STRUCTURE OF NERVE FIBRES AND SYNAPSES IN SOME INVERTEBRATES

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Although our knowledge of biological structure has long ceased to be limited to analysis into parts which can be seen directly with the eyes, and has been extended to the study of molecular structure, yet there are still large divisions of physiology whose development is being retarded by lack of histological data in the old sense, data about the visible structure of tissues. In the present state of nerve physiology there are a great many subjects about which such histological information is urgently needed. For instance, we are still far from agreement even as to the visible structure of axons, and the histologist may have an important contribution to make to the search for surfaces, on the outside of, or within, the neuron, which are likely to provide polarised membranes whose progressive depolarisation might constitute the propagated nerve impulse. The study of the localisation of inorganic ions or other substances at such membranes and at synaptic junctions is another very attractive but difficult field of work, in which little progress has so far been made.

Less spectacular but equally important work is the obtaining of data as to the numbers and sizes of fibres in different nerves, data which are still badly needed, especially for the lesser known invertebrate nerves. Estimates of the proportions in which axoplasm, myelin, connective tissues and lymph occur in different nerves are likely to be very useful in connection with studies of their chemical make-up and metabolism.

The investigation both of the normal functioning of nerve and of its excitation by electrical and other means requires knowledge of the structure of the sheaths surrounding the axon. Here the work of the histologist is especially necessary. By comparison of the structure of various types of nerve he may be able to suggest preparations capable of solving crucial problems in this field, such as the part played by the myelin sheath and the nodes of Ranvier.

These are all problems connected with the functioning of peripheral nerve trunks; a host of further histological questions arise in the study of synaptic functioning and of the relation of the cell body to the rest of the neuron. There is still no certainty as to the structural relationships of the cells even in such well known regions as the ventral horn of the mammalian spinal cord or in the sympathetic ganglia, and microscopical analysis will certainly be an essential part of further study of synaptic excitation.

In this paper there are described some nerves and synapses which have been investigated during the past few years with considerations of this sort in mind, and it is hoped that these comparative studies may serve to throw into relief certain points of interest for the study of the general physiology of nerve.

The Nerves of Arthropods

There is still considerable uncertainty as to the structure of the fibres in the peripheral nerves of arthropods, although the decapod crustaceans (crayfish, lobsters, crabs, etc.) and the arachnid *Limulus* have been used for many years for the study of non-medullated nerve fibres. In most arthropods the muscles are controlled by relatively few large motor axons, and these make very suitable material for microscopic study. Many details about their structure were known in the last century to such workers as Huxley (1880), Retzius (1890) and Hardy (1894), who described them as "nerves tubes" composed of an outer nucleated sheath enclosing a relatively fluid axoplasm.

In the larger axons of the leg nerves of the spider crab, *Maia*, these sheaths are surprisingly thick. Thus around an axon of 15 or 20 microns diameter there may be a sheath which gives the whole fibre a diameter of 80 microns. The sheath is composed of a substance which stains similarly to the collagen of vertebrates, nuclei being scattered throughout its thickness. Some of these nuclei lie in the very innermost layers of the sheath, and may actually indent the surface of the axon, leaving no space for a special fatty layer free of nuclei between the sheath and the axon.

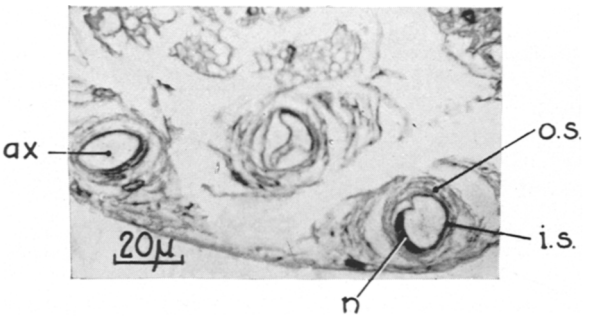

Fig. 1. Transverse section of large axons in the leg nerves of Maia. Fixation Flemming's fluid, embedding paraffin, stain azan. ax.: axoplasm; i.s.: dark-staining inner sheath.; o.s.: lighter staining outer sheath; n.: nucleus of sheath.

Although there is, therefore, no layer exactly comparable with the medullary sheath of vertebrates, yet in some preparations it can be seen that the innermost layer of the sheath differs somewhat from the layers outside it, having a smoother aspect, and blackening somewhat more

readily with osmium tetroxide (Young, 1935b). This is of great interest in view of the fact that there is a layer in the sheath of these axons which becomes negatively birefringent after treatment with glycerine (Göthlin, 1913). It seems probable that this layer is more intimately related to the functioning of the axon than are the outer sheets, which may be merely supporting structures.

The thickness of the whole sheath seems to increase with that of the axon, the largest fibres, with the thickest sheaths, lying near the base of the limb. It is impossible at present to decide whether this variation in the thickness of the outer sheath has any physiological significance.

From a physiological point of view the interest of these sheaths lies in the fact that although they are not medullated in the vertebrate sense, that is to say do not have a homogeneous layer without nuclei between the sheath and the axon, yet there is a layer which becomes negatively birefringent after treatment with glycerine and presumably contains oriented lipoids (Schmitt, Bear, and Clark, 1935). Secondly, there is the important fact that the sheaths do not show any breaks comparable to the nodes of Ranvier.

Although no homogeneous fatty sheath without nuclei can be detected around the axons of the reptant decapods which we have been considering, yet Retzius (1888) showed that such distinct fatty layers of "myelin" are present in shrimps, prawns, and mysids, and Göthlin (1913) and Nageotte (1922) have also studied these "myelin" sheaths in a variety of Natant Decapods, other groups of primitive Malacostraca and the still more primitive Barnacles. According to these workers there is a distinct osmiophil, negatively birefringent layer, which lies not, as in vertebrates, immediately against the axon, but separated from the latter by a nucleated "Schwann sheath". There are said to be constrictions forming structures resembling nodes of Ranvier, but even at these points the fatty layer, though thinned, is not completely interrupted. Further investigation of the anatomy, embryology, and physiology of these sheaths is highly desirable, in order to discover whether there is a difference only of degree between their structure and that of the nerves of higher Crustacea.

Giant Nerve Fibres of Annelids

The ventral nerve cord of many Annelid worms contains giant nerve fibres which have been proved in the case of the earthworm to produce the rapid contractions by which the animal retreats either forwards or backwards when suddenly stimulated. In the earthworms there are three of these fibres and they are segmented, that is to say divided longitudinally into units each separated from its neighbours by complete membranes, the 'septa' or synapses (Fig. 2).

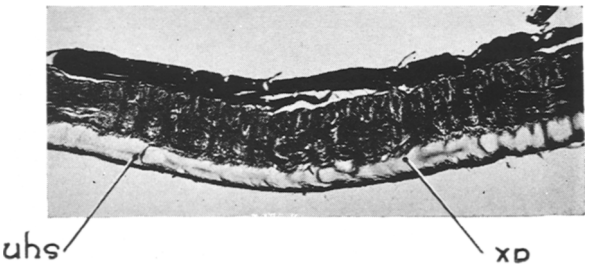

Fig. 2. Sagittal section through ventral nerve cord of earthworm to show median giant fibre. Fixation and staining as in Fig. 1. ax.: axoplasm; syn.: synapse.

Stough (1926, 1930) showed that in life the median fibre of an earthworm conducts anteroposteriorly and the lateral fibres poster-anteriorly, and that there is a constant difference in the staining reaction on the two sides of each synapse, certain granules occurring on the anterior face of the synapses in the median fibre and the posterior face of those of the lateral fibres. However, Eccles, Granit, and Young (1933) found that by means of electrical stimulation impulses can be set up at either end of the median or lateral fibres, and that once set up they travel with equal speed in *either direction* across the synapses. The difference between this case of reversible conduction between units separated by complete membranes and the normal irreversible synapse may perhaps be connected with the fact that in the case of the earthworm the two membranes in contact are of similar dimensions, whereas in, say, a vertebrate ventral horn cell the end foot and dendrite which form the two members of the synapse are of very different shapes.

Nerve Fibres and Synapses in Cephalopods

The nerves of the Decapod Cephalopods such as the squid, *Loligo*, or the cuttlefish, *Sepia*, are particularly interesting because they contain enormous axons, the largest of them in a large Atlantic squid (*Loligo pealii*) being as much as one millimetre in diameter. In spite of their great size these axons seem to have been referred to previously only by Williams (1909), who described them very briefly.

The large fibres do not differ in any essential point of structure from the smaller nerve fibres of these animals, and indeed axons of all diameters from less than one micron to nearly a millimetre can be found. Each fibre in the peripheral nerves consists of a mass of axoplasm surrounded by a sheath which stains like vertebrate connective tissue. This sheath consists of a few layers of what appears to be collagenous material interspersed

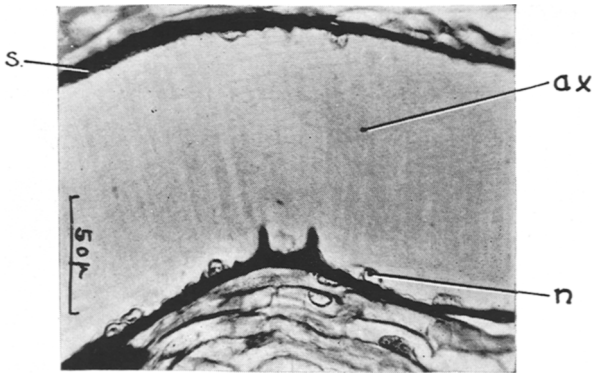

Fig. 3. Longitudinal section through a giant nerve fibre in stellar nerve of Sepia. Fixed and stained as Fig. 1. ax.: axoplasm showing faint longitudinal striations; n.: sheath nucleus; s.: sheath, deeply stained with anilin blue.

with nuclei (Figs. 3 and 4). As in the case of *Maia* the nuclei often lie so close to the axon as to indent the latter. The sheaths appear to contain fibrils running in a radial direction around the axon (Fig. 4), and in this way there is formed a continuous investment over the whole surface, there being no breaks comparable to the nodes of Ranvier. The whole sheath stains faintly with osmium tetroxide, and no doubt contains fatty substances, but there is no histological evidence for the existence of any especially fatty layers. However, recent studies with polarised light (Bear, Schmitt and Young, 1936) have shown that such a layer is present even though it cannot be recognized in fixed and stained preparations.

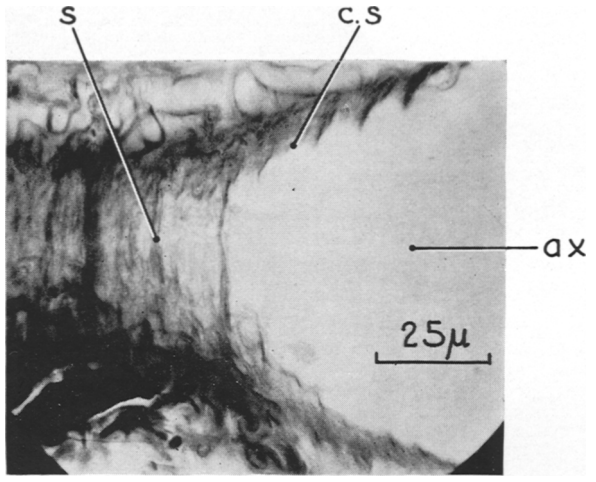

Fig. 4. Oblique longitudinal section through giant axon in stellar nerve of Sepia. Fixation and staining as Fig. 1. ax.: axoplasm; c.s.: cut edge of sheath; s.: sheath seen in surface view, showing transverse striations.

The structure of the individual axons is of the greatest interest. If a fibre be cut in such a way that the end is not closed off, then the contents pour out in a stream from the sheath (Young 1935a). It is therefore necessary when removing the nerves for experimental purposes to ligate the ends in order to prevent the axon from escaping.

The anatomy of the giant fibre system will be described in full detail in a future publication. Large nerve fibres run to the mantle muscles, retractor muscles of the head and muscles of the funnel, and the system probably operates the quick contractions by means of which the animal expels a jet of water violently from the mantle and darts backwards or forwards through the water with such remarkable speed.

A point which is of general neurological interest is that in two parts of the system axons arising from separate cell bodies are fused, that is to say in protoplasmic continuity with each other. As shown diagrammatically in Fig. 5 the axons of the two large cells in the central nervous system from which the system may be said to begin are joined completely across the middle line by an interaxonic bridge. That this is a complete fusion and not a chiasma is shown by the fact that both the axoplasms and the sheaths of the two fibres are continuous. The two sheaths undoubtedly join to form a single cylinder across the middle line, and in view of the fact that the sheath forms an integral part of the fibre, without which it fails to maintain its shape, it is difficult to see how two separate fibres could exist within one sheath. If there were two fibres forming a chiasma one would expect to see some evidence of a line of separation between them, but study of preparations fixed and stained in a variety of ways has failed to show any traces of such membranes, though the axoplasm can easily be seen as a faintly striated substance filling the sheath across the "bridge". The only possible conclusion is that at this point there is complete fusion between the two axons. This presumably means that impulses set up in one of them would always be conducted to the other. It is not difficult to see how this arrangement may be of value to the animal if it be important for both sides to contract simultaneously.

Behind the interaxonic bridge the two axons make endings in contact, but not continuity, with axons of the second order, which pass out to the funnel, retractor muscles of the head and stellate ganglia. The single fibre which runs to each stellate ganglion breaks up and ends there, making synapse with third order axons. In *Loligo* each of the stellar nerves contains one of these third order axons and this branches peripherally among the muscles. The fibres differ in size, that in the hindermost and longest stellar nerve being the largest and the diameters decreasing regularly passing forwards, so that the shorter nerves contain the smaller fibres. It remains to be discovered

whether these fibres of differing diameters also conduct at different speeds.

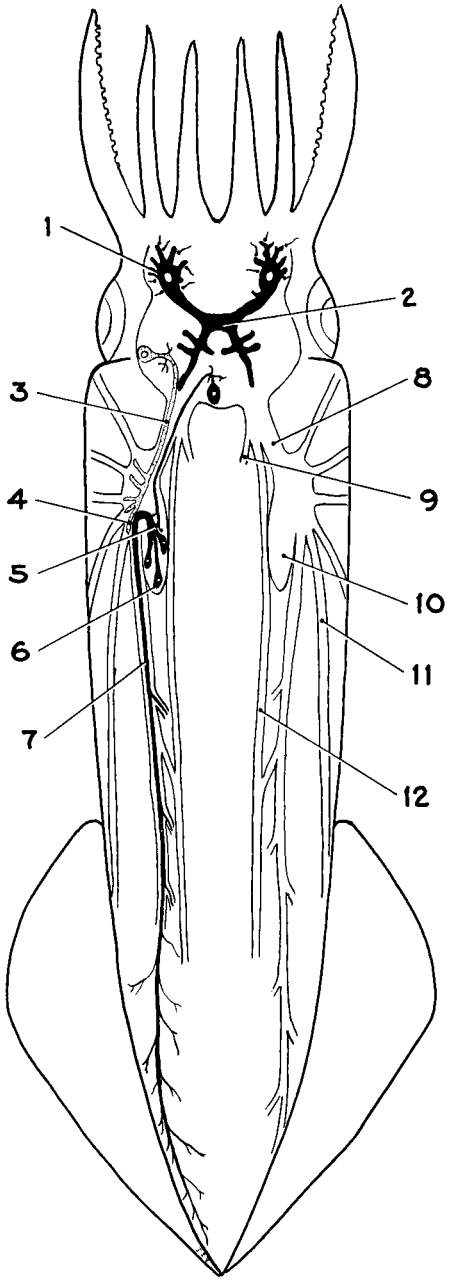

Fig. 5. Diagram of giant fibre system of **Loligo**, as seen from above, the ganglia shown disproportionately large. The nerves are shown in outline on the right and the giant fibres filled in on the left. 1: giant cell, with end feet attached to its dendrites. 2: interaxonic bridge. 3: 2nd order giant axon. 4: distal synapse between 2nd and 3rd order axons. 5: proximal synapse between fibre arising independently in the C. N. S. and 3rd order axon. 6: cell bodies of 3rd order axons. 7: 3rd order axon. 8: mantle connective (joining C. N. S. to stellate ganglion). 9: nerve to retractor muscle of head. 10: giant fibre lobe of stellate ganglion. 11: stellar nerve. 12: fin nerve.

These large fibres in the stellar nerves are not the processes of single giant cells but are syncytia, each formed by fusion of the processes of numerous small neurons. In *Loligo* the cells of origin are all collected together to form a peculiar pointed mass, the giant fibre lobe, easily visible with the naked eye at the hind end of the stellate ganglion. In serial sections the giant fibres can be traced from the stellar nerves into this lobe, where they break up into fine branches which are the processes of the cells of the lobe.

The fusion of these axons seems to be complete, so that the resulting giant axon is a single neural unit and not a number of separate axons inclosed in a common sheath. That this is the case is shown by the fact that no separate axons can be seen beyond the point of fusion, either in the living state or in material fixed in a variety of ways and cut into transverse or longitudinal sections. The axoplasm when examined alive appears as a homogeneous, rather fluid, substance showing a very faint longitudinal striation but no definite separate fibrils. The whole giant fibre is surrounded by a sheath similar in histological structure and optical properties to that which surrounds the smaller axons of the nerve. It is unlikely that a sheath with such peculiar properties would surround any but a single nervous unit. Furthermore, it has recently been found (Bear, Schmitt and Young, 1936) that the axoplasm itself is positively birefringent. Other evidence that the giant fibre functions as a whole is that it gives off collaterals to form synapses in the stellate ganglion, and it is very difficult to see how a composite could do this, since the collaterals arise from a part only of the surface. Perhaps the most striking fact of all is that each giant fibre behaves functionally as a single unit. Stimulation of one of the stellar nerves with condenser discharges or break induction shocks causes contraction of the mantle muscles even after all the axons in the nerve except the giant fibre have been cut. By recording the twitches produced by the muscle it has been found that the response is always maximal. Increasing the strength of the shock above threshold never increases the response. Although the fibre arises by the fusion of so many axons, it nevertheless conducts in an all-or-none manner as a single unit.

There are, then, two situations in the Cephalopod giant fibre system in which there is continuity between the axons of two or more nerve cells. It is a striking fact that in the same system there are also discontinuous synapses, namely on the dendrites of the first order giant cells and between the first and second, and second and third, order fibres (Fig. 5). In the stellate ganglion the second order axon sends one branch to each of the third order fibres, which as already mentioned

give off axonic collaterals, forming what may be called the distal synapse. In addition, there is on each side another large fibre running into the stellate ganglion, this fibre having connections in the C. N. S. quite different from those of the second order axon. This other fibre breaks up in the giant fibre lobe into a great number of fine knobs, the proximal synapses, in contact with the third order axons.

It is impossible here to describe the structure of these interesting synapses in detail. The point to be stressed is that even in a system in which axons which always work together are completely fused there are, nevertheless, discontinuous synapses at points where it is important that not every impulse be passed onwards. The second, as well as the third, order neurons, besides the distal synapses which they receive from the main giant fibre system, also receive fibres from other sources which make proximal synapses, lying close to the cell bodies (Fig. 5). Presumably these are correlation points in the system, at which impulses are set up or inhibited, and it would be interesting to know whether there is any special significance in the position of the endings.

The giant fibre system of *Loligo* seems, therefore, to show that where a number of nerve fibres always act together they may become fused, but wherever a correlation point is reached at which a balance of excitation and inhibition is struck and impulses do not invariably pass on, there discontinuous synapses are found.

This emphasises rather than weakens the essention point of the neuron theory in its physiological aspect; namely, that impulses set up anywhere on a neuron will be propagated without decrement over the whole surface with which the stimulated point is in protoplasmic continuity. Synapses are therefore necessary since all the neurons are not equally excited in every act of behaviour and there must be points of discontinuity at which the next unit may or may not be activated.

The case thus throws into sharp relief the distinction between excitation of one part of a protoplasmic unit by another part of the same continuous mass, and excitation across membranes separating two such units. Though the distinction has long been recognized it is still of great importance that it be stressed. We know from the difference between conduction in nerve trunks and across synapses that processes are occurring at the latter which are not present in the former, but we still have all too little information as to the nature of these complicating synaptic factors. The fact that there is a discontinuity of membranes at the points where impulses are set up or inhibited still remains one of the few clues which are available to help us in the understanding of this important aspect of the problem of nerve excitation.

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DISCUSSION

Dr. Schmitt: I agree with Young's view regarding the necessity for further neurohistological work. The literature on the structure of living crustacean fibres is very sketchy, and in many instances altogether unreliable. I agree also with Young regarding the essential similarity of the sheath immediately surrounding the axis cylinder and the remaining loosely applied sheaths, at least in respect to protein structures. We distinguish between these two sheaths, however, as to chemical composition, particularly with respect to lipoids.

I should be much interested in Young's estimate of the relative amount of connective tissue in, let us say, the leg nerve of a lobster. In our work on nerve proteins it is essential to know the approximate proportions of lymph, connective tissue, and axon. I should also like to know whether these interesting sheaths on the giant axons are applied also upon the cell body. From our work on the structure and possible significance of the inner sheath, this will be of obvious importance.

Dr. Young: With regard to the quantitative data, the amount of connective tissue differs very considerably in different parts of the leg nerve. At the base of the leg nerve there are fibres with thick sheaths, and although there are not very many of them, they affect the proportion. In the claw there are purely afferent nerves giving a different proportion. The level I have examined most carefully is at the middle of the leg where the cross sectional area of the nerve contains 65 per cent of axon as against 35 per cent of all other tissues. I have not been able to estimate the quantity of lymph separately from that of other tissues. These crab nerves are very difficult to fix well and I hesitate to make any statement. I would say that the proportion of lymph spaces is

very small. There are blood vessels here and there, which complicates matters. Of lymph spaces I would say there is not more than 10 per cent of the cross-sectional area of the nerve, but I would not like to put any great reliance in that figure.

The second question—one which has been greatly neglected—is the nature of the sheath around the perikaryon. Around nerve cells of *Sepia* and *Loligo* there is a very complete connective tissue sheath. Sheaths of the same general sort as that surrounding the axon appear to surround the entire perikaryon. Most invertebrate nerve cells are of the unipolar type and the perikaryon does not usually carry endings, though it may do so more often perhaps than is generally supposed, and yet it is surrounded by a network which is continuous with sheaths of the axon. Whether or not this is typical of other neurons in which there are more endings on the perikaryon, I would not like to say at the moment.

Dr. Davenport: Have any chemical studies been made of the fluid in the axons?

Dr. Young: I hope to get the fluid uncontaminated by the sheath material. It would certainly be valuable material for chemical study, and the biggest fibres should provide considerable amounts of substance for analysis.

Dr. Ponder: Does the axoplasm coagulate when it flows out?

Dr. Young: The material which flows out usually remains liquid and the particles in it show active Brownian movement. It does not at once mix with the sea water. The outer layers contain fewer granules than the inner, but there is no definite ectoplasmic layer. Yet it does not mix completely or flow freely in the sea water, unless shaken or otherwise disturbed.

Dr. Walzl: If a nerve of a vertebrate is sectioned the central stump degenerates as far as the first node of Ranvier. How far does degeneration proceed in the forms that lack these nodes?

Dr. Young: That was the original purpose of this whole study. I started in 1928 to study regeneration of Octopods and it was in the course of that work that I came across the giant fibres. My impression is that in most cases there is very little back degeneration. After a fibre has been cut the axon flows out and then divides. Little buds are thrown off and they sprout out in all directions, very much as in vertebrates. In the summer the fibres grow at rates up to 20 μ per hour, in winter much more slowly.

Peripherally there is rapid degeneration. The muscles of the mantle become free of fibres four days after section of their nerve.

Dr. Schmitt: Have you ever observed a disappearance of the neurofibrils after they have once been formed, let us say by a minimal amount of manipulation? de Rényi observed such a reversible production of neurofibrils as a result of stretching vertebrate axons, but there is little other evidence, so far as I know, for such a reversible protein degeneration in the axis cylinder.

Dr. Young: I have never looked for it so I cannot say whether it occurs or not. The nerves can be stretched considerably. Carlson, in his work on slugs, stretched the nerves of the foot and found that the conduction time was increased. These Cephalopod nerves are certainly capable of very considerable extension, but I have no data as to the effect of this on the micro-structure.

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