

THE PATTERN OF MOTOR INNERVATION IN MAMMALIAN STRIATED MUSCLE

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INTRODUCTION

It has long been known that the motor nerve fibres entering a muscle may branch profusely to supply many muscle fibres (Ramón y Cajal, 1881, 1909), but little information is yet available on the mode of distribution or the extent of overlap and intermingling effected within a muscle by these nerve fibres and their terminals. Further knowledge of these features of the pattern of innervation in mammalian striated muscles seems desirable in order to provide a more precise anatomical background for studies of muscle function.

The present report is based upon the examination of the neural pattern in selected muscles of the rabbit and in the extrinsic ocular muscles of two macaque monkeys.

MATERIALS AND METHODS

The anatomical material was obtained from eighteen young adult rabbits and from two macaque monkeys.

The muscles examined include the lumbrical and other intrinsic plantar muscles, the extensor digitorum longus, tibialis anterior, soleus, diaphragm, sterno-mastoid, crico-thyroid and the extrinsic ocular muscles.

The main histological method used was the technique of slow intravenous perfusion of methylene blue into the anaesthetized animal. The details of this technique and its application to neuro-anatomical studies have already been described (Feindel, Sinclair & Weddell, 1947; Feindel, 1948). This method, if conditions are optimal, gives selective staining of intramuscular axis cylinders and nerve endings. It is thus possible, after fixation and clearing, to map out the general neural pattern and to examine details of innervation in whole preparations of small muscles such as the lumbricals and extrinsic ocular muscles. Because the dye is perfused throughout the animal a range of preparations illustrating the innervation of muscles from different parts of the body can be obtained and compared. The larger muscles, however, must be divided into smaller segments before the details of their innervation can be determined. The segments which can be usefully examined are, nevertheless, relatively large compared with the extent of tissue available for study in the usual paraffin or frozen sections.

The neural elements in the crico-thyroid and sterno-mastoid muscles were stained by injecting a dilute solution of methylene blue directly into the muscle (Weddell, Harpman, Lambley & Young, 1940). Although this method inevitably gives considerable mechanical distortion of the neural pattern in such a tissue, material

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so obtained was nevertheless of considerable value for examining the arrangement of pre-terminal motor fibres and of end-plates.

In muscles too large to be examined histologically in the form of whole preparations, the neural patterns were examined by dissection under a low power binocular microscope. The muscle fibres were carefully teased apart to display the main nerve trunk and many of its smaller branches. The exposed nerves and the muscle fibres around them were then moistened at intervals with a 0.25% solution of osmium tetroxide, which blackens the nerves and renders visible the finer nerve bundles which proceed from them. The muscles were then allowed to clear in pure glycerol. After clearing it is possible, particularly in strap-like muscles, to display extremely fine nerve bundles by carefully teasing the muscle fibres off their surface.

The crico-thyroid muscles from two rabbits were cut in serial sections and impregnated by the protargol silver method of Bodian after the following experimental procedures had been carried out. The muscles were prepared in one animal 6 weeks after section and removal of a segment from the left superior laryngeal nerve; in the other animal, 6 weeks following unilateral section and removal of a segment of the motor branch to the muscle from the pharyngeal plexus. Immediately before the animals were killed, the crico-thyroid muscles were examined electro-myographically.

OBSERVATIONS

Intrinsic plantar muscles

In intact lumbrical muscles prepared by the intravenous staining technique the course of nerve fibres can be examined from a point 25 mm. proximal to the muscle, up to their final terminations within the muscle. The remarkable complexity of the neural pattern as illustrated by an area of lumbrical muscle shown in Pl. 1, fig. 1, precludes the tracing of individual axons over any great distance. However, since such preparations provide for study considerably larger areas of the intramuscular neural plexus than have been previously reported in the literature, it seems worth while to describe an example in detail.

The general scheme of the neural pattern in the area of lumbrical muscle (Pl. 1, fig. 1) is brought out more clearly in the diagram (Pl. 1, fig. 2) based on the photomicrograph.

The course of the main nerve trunk is represented by the heavy black line, *A-X-B*, running through the centre of the diagram. A number of large nerve bundles branch off from this main nerve stem to form an intricate plexiform pattern. Near the mid-point (*X*) of this main nerve stem one large nerve bundle branches off to the left, and divides in turn to form smaller bundles, *D* and *E*, running to right and left. Another bundle of the plexus, marked *C*, is represented near the top left of the diagram. Still another bundle, labelled *F*, comes off the deep-lying portion of the main stem, between *X* and *B*. It may be noted that these secondary bundles continue beyond the field shown in the photomicrograph.

In this zone of muscle, twelve bundles of terminal fibres (indicated in the diagram, Pl. 1, fig. 2, by arrowed lines), which are derived from the main nerve stem and its secondary branches, give rise to sprays of motor end-plates.

All the nerve fibres do not travel to their terminations in the direction *A* to *B*,

a fact suggested by the angle at which the individual fibres or small bundles leave the main stem or its branches. Examination under high magnification shows that some of the branches are made up chiefly of nerve fibres which have an upward direction, i.e. from *B* to *A*.

The plexiform nature of the pattern is illustrated at the forks of the main nerve trunk and its secondary branches. At such forks, axon bifurcation frequently takes place and each collateral so formed can often be traced into different branches of the fork. This clearly makes it possible for one nerve fibre to supply endings which may lie in different regions of the muscle. Axon branching also takes place in the course of a nerve bundle not in the region of a fork. This is illustrated by Pl. 3, fig. 11, where the arrow indicates branches which continue for a distance in the same nerve bundle. It can also be seen that some of the fibres in the branches of the main nerve stem (Pl. 1, fig. 1) are derived from sources other than the main stem. For example, the branches *D* and *E* contain in common some nerve fibres not derived from *A-X-B*.

The terminal fibres and motor end-plates are disposed for the most part in groups which may consist of as many as fifteen or twenty endings. Less commonly, groups of two or three endings are seen: these are usually close to the larger groups of terminals. Isolated endings are rare, and when present, are borne upon fibres which have their origin near the terminal distribution of a small nerve bundle, and only unusually from a large plexus bundle. The sprays of motor end-plates are usually grouped in circumscribed zones at some distance from one another, the intervening zones being free from end-plates and often from nerve fibres as well.

The pattern shown by these terminal sprays of motor endings is such that several different axons of a nerve bundle supply end-plates in the same zone of muscle. Many of these axons, when traced back from the end-plates, are seen to remain separate from each other, although each axon may commonly give off collaterals which run in other nerve bundles to other zones of the muscle. Some of the axons, however, branch in their preterminal course (Pl. 3, fig. 11), so that the number of end-plates in any given zone of muscle is greater than the number of axons in the nerve bundle supplying that zone.

The motor end-plates derived from any one axon supplying terminals in that zone may be arranged in a variety of ways. Most frequently, however, the individual endings of one axon terminate on scattered muscle fibres separated by intervening muscle fibres which are supplied by other axons of the terminal nerve bundle to that zone.

The resulting arrangement is such that each axon of the terminal nerve bundle appears to fan out and branch to supply throughout the zone end-plates which interweave with the endings of other axons of the same bundle. Conversely, if several adjacent motor end-plates are picked at random in a zone of muscle and their axons of supply traced proximally, it is usual to find that they are derived from different axons of the nerve bundle.

A similar arrangement of terminal sprays of motor end-plates is found in the crico-thyroid muscle (Pl. 2, fig. 6). Here again the muscle fibres are innervated by nerve bundles the axons of which fan out, divide and end on muscle fibres so that

endings derived from one axon interweave with those derived from other axons of the same nerve bundle.

Additional examples of grouped end-plates from another lumbrical muscle are shown in Pl. 2, fig. 5. Nerve fibres from three different nerve bundles, labelled *A*, *B* and *C*, supply motor endings to muscle fibres in this region. Each nerve bundle approaches the area from a somewhat different direction as indicated by the arrows. The endings interweave in relation to the distribution of the muscle fibres which they supply. At the optical plane represented, for example, two terminals from bundle *A* supply muscle fibres on either side of a third muscle fibre which receives a terminal from bundle *B*. The terminal fibres to this zone of muscle are also arranged in sprays, the nerve bundles *A*, *B* and *C* giving off respectively four, two, and five end-plates. Careful examination showed no evidence of a muscle fibre in this zone having more than one axon supplying it. As in the preceding examples, it is to be noted that there is no arrangement whereby a solitary axon approaches a local area to supply end-plates to a group of contiguous muscle fibres.

Although this report is devoted primarily to a consideration of the pattern of motor nerve fibres in muscle, it need hardly be mentioned that many of the intramuscular nerve fibres are afferent in function. In nerve bundles of the intramuscular plexus these may be indistinguishable from nerve fibres derived from ventral roots. Nevertheless, two types of sensory fibres may be identified with reasonable certainty.

Scrutiny of the large nerve bundles shown in Pl. 1, fig. 1, will disclose occasional nerve fibres of larger diameter, which are 'giant' afferent fibres supplying muscle-spindles and tendon-organs.

A second type of fibre is indicated by arrows in Pl. 1, fig. 1. It is distinguished by its small size and its beaded appearance and by the fact that, unlike the larger intramuscular nerve fibres, it does not supply organized endings. Such fine beaded fibres occur infrequently in the muscle tissue proper, this being the only one found in the area of muscle pictured in Pl. 1, fig. 1. They may be seen more often in the connective tissue septa or sheath of the muscle.

Neural pattern in larger muscles

The general neural pattern in different muscles as demonstrated by methylene blue staining shows considerable variation. Neither the size and number of the nerves entering a muscle nor their sites of entry give much indication of their intramuscular distribution and pattern. In one muscle a small nerve entering as a single trunk at one point may arborize to cover a wide intramuscular region. In other muscles, as is well known, the zone of innervation may be restricted to part of the muscle so that other regions are almost lacking in nerve fibres and terminals.

A portion of the gross neural pattern within the sterno-mastoid muscle as displayed by dissection under a binocular microscope is shown in Pl. 1, fig. 4. The main nerve runs lengthwise through the muscle and at intervals gives off lateral branches. Some of these branches leave the main nerve trunk almost at right angles and subsequently divide into smaller nerve bundles. A proportion of these smaller nerve bundles take a recurrent course and 'anastomose' with bundles derived from neighbouring branches of the main nerve trunk (Pl. 1, fig. 4, arrows). It

should be noted that such 'anastomoses' occur in relation to nerve trunks and nerve bundles, and not in relation to single nerve fibres, which retain their individuality within the intramuscular plexus. It is clear that not all the nerve fibres in one of the lateral branches are destined to end in the zone of muscle to which they are at first directed, since some may be recircuited by way of these anastomotic bundles to other parts of the muscle.

The gross neural pattern in the other large muscles which have been examined in this study (extensor digitorum longus (Pl. 1, fig. 3), tibialis anterior, soleus, and the diaphragm) varies to a greater or lesser degree from that just described, according to the arrangement of the muscle fibres within the muscle as a whole. 'Loops' formed by recurrent nerve fibres are seen in all these muscles although the number and size of such 'loops' varies considerably.

In segments of larger muscles stained by methylene blue, it is possible to trace the course of some individual axons for a considerable distance. Single axons have been seen giving rise to numerous branches which pursue a complicated, at first usually divergent, but later sometimes recurrent course through the intramuscular plexus. Such single axons thus give rise to end-plates in widely separated positions throughout the muscle segment. It is also possible to determine that the motor end-plates supplying contiguous muscle fibres in any given zone of innervation may be supplied by axons which approach by different routes through the intramuscular plexus as described with reference to the lumbrical muscles (Pl. 2, fig. 5). In all segments examined it is rare to find isolated motor fibres and end-plates.

The neural pattern in these larger muscles of the rabbit is therefore similar in essential features to that in the lumbrical muscles.

Crico-thyroid muscle

The crico-thyroid muscle, which is supplied by two separate nerves, the superior laryngeal and a branch from the pharyngeal plexus, has been included in this report because it has been stated that single muscle fibres in this muscle receive a dual motor supply (Exner, 1885).

Both main nerves to the muscle take part in the formation of a common intramuscular plexus, and histological examination of the crico-thyroid muscle suggests that its neural pattern is similar to that of the lumbrical muscles. Unfortunately, since the specimens of crico-thyroid muscle were stained by the local injection technique, it was not possible to define the course of the axons in relation to the whole neural pattern as in the case of the lumbrical muscles. However, well-stained sprays of motor end-plates in local zones of crico-thyroid muscle (Pl. 2, fig. 6) are similar to those found in lumbrical muscles. In the light of the analysis of these terminal groups in whole preparations of lumbrical muscles it seems justifiable to assume that, in the crico-thyroid muscle as well, these terminal sprays represent intermingling of end-plates which, in some instances at least, are derived from different parent axons of the intramuscular plexus.

Six weeks after unilateral section of the branch of supply from the pharyngeal plexus, normal motor end-plates were seen scattered throughout the partially denervated muscle. In a second experimental animal, 6 weeks following section of the superior laryngeal nerve, end-plates were again found scattered throughout the

muscle. Each nerve thus gives rise to terminals which are distributed throughout the muscle, rather than to any particular zone of it.

Multiple end-plates on single muscle fibres

From time to time, evidence of a morphological or physiological nature has suggested the innervation of individual muscle fibres by more than one axon. In the present study no direct histological evidence for this was found. Certain observations have been made, however, which have a bearing on this problem.

Where a group of muscle fibres innervated by a spray of fifteen to twenty intermingled end-plates is examined under the low power of the microscope, it often appears that one muscle fibre is supplied by two or even more individual axons and end-plates (Pl. 2, fig. 5). However, critical examination showed that, in the majority of cases, each end-plate was associated with a separate muscle fibre. This could be determined by examination of thick, cleared preparations under high magnification, when each individual end-plate, whether seen in surface (Pl. 2, figs. 9 and 10) or side view (Pl. 2, figs. 7 and 8), could be clearly defined in relation to its corresponding muscle fibre.

Nevertheless, in a few instances, single muscle fibres associated with two or more end-plates have been observed in preparations of crico-thyroid muscles (Pl. 3, fig. 11) and limb muscles (Pl. 3, figs. 12 and 13).

It is perhaps worth while to emphasize again that the occurrence of two or more end-plates on a single muscle fibre does not necessarily provide histological evidence for true multiple innervation by different parent axons. In examples observed in this study, such end-plates were always supplied from the same parent axon. In Pl. 3, figs. 12 and 13, the double end-plates are situated close together on the muscle fibre. The axon either divides near its termination (Pl. 3, fig. 13) or supplies two end-plates 'in series'. In an unusual example taken from the crico-thyroid muscle (Pl. 3, fig. 11), the terminal fibres supplying a double end-plate could be readily followed back to their point of division.

In whole specimens or larger areas of muscle prepared by the methylene blue techniques we have employed, it is usually possible to trace out the exact arrangement of such double terminals: in material prepared in the form of thin serial sections, it is rarely possible to do this. A picture illustrating this is taken from a paraffin section of muscle stained by the protargol method of Bodian (Pl. 3, fig. 14). The lower terminal is a double end-plate obviously supplied by one axon. The origin of the upper end-plate which lies on the same muscle fibre cannot be determined. In the light of the previous examples, it is not possible to assume that the multiple end-plates on this single muscle fibre are necessarily derived from different parent axons.

Nevertheless, the possibility that single fibres in the muscle have a dual innervation cannot be wholly excluded on histological evidence. In an attempt to throw further light on the matter, in two animals one or other of the motor nerves to the crico-thyroid muscle was sectioned unilaterally. Six weeks after these operations electromyographic studies of the partially denervated muscles were made immediately after sectioning the intact nerve. Spontaneous fibrillation action potentials were recorded from all parts of the affected muscles by using either very fine

monopolar or concentric needle electrodes. At no point on or within the muscle was there electrical 'silence'. Immediately after section of both motor nerves no action potentials could be obtained from any position on or within the muscles on the unoperated side.

Neural pattern of extrinsic ocular muscles

The extrinsic ocular muscles differ distinctly in their neural pattern from the muscles considered above. The pre-terminal nerve fibres usually run in a direction approximately parallel to the muscle fibres. There appears to be less terminal intermingling of the nerve bundles and more variation in size of individual terminal fibres (Pl. 4, fig. 18) than in other muscles examined in this study. The density of nerve fibres and endings in some zones of the muscle is particularly striking.

The nerves supplying the ocular muscles are also distinguished by their mode of terminal branching. The terminal branches in the limb muscles are most commonly arranged as small bundles of intermingling nerve fibres each of which supplies a spray of end-plates in a zone of muscle (Pl. 2, figs. 5 and 6). Isolated terminal fibres and end-plates are unusual and an axon supplying more than one end-plate to the same muscle fibre is a rarity.

In contrast, both these features are frequently observed in the ocular muscles. Single isolated nerve fibres are as common as small nerve bundles (Pl. 4, figs. 15, 16 and 20). Characteristically, a nerve fibre supplies a number of pre-terminal fibrils ending in plaque-like terminals, many of which are applied to the same muscle fibre (Pl. 4, figs. 15 and 16). Other nerve fibres can be traced along a muscle fibre to their termination in complex endings composed of a series of irregular knobs (Pl. 4, fig. 15, fibres 2 and 3; and fig. 17). Endings of much the same appearance as ordinary motor end-plates found in limb muscles are also present in the ocular muscles (Pl. 4, fig. 21). Near the insertion of the muscle a third, more complex, type of ending is found, in the form of terminal fibrils which split repeatedly to supply plaques and knob-like endings (Pl. 4, figs. 18 and 19). As with the nerve endings noted in Pl. 4, figs. 15 and 16, these also extend for a considerable distance along the muscle fibre.

DISCUSSION

General features of the neural pattern

With the exception of the extrinsic ocular muscles, all the muscles examined in this study appear to have neural patterns which are essentially similar in their main features. The intricate plexiform pattern formed by the intramuscular nerve trunks and bundles provides anatomical pathways for much mixing and interweaving of the nerve fibres during their distribution within the muscle. At the points of subdivision of the nerve trunks making up the intramuscular plexus, groups of individual fibres become segregated into nerve bundles which course in divergent directions. Very commonly fibres undergo division immediately proximal to such a fork and the collaterals take divergent pathways.

An increase of the fibre-counts immediately after division of a nerve trunk, so that the sum of the fibres in the two smaller bundles is greater than the number of fibres in the parent nerve trunk, does not necessarily imply that the collaterals

formed by dichotomy take divergent paths along two different nerve trunks. As noted by Eccles & Sherrington (1930), increase of fibres by division occurs along parts of a nerve trunk not undergoing separation into smaller bundles. Occasionally geminal fibres can be seen coursing together for short distances or even running into the same nerve bundle when an alternative diverging bundle is available. However, the most common event when the fibres of a nerve trunk are sorted out into two different bundles appears to be a divergence of collaterals, and certain knowledge of this can be obtained by directly tracing the divergent path of the fibres, as in the present studies, or by showing that action potentials can be conducted from one collateral branch to the other by way of their common junctional stem (Adrian, 1925; Cooper, 1929).

These anatomical features, the dichotomy of fibres and the divergence of their collateral branches, make it possible for one parent fibre in the main nerve to supply many terminals, some of which may be applied to individual muscle fibres scattered throughout different parts of the muscle. The anastomoses of intramuscular nerve trunks and branches, depending upon the extent to which these are present in any particular muscle, enable an even more complex distribution of nerve fibres to take place.

The sprays of pre-terminal fibres and end-plates given off from the intramuscular nerve bundles usually innervate a group of contiguous muscle fibres. The individual endings of such sprays are, however, frequently derived from different axons.

It may be noted in passing that a different pattern of innervation is presented by the large sensory receptors in muscle, of which the neuromuscular spindles and the tendon end-organs are the main types. In contrast to the complexity of branching and intermingling of the motor fibres, the large afferent nerve fibres which supply these end-organs are distinguished by absence or paucity of branching (Feindel, 1949). These sensory nerve fibres therefore provide a direct restricted pathway between a local zone of muscle and the spinal cord. The fine, beaded, non-myelinated fibres which supply free nerve endings to the connective tissue septa or sheath of a muscle and to muscle spindles are probably related to pain sensibility in muscle (Feindel, Weddell & Sinclair, 1948).

Overlap and intermingling of nerve endings

The terminals derived from different axons intermingle and overlap to a marked degree, somewhat more in the intrinsic plantar than in the lumbrical muscles. Such overlapping is probably at its greatest in the distal muscles of the limb. These findings provide an anatomical basis for the physiological observations of Sherrington (1892).

Sherrington found that in the macaque monkey the lumbricals and interossei of the foot receive a segmental supply from the 7th, 8th and 9th subthoracic roots of a post-fixed plexus and from the 7th and 8th of a pre-fixed plexus. Stimulation of the anterior roots of any of these segments resulted in contraction of the lumbricals and interossei. He also observed that in the monkey, cat and rabbit the neural segments supplying the distal part of the limb 'are so intimately fused in the plantar region that it is difficult to pick out from the rest any one muscle which shows more of this or that constituent'. Sherrington further demonstrated that

this intermingling could be defined in terms of even smaller units, since stimulation of different filaments of the roots reproduced visible contraction in the same muscles as did stimulation of the entire root. Indeed, in the monkey, stimulation of the highest of nine rootlets of the 8th subthoracic segment produced the same type of movement of the hallux as stimulation of the lowest of four rootlets of the 9th segment (Sherrington, 1892).

From this evidence it would seem necessary for intermingling of the motor fibres in such distal muscles to occur to such an extent that all parts of the muscles would be supplied by terminals from different rootlets. From the present histological study it is apparent that the neural pattern of these muscles features a degree of overlap and intermingling of intramuscular nerve fibres which is in keeping with the diffuse physiological representation of each motor rootlet throughout these muscles.

So far as they go, the histological observations made in this study on larger muscles also would support the idea of interweaving of axonal terminals and the separation and scattering of muscle fibres belonging to the same motor unit.

The gross neural pattern certainly varies in the different muscles we have examined, but it remains approximately the same in a given muscle from different rabbits. Although the number of muscles we have studied is small, in all of them the gross neural pattern seems to adapt itself to the arrangement of the muscle fibres with the muscle as a whole, and at the same time allows, by the provision of 'loops', for the wide distribution and interweaving of motor units.

That electrical stimulation of single axons may give contraction of muscle fibres in separated parts of a muscle has been shown in the cat by Adrian (1925) for the tenuissimus muscle and by Cooper (1929) for the sartorius muscle. Van Harreveld (1946) avulsed one of the spinal nerves containing fibres supplying the sartorius muscle in the cat and removed the muscle 2 weeks later. Transverse sections showed intermingling of normal and atrophic muscle fibres. He concluded that muscle fibres of motor units in the sartorius muscle do not form a single close bundle, but that elements of the unit are scattered, and intermingled with the muscle fibres of other units.

The innervation of single muscle fibres by more than one axon, whether from the same or from a different spinal segment, does not appear to have been substantiated physiologically or histologically in mammalian muscle (Fulton, 1926; Hinsey, 1934; Denny-Brown & Pennybacker, 1938). In the present study, in spite of the intimate interweaving of terminals supplied by different axons, no histological evidence has been obtained that individual mammalian muscle fibres receive end-plates derived from more than one axon. On the contrary, multiple end-plates associated as a rarity with the same muscle fibre were always found to be derived from the same parent axon. In sectioned material the ease with which multiple end-plates may be mistaken for 'multiple' or plurisegmental innervation has already been pointed out by Kulchitsky (1924) and Denny-Brown & Pennybacker (1938).

Neural pattern and electromyography

Although anatomical information relating to the mode of innervation of human skeletal muscle is meagre, there is some evidence to suggest that in many human muscles the arrangements may resemble those just shown to exist in certain muscles

in the rabbit. It was shown by Duchenne (1867) and Frohse (1898) that there are in many human muscles complicated intramuscular plexuses of nerve bundles which resemble those described in the rabbit. It has also been shown by Wohlfart (1935, 1949) that in denervation of human muscle due to disease of the motor neurons the unit of muscular atrophy does not correspond to the muscle fasciculus.

The mode of distribution of motor units in large human muscles cannot be determined by the anatomical methods used in this investigation. The technique of electromyography may possibly prove of particular value in this connexion. The present study suggests, however, that anatomical features of the neural pattern of striate muscles may render the interpretation of electromyographic records more difficult than has generally been supposed.

This is illustrated by a consideration of the relation of electromyographic electrodes to the neural pattern in a lumbrical muscle. In Pl. 1, fig. 1, the small circle *M* has a diameter equivalent to 80μ which is somewhat greater than the tip of the monopolar electrode used by Jasper (1946). The larger circle *B* has a diameter equivalent to 500μ which would correspond to the outside diameter of a No. 23 hypodermic needle, which is commonly employed in making co-axial electrodes. Regardless of the exact positions of such electrodes it is clear that in the lumbrical muscle they would rest among a number of motor units and would frequently contact large and small nerve bundles conveying impulses to or from distant regions of the muscle. Both types of needles would clearly sample the electrical activity derived from contracting muscle fibres for a much greater distance around their tips. In the case of the co-axial electrode this distance is as much as 1 cm. (Weddell, Feinstein & Pattle, 1944). The total width of the area of muscle illustrated in Pl. 1, fig. 1 measures only about 0.1 cm., so that on the basis of a recording zone 1 cm. in diameter, the co-axial electrode would be capable of sampling a volume of muscle tissue of which the diameter would be ten times that of the total muscle area represented in Pl. 1, fig. 1.

It may be argued that the density of the neural elements at any given point in many human muscles will be less than in the lumbrical muscle of the rabbit. However, these anatomical observations would indicate that the features of the neural pattern are of significance in determining the nature and particularly the variability of the electrical record obtained from different muscles and from adjacent zones of the same muscle. They should be borne in mind when attempting to analyse complex electromyographic recordings.

For example, it is known (Weddell *et al.* 1944; Feinstein, 1946; Petersén & Kugelberg, 1949) that discrete single action potentials are obtained only with difficulty from the facial and laryngeal muscles. Records from these muscles are characterised by complex polyphasic discharges. This suggests that the axonal terminals of the different motor units within these muscles can overlap to such a degree as to prevent the activity of any one unit from being isolated by the electrode probe. The same is true to a lesser extent of most normal human muscles, since it is under conditions of minimal contraction that single repetitive action potentials or 'motor units' can best be obtained. Stronger contraction produces a complex record by bringing in adjacent and, as the present studies now suggest, overlapping motor units. Overlapping of anatomical motor units has also been

suggested by Jasper & Ballem (1949) to explain the observation that a single unit may continue to be recorded with little change in amplitude while the electrode is moved into the field of a second active motor unit.

Neural pattern in extrinsic ocular muscles

The neuro-anatomical arrangement in the extrinsic ocular muscles is quite distinct from that in any of the other muscles examined. The density of innervation appears to be much greater and is no doubt related to the much higher ratio of nerve fibres to muscle fibres, which in eye muscles has been estimated at 1:5 as compared to 1:50 in the semitendinosus muscle (Bors, 1926) and 1:120 in the soleus muscle (Clark, 1931). Our observations on the mode of innervation of the rabbit's extrinsic ocular muscles agree with the clear description of Hines (1931). In addition, we have shown that the innervation of these muscles in the macaque monkey is similar to that in the rabbit. A distinctive finding is the presence of terminal nerve fibres which give off multiple endings applied in succession along a considerable length of a single muscle fibre. The various types of nerve terminals are described with reference only to their anatomical pattern and not to their functional role. The distinctive nature of the neuro-anatomical arrangements in the extrinsic ocular muscles is of interest, however, in view of the fact that they also differ from other skeletal muscles in the rapidity of their contraction (Cooper & Eccles, 1930), in the higher frequency at which their motor units are capable of discharging (Reid & Whitteridge, 1949) and in their unusual sensitivity to certain drugs (Brown & Harvey, 1941).

SUMMARY

1. Some features of the neural pattern in various striated muscles of the rabbit and macaque monkey have been studied with the aid of intravital methylene blue staining.
2. In the lumbrical muscles of the rabbit the intricate plexiform pattern and the frequent occurrence of axon bifurcation with formation of divergent and recurrent collaterals provide an anatomical means for much commingling and interweaving of the nerve fibres during their distribution within the muscle. As a result of this, one axon entering the muscle may divide to supply end-plates in different parts of the muscle. Conversely, individual end-plates in a given zone of the muscle are frequently derived from intermingling terminal fibres from different parent axons.
3. In other muscles of the rabbit (excluding the extrinsic ocular muscles), although histological examination was not as detailed as for the lumbricals, the neural arrangements appear to be similar.
4. No anatomical evidence has been obtained in support of innervation of single mammalian muscle fibres by more than one axon. In preparations stained with methylene blue, in every instance in which more than one motor end-plate was seen to be related to a single muscle fibre, the end-plates concerned were found to be derived from a common axon.
5. There is evidence that the main features of the neural pattern as described in rabbit muscles may also be found in human muscles. The area of contact and

sampling of electromyographic recording needles is discussed in relation to the intramuscular plexiform arrangement of nerve bundles and the overlap of axonal terminals. The difficulty of recording discrete motor unit action potentials from some muscles is no doubt partly due to the extensive anatomical overlap of axonal terminals from different motor units.

6. The extrinsic ocular muscles show a neural pattern which contrasts with that in most other muscles in that terminal nerve fibres more often run singly and effect less terminal overlap. In addition, a single nerve fibre commonly gives off multiple endings which are deployed along a considerable length of one muscle fibre. These anatomical features may be related to the unique physiological responses exhibited by the ocular muscles.

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EXPLANATION OF PLATES

PLATE 1

All illustrations are from rabbit muscles.

- Figs. 1 and 2. A region of a lumbrical muscle showing the complex pattern of nerve bundles and terminals. Fig. 2 is an outline diagram of the main features of the photomicrograph. The small circle *M* represents the area of contact, 80μ in diameter, of a monopolar microelectrode used in electromyography. The large circle *B* represents the area of contact, 500μ in diameter, of a bipolar concentric electrode made from a No. 23 hypodermic needle. A single beaded fibre, of the morphological type which subserves pain, is indicated by the arrows. Methylene blue, intravenous method; whole muscle preparation. $\times 110$.
- Fig. 3. Schematic representation of the gross neural pattern in the extensor digitorum longus muscle as revealed by dissection. Approx. $\times 2$.
- Fig. 4. Dissection of sterno-mastoid muscle to show the gross neural pattern. Fine anastomotic nerve bundles which make up an intramuscular nerve plexus are indicated by arrows. Approx. $\times 3$.

PLATE 2

All illustrations are from methylene blue stained muscles from rabbit.

- Fig. 5. A small zone of lumbrical muscle showing muscle fibres innervated by sprays of terminals from three different nerve bundles—*A*, *B* and *C*. Arrows indicate direction of approach of these bundles. Intravenous method. $\times 400$.
- Fig. 6. Sprays of motor endings in crico-thyroid muscle. Note that each spray is made up of multiple nerve fibres which fan out to supply end-plates to a small group of muscle fibres. Local injection method. $\times 225$.

- Fig. 7. Intrinsic plantar muscle. Side-view of end-plate showing the sarcolemmal membrane bulging to surround it. Intravenous method. $\times 900$.
- Fig. 8. Three axons, one of which gives off a collateral to a motor end-plate supplying a muscle fibre of an intrinsic plantar muscle. Intravenous method. $\times 900$.
- Fig. 9. Motor end-plate from crico-thyroid muscle. Local injection method. $\times 1000$.
- Fig. 10. Surface view of motor end-plate from crico-thyroid muscle. Local injection methods. $\times 2900$.

PLATE 3

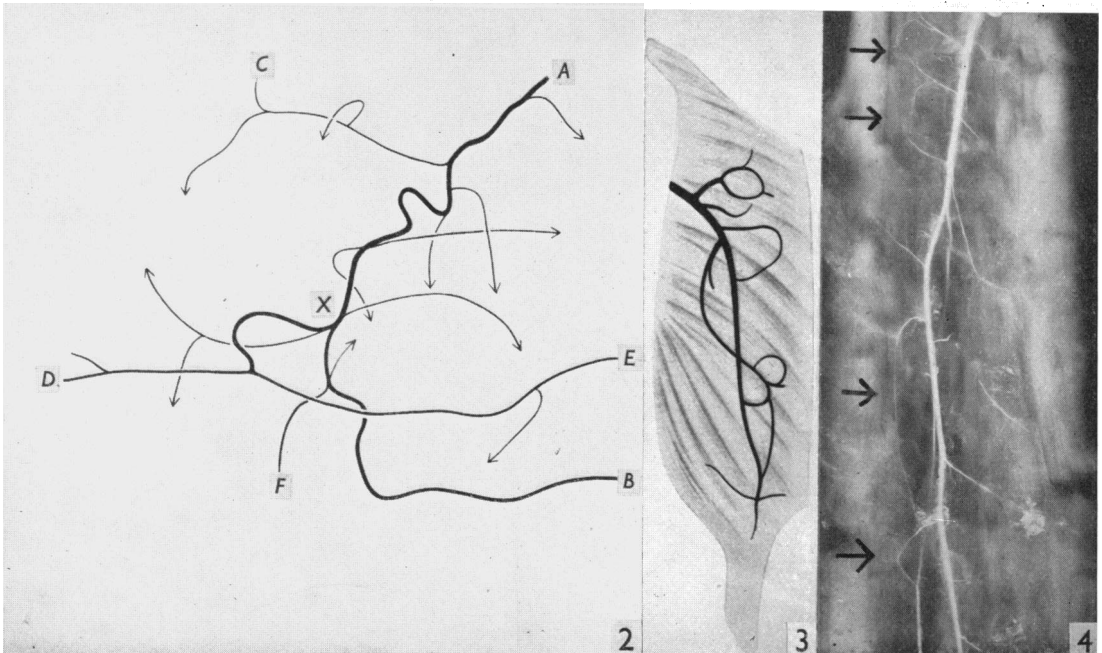
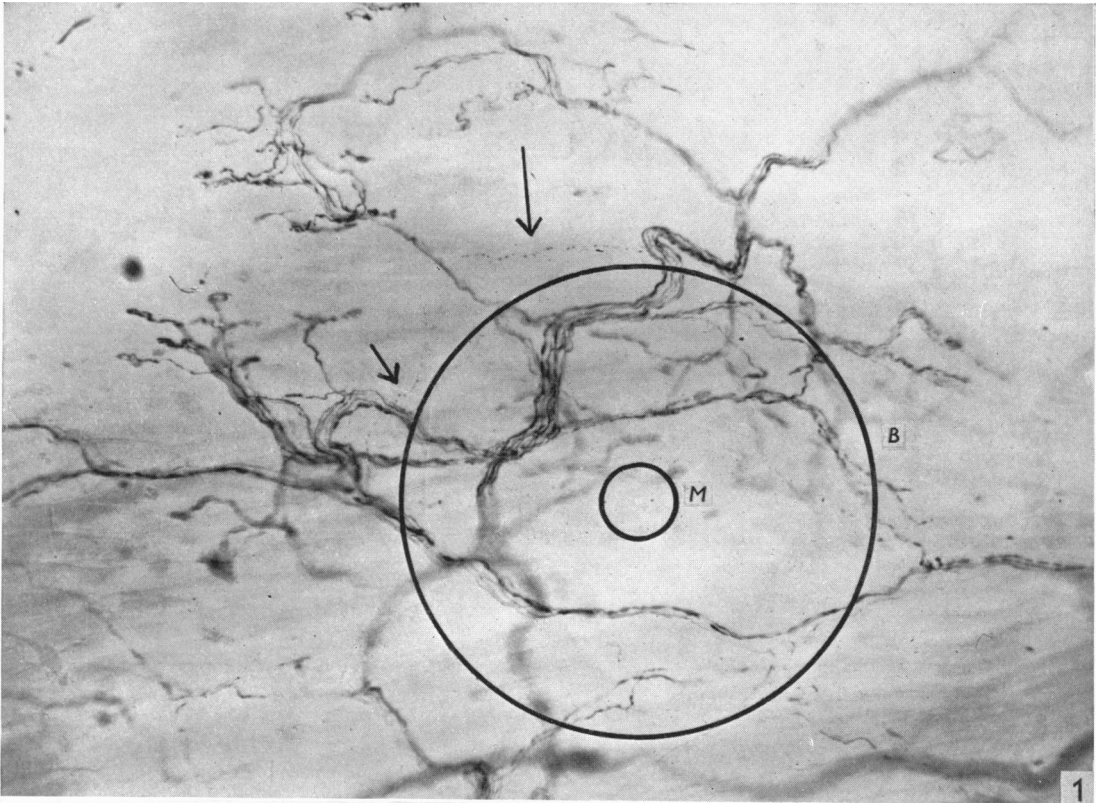
All illustrations are from rabbit muscles.

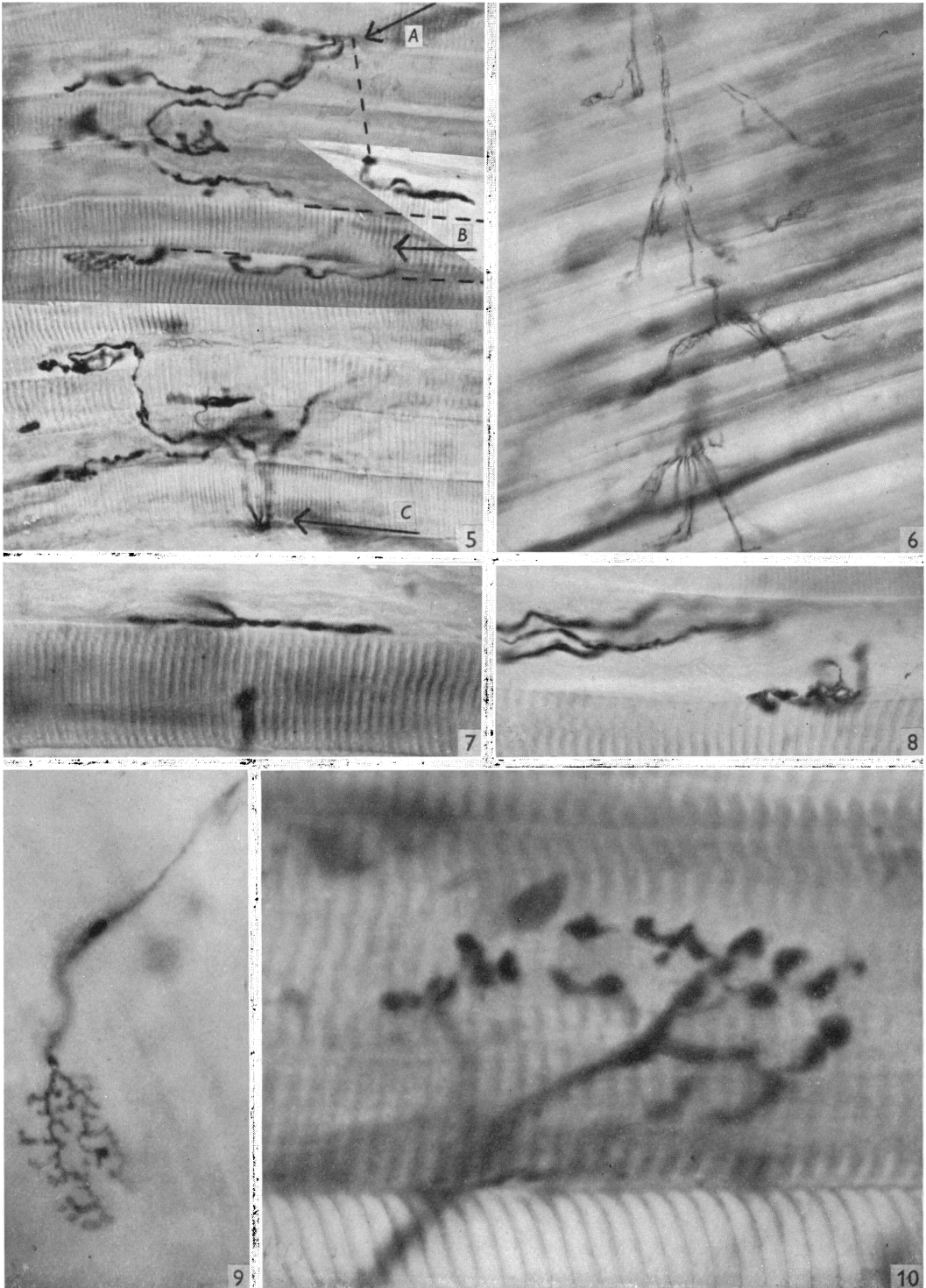
- Fig. 11. Double motor ending lying on one muscle fibre and supplied by twin branches of a single axon. The arrow indicates a single parent axon dividing into daughter axons. Crico-thyroid muscle. Methylene blue, local injection method. $\times 1400$.
- Fig. 12. Double end-plate derived from one axonal branch and applied 'in series' to a single muscle fibre. Intrinsic plantar muscle. Methylene blue, intravenous method. $\times 1500$.
- Fig. 13. Another form of double end-plate applied to a single muscle fibre. Note difference in diameter of preterminal axons. Intrinsic plantar muscle. Methylene blue, intravenous method. $\times 1300$.
- Fig. 14. Two end-plates on the same muscle fibre, as seen in a paraffin section of muscle. The lower end-plate is double. Compare with Fig. 11. Crico-thyroid muscle. Silver impregnation, Bodian protargol method. $\times 600$.

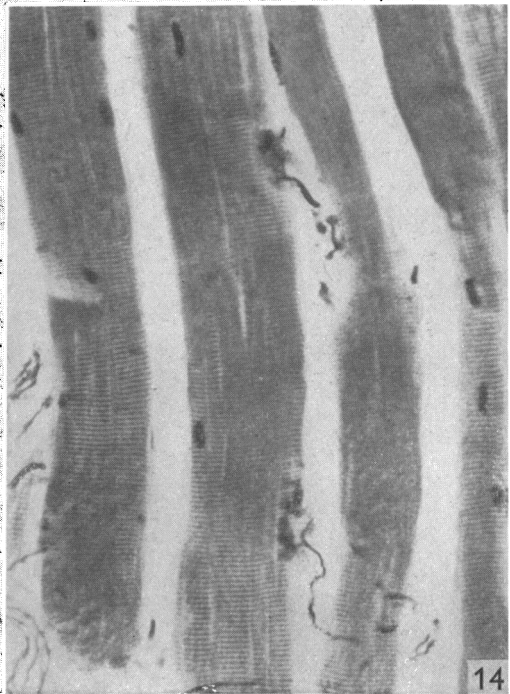
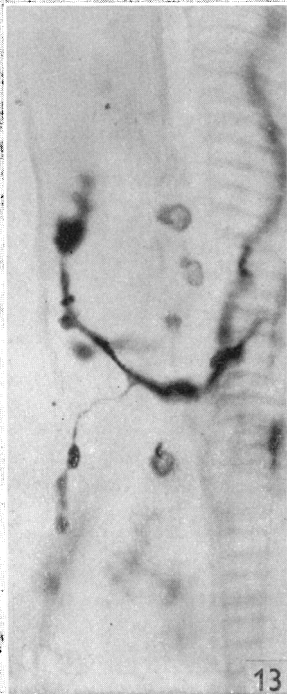
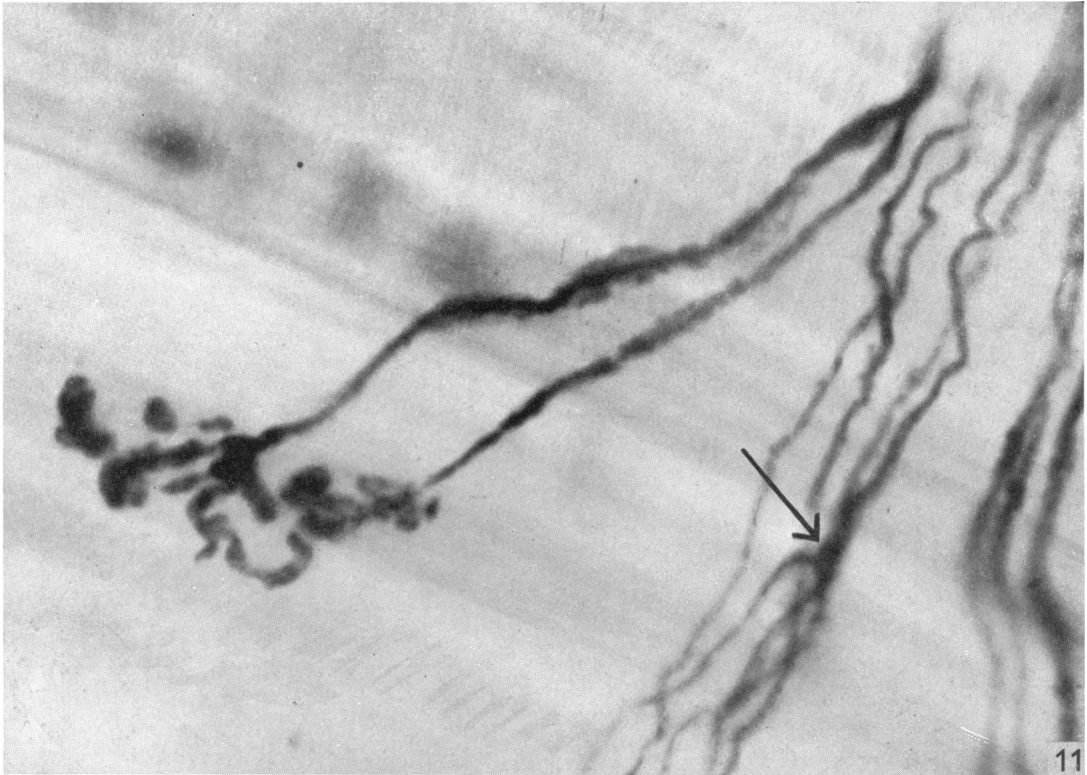
PLATE 4

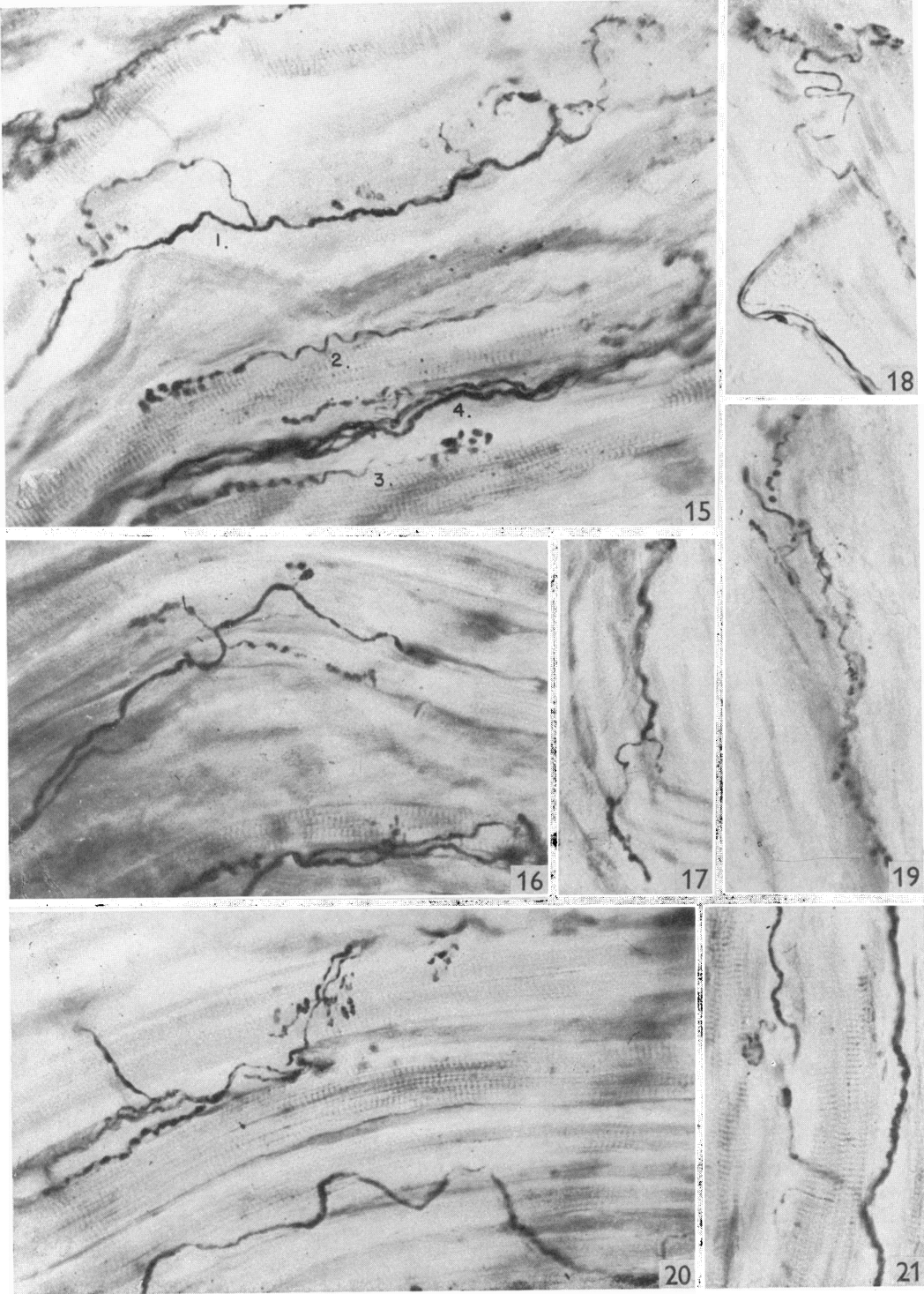
All illustrations are from extrinsic ocular muscles of macaque monkey, stained by intravenous methylene blue.

- Fig. 15. The single fibre (1) gives off multiple endings, which in this region are all applied to the same muscle fibre. Note fibres (2) and (3), which supply moniliform endings, and the small bundle of preterminal fibres of different sizes (4). The nerve fibres run parallel to the muscle fibres. $\times 400$.
- Fig. 16. A terminal nerve fibre of large diameter gives off three endings to the same muscle fibre and a fourth ending to an adjacent muscle fibre. Similar endings are present on the muscle fibres in the lower part of the photograph. $\times 400$.
- Fig. 17. An isolated fibre giving rise to two endings applied to different muscle fibres. $\times 400$.
- Fig. 18. Complex endings near the insertion of the muscle. Note the variation in diameter of the nerve fibres in the small nerve bundle. $\times 200$.
- Fig. 19. Complex endings near the insertion of the muscle. $\times 200$.
- Fig. 20. The small bundle of nerve fibres supplies endings to several neighbouring muscle fibres. Note the single large fibre which divides into three branches, at lower right-hand corner. $\times 400$.
- Fig. 21. A single terminal which resembles motor end-plates found in limb muscles. $\times 400$.









FEINDEL, HINSHAW AND WEDDELL—THE PATTERN OF MOTOR INNERVATION IN MAMMALIAN STRIATED MUSCLE