann (1907*b*). He suggested that anastomosing is probable in the subcutaneous plexus of the fin of *Sepia*, but that if a net is present it conducts with strong decrement. Cate (1929*a*) showed, on the one hand, the lack of dependence of the peristaltic waves on the continuity of a peripheral net in *Sepia* fin. On the other hand, he found that with local stimulation of the isolated fin excitation was conducted a certain distance.

The nerve supply of the mantle and fin, hence of the swimming and respiratory muscles, arises from the stellate ganglion (Fig. 1). The anatomy of the stellate ganglion is relatively well known. Reference will be made later to the works of Sereni & Young on *Octopus*. Graziadei (1959) has recently described the stellate ganglion of *Sepia*.

The aspects of control of movement of the cephalopod mantle treated here may be divided into two parts: the role of the stellate ganglion and neuromuscular trans-

mission. Concepts of the function of the stellate ganglion have had a controversial history. Von Uexküll (1894) viewed the ganglion as a motor relay centre without reflex function. The same is true of Baglioni (1905) who compared it to the ventral horn with lower motor neurons of vertebrates. However, he did not credit it with sensory feedback. A. Fröhlich & Loewi (1907) found spread of excitation over the mantle even after section of the mantle nerve, but not after removal of the ganglion. F. Fröhlich (1910*a*) stated that old or anoxic preparations lose this function quickly. More recently, the question as to whether the stellate ganglion is a reflex centre has been answered negatively by Bozler (1927), and positively by Cate (1929*b*) and Sereni & Young (1932). Early studies of transmission through the ganglion were made by F. Fröhlich (1910*b*) who demonstrated physiological synapses which facilitate and result in a long ganglionic delay.

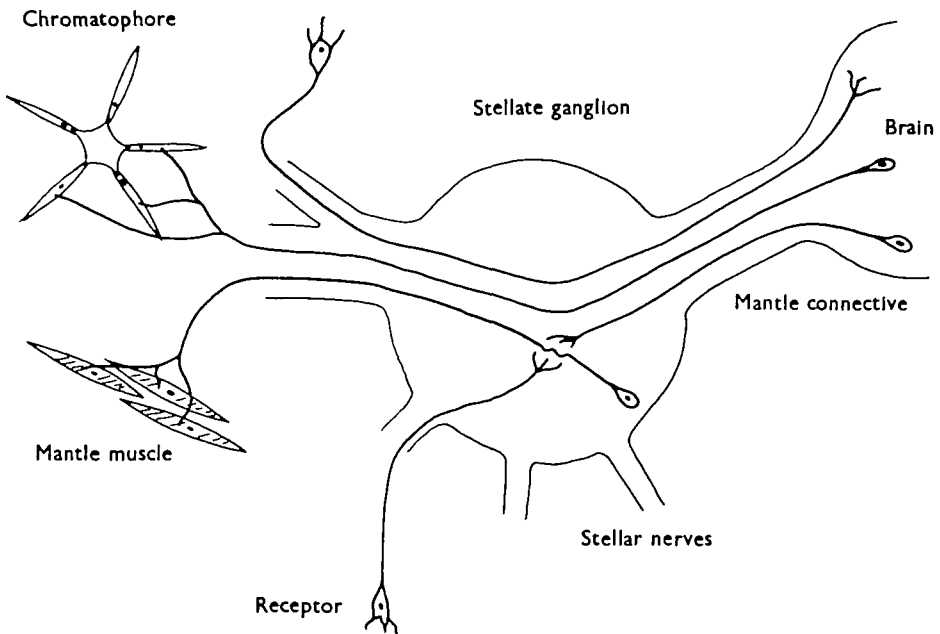


Fig. 1. Diagram of the stellate ganglion and associated nerves in *Octopus*.

A nerve-muscle preparation of the mantle and the stellar ganglion and nerves of octopuses was described by Burian (1908). F. Fröhlich used this both for the above-mentioned studies and to show some of the properties of the mantle muscle. He found summation of mechanical responses when stimulating the stellar nerves and facilitation when stimulating the mantle connective. Stimulation of the connective also resulted in inhibition of tonus in the mantle muscle.

As a result of the studies of Prosser & Young (1937) and Young (1938) the control of the squid mantle is better known than other aspects of cephalopod neuromuscular physiology. Each giant axon innervates a large area and produces non-

facilitating, virtually non-summating responses to each impulse. Many small fibres in the stellar nerves supply the same areas and produce contractions which vary with stimulus intensity and frequency.

EXPERIMENTS ON OCTOPUSES

Material and Methods

Two-spotted octopuses were obtained from southern California beaches. These were *Octopus bimaculatus* Verrill and *O. bimaculoides* Pickford & McConnaughey, two similar species separated most easily on the basis of egg size. No species differences are evident in the experimental results.

The nerve-muscle preparation was made by isolating one half of the mantle including one stellate ganglion and its stellar nerves. The mantle was pinned to a wax dish and flooded with sea water. Stimulation was either by means of paired silver wire electrodes holding a nerve out of the bath, or through a semi-micro tungsten needle plunged into the mantle or nerve. Electrical recording in the mantle was also through a tungsten needle probe. The tungsten electrodes were tapered by electrolytic etching in potassium nitrate (Hubel, 1957), and insulated to a point near the tips with insulex or glyptal. These electrodes were a few micra in diameter at the tip and thus suited to extracellular recording from a number of muscle cells but within a small area. For tension recording connexion was made to an RCA 5734 mechano-transducer tube. The recording device allowed only a very small amount of length change.

Anatomy

Description of the mantle muscle

According to the description of Winkler & Ashley (1954) the mantle of *Octopus vulgaris* is composed of three layers, an outer longitudinal layer and two circular layers within. Burian (1908) lists, in addition, transverse or radial fibres in the mantle. In the species used in this study all four muscle groups exist. The outer longitudinal layer is thin, amounting to only about one-eighth of the whole thickness of the mantle. The two circular layers have the same direction, but are separated by a thin layer of connective tissue through which course the stellar nerves and their branches. All three layers are crossed by parallel bands of radially oriented fibres. The bands are only one to a few cells in thickness. Individual fibres in these bands cross the boundaries between the main layers.

Muscle fibres and nerve fibres

Using an octopus about average in size for this study (about 250 g.), estimates were made of the numbers of muscle fibres and nerve fibres. The muscle count was based on a relaxed mantle length of 6 cm. Muscle fibre size was observed after maceration in MaCallum's fluid (Guyer, 1953). In this, as in other cephalopods (Ballowitz, 1892; Plenk, 1933), the fibres are uninucleate and spirally striated. They have a length of 1–2 mm. and a maximum thickness of 8–9 μ and taper toward both ends. Allowing 50% for connective tissue and dividing the remaining mantle

muscle volume by muscle cell volume the mantle is shown to contain at least 2×10^8 fibres. The same mantle must have at least 1–2 million chromatophores.

The number of light-microscopically visible fibres in the stellar nerves was determined from sections prepared according to the technique of Sereni & Young (1932). The number in different nerves varied from several hundred to several thousand. No size classes were observed; instead a continuous spectrum of fibres having connective tissue sheath diameters of 3–15 μ was found. Electrical observations show a smoothly graded response having maximum velocity of 1.75 m/sec. at 24° C. Fröhlich (1910c) found a velocity of about 1 m/sec. at 15° C. The sum of the fibres of all the nerves was estimated at 20,000.

According to Sereni & Young (1932) only a few of the total nerve fibres in the mantle are sensory. In view of the fine control of colour pattern possessed by octopuses it may be postulated that a large proportion of the motor fibres innervate the chromatophores. As a conservative estimate, and without considering the possibility of multiple innervation, the motor unit in the mantle comprises tens of thousands of muscle fibres.

Physiology

Responses of the mantle muscle to stimulation of the stellar nerves

These observations may be divided into three parts: visual observations of gross movement, recordings of electrical events, and recordings of tension changes.

Most of the tests were made at room temperature, about 23° C. Although the natural environment is cooler, around 16° C., these animals can be maintained in apparent good health for months at room temperatures. The effect of temperature on neuromuscular control was checked once. At 15°, 22°, and 28° C. the responses were similar. Above 30° C., the responses were weaker, but not qualitatively different. The kind of response is not dependent upon temperature in this range.

Stimulation of the stellar nerves usually resulted in contractions which *reduced the area* of the mantle. With high intensity stimulation the contraction involved a sector of the mantle corresponding to the area innervated by the stellar nerve. At low intensities of stimulation discrete patches of contracting tissue responded in an all-or-none fashion. Repetition caused stronger contractions. Increases in intensity brought in new patches. Strips cut in the direction of the longitudinal or circular layers were stimulated directly and tested for differential activity in these two layers. Regardless of the direction of the strips stimulation resulted in some shortening. It was not possible to separate longitudinal and circular muscle contractions.

A different response was observed on a few preparations. Above a threshold value of stimulation, a *small patch of mantle thinned* conspicuously, causing the mantle to spread. The movement was not graded with intensity, but became greater with repetition. It is interpreted as due to selective activation of the radial muscle fibres. The existence of this kind of muscle activity is predictable from observations of the whole animal. Without the aid of peristaltic waves and with the aperture of the mantle open, the octopus may increase the area of its mantle and the volume of the mantle cavity.

The *electrical events* in the circular muscle layers of the mantle resulting from stimulation of the stellar nerves were of two kinds. At lowest intensity of stimulation the all-or-none patches could be mapped by means of the probing electrode. These patches had fairly sharp boundaries which did not change with repetition. In a specimen of 8 cm. mantle length the patches were 5–10 mm. in diameter. By changing the intensity and site of stimulation of the nerve different reactive units may be identified in the same preparation.

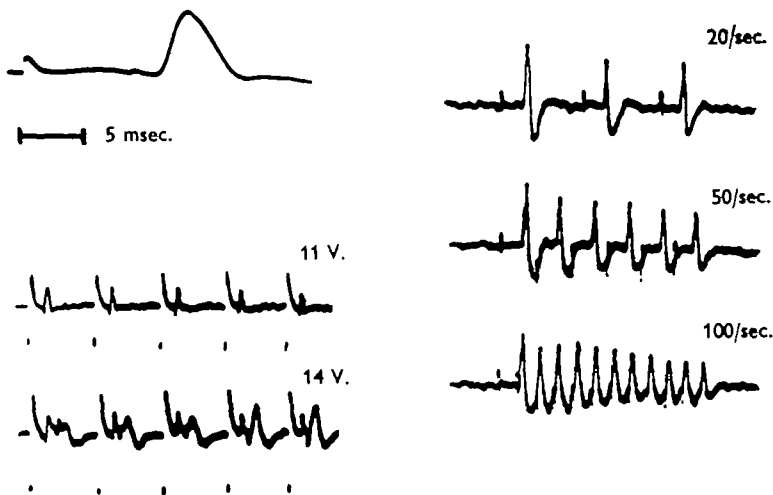


Fig. 2. All-or-none, threshold electrical responses in the octopus mantle. Left. Top: nerve potential and muscle action potential of fast response. Time marker, 5 msec. Bottom: response to varying voltage of stimulation of stellar nerve. At the higher voltage a slower, facilitating response occurs. Large initial deflexion is stimulus artifact. Frequency 20/sec. Right. Fast response at 20, 50, and 100/sec.

The electrical record in one all-or-none area is shown in Fig. 2. The response to a single shock was a muscle action potential having a duration of about 6 msec., the rising phase lasting about 2 msec. A small wave preceding the muscle action potential (Fig. 2) was interpreted as nervous activity. This would indicate a neuromuscular delay of nearly 2 msec. Repetition above 10/sec. resulted in slightly *decreasing action potentials*. The decrease was greater at greater frequencies, the response waning to one-half after about 10 shocks at 100/sec. At frequencies above 100/sec. action potentials did not follow long trains of stimuli in a one-to-one relationship. However, for pairs of stimuli the nerve-muscle preparation follows to very high frequencies. With stimulation of the stellar nerve the absolute refractory period for an all-or-none muscle unit was never greater than 1.6 msec. When the interval between stimuli was increased beyond the refractory period a second electrical response appeared suddenly, but about 1 msec. later than expected. It is suggested that this absolute refractory period is in the nerve and that the lateness of the second muscle response is due to slowing of nervous conduction. The second response fuses with the first, but the increment it adds becomes smaller with higher frequencies so that it never produces a peak higher than the first. The muscle

potential due to this fast innervation is maximal with the first excitation, but it has no absolute refractory period which outlasts the rising phase as is the case with spike potentials.

At higher intensities of stimulation complex action potentials appeared at a single electrode position (Fig. 2). In the simplest form these included two waves, one corresponding to that just described, and a later wave of longer duration and initially lower magnitude. This second wave increased rather than decreased with repetition, that is, showed facilitation, and at fusion frequency summated as well.

In most preparations, especially old ones in which the nerve had dried, some patches were found which *responded with facilitation*. These were presumably innervated by smaller nerve fibres which had outlived the larger low threshold ones. In a few cases these have also been found as unit-responding preparations, but usually they are compound. The area of innervation was much smaller than for the faster fibres, perhaps 2 to 3 mm. in diameter, but was not carefully mapped. The boundaries appeared to be diffuse rather than sharp. Different fibres gave different frequency-response relationships. Some showed facilitation at 5/sec. with a maximum at 30/sec.; others showed conspicuous augmentation only at 50/sec. or more. Maximum amplitude was reached after a few shocks, usually more than three but less than ten. The *minimum number of motor units* may be calculated assuming overlap of fast and slow motor units but no overlap of the same kind, and taking the average diameters of these units to be, respectively, 5 and 2 mm. in an octopus of average size for this study. This calculation indicates space for about 600 of the first kind and 4000 of the second. Together with the estimated total of 20,000 nerve fibres to the mantle, these figures suggest overlap of motor unit areas, but the level of error possible in these estimates does not permit a conclusive statement.

Tension records for the all-or-none fast type of response in the circular muscle began about 16 msec. after the start of electrical activity. The rising phase of the single twitch lasted about 0.16 sec. The relaxation was about 3 times as long. At frequencies between 2 and 4/sec. the twitches began to fuse, but the second was smaller than the first. Above 4/sec. summation occurred. Smooth tetanus occurred above 15 impulses/sec. The tetanus:twitch ratio was large, varying in different preparations between 20 and 100 to 1. Fig. 3 shows records of these responses.

Maximal stimulation of fresh preparations produced effects similar to those described for the single fast fibre. Paired shocks gave a maximal response about twice the amplitude of a single twitch. This maximum occurred at 30/sec. and was conspicuously reduced only below 10/sec. and above 60/sec. With maintained stimulation, tension increased rapidly at first and then more slowly for several seconds. The rate of increase in tension continued to rise with increased frequency to about 150/sec. The two separate phases of increase were the striking feature of this tetanus. Although this fast type of response shows a diminution rather than a facilitation of twitches, a post-tetanic potentiation occurred with maximal stimulation so that successive tetani were larger, within certain time limits (Fig. 3).

The mechanical response of the slow facilitating type was not within the range of the recording apparatus at threshold, but in old preparations, after the nerve had

partially dried, facilitated mechanical responses sometimes could be recorded with maximal stimulation. The response to two shocks was sometimes more than three times greater than to one. The facilitation began at 10–15/sec., fell off above 100/sec., and disappeared at approximately 160/sec.

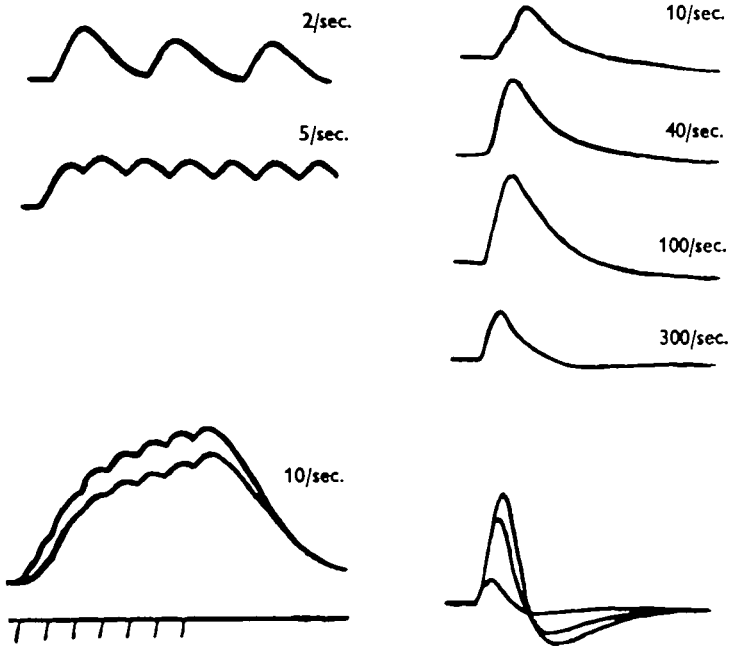


Fig. 3. Left. Tension in mantle with just above threshold stimulation of fast system in octopuses. Top: fast response in *Octopus* at 2 and 5/sec. Bottom: fast response in *Octopus* at 10/sec. repeated after 1 sec. Greater response is second series. Right. Mantle tension resulting from stimulation of many units of the slow systems of octopuses. Top: slow response of *Octopus* to pairs of shocks direct to the mantle at 10, 40, 100 and 300/sec. Bottom: slow response of *Octopus* at 30/sec. Superimposed records of 1, 2 and 3 shocks. Recorded with a.c. coupling to insure constant base-line.

Response to direct stimulation of the mantle

Small waves of electrical activity were propagated with a conduction velocity of less than 1 m/sec. away from stimulating electrodes within the mantle muscle. The maximum range of spread obtained was about 6 mm. The amplitude of the response was dependent upon frequency, but the degree of spread apparently was not. The mechanical response to this type of stimulation was always facilitated by repetition at near threshold values of stimulation. Thus there appeared a reversal of the thresholds of stimulation of the fast and slow systems between the stellar nerves and the periphery. The frequency response relationships of the slow system were more easily determined with the more peripheral stimulation because of this threshold reversal. The response to pairs of shocks was somewhat more than doubled over a single shock at 10/sec. The degree of facilitation increased to about five times

at 100/sec. At higher frequencies it waned. The facilitation for the third shock was less and at frequencies above 30/sec. the tension added by the third shock was no greater than the first (Fig. 3). At the lowest frequencies maximum facilitation was reached within four shocks.

Effect of previous section of stellar nerves

Mantles isolated from the central nervous system, either for a few minutes or many days, show considerable spontaneity in the form of weak local contractions. Mechanical stimulation produces stronger local contractions.

No physiological evidence of degeneration in the muscle innervation was found after section of the stellar nerves or removal of the stellate ganglion. Stimulation of the central ends of the stellar nerve stumps in octopuses kept at 15° C. for up to 34 days after removal of the ganglion gave normal non-facilitating electrical responses. In a 17-day-old preparation these responses fatigued more rapidly than in normal animals and were replaced by facilitating potentials like those of the slow system. Response to stimulation direct to the mantle was normal. In the oldest preparation, however, no facilitating responses were found; stimulation either at the nerve stump or more peripherally resulted in fast responses even after fatigue.

Sereni & Young (1932) found histological degeneration of the motor fibres within a few days after their section in octopuses kept at 25° C. Besides the possibility that such considerable difference in degeneration could be due to the 10° C. difference in temperature, several possible but untested explanations exist: (1) histological and physiological criteria of degeneration do not coincide and functional degeneration is slower; (2) central motor axons have nutritive contact with peripheral cell bodies; (3) only the slow fibres have such contact and hypersensitivity results in maximal electrical response to single shocks even though the fast fibres are inactive.

Motor functions of the stellate ganglion

Synaptic relay of brain efferents. Electrical stimulation in the mantle connective and recording in a stellar nerve gave the following results. At the lowest intensity producing any response a small electrical wave appeared in the stellar nerve. This was accompanied by a small contraction of the mantle. Repetition was followed by increasing potentials indicating synaptic integrative processes in the ganglion. At higher intensity an earlier wave appeared, associated with expansion of the chromatophores. It did not show frequency augmentation. The higher intensity necessary for stimulation of these fibres, indicating that they are smaller than the former, together with the shorter latency and lack of frequency grading, suggest that they pass without synapse through the ganglion. Long-continued stimulation resulted in much earlier fatigue of the mantle motor response than the chromatophore response. After this fatigue, stimulation distal to the ganglion resulted in contractions again, but not so in the case of fatigue of the chromatophore response. These results agree with the anatomical studies of Sereni & Young (1932). Excitatory motor fibres to the chromatophores travel from the brain directly to the

periphery, the mantle motor fibres synapse in the stellate ganglion where facilitation to repetition and perhaps after-discharge occurs.

Stimulation of the mantle connective resulted in a complex wave of electrical activity in the mantle muscle. At frequencies around 1/sec. the latency decreased slightly and the magnitude increased with repetition. The resulting facilitation lasted several seconds. Contractions in the mantle due to stimulation of the mantle connective showed a larger degree of facilitation than stimulation more peripherally.

Reflex function from peripheral stimulation

Stimulation of the proximal stump of the largest stellar nerve also showed greater facilitation than more peripheral stimulation. Such afferent stimulation caused mild contractions over much of the same half of the mantle even when the mantle connective was cut. Stimulation of the mantle connective and the proximal stump of a stellar nerve simultaneously, or nearly so, resulted in a summated response.

It is unlikely that the contribution from the stellar nerve was via an axonal reflex or other through-conducting pathway. Its latency was longer than would be predicted by the increase in conduction distance across the ganglion as compared to efferent stimulation of a stellar nerve. The duration of the response to a single stimulus was much longer than that resulting from stimulation not across the ganglion. It seemed to fatigue more rapidly than the simple nerve muscle preparation. In the partially fatigued preparation there was no recordable response to one or a few shocks, or to frequencies as low as one or two per second, but large responses followed long and high-frequency bursts. The response did not have a simple relationship to the stimulus, probably due to the interaction of antagonistic muscles. Often the relaxation following cessation of stimulation was extraordinarily rapid and showed considerable and long-lasting overshoot. This is reminiscent of the inhibition of tonus reported by Fröhlich (1910*d*), but might also be explained as belated or more enduring reaction of antagonistic fibres. The possibility that these reflexes are mediated by an artificial synapse at the cut end of the mantle connective is slight since the sea-water bath would provide a short circuit.

Degeneration studies done by Sereni & Young (1932) demonstrate that most of the fibres present in the stellar nerves and visible in the light microscope are motor. Exact counts are not given, but the authors state that only a very small percentage of the fibres are sensory. The modalities of the fibres which cause the reflex activity are unknown. There was no evidence in reflexes of the negative feedback which might be expected in the case of stretch receptors.

EXPERIMENTS ON SQUIDS

Squid preparation

Loligo pealeii (Lesueur) were studied at Woods Hole and *L. opalescens* Berry in Los Angeles. The preparation and recording techniques were the same as for octopuses. The effect of small fibre stimulation was determined either by using the fin nerve or by damaging the giant fibre in a stellar nerve.

Response of the mantle to giant fibre stimulation

Mechanical responses were obtained which agree in every respect with those of Prosser & Young (1937). At threshold, all-or-none muscle twitches occurred to each stimulus. These did not facilitate nor even sum noticeably, but did fuse at frequencies above about 5–10/sec., giving a small tetanus:twitch ratio (*c.* 1).

Electrical recording produced additional information about the giant fibre system. Muscle action potentials lasting about 10 msec. follow giant fibre stimulation. These *decreased in amplitude* with repetition over 20/sec. Pairs at intervals shorter than 10 msec. fused, at least in partially fatigued specimens, but the second response was always lower than the first. At no frequency could uphill *treppe* be produced.

After fatigue to 5 or 10% of its original fresh level the giant muscle action potential takes on the characteristics of a slow innervation system. In the fatigued condition successive responses grew slightly at repetition rates above 10/sec. These responses were associated with a low threshold all-or-none unit in the stellar nerve which could not be other than the giant fibre.

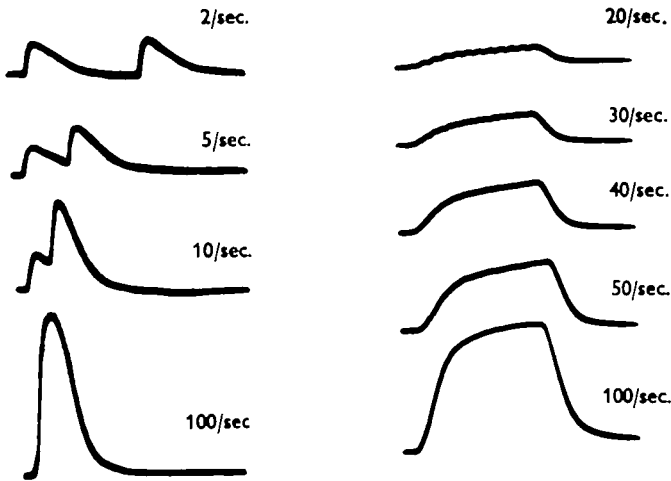


Fig. 4. Tension in the squid mantle due to stimulation of many small fibres of a stellar nerve. Left. Slow response at 2, 5, 10 and 100/sec. near maximum paired stimulation of stellar nerve with damaged giant fibre. Right. Response at 20, 30, 40, 50 and 100/sec. maintained for $\frac{1}{4}$ sec.

Responses to small fibre stimulation

Facilitating potentials with higher threshold and longer latency could be produced by stimulation of the same nerve in which the giant axon response was fatigued. Similar facilitating muscle-action potentials occurred when fin nerve or stellar nerve with damaged giant axon was stimulated. This facilitating response corresponds to the frequency-graded responses found by Young (1938). The degree of facilitation was larger than for fatigued giant axon stimulation. Facilitation followed only the first few shocks at frequencies greater than 4/sec. Fusion began at 100/sec.

Mechanical records during small nerve fibre stimulation were similar to the slow responses of octopuses (Fig. 4). Facilitation occurred above 5/sec. Response to pairs was maximal at 100/sec. and decreased slightly to above 300/sec. when it fell off abruptly, presumably due to failure of nerve conduction. Maximum facilitation was reached after 4 or 5 shocks at low frequency (Fig. 5) and in fewer shocks at higher frequencies.

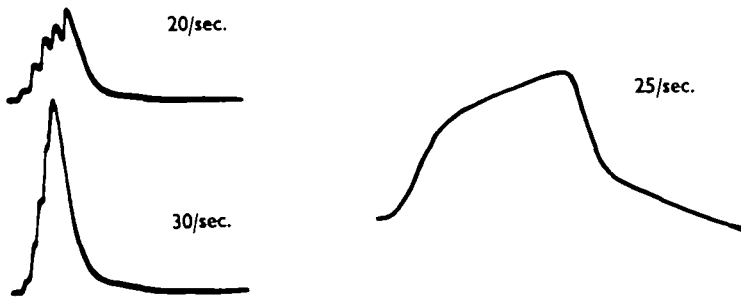


Fig. 5. Tension in the squid mantle due to stimulation of many small fibres of a stellar nerve. Left. Response to 5 shocks at 20 and 30/sec. Right. Response at 25/sec. for $\frac{1}{2}$ sec.

Long trains of stimuli resulted in tetanic contractions with smooth fusion of responses above 30/sec. (Fig. 4). The tetanus:twitch ratio at 30/sec. was about 100:1. The strength of the contraction increased disproportionately with increase in frequency at least to 100/sec. As in *Octopus* mantle, two phases of the tetanus could be seen (Fig. 5). A rapid initial rise was followed by a slower and long continued increase in tension. Relaxation was likewise rapid at first and then slower.

Stimulation of the small fibres of the stellar nerves *never resulted in fast type responses*, although wide ranges of intensity and duration of stimulus were employed.

DISCUSSION

Organization of the motor apparatus

The breathing and swimming movements of octopuses and squids involve alternate increases and decreases in the mantle volume. The muscular movements which effect these changes consist in reciprocal activity between antagonistic fibres in the mantle. Since the mantle cavity is normally open, the necessary changes in shape cannot result from the activity of circular and longitudinal muscles operating alone against a turgor skeleton, but probably are due also to radial musculature making use of some rigidity of the mantle itself. Radial fibres have been described for squids (Young, 1938) and were found for octopuses by Burian (1908). In octopuses, nerve fibres were found which selectively activated muscle fibres causing thinning and expansion of the mantle. These muscle fibres are undoubtedly the radial ones. These radial fibres oppose both the circular and longitudinal fibres which usually appear to act together. In this study no means could be found to separate the action of the last two layers and it is suspected that they have overlapping innervation. The suggestion has been made by Hofmann (1907*b*) that the layers of the fin of *Sepia*

are nervously linked. However, Fröhlich (1910*d*) was able to elicit separate contractions of the longitudinal and circular layers of *Octopus macropus* by stimulation of the intact animal. It is not impossible that the two systems of innervation, facilitating and non-facilitating, differ in this respect, with perhaps one system activating the layers separately and the other activating them together. The several histological studies do not elucidate these matters, nor do they exclude or support a double innervation of muscle fibres.

Normal use of the mantle depends on its *connexion to the central nervous system*. Since the two halves of the mantle normally act together, but are not dependent upon each other (cutting one mantle connective and therefore the commissure between the ganglia does not affect the other side), control of respiration and swimming is probably from higher centres than the stellate ganglion. However, it is clear that the stellate ganglion is involved in integrative processes affecting the activity of the mantle muscle. The older anatomical and physiological findings (Fröhlich, 1910*b*; Bullock, 1952), as well as the present evidence, demonstrate the presence of synapses along the motor pathway to the mantle. These synapses are not one-to-one but are facilitated to shorter latency and larger discharge, and probably after-discharge, by repetition of presynaptic stimulation. This activity of the ganglion may result in a smooth grading of a simple central command analogous to that made possible by the high degree of summation and facilitation found more peripherally in arthropods. The evidence is strongly in favour of a reflex function in the ganglion as well. It is not possible to say yet whether this is primarily proprioceptive and feedback in nature, or whether it is primarily exteroceptive and therefore relatively independent of more central control. It seems probable that in the intact animal the command for movement of the mantle operates through the brain and that the stellate ganglion mainly brings about a smoothing of control.

Gradual contractions superimposed on faster ones were found in the case of tetanic stimulation. These resulted in sloping tetanic plateaux following stimulation of the small fibres of squid or octopuses. They do not occur with pure giant fibre stimulation. Tonic contractions were never observed by themselves and it is unknown whether they can be elicited centrally or whether they are a local phenomenon elicited by faster contraction. No evidence was found of fibres in the stellar nerves which inhibit peripheral tonus. However, the evidence of Bozler (1928) on the chromatophores and the findings of Fröhlich lead one to expect such inhibitory mechanisms in the mantle. The extraordinarily rapid relaxations, sometimes with overshoot, which often followed preganglionic stimulation, resemble Fröhlich's results, but are here interpreted as due to interactions of antagonistic muscles.

The *reversal of threshold* between the fast and slow innervation systems dependent on whether stimulation is in the proximal part of the nerves or in the muscles takes place in *Octopus* but not in *Loligo*. The giant fibres, the only ones which mediate a fast response in squids, apparently remain relatively large even within a small distance of their terminations. In the octopus mantle the slow fibres may be associated with some larger structures such as peripheral cells or the neural tubes described by Rossi & Graziadei (1958). Some nervous structure must account for

the slow type of excitation which takes place when the mantle is stimulated directly. The constant limit to which such excitation will spread suggests that the stimulus does not directly affect the muscle cells and then conduct from muscle fibre to muscle fibre.

The present results agree with those of Hofmann and Cate on the presence of *peripheral conduction* of excitation. All concur that spread of contraction with repeated stimulation takes place over short distances. Limited spread cannot, by itself, be taken as proof that no nerve net is functioning (Horridge, 1957), but other evidence does suggest this. The actual distances found in this study are similar to the diameter of the fields of influence of single units in the stellar nerves. The conduction velocity for the electrical wave, nearly 1 m./sec., is high for a diffuse network. The simplest interpretation is that no physiological nerve net exists, that local reactions spread only along the branches of a single fibre, and that the histologically evident plexus does not represent anastomosing terminal branches of many units.

No physiological evidence has been advanced on the peripheral neurons supposed by several anatomists to be motor neurons.

Neuromuscular relationships

The large number of muscle fibres innervated by a single nerve fibre in cephalopods does not raise the same question that it does in the case of the arthropods. In the case of arthropods a single axon or a few axons must control the movement of whole muscles and gradation of response cannot be like that of vertebrate motor units. In cephalopods, while the ratio of muscle fibres to nerves is even higher, the number of nerve fibres to a large muscle is considerable. On the evidence up to this study, any of the following means of grading tension was possible: (a) strictly all-or-none activation of the entire field of innervation of individual axons such as is typically found in vertebrates with grading by recruitment; (b) all-or-none activation of each muscle fibre but with different amounts of repetition required to produce this activation in different fibres, as is found in curarized vertebrate muscle (Adrian & Lucas, 1912); (c) graded activity in single muscle fibres dependent on local phenomena which increase or spread with repetition, as is typical of arthropods; and (d) peripheral inhibition superimposed on any of the above.

Two kinds of response are found in octopuses and squids. The slower, *facilitating responses* are so similar in the two forms, both in degree and time-course of facilitation, that it seems probable the two systems are homologous. In these animals the degree of facilitation is not large. Minimal contraction does not require two stimuli as in some coelenterates (Pantin, 1935) nor does the facilitation increase beyond the first few stimuli as it does in arthropods (Pantin, 1934). The magnitude of neuromuscular facilitation in cephalopods seems to be more like that of annelids (Wilson, 1960) than other groups which have been studied.

The *non-facilitating reaction* of octopuses and the giant response of squids are similar in many ways. The motor unit area is, however, of the order of 100 times larger in squids. In each case the electrical response to the first stimulus is as large

as, or larger than, that to later stimuli. The mechanical response may show summation as in *Octopus*, but not facilitation, or may be almost maximal to a single impulse in *Loligo*. In both *Octopus* and *Loligo* stimulation of the fast systems at relatively low frequencies (10/sec.) results in diminishing action potentials, but this characteristic is not as marked as in polychaetes. Such diminishing potentials in whole muscle might be due either to gross failure of some number of units to respond after a few repetitions or to smaller electrical response by each unit. The apparent lack of refractory period during the muscle potential argues against propagating spikes, and it appears unlikely that failure in single fibres is all-or-none; these considerations support the second alternative. The anatomical evidence available includes descriptions of broad or multiterminal neuromuscular endings and allows the notion that local potentials could excite whole muscle cells. Another promising case for local response in cephalopod muscle is that of the denervated chromatophore muscle. This preparation was found by Bozler (1928) to respond in proportion to stimulus intensity, and although he was unwilling to accept this as a property of a pure single unit, this seems at least as likely as an alternative.

The two kinds of muscle excitation in cephalopods bear many similarities to those of the snail. Ramsay (1940) found two kinds of contraction in the buccal retractor. Electrical records showed an early potential which declined with repetition followed by a later augmenting potential. The first response was not maximal to the first stimulus as it is in cephalopods, and did have an absolute refractory period. Physiological evidence of double excitatory systems have been found in the clam (Pumphrey, 1938) and both tonic and phasic excitatory innervation as well as tonus inhibitory innervation are indicated in *Mytilus* (Hoyle & Lowy, 1956). Schmandt & Sleator (1955) found that action potentials in *Mytilus* muscle conducted with decrement in individual fibres, facilitated with repetition, and had little or no absolute refractory period. They found post-tetanic potentiation lasting minutes. The neuromuscular mechanisms found in cephalopods are at least represented, and are probably general, in the other mollusc groups.

Probably both the fast and slow systems in the cephalopods studied, and in *Neanthes* (Wilson, 1960), operate by means of *local potentials* and grading in individual fibres rather than spike potentials and recruitment. The fast system in cephalopods at least normally operates with a maximum electrical response to the first nerve impulse.

SUMMARY

1. Nerve muscle preparations have been made of the mantle and stellar nerves of octopuses and squids.
2. Two motor innervation systems have been found in each. Both have been observed as unit preparations. The possibility of double innervation of the same muscle cells exists but has not been directly checked.
3. The fast innervations produce electrical responses which are maximal to the first stimulus and which have little or no absolute refractory period. They appear to be local rather than spike potentials. Fatigue is very rapid. The mechanical response sums in *Octopus*, but not in *Loligo*.

4. The slow innervations produce electrical and mechanical responses which facilitate with repetition. The fast system of *Loligo* does likewise after fatigue to a low level of response.

5. No evidence was found for a functional nerve net in the mantle.

6. Organizational features of the stellate ganglion have been identified physiologically in *Octopus*. The ganglion acts both as an integrating motor centre and as a reflex centre.

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