POLYNEURONAL INNERVATION OF THE FAST MUSCLES OF THE MARINE TELEOST *COTTUS SCORPIUS* L.

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**Introduction**

Two distinct patterns of innervation of the myotomal musculature of teleosts have been described (Barets, 1961). The fast muscles of the majority of teleosts—for example, tench—receive numerous distributed endings from a plexus of nerves spread over the surface of the myotome and between the muscle fibres, as do the slow muscles of all fish (see also Bone, 1964). In only a few teleost species—for instance, catfish—but characteristically in elasmobranchs (Bone, 1964, 1966) and hagfish (Jansen, Andersen & Jansen, 1962), the fast muscle fibres are focally innervated from nerves confined to the myosepta. Barets further indicated that in the case of a distributed innervation each localized terminal region comprises numerous discrete neurofibrillar annuli, and each axon terminates generally in the formation of a single annulus. Less commonly a number of annuli, which may be on separate muscle fibres, are connected in series by a fine unmyelinated axon of decreasing thickness. Individual muscle fibres, therefore, either receive a considerable polyneuronal innervation, or else extensive branching of the motor axons occurs and each fibre is multiterminally innervated.

Multiterminal innervation can be identified only if the full extent of polyneuronal innervation and the total number of nerve terminations per fibre are known. No information is available about the latter, whilst little enough is known about polyneuronal innervation. Quantized junction potentials—that is, junction potentials that increase in amplitude by discrete steps—recorded intracellularly from the fast somatic muscles of tench and catfish (Barets, 1961), and the fast pectoral fin muscles of snakefish (Takeuchi, 1959) provide direct evidence of a polyneuronal innervation by 2–3 axons from a single spinal nerve. Cuypers & Fessard (1954) and later Barets (1961) suggested, on the basis of extracellular recordings, that two spinal nerves innervate the muscles of a myotome. The effects of stimulating more than one spinal nerve on the intracellular response recorded from a single teleost muscle fibre have not, however, been determined. Thus, no accurate figure is available as to the total number of axons that innervate a single fibre, and it is not known whether each fibre receives a similar axonic complement both in number and conduction velocity.

The ventral roots of tench and catfish (Barets, 1961) and carp (Kiss & Mihalik, 1928) contain axons in two size groups. Barets showed that posterior to the anus the number of small axons diminished rapidly and were absent altogether from the ventral roots adjacent to the caudal fin. In addition he found that the relative propor-
tions of slow to fast muscle increased caudally. On this slender evidence he concluded that the class of large axons was motor, and that the small axons were sympathetic. By analogy with the frog motor system it might be expected that the fast muscles of fish are innervated by axons of the large-diameter class, and the slow fibres by axons of the small-diameter class. Since certain parallels have been drawn between tench and crustacean muscles (Barets, 1961), it is possible that the fast muscle fibres may also be innervated by small-diameter axons.

As the response of a nerve trunk is determined by the diameters of its constituent axons (Gasser & Erlanger, 1927) an indication of the types of motor axons should be obtained by an analysis of the electrophysiological properties of the spinal nerves and roots. Although the spinal nerves (Campbell, 1946; Cragg & Thomas, 1957; Roots & Prosser, 1962; Thomas & Young, 1949) and roots (Bennett, Crain & Grundfest, 1959) of fish have received some attention, an analysis of this nature has not previously been made.

In the present paper an attempt is made to define the nature and extent of polynuveal innervation of teleost fast muscles that receive a distributed innervation by histological and electrophysiological study of the components of a nerve-muscle preparation.

MATERIALS AND METHODS

Specimens of the marine teleost Cottus scorpius L. were obtained from local fishermen, kept in running sea-water aquaria at an average seasonal temperature of 10°C. (range 5–14°C.), and in most instances used within a few days of capture.

Short lengths of nerves and roots were lightly stretched on card and fixed in Flemming's solution for 24 hr. Paraffin sections 5–6 μm thick were stained with Wolter's haematoxylin according to the method described by Williams & Wendell-Smith (1960). Araldite sections 0.25–0.5 μm thick, cut with glass knives on a Porter–Blum Mk. I ultramicrotome, were stained with toluidine blue.

Strips of the abdominal wall musculature were stained with methylene blue, fixed in saturated ammonium molybdate, washed in tap water, squashed between slides and dehydrated in three rapid changes of absolute alcohol. Preparations were cleared in xylene, thinned to a sheet 1–3 fibres thick, and mounted in Damar.

Pre-terminal axons and neuromuscular junctions were demonstrated by the acetylthiocholine technique described by Naik (1963). Small strips of intact muscle fibres fixed in 10% formalin at pH 5.2 for 2 hr. at 15°C. stained maximally after incubation for more than 4 hr. at 37°C. in a pH 5.2 incubative solution. Non-specific cholinesterases were inhibited by the addition of ethopropazine hydrochloride to the incubating solution. Muscle strips incubated in eserine sulphate served as controls. Single fibres and small groups of fibres were teased out from stained muscle strips, and cleared and mounted in glycerine.

The spinal nerve preparation consisted of portions of nerve 5–8 cm. long isolated from the abdominal wall. The isolated nerve was placed across movable electrodes in a small Perspex chamber containing a shallow layer of Ringer solution. The portion of nerve between the recording and stimulating electrodes was earthed via the Ringer. The suspended portions of nerve were periodically immersed to prevent desiccation. The leading stimulating electrode was always the cathode.
The nerve-muscle preparation was taken from the 2–3 mm. thick sheet of muscle fibres in the anterior ventral region of the abdominal wall. The ventral spinal nerve that issues from the cord just posterior to the innervation of the paired fins and a portion of myotome posterior to its course were used, except where otherwise indicated. The isolated myotome strip with centrally situated muscle fibres and their innervation intact was mounted under tension in the experimental dish. Fresh Ringer bathing the preparation was changed at frequent intervals. The nerve was placed across stimulating, earth, and recording electrodes under paraffin-oil in a separate compartment.

Extracellular recordings of the nerve response were made diphasically using two silver-wire electrodes with a fixed separation of 6 mm. The responses were recorded via a cathode follower and displayed on one beam of an oscilloscope after differential a.c. amplification. The responses of the muscle fibres were recorded with conventional intracellular recording techniques via a unity-gain cathode follower (Bak, 1958) with adjustment for zero grid current. An incorporated circuit allowed the measurement of electrode resistance and a sufficiency of capacity neutralization whilst the electrodes were in use (Lettvin, Howland & Gesteland, 1958). Pipettes of 3 M-KCl with stable resistances of 10–30 MΩ were drawn with long thin flexible tips to allow for move-
ment. A greater measure of success was achieved using pipettes of this type, although movement frequently displaced the electrode.

All experiments were carried out at 10–12°C in a Ringer solution specifically prepared for Cottus (Hudson, 1968). The temperature of the water bath surrounding the preparation dish was controlled to within 1°C by means of the circuit illustrated in Text-fig. 1, and a head reservoir of iced water.

RESULTS

A. Histology

At proximal, medial, and distal levels (Pl. 1, fig. 1(a)–(c)) nerves contain mixed large and small myelinated axons, and a large number of small unmyelinated axons (Pl. 1, fig. 1(d)). The motor and sensory axons are not separated into distinct bundles as found in elasmobranchs (Roberts, 1965). The ventral root (Pl. 1, fig. 2(a)) contains principally large diameter axons, but situated to one side there is, in addition, a group of small axons. The dorsal root (Pl. 1, fig. 2(b)) contains both large and small axons intermixed, and appears much like the peripheral nerve.

Myelinated axon diameters at proximal and distal levels of a peripheral nerve and in the spinal roots of the same nerve were measured from photographs giving a total magnification of 1750, and size frequency histograms were plotted. The external diameters of the axons were measured since the thickness of the myelin sheath is more variable than the external diameter (Williams & Wendell-Smith, 1960). Due to shrinkage and distortion of the sheath it was not possible to measure axon diameters with any validity in groupings of less than 2 μm. Attempts to reduce distortion of the myelin profile were unsuccessful. Flemming’s fixative made up with Ringer solution or sea water in place of distilled water produced markedly greater shrinkage and distortion.

At the proximal level (Text-fig. 2A) the nerve contained 781 axons in a single class 1–18 μm. in diameter with a mean at 3.4 μm. The total number of axons at the distal level (Text-fig. 2B) was less (153) but they retained essentially the same distribution. There is a roughly proportional decrease in the frequency of each interval, inclusive of the loss of some of the larger diameter axons.

There is a clear difference between the distribution of axon diameters in the two spinal roots of the same nerve (Text-fig. 3). The ventral root contained 306 axons in two classes from 2–22 μm. in diameter with means at 4.6 and 12–14 μm., a result analogous to those of Kiss & Mihalik (1928) and Barets (1961). On the other hand, the dorsal root contained 853 axons in a single class from 1–16 μm. with a mean at 2–4 μm.

Muscle innervation

Methylene blue stained the nerves effectively but not the fine pre-terminal axons or the nerve endings. In contrast, the latter were clearly stained by the acetylthiocholine method. By comparison with muscles incubated in eserine sulphate, and ethopropazine hydrochloride, it is evident that the principal or only transmitter at the neuromuscular junction is acetylcholine. Nerve endings failed to stain when muscle strips were incubated in solutions containing eserine sulphate. The intensity of staining of the
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Text-fig. 2. Frequency distribution spectra of axon diameters in a ventral spinal nerve from the anterior region of the abdominal cavity. A, Proximal level; B, distal level.

Text-fig. 3. Frequency distribution spectra of axon diameters in the ventral root (A) and dorsal root (B) of one nerve from the anterior region of the abdominal cavity.
nerve endings incubated in solutions containing ethopropazine hydrochloride was not appreciably reduced.

The two staining procedures demonstrated that the fast muscles receive a distributed innervation (Text-fig. 4; Pl. 2). As a result of extensive peripheral branching a diffuse and complex axon network is formed over the surface of each myotome and between the muscle fibres. Each spinal nerve that courses for the most part along a myoseptum gives off branches at irregular intervals that course anteriorly or posteriorly and contribute to the axon networks of both the adjacent myotomes. Axons in a number of these branches extend further, cross myosepta, and join the axon networks of the subadjacent myotomes (see Text-fig. 4). Axons could not be traced for further distances to determine whether more remote myotomes are also innervated by one spinal nerve.

Small branches that contain a variable number of axons are given off from the axon network and run for short distances between the muscle fibres. The axons in these bundles do not all terminate on the same muscle fibre (Pl. 2(a)).

Each muscle fibre has distributed along its entire length a variable number of terminal regions (Pl. 2(b)). These regions are multiple and very varied in extent and form (Pl. 3). Whilst they are discrete from one another on the same fibre some were frequently observed to overlap on to adjacent fibres. Axons terminate forming sequential bead-like expansions (Pl. 3(a)) that are presumably synaptic sites since they stain more intensely for acetylcholinesterase. Individual terminal regions may extend up to 900 μm. (Pl. 3(b)) but are more commonly between 300 and 400 μm. A feature
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of particular interest is that each terminal region appears to consist of the terminations of two axons whose terminal processes intertwine and run adjacent to each other. In many instances these regions were actually found to be supplied by the branches of one axon (Pl. 3(c)). In others (for example, Pl. 3(d)) this could not be ascertained because the pre-terminal axons could not be traced for sufficient distances. However, as no apparent structural difference was found between the diverse regions it is supposed that each terminal region is, in fact, the termination of a single axon. What is extremely difficult to determine from histological studies is whether different terminations on the same muscle fibre are supplied by different motoneurones, or by branches of one or more motoneurones as found for crustacean muscles (van Harreveld, 1939; Hoyle & Wiersma, 1958) for example.

The number of terminal regions per fibre ranged from 8 to 22 with an average of 14.5 as measured on 52 isolated single fibres. On the reasonable assumption that the length of the muscle fibres is 1 cm., the average distance between ending centres is 0.75 mm. The interterminal distance measured directly varied between 0.094 and 2.05 mm. The average separation determined in this way is 0.64 mm.

Text-fig. 5. Compound action potentials to supramaximal stimulation recorded from the same nerve at increasing distances of conduction. Stimulus artifacts indicated by arrows. (a) Conduction distance 15 mm.: the action potential consists of three principal peaks, with the possible presence of a considerably slower fourth peak. (b) Conduction distance 30 mm.: subdivision of the second and third peaks into small potential waves is beginning to occur. (c) Conduction distance 40 mm.: the second and third peaks are now clearly divided into a number of small potential waves.

B. Electrophysiology

The electrophysiological properties of the spinal nerves were studied by stimulating the proximal end and recording the diphasic compound action potential at different distal levels in the same preparation.

Conduction properties. Maximal responses after conduction along increasing lengths of one nerve are illustrated in Text-fig. 5. Over short conduction distances of about 15 mm. the action potential has three principal peaks with, rarely, a very slow fourth peak. These peaks travel with different conduction velocities and represent the activi-
ties of separate axon groups. At more distal recording levels the second and third peaks, but particularly the latter, become subdivided into a number of small potential waves. As conduction velocity is directly related to axon diameter (Gasser & Grundfest, 1939; Hursh, 1939; Tasaki, Ishii & Ito, 1943) potential waves of slow velocities separate into subwaves more rapidly than fast ones (Gasser & Erlanger, 1927).

Conduction velocities for 3–6 and in one instance 7 peaks were determined from 21 nerves. When these are presented in the form of a histogram (Text-fig. 6) two principal velocity components, the slower of which is divisible into three, are revealed.

As pointed out earlier, the compound action potential of any one nerve comprises three principal peaks, of which the latter two in temporal sequence in turn comprise a variable number. The velocity of the initial peak appeared to determine the velocities of subsequent slower peaks. This would be expected if nerve conduction velocities are positively related to fish (or nerve) length as suggested by the work of Thomas & Young (1949) and Cragg & Thomas (1957) and would account for masking of the real phenomena by a graphical analysis. As shown later, only the fast velocity component was of significance in studying the electrophysiology of the fast muscle fibres. This component had a velocity of 17·0–23·8 m./sec. at 10–12° C. The second component had maximum and minimum velocities of 12·2 and 1·5 m./sec. respectively, also at 10–12° C. It is clearly separable from the first and is given no further consideration.

Threshold phenomena. As the stimulus intensity applied to the complete nerve is increased, successively more axons are recruited (Text-fig. 7).

There is a gradation of threshold from the first peak with the lowest threshold to the third with the highest in the approximate ratio of 1:3:7. The threshold values were determined by increasing the stimulus intensity until the first peak just started to appear and similarly for the second and third peaks. The stimulus intensity (voltage at electrodes) in each instance was recorded.

Anodal block. Attempts to obtain anodal block were largely unsuccessful. Partial block of the first peak of the compound action potential could be achieved but this was
capricious. Block of the second and third peaks was never achieved. Kuffler & Vaughan-Williams (1953) experienced the same difficulty with the peripheral nerves of frogs, and the same argument for their failure to obtain anodal block can be applied in this case: 'Large nerve trunks are surrounded by a connective tissue sheath and contain sensory fibres also. It is likely that in such a situation the entry and exit of current is less sharply localized in relation to the elements which it is desired to block.'

Refractory periods. Since it was not possible to apply anodal block to the nerve satisfactorily, only the refractory periods of the first peak could be determined with any validity. The absolute refractory period was determined by increasing the delay between two equal pulses until the second response just started to appear. The relative

refractory periods were determined by increasing the delay between two similar pulses until the second response just started to diminish in amplitude. The average values for the absolute and relative refractory periods determined from twenty-one nerves are 2.0 msec. (S.D. 0.08) and 13.1 msec. (S.D. 5.94) respectively.

Dorsal and ventral root responses. In order to assess the motor and sensory contributions to the compound action potential of the peripheral nerve, the responses in the dorsal and ventral roots were investigated separately.

The roots were exposed by dissection, left in situ, and their connexions to the cord were severed. The arrangement of the electrodes and the preparation are diagrammatically illustrated in Text-fig. 8.

The responses illustrated in Text-fig. 8 indicate that with orthodromic or antidromic stimulation the ventral root shows only one large peak with the possibility of a slow very small second one, while the dorsal root shows three principal peaks. Similar results were obtained by Bennett et al. (1959) from the roots of the puffer fish. The first wave in the dorsal root has a conduction velocity approximately 3 m./sec. slower than that in the ventral root.

A comparison of the separate records (see Text-figs. 5, 8) shows that the first peak
of the complete nerve response represents the activity of both motor and sensory axons, whilst the second and third represent the activity of sensory axons only, with the possibility that a few small motor axons contribute to the second peak. Muscle fibres of the abdominal wall, therefore, appear to be innervated by axons of a single fast class, and possibly also by a few axons of a slow class.

Selective stimulation of the motor axons can only be obtained by working at threshold as anodal block cannot be applied satisfactorily, and the isolation of single units is impracticable.

Muscle

Membrane potentials of muscle fibres exposed on the internal surface of the abdominal wall fall within a single class. The average value of 75.3 mV. (s.d. 4.55 mV.) did not differ significantly from that determined in Cottus serum (Hudson, 1968).

Neuromuscular transmission. Electrical stimulation of the spinal nerves elicits two types of electrical response from the muscle fibres—spike potentials and junction potentials.

Spike potentials. The response to supramaximal stimulation is an all-or-none spike potential which overshoots zero potential by as much as 20 mV. (Text-fig. 9). Repolarization consisted of a short fast phase followed by a much slower one as in frog muscles (Nastuk & Hodgkin, 1950). The duration of the fast phase of the spike-potential was 1.5-2.5 msec. Afterhyperpolarization was never observed. After several hours of experimentation spike potential amplitude would diminish and in many cases disappear altogether, leaving junction potentials only.

Junction potentials. At stimulus intensities below those required to elicit spike
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Potentials junction potentials (j.p.s) are recorded (Text-fig. 10(a)). By variations in the stimulus intensity junction potential amplitude may be quantized in discrete steps (Fig. 10(b)) until a critical level of depolarization is exceeded and spike potentials are elicited. Generally the maximum junctional response does not exceed 35 mV. Frequently it was not possible to record j.p.s in the absence of spike potentials, particularly in fresh preparations. This suggests that spike potentials are normally...
elicited by a single j.p. In a few preparations and from some muscle fibres in others, j.p.s were the only responses obtained. In these fibres, a second stimulus following a short interval after the first frequently elicited a spike potential by summation of two j.p.s to exceed a critical threshold.

The distributed nature of the neuromuscular junctions along the length of the muscle fibres as seen histologically is verified by the electrophysiological findings. Junction potentials of variable amplitude and form that always precede spike potentials (see Text-fig. 9) and those obtained by threshold stimulation were recorded from any point across a myotome. The space constant of the muscle fibres is 3.1 mm, approximately one third of their length. Further, spike potentials are recorded from any point in a myotome without appreciable latency differences. As shown later, spike potentials are propagated with an approximate conduction velocity of 1.1 m/sec. which is more than 10 times slower than that of the motor axons. The quantized nature of the junction potentials indicates that the muscle fibres are polyneuronally innervated. These two conclusions accord with those of Takeuchi (1959) for snakefish, and Barets (1961) for tench, on other evidence.

Table 1. Latencies of j.p.s and spike potentials (msec.), measured with respect to the stimulus artifact, at three different transaxial levels along a narrow bandwidth of muscle fibres (posterior adjacent nerve stimulated)

<table>
<thead>
<tr>
<th>Distance along muscle</th>
<th>(A) One-third</th>
<th>(B) Two-thirds</th>
<th>(C) Seven-eighths</th>
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</thead>
<tbody>
<tr>
<td>7.00</td>
<td>10.50</td>
<td>6.50</td>
<td>17.00</td>
</tr>
<tr>
<td>7.00</td>
<td>10.25</td>
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<td>6.50</td>
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<tr>
<td>7.00</td>
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<td>6.50</td>
<td>11.25</td>
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<tr>
<td>7.75</td>
<td>10.75</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Average 6.72</td>
<td>11.50</td>
<td>6.42</td>
<td>14.08</td>
</tr>
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</table>

Propagation of the spike potential. In a unique preparation the observation that spike potentials appeared to arise with different latencies at either side of the myotome suggested that these potentials are propagated. As single fibres cannot be traced for more than a short distance this possibility was examined by recording the responses at three levels across a narrow bandwidth of muscle fibres (see insert Text-fig. 11). The latencies of the j.p.s and spike potentials, with respect to the stimulus artifact, recorded at these levels are presented in Table 1. Typical responses at the two extreme levels are illustrated in Text-fig. 11. Junction and spike potentials at level A occurred with average latencies of 6.72 and 11.50 msec. respectively. At level C the average latencies were 7.2 and 16.4 msec.

The different latencies of the junction potentials at levels A and C do not indicate axons of different conduction velocities as the motor axons fall into a single fast class (see p. 60). Fatt & Katz (1951) showed that at increasing distances from the end-plate
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region on frog sartorius muscle the intracellular end-plate potential occurs with increasing latency, slower time course, and greatly diminished amplitude. The similarity of j.p.s preceding spike potentials at C, illustrated in Text-fig. 11 (c), (d), with those at distant recording sites in frog muscle is obvious. Confirmation of this analogy is provided by the short space constant of Cottus muscle. The different latencies of j.p.s at A and C are thus of no significance concerning the temporal initiation of spike potentials, but rather indicate that they have been conducted from distant sites.

Spike potentials recorded at A and C have an average latency difference of 4.95 msec. This finding can only be explained on the assumption that spike potentials are propagated. The average latency of spike potentials at B is 14.08 msec., but is based on three readings only. This latency is intermediate to the above quoted and suggests that spike potentials were initiated at points proximal to B and conducted towards C. On this assumption and that the muscle fibre length is 1 cm. the calculated conduction velocity of the spike potentials is approximately 1.1 m./sec. at 10–12° C.

Polyneuronal innervation. Polyneuronal innervation is identified by the quantized nature of junction potentials where each jump in amplitude, as the stimulus intensity is increased, represents the activation of an additional axon. The number of jumps is thus a direct measure of the number of separate axons stimulated, assuming that only one additional axon has been recruited for each jump. If junction potentials occur with

Text-fig. 11. Evidence for propagation of spike potentials. Representative responses recorded at two different levels along a narrow bandwidth of muscle fibres (see diagram). Responses (a) and (b) were recorded at level A; responses (c) and (d) at level C. The nerve stimulated was to the left of the diagram. Note the increased latency of the spike potentials and the small amplitude of the junction potentials at level C. The horizontal arrow in the diagram indicates the direction of spike propagation. Stimulus artifacts indicated by white spots.
different latencies, then axons of different diameters and conduction velocities are involved.

Since spike potentials mask junction responses it was not possible to determine accurately the number of axons that supply a single muscle fibre in which both responses occurred. The extent of polyneuronal innervation was, therefore, determined from those fibres that gave j.p.s only in fresh and fatigued preparations.

Simultaneous recordings of the response from the nerve and the resultant activity in muscle fibres of the posterior adjacent myotome, to small variations in stimulus intensity, were made to classify the motor axons and to determine the degree of poly-

Text-fig. 12. Simultaneous recordings of the nerve compound action potential (upper trace) and the concomitant response from a single muscle fibre (lower trace) to variations in the stimulus intensity. The difference between the two traces represents the membrane potential of the muscle fibre except in (f). Spike potentials failed to be elicited by supramaximal stimulation. The experimental situation is illustrated. S, Stimulating electrodes; E, earth electrode. Nerve responses were monitored extracellularly at the distal end. Muscle responses recorded intracellularly. (a)–(d) Separate responses to small increments in stimulus intensity at and just above threshold. (e) Responses to supramaximal stimulation. The muscle responses are associated with the first peak of the compound action potential. (f) Superimposed responses recorded from the same muscle fibre to small increments in stimulus intensity. Note that five responses were obtained from the muscle fibre and that, except for the first, they occur with the same latency. Nerve conduction distance c. 60 mm.

neuronal innervation. First, the motor axons were found to be contained within a single fast class. Responses obtained from single muscle fibres were in all cases correlated with the first peak of the compound action potential (Text-fig. 12, 13). More conclusively, jumps in junction potential amplitude obtained to increasing stimulus
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intensity appeared with the same, or only slightly different latencies, even with nerve lengths of up to 60 mm. (Text-figs. 12(f), 13(e)). The latency of the first response in Text-fig. 12(f) is not considered to be due to excitation of a slower axon since at threshold for nerve excitation there is considerable variation in shock-to-spike initiation time (Blair & Erlanger, 1933).

Secondly, each muscle fibre was found to be polyneuronally innervated by 2–5 axons. An innervation by less than 2 axons was never observed. The responses illustrated in Text-figs. 13(e), 10(b) and 12(f) indicate that 3 different muscle fibres were innervated by 3, 4, and 5 axons respectively. The different time courses of the responses illustrated in Text-fig. 12(f) suggest that one or more abortive action potentials were elicited. As stimuli were applied to the nerve at minimal intervals of 1 sec., and the responses were repeatable, these potentials are considered to represent the activation of separate axons whose myoneural contacts are situated at different distances from the recording site.

More commonly an innervation by 2–3 axons was found. This is considered to be,
on the whole, a low figure even though some muscle fibres are undoubtedly innervated by two or three axons only. For the same reasons as mentioned below the possibility exists that more than five axons innervate single fibres. As the differences between stimulus intensities required to elicit the separate responses is extremely small and fractional variations difficult to obtain, it is likely that in many instances two or more axons were stimulated simultaneously. Moreover, as the space constant is short, j.p.s from distant sites will not be recorded above noise due to electrotonic decay, or will be masked by the activity of junctions in closer proximity to the recording site.

Text-fig. 14. Diagrammatic illustration of the preparation investigated to determine whether more than one spinal nerve innervates the muscles of one myotome. The adjacent (ad) and sub-adjacent (s-ad) nerves of the myotome probed are indicated.

All the muscle fibres of a myotome are polyneuronally innervated, as shown above, by axons of the anterior adjacent spinal nerve. To test whether a single myotome is innervated by axons from more than one spinal nerve the responses to stimulation of the posterior and the subadjacent nerves were determined (see Text-fig. 14). Intracellular recordings from more than 150 fibres indicated that all the fibres in a myotome are polyneuronally innervated by at least 2–4 axons emanating from the posterior adjacent nerve. Responses recorded from 90 fibres in five preparations showed that, in addition, all the muscle fibres are innervated also by at least 2–4 axons from each subadjacent nerve. No results were obtained that suggested that more than four spinal nerves innervate the muscles of a myotome.

Identical responses were recorded from muscle fibres in a myotome to stimulation of the anterior and posterior adjacent nerves. In contrast, junction potentials recorded in subadjacent myotomes differed from those following stimulation of the adjacent nerves in that their amplitudes were generally less than 10 mV. A comparison of junction and spike potential latencies and the latencies of movement artifacts in adjacent and the subadjacent myotomes excluded the possibility that the subadjacent muscle responses were artifactual. Moreover, their quantized nature supports the contention that these responses reflect neuromuscular events and confirms the histological evidence.
DISCUSSION

The electrophysiological properties of the spinal nerves do not differ from those obtained by Gasser, Erlanger, Blair and others for the spinal nerves of amphibians and mammals, but results obtained by the diphasic recording method are open to a number of well-documented criticisms. Principal amongst these is that, when the electrode separation is less than the wavelength of an action potential, distortion of the time course and amplitude of the electrical events at each electrode occurs because activity reaches the distal electrode before repolarization has occurred at the proximal one. This distortion is particularly serious when the response of a whole nerve trunk is recorded since the contributions of both the slow and fast conducting elements will tend to cancel each other out, and the method only records the difference in potential between the two electrodes (Ruch, Patton, Woodbury & Towe, 1965). In connexion with the present findings that the fast muscles are innervated by only large diameter axons (cf. Text-figs. 6, 12), these criticisms can safely be discounted.

The conduction velocities of 17-23.8 m./sec. at 10-12°C and diameters of 10-14 μm. of the fast motor axons compare favourably with those of frog fast motor axons which have a conduction velocity of 8-40 m./sec. (Kuffler & Vaughan-Williams, 1953) and a diameter of about 12 μm. (Tasaki & Mitzutani, 1944; Tasaki & Tsukagoshi, 1944). Tench motor axons about 13 μm. in diameter conduct with velocities of 15-24 m./sec. at 22°C. (Barets, 1961) somewhat slower than those obtained here on the basis of a Q₁₀ of 1.4 for fish nerves (Döving & Gemne, 1965).

Data are presented to indicate that muscle spike potentials are propagated. Although based on evidence of an indirect nature the conduction velocity of approximately 1.1 m./sec. at 10-12°C compares favourably with that of frog sartorius muscle which is 1.6 m./sec. at 20°C. (Eccles, Katz & Kuffler, 1941) and active chelonian muscle which is 1.3 m./sec. at 15-24°C. (Levine, 1966).

Previous investigators found that the fast muscles of teleosts that receive a focal innervation give all-or-none spike potentials which overshoot (Barets, 1961; Hagiwara & Takahashi, 1967), whilst those that have a distributed innervation give either abortive spike potentials (Barets, 1961) or spikes that generally fail to overshoot (Barets, 1961; Hagiwara & Takahashi, 1967; Takeuchi, 1959). The present findings suggest that there is no correlation between the pattern of innervation and the type of electrical response, but rather that fast muscles receiving a distributed innervation also give all-or-none spike potentials that show a marked positive overshoot (see also Hudson, 1968).

The extent of polyneuronal innervation of the fast muscles is considerably greater than that previously indicated in fish (Barets, 1961; Takeuchi, 1959) or any other animal group. Each muscle fibre is polyneuronally innervated by four segmental nerves, and receives an innervation from between two and five axons or more (see p. 61) from a single nerve. Segmental overlap of the motor innervation originally proposed by Müller (1909), and later supported by ten Cate (1935), in two different elasmobranchs is thus confirmed, at least for teleosts. Whether this overlap is trapezoidal in shape, being wider ventrally than dorsally as found for the sensory dermatomes (e.g. ten Cate, 1927), is not known.

In the case of muscle fibres with distributed myoneural junctions, three basic types
of innervation (Text-fig. 15) are possible—multiterminal, polyneuronal, or a combination of both. Since polyneuronal innervation has been demonstrated the first can be eliminated. To distinguish between the latter two the precise number of nerve terminations per fibre and the full extent of polyneuronal innervation must be known. Each muscle fibre in the present studies was found to be polyneuronally innervated by 8–20 different axons, and to possess distributed along its length 8–22 discrete nerve terminal regions. Whilst some terminal regions are clearly supplied by single axons, others appear to be supplied by up to three. In the latter instances it was not possible to determine whether these were branches of a common motoneurone. However, it was earlier argued (p. 53) that each terminal region is supplied by a single axon. On this basis the results of the present research favour the hypothesis that the fast muscles of *Cottus* are polyneuronally innervated in the absence of multiterminal innervation.

Text-fig. 15. Diagrammatic illustration of three basic possible types of innervation that pertain to muscle fibres that have nerve terminations distributed along their length. These are multiterminal (A), polyneuronal (B), and a combination of both multiterminal and polyneuronal innervation (C).

Can multiterminal innervation be ruled out? Although there is a remarkably good correlation between the number of terminal regions per fibre and the extent of polyneuronal innervation the answer to this question is no. A number of the terminal regions may in fact be supplied by more than one axon even though this is not at present considered to be the case. Motor axons may branch within the spinal nerve trunk at more central levels than those at which stimuli were applied to the nerve. Further, the earlier conclusion favouring polyneuronal innervation only is based on the result of averaging available data. The possibility, which cannot be ruled out, that a single fibre may be innervated by fourteen axons yet have eighteen terminal regions, for instance, would be hidden by such treatment.

As regards the functional properties of teleost fast muscles, it is important that the question whether or not multiterminal innervation is present should be resolved. If the fast muscles are polyneuronally innervated only, a wide range of contractile responses
would be expected. This would not be true if the muscle fibres were both multi-
terminally and polyneuronally innervated. Efforts to resolve this question are in pro-
gress at the present time, and until this has been achieved it is premature to speculate
further on the functional properties and roles of the fast muscles in the swimming
behaviour of fish.

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SUMMARY

1. Histological and electrophysiological studies of the spinal nerves, nerve roots and
muscles of the abdominal wall of the marine teleost Cottus scorpius have been under-
taken to determine the extent and nature of polyneuronal innervation of the fast
muscles.

2. Spinal nerves at proximal and distal levels, and the dorsal roots, contain axons in
a single mixed population with a mean diameter of 2.4 µm., while the ventral roots
contain axons in two diameter classes with means at 4.6 and 12.14 µm.

3. Between 8 and 22 distributed nerve terminations were counted on fifty-two
teased intact single muscle fibres stained for acetylcholinesterase activity. The average
distance between the terminals is 0.64 mm. (range 0.09 ± 2.05 mm.).

4. The compound action potential of the nerve comprises two principal peaks with
conduction velocities of 17.0—23.8 m./sec. and 1.5—12.2 m./sec. at 10—12°C.

5. Fast muscle fibres gave two types of electrical response—all-or-none spike
potentials that are propagated with a conduction velocity of c. 1.1 m./sec. at 10—12°C.,
and quantized distributed junction potentials.

6. The electrical properties of the nerves and roots suggest that the fast muscles are
innervated by a single class of fast axons and possibly by a few slow axons.

7. Simultaneous recordings of nerve and muscle activities were made at different
stimulus intensities. In all cases muscle responses were correlated with the first peak
of the compound action potential, and appeared with the same or only slightly dif-
ferent latencies.

8. Each muscle fibre is shown electrophysiologically to be polyneuronally inner-
vated by 2—5 axons from a single spinal nerve, and to receive a similar axonic comple-
ment from each of four spinal nerves.

9. Polyneuronal innervation of the muscle fibres by 8—22 different axons in the
absence of multiterminal innervation is postulated.
REFERENCES


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(Facing p. 66)


**EXPLANATION OF PLATES**

**PLATE 1**

Fig. 1. Transverse sections through one spinal nerve at proximal (a), medial (b), and distal (c) levels. Stained with Wolter's haematoxylin. The nerve is seen to contain myelinated axons of various diameters. A large number of small unmyelinated axons 1–2 μm, grouped in a bundle to the left in (d) (stained with toluidine blue), is also present.

Fig. 2. Transverse sections of ventral (a) and dorsal (b) spinal nerve roots. Note the group of small-diameter axons situated to one side in the ventral root. Stained with toluidine blue.

**PLATE 2**

Nerve terminations on groups of teased muscle fibres from the abdominal wall. Stained for acetylcholinesterase activity. Axons in a nerve bundle (a) do not all terminate on the same muscle fibre. The distributed nature of the nerve terminations along the muscle fibres and their density (b) are illustrated. Each muscle fibre is innervated at a number of different sites.

**PLATE 3**

Examples of nerve endings commonly observed distributed along the length of teased single muscle fibres. Stained for acetylcholinesterase activity. Axons branch pre-terminally and terminally and synapse extensively with a muscle fibre at a large number of sequential bead-like expansions as indicated by arrows in (a). In (c) one axon only innervates the terminal region, and this appears also to be the case in (b). Two axons appear to innervate the terminal region in (c). Note the double nature of each terminal region.