Proceedings of the Iowa Academy of Science

Volume 13 | Annual Issue Article 40

1906

Relation of the Motor Nerve Endings to Voluntary Muscle in the Frog

B. A. Place

RELATION OF THE MOTOR NERVE ENDINGS TO VOLUNTARY MUSCLE IN THE FROG.

BY B. A. PLACE.

Plate XXVIII.

This is a subject upon which most authorities differ widely, and few, if any, agree upon all points entirely. It is therefore, an inviting field for research. Because of the extreme diversity of opinions current it has seemed best to me to formulate them in a brief way before giving the results of my own observations.

It seems to be generally agreed that there are at least some nerve endings distributed to every voluntary muscle fibre. As nothing to the contrary has been found in my work this matter will not be referred to again in this paper. The points on which difference of opinion are based are as follows, and will be discussed in the same order. (1). Is there an end plate or localized accumulation of specialized muscular tissue in which the branches of the axis-cylinder terminate? (2). What is the relation of the ultimate branches of the axis-cylinder to the sarcolemma? (3). Where does the sheath of Henle stop, also the medullary sheath and the neurilemma? (4). What is the appearance and disposition of the ultimate branches of the axis-cylinder?

The Huber-Dewitt paper states that "Kühne deserves the credit of the discovery that the axis-cylinder terminates under the sarcolemma in the nucleated granular substance which he describes as the sole (Sohle); the nuclei as sole nuclei (Sohlenkerne)". It also states that Kuhne makes the hypothesis that the sole is "muscle protoplasm sarcoglia or sarcoplasm, while the nuclei of the sole might be likened to muscle nuclei". To quote them still further: "This interpretation of the granular sole and its nuclei suggested itself to us before Kühne's similar observation was definitely understood." They add this feature to its description: "As is well known in Amphibia, the axis-cylinder of the motor fibre terminating in striped muscle does not end in a localized area (birds, reptilia and mammalia), but ramifies over a proportionately much larger area. Conditions here presented are therefore very similar to those in such as present a localized motorial ending with the distinction that in the later the axis-cylinder terminates in a localized elevation—the sole—which has been interpreted as a circumscribed accumulation of sarcoplasm surrounding the ramified ending of the axis-cylinder and extending like it over a larger proportionate area of the muscle fibre." Wilson makes this statement denying the existence of any ground plate at all: "In the frog's muscle the nerve ending has no ground plate in

which the branches ramify." Sihler thinks that what has been seen in this regard by Kühne and others is derived from Henle's sheath. To quote: "I find that the so-called 'Sohlenstanz' of Kühne is derived from Henle's sheath."

Huber-Dewitt conclude that the end branches of the axis-cylinder are entirely under the sarcolemma. To use their own language: "Two cross sections are shown in Figs. 13 and 14. It may here be seen that the ramifications of the axis-cylinder are under the sarcolemma terminating in a relatively thin layer of sarcoplasma." Kühne is mentioned by them as holding a similar view. According to Sihler, Engelmann, Klein, Gerlach, Frey, and Waldeyer are also of this same opinion. Sihler himself, however, concludes that with perhaps some qualifications these end-branches are epilemmal. The following statements from him will serve to show his position: "The endings of motor nerves in striped muscle remain on the outside of the sarcolemma. What may be the exact condition of things at the points where muscle and nerve fibre are in actual contact—whether the sarcolemma and neurilemma are wanting there or perforations exist—I cannot say. The precise relation of muscle to nerve here is an unsolved and difficult problem." Dogiel in the following language states that they are hypolemmal "die Marksubstanz aber sammt dem Achsencylinder tritt unter das Sarcolemma". Huber-Dewitt quote Rhetzius as being in doubt whether the end branches are epi- or hypolemmal. Wilson takes the view that the larger branches of the endings lie over the sarcolemma and at some distance from it, while small fibrillae possessing a terminal knob come off lying either within or under the sarcolemma.

Sihler states that Henle's sheath ends before the muscle fibre is reached and is open so that the axis-cylinder with its neurilemma appears like an arm emerging from a sleeve, while the sheath of Schwan covers the end fibres down to their tips and is provided with nuclei. Huber-Dewitt thinks that the sarcolemma and neurilemma become continuous with each other at the point where the nerve fibre, as they claim, pierces the sarcolemma. This small area made up of both sheaths they call telolemma and its nuclei if any are found at this point, telolemma nuclei. The following statement from Dogiel will show his attitude: "In ersten Falle tritt die markhaltige Nervenfaser an irgend eine Stelle der Muskelfaser heran, verliert in dem Sarkolemma ihre Schwan, 'sche und Henlische Scheide" which practically agrees with the last preceding writer.

As to the appearance and disposition of these ultimate branches, Dogiel thinks they are devoid of any sheath, are thickened, toothed and run along the muscle fibre frequently ending in a knob. He thinks they neither go to any neighboring muscle fibres nor form any plexuses. Wilson thinks on the other hand that they are covered by a sort of a sheath formed from the ordinary nerve coverings and the sarcolemma,but are non-medullated. He also thinks that some of them end in the intermuscular connective tissue, and that others go to neighboring fibres. Plexuses are found at times also, he thinks. Sihler remarks that the

nuclei of the neurilemma on these fibrils have more of a tendency to roundness and that the individual fibrils end as such and not in a bulb. He states that the endings have little of the medullary sheath.

This investigation was begun in 1904, and was presented in somewhat different form to the faculty of Ohio University in June, 1905, as a thesis for the degree of A. M. Research has been continued since then as time would permit. The animal thus far used for observation was the common North American toad (*Bufo lentiginosus*) and the method employed essentially that of Sihler's. The microscope chiefly used has a Zeiss aprochromatic oil immersion two millimeter lens.

Fresh tissue was macerated, stained, teased, and mounted in glycerine on slides.

The macerating fluid is made as follows:

Ordinary acetic acid .....1 part
Glycerine .....1 part
Chloral hydrate 1 per cent. solution in distilled water.....6 parts

The muscles should be in bundles of not more than three or four millimeters in diameter. Those of the foot or fore limb of the toad or frog are already of convenient size.

The staining material is prepared as follows:

Well ripened Erlich's Haematoxylin .....1 part
Glycerine .....1 part
Chloral 1 per cent. solution in distilled water .....6 parts

The muscle should be macerated from 12 to 20 hours depending upon the age of the animal. It would do no harm to use stronger acetic acid for older animals. The object is to dissolve the inter-muscular connective tissue, which is accomplished when the muscle no longer offers much resistance to teasing. One should begin testing the matter as soon as 12 hours have passed. When properly macerated the tissue should be left in glycerine until it becomes saturated, which requires about two hours.

Before putting the tissue into the staining fluid it should be further teased into bundles not more than one or two millimeters in diameter. All teasing should be done in glycerine. Staining requires from 3 to 10 days. When properly stained the muscle fibres have a color ranging from wine color to nearly black. Nervous and muscular material are stained almost black, while connective tissue remains quite pale. The darkened nuclei of the capillaries which are everywhere so abundant are a good index to the intensity of the staining process, since nerve structures take the stain at about the same rate with them. All the parts do not stain uniformly. Parts that are over-stained can be readily reduced in color by subjecting them to a weak solution of acetic acid. A convenient way to accomplish this is to immerse them in glycerine, to which has been added a small amount of acetic acid. The stained tissue may be kept unchanged almost indefinitely in glycerine.

A convenient way to find nerve structure is to tease quite a number of muscle bundles still smaller until each remaining part contains perhaps half a dozen fibres. These in a shallow glass dish may be examined

under 50 to 100 diameters and the ones best showing nerve structures sorted out. These latter mounted in glycerine on slides will flatten out under slight pressure upon the cover glass so as to enable one to continue the examination with higher power of the microscope. These structures are quite delicate and when once mounted cannot be further manipulated for different views. For this reason there is an advantage in mounting material between large cover glasses in as much as both sides of the preparation may then be viewed. There is this disadvantage, however, that such a preparation cannot be preserved long.

In addition to the above process stained tissue was hardened in alcohol, embedded in paraffin, sectioned and examined in series. Various counter stains were employed but with very little success thus far.

As to my own results I am convinced that the primary nerve fibre neither terminates in an end or ground plate, or granular sole, nor pierces the sarcolemma, at least at that point. It would seem to me that the sole nuclei described by Huber-Dewitt and others were end bulbs belonging to the ultimate branches of the axis-cylinder. For by this method unless a particular preparation is stained quite perfectly only a few of the end fibrils can be traced, while their granular nuclear-appearing bunches or knobs appear quite conspicuous, but disconnected. I was for some time of the opinion myself that these apparently isolated knobs belonged to the muscle fibre, and that the muscle fibre was consequently specialized at these places for receiving the nerve endings. But by observing that the best stained preparations showed all of these knobs or bulbs to be either connected, or partly so, by fine nerve fibrils, I concluded that such would be the case with all of them if the staining was sufficiently perfect. Later observations confirm the belief. There seems to be no regular order, size, shape or number of either the end fibrils or end bulbs. Generally the fibrils are relatively short and the bulbs are elongated and more pointed at the distal ends. Plexuses among the fibrils are occasionally found. The fibrils show by their pale outline down to their tips a covering of connective tissue which is probably a continuation of the neurilemma. In my observation no neurilemma nuclei have been seen upon them, but this does not argue much for the absence of neurilemma here, in as much as neurilemma nuclei of the main fibre are generally much farther apart than the entire length of these fibrils. These structures seem to be connected more or less with the sarcolemma, as may be seen by their adhesion to that membrane when being torn off. The relation seems to be either that of intermuscular connective tissue joining fibrils and bulbs to the sarcolemma, or a superficial coalescence between neurilemma and sarcolemma. In the examination of a very great number of cross and longitudinal sections no point could be found where either bulbs or fibrils were beneath the sarcolemma. In the fibrils the medullary sheath is either present in very slight amount or is entirely wanting. Figure 1 is fairly typical of this structure. Exceptional forms are occasionally found differing widely from the typical.

The sheath of Henle is a rather robust structure of the primary nerve fibre presenting nuclei similar to those of the neurilemma. The nuclei of neither of these structures seem to have any regular order or position. These two sheaths appear to coalesce at, or slightly distal to, the point where the axis-cylinder breaks up into its fibrils. More evidence, however, is desirable upon this point. The myelin or medullary sheath stains dark, about the same as the axis-cylinder, which would tend to show that this structure was nervous and not connective tissue. Incidentally this helps to settle the question—from what is the myeline sheath derived, whether from the axis-cylinder or from the sheath of Schwann? It is wanting, or nearly so, at intervals known as nodes of Ranvier. Divisions of the axis-cylinder occur at these nodes. These structures are shown in Figure 2.

WORKS CONSULTED.

Piersol. Normal Histology.

Dogiel. Methylenblautinktion der motorischen Nervenendigungen in den Muskel der Amphibien und Reptilien. Archiv für Mikroskopische Anatomie. 1890.

Sihler. On the Endings of the Motor Nerves in the Voluntary Muscle of the Frog. Studies from the Biological Laboratory of Johns-Hopkins University, Vol. III, 1885.

Sihler. Neu Untersuchungen über die Nerven der Muskeln mit besonder Borucksichtigung umstreittener Fragen. Zeitschrift für wissenschaftliche Zoologie. LXVIII. 3.

Sihler. The nerves of the Capillaries with remarks on Nerve-endings in Muscle. Journal of Experimental Medicine. Vol. V, No. 5.

Sihler. Verbandlugen der physiologischen Gesellschaft. Jahrung, 1894-5.

Sihler. Die Muskelspindeln, Kerne und Lage der motorischen Nervenendigungen. Archiv fur microskopische Anatomie und Entwicklungsgeschichte. Band. 56. 1900.

Huber-Dewitt. A contribution on the Motor Nerve-endings and on the Nerve-endings in the Muscle-spindles. Journal of Comparative Neurology, March, 1898.

Wilson. The Relation of Motor Endings on the Muscle of the Frog to the Neighboring Structures. Journal of Comparative Neurology and Psychology, March, 1904.

KEY TO THE DRAWINGS.

A and B, muscle fibres.

a and a′, nerve fibres.

c and d, branchings other than at points where the axis-cylinder breaks up into its ultimate fibrils.

b—The point where the nerve fibre breaks up into its ultimate fibrils, which is usual soon after the primary fibre comes upon the muscle fibre.

Med. S.—Medullary sheath.
Axis-C.—Axis-cylinder.
Neu.—Neurilemma.
Hen.—Henle's sheath.
Hen.-N.—Nuclei of Henle's sheath .
End. f.—End fibrils.
End. b.—End bulbs.
Plex.—Plexus.
The size of the fibres are exaggerated.